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Spectral sensitivity and retinal anatomy of the weakly electric fish, *Gnathonemus petersii*

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SPECTRAL SENSITIVITY AND RETINAL ANATOMY OF THE WEAKLY ELECTRIC FISH, *Gnathonemus petersii*.

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

SPECTRAL SENSITIVITY AND RETINAL ANATOMY IN THE WEAKLY ELECTRIC FISH, Gnathonemus petersii.

by

Samuel P. Ciali

Advisor: Professor Peter Moller

The current project explored spectral sensitivity in Gnathonemus petersii under two conditions of light adaptation. Two groups of four fish were exposed to brief (500 msec) flashes of monochromatic light stimuli. The startle response, an immediate and temporary acceleration in electric organ discharge (EOD) rate, was the dependent measure and served as the indice of detection and/or response by the fish.

In both conditions of light adaptation, the fish demonstrated greatest sensitivity to a 525 nm light (λ max). This finding was consistent with Bridges' (1972) nomogram for a freshwater fish species under conditions of dark-adaptation. The fish also exhibited relatively acute sensitivity to long wavelength light. Relative sensitivity among wavelengths

varied between the two conditions of light adaptation with light-adapted fish demonstrating less pronounced relative sensitivity differences among wavelengths compared to dark-adapted fish.

These results suggest that the rod photoreceptors contain visual pigment which is most sensitive to a 525 nm light (i.e., a porphyropsin, vitamin A_2). As such, peak sensitivity (spectral absorbance) is red-shifted to wavelengths longer than 500 nm, the typical λ max for rhodopsinoid, vitamin A_1 pigments found in most marine species. The presence of a second visual pigment type most sensitive to light of about 625 nm is suggested by a second peak located at the long wavelengths and the broad shoulder of relatively acute sensitivity to long-wavelength light. The startle response, as the dependent measure, was found to be graded such that the amount of acceleration in rate of EOD varied as a function of light intensity.

Investigation of retinal anatomy was performed at the level of light microscopy. The results of the histological investigation confirmed McEwan's (1938) original findings for related mormyrid species. The retina contained photoreceptors which were grouped into bundles with some 15-30 receptors per group.

The effects of the light-dark cycle, testing in clear-water conditions, and the intensity of the adapting light were discussed. Environmental conditions and

behavioral ecology were viewed as correlated with the visual system of G. petersii. Mormyrid fish are able to integrate across visual and electric senses environmental information critical to their survival.

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[1] INTRODUCTION

This project explored several aspects of the visual system of Gnathonemus petersii, a weakly electric African freshwater teleost belonging to the family Mormyridae: 1) The spectral sensitivity of dark-adapted and light-adapted animals and 2) photoreceptor anatomy. In light of previous findings regarding the interaction between visual functioning and environmental constraints (Levine and MacNichol, 1980) and known retinal anatomy in representative species within the family Mormyridae (McEwan, 1938), it is possible to generate hypotheses pertaining to the visual capacities of G. petersii:

1) Under dark-adapted conditions, spectral sensitivity curves should approximate curves for other freshwater teleosts employing porphyropsin pigment (Bridges, 1972). These curves show a peak (λ max) at 525 nm with a negative acceleration to 425 nm and a more pronounced negative acceleration to 675 nm. 2) Under light-adapted conditions, the spectral sensitivity curves should resemble those obtained under dark-adapted conditions in terms of peak sensitivity occurring at the same wavelength and the same relative sensitivity among wavelengths. It is assumed that the retina of G. petersii contains a single visual pigment, a porphyropsin (Vitamin A_2 based), which would account for

the correspondance between relative spectral sensitivity curves obtained under different conditions of light adaptation. Curves should differ quantitatively, however, with reduced sensitivity at all wavelengths in the light-adapted condition due to the higher rod threshold.

3) The composition of photoreceptors should be of the bundle-type (as shown by McEwan, 1938) with a single cone cell surrounded by some eight rod cells. Levine and MacNichol (1980) have stated that perhaps an evolutionary advantage of rod/cone retinas, other than color vision, is to provide contrast sensitivity.

The following will provide a general review of the visual system with emphasis on vision in fish, visual adaptations to environmental conditions, and methodologies used to study the visual system in animals.

Adaptation to a unique set of environmental conditions may be inferred most readily in the adaptive sensory specializations of animals. Such specialization can be of two kinds. First, a highly specialized mode of life may be realized via successful integration of an entire range of sensory modalities (the social hymenoptera, for example, are an extreme example of this form of sensory specialization and interdependence).

Sensory specialization may also be confined to a single sensory modality only. This second type of specialization is apparent in the eyes of deep-sea or

cave animals which have to contend with inordinately minuscule amounts of light, or in the electroreceptors of certain fishes, such as the African freshwater mormyrids, which possess the ability to electrolocate and electrocommunicate via self-generated electric organ discharges (Moller, 1980; Hopkins, 1986). A comparative analysis of sensitivity limits may reveal instances of adaptation to special environmental conditions.

Although a visual sense is common to many creatures, the particular morphologies of the visual organs and the unique modes of visual processing vary, in large part, as a function of the demands of each organism's environment. In investigating the qualitative and quantitative aspects of visual systems, emphasis has been placed on assaying the mechanisms underlying absolute light sensitivity, spectral sensitivity, and the ability to make wavelength discriminations.

Although a vast diversity of structures exists, the problem of transducing one form of energy (electromagnetic) to another form (neuronal activity) which can be perceived and utilized by the organism, is common to all. For any visual system ambient light is the stimulus. Mere detection of light energy can be accomplished in less complex life forms (e.g., jellyfish) via ocelli which are no more than groupings of photosensitive pigment cells into vesicles innervated by nerve fibers. The perceptual capabilities of such a

system are limited but the presence of multiple ocelli, each directionally selective, permits increased detection of movements in the environment and greater control over locomotion. Evolution has selected for increased numbers of such visual units. Arthropods developed compound eyes by multiplying ocelli whereas the early vertebrates developed eyes by enlarging ocelli. Enlargement also entailed diversification of structure. The functional vertebrate eye requires a large lens, pupil, and a multicellular retina for reasonable acuity. The advanced vertebrate retina contains various groupings of photo-sensitive elements which selectively respond to different visual stimuli. Such groupings specify particular information, for example, directed/oriented motion in space, or in animals with color vision, the perception of light of particular wavelengths. The dynamics of the visual sensory modality will be discussed further with emphasis on vision in fish.

1.1] VISION IN FISH

The unique aqueous habitat of fish prescribes adaptive morphological and physiological strategies which contrast with those of terrestrial vertebrates. Major support is afforded by a water medium which permits a greater range of size and shape in the eyes. Such support permits long-stalked eyes in deep-sea fish for

example.

1/ Anatomy

The eyes of all vertebrates are set in sockets of the skull called orbits which allows the position of the eyes to differ relative to the posture of the head. In fish, the lateral positioning of the eyes on the body permits a near-panoramic field of view. Each eye embraces more than a hemisphere of the ambient light array such that binocular overlap exists in front, permitting a double registration of the forward field. Light enters the eyes via the cornea, the clear portion of the tough, primarily opaque sclera, the outer covering of the eye. The cornea is a more complex structure in fish than in terrestrial animals consisting of three layers: the autochthonous, the scleral, and the dermal layers. In terrestrial animals, the cornea represents the primary refractive element which, in conjunction with the lens, functions to bend the ambient light rays and focus them onto the back of the eye. In fish, however, the focusing powers of the cornea are minimal for its refractive index is approximately equal to that of the aqueous medium. Instead, the primary refractive element is the lens which is more spherical than in terrestrial animals, an adaptation to magnify its refractive capacity. In fish living close to the water surface the

lens often contains a pigment absorbing in the blue and near ultra-violet range. In deep-sea fish these pigments are not present. In general, freshwater fish possess purple-colored pigments while marine fish possess rose- and golden-colored lens pigments (Bogatyrev, 1966). Accommodation to varying distances of the object can be accomplished by moving the lens towards or away from the retina. In contrast, mammals accommodate by altering the curvature of the lens. Accommodation, regardless of the manner in which it is accomplished, is a muscular reaction to adjust to the 'blur' of the retinal image, a problem with which both terrestrial and aquatic organisms must contend. Bogatyrev (1966), believes that fish are capable of sharp vision over a wide range of distances. By focusing, that is, varying the distance from lens to retina, objects from a near point of 5 cm or less out to infinity can be viewed clearly. It is as yet inconclusive whether most teleosts are emmetropic or hypermetropic in the unaccommodated eye. Pumphrey (1961) states that teleosts are normal-sighted (emmetropic) and that even quite nearby objects viewed laterally should be sharply imaged on the retina, in part, due to the short focal length of the lens. The distance from lens to retina is slightly greater for objects viewed in front of the fish because of the ellipsoid shape of the retina. In order that forward vision be made sharper the distance from lens to retina must be decreased via accommodation

in which the lens is moved posteriorly by the action of the retractor lentis muscle.

Light passes beyond the lens through the vitreous humor which in different fishes ranges in consistency from a liquid to a firm gel. Other ocular structures resemble those in other vertebrates; however, in many fish the choroid contains pigment. The choroid combines the functions of nourishing the retina and of absorbing stray light or reflecting it via a tapetum lucidum back through the retina; a highly adaptive function in various deep-sea and murky-water dwelling fish which results in greater utilization of available light.

The retina is part of the Central Nervous System and is a laminated structure of several cell layers each separated one from the other yet interacting synaptically. The pigment epithelial cells contain melanin granules and in some instances reflecting material. The melanin serves to absorb light quanta which have not already been absorbed by the visual pigment contained in the photoreceptors. In a majority of teleosts the melanin is capable of responding to light by migrating from the epithelial cell nuclei into the outer cell processes forming a pigmented sheath around the photoreceptors. Guanine, a white reflecting pigment, is also often found in teleost retinas. It functions to reflect and scatter light but does not absorb it. Guanine appears to act as a retinal tapetum

in certain species, reflecting light back into the ocular interior.

Bordering the pigment epithelium are the visual cells themselves: the rods and cones. The division of photoreceptors into rods and cones based on morphological and functional distinctions is known as the duplex theory of vision. This distinction of photoreceptor types is apparent throughout the majority of vertebrate retinas including most shallow-water teleosts. In contrast, the retinas of deep-sea fish possess only rods. The presence of multiple cone types (e.g., single and double varieties) is characteristic of a number of fish species as well as of other vertebrates. The distribution of photoreceptors in the retina varies greatly from species to species. In animals with binocular vision a characteristic feature is the presence of a fovea or area of high acuity on the retina. Organisms which possess a center of acute vision normally scan the visual array by successive fixations and eventually fixate the image of interest upon the retina at the point where visual acuity is optimal. In those rare fish species possessing a true fovea, this point is usually located on the posterior border of the retina (an adaptation to laterally positioned eyes) and consists of a concentration of single cones (Pumphrey, 1961). The majority of teleosts do possess some specialized area on the retina in which there typically exists a higher

concentration of cones although such '*area centrali*' would not be classified as foveas in the true sense. Also, pure-rod foveas exist in the retinas of several deep-sea fishes (Pumphrey, 1961).

2/ Visual Pigments

The visual pigment within photoreceptors is the essential and primary constituent in the process of photoreception, for its function is the capture of light quanta. It is the capture of light quanta by photoreceptors which eventuates a conformational change in the photopigment (i.e., bleaching) and eventually results in the hyperpolarization of the photoreceptor membrane. The kinetics of bleaching and regeneration differ between rod and cone photopigments. Visual pigments differ in their spectral absorbance rates and consequently their responses to light of various wavelengths. The dual classification of photoreceptors as rods or cones as well as distinctions among cones is in part due to differences in spectral absorbance rates of the contained pigment. However, rods and cones cannot be differentiated by their spectral absorbance properties alone.

Since little is known regarding cone pigments in general, the following discussion will be confined to rod pigments only. The photopigments found in all mammals,

reptiles, birds and most marine fish are rhodopsins based on retinol (Vitamin A_1) while those found among freshwater fish are porphyropsins based on 3-dihydroretinal (Vitamin A_2). Some fish retinas contain a mixture of both, and in certain species which migrate between fresh and saltwater the pigment is characterized as either porphyropsinoid or rhodopsinoid depending on the current environment.

Spectral absorbance peaks differ across species for the two classes of photopigments and the λ max values are not normally distributed about a single particular wavelength. Rather, λ max values cluster at multiple wavelengths with freshwater fish showing peaks within a range from about 500 to 530 nm while marine fish exhibit peaks between about 475 to 520 nm (Munz, 1971). Porphyropsin pigments often occur in mixtures with rhodopsin pigments and the proportions of the two pigments can be altered within individual fish in response to environmental light levels. Munz (1971), hypothesized that due to the instability of freshwater photic environments, an adaptive visual system may have selective advantage for some freshwater fish. He proposed that since a variable freshwater environment is probably richer in long wavelength light an increased sensitivity to such light (characteristic of porphyropsin pigments) would be adaptive.

The sensation of color is entirely a product of

visual functioning. It is logical to assume therefore that due to the myriad representation of structure and function found within the animal kingdom that not all organisms possess the capacity for sensing color. Furthermore, even within those animals possessing color vision, limitations in sensitivity to different wavelengths provide for tremendous variation in terms of the 'richness' of the color sense for any given species. As always, the role of the environment in imposing restrictions upon phenotypic representation is important to a consideration of color vision. The visual capacities of many organisms, including color vision, have been selected for during the evolutionary history of the species. The parameters of color sense (e.g., the organism's spectral sensitivity) can be viewed as a function of the environmental constraints operating, in part the ambient light array present in the organism's niche.

3/ Photoreceptors

The cone cell is associated with photopic vision, the system employed in bright light, while the rod cell is associated with scotopic vision, a dim-light visual system. The essentially distinct operation of the two systems (duplex theory) has been demonstrated in the dark adaptation curve for human subjects (Hecht, Haig, and

Chase, 1937). During the photopic period of the curve (initial 8-10 min in reduced light) the cone cells reach their maximum sensitivity. The threshold of the rod cells is as yet higher than that of the cones such that the stimulus light is detected by the cones. After 8-10 min in the dark the sensitivity of the rod cells exceeds that of the cones and so the rods are what determine threshold. The ultimate absolute threshold is reached after about 30 min in the dark. During this scotopic period a lack of color vision is evidenced while sensitivity to dim light is maximized. The dark adaptation experiment emphasizes the relative importance of the two photoreceptor systems in bright and dim light.

Rod and cone cells share the same general structural components including an outer segment which projects into the space between the retina and the pigment epithelium; an inner segment containing various organelles; and a synaptic region in the terminal portion of the cell. In most typical cone cells (an exception would be human foveal cones) the outer segment tapers throughout its length such that the relative diameters of the inner and outer segments differ. The result is that cone cells do not appear uniformly cylindrical as do rod cells. Within the outer segment of both rods and cones are found membrane-limited disks which contain the visual photopigments. In part, rods and cones can be distinguished via dissimilarities in their respective

disks. Those of the cones are found to be physically continuous with the outer segment cell membrane while those of the rods are discontinuous with the membrane such that a single disk is distinct from other disks, at least in the outer two-thirds of the outer segment. A constant process of disk renewal occurs in rod cells with older disks being sloughed off at the tip and phagocytized by epithelial cells. Portions of cone cells are known to be phagocytized, as well, however disk renewal does not occur. The terminal segments of cones tend to be large and flattened while those of the rods are small and rounded. Synapses with bipolar and horizontal cells occur within multiple invaginations of the cone terminal but only within a single invagination in rods. Such a clear-cut distinction between rod and cone terminal portions does not exist across species. Some cone cells of most vertebrate groups, including some placental mammals, are joined through part of their length producing what has been termed 'double cones'. The members of each pair of double cones are morphologically distinct and they can normally be distinguished as chief and accessory cones. When the two cone cells of a pair are so morphologically similar as to be indistinguishable they are termed 'twin cones.' Twin cones appear to be a later evolutionary adaptation as they are only found in advanced bony fishes. There also appears to be some correlation between a species

possessing twin cones and being native to a bright-light surface habitat (Walls, 1942). The arrangement of cone cells in the retinas of many fish is often observed to be of some regular mosaic pattern. For example, single cone cells are often found central to a grouping of double cones (McEwan, 1938). Savetichin, Negishi, and Fatehchand (1965) suggest that such organization may be an adaptation to pattern and movement recognition. Numerous other arrangements exist as well. Within the same retina both short single cones and long single cones may be found, as for example in the family Cyprinidae (Savetechin et al , 1965), which further adds to the variety and complexity.

Stell (1975) and Marc and Sperling (1976) found that in goldfish the chief and accessory cones of a double cone were each associated with a different visual pigment based on vitamin A_2. The longer, chief member contained a pigment which maximally absorbed higher wavelengths while the shorter accessory member contained a pigment which maximally absorbed intermediate wavelength light. In addition, goldfish possess short single cone cells which maximally absorb short wavelength light. Microspectrophotometric studies on single cones of goldfish have demonstrated that three types of cones exist and that most likely each type possesses a single visual pigment (Marks 1963, 1965 and MacNichol, 1964). The visual pigments were found to absorb maximally at

about 455, 535, and 625 nm.

The amount and spectral quality of the light which eventually impinges upon the photoreceptors is a function of the amount of absorption, reflection, and scattering ofthe light that has occurred in both the environmental and ocular media. As much as 50% of the light passing through the ocular media does not contribute to vision due to these intervening processes not to mention the extent of primary attenuation due to environmental conditions (e.g., various aqueous suspensions which preferentially absorb some wavelength and not others).

Whether a response (i.e., hyperpolarization) is generated by a photoreceptor depends on the quantity of light-energy photons absorbed. The quantity of photons absorbed is a function of the number delivered and the wavelength. If the photons delivered are of a wavelength to which a given visual pigment is maximally sensitive then a fewer number of such photons will be required to produce a response. The converse is also true; more photons of wavelengths other than λ max are required to produce a response. As such, any photoreceptor will respond to light of various wavelengths (other than λ max) if a sufficient number of photons are delivered. The wavelength of light is of importance only in terms of the probability of absorption being higher at some wavelengths than at others. The response produced by a photoreceptor is not all-or-none but graded such that

response magnitude of the photoreceptor differs as a function of the number of quanta absorbed, and, the probability of absorption is different for different wavelengths. In essence, a visual photopigment is merely a receptive device incapable of providing information as to the spectral distribution of the light stimulus.

1.2] VISUAL ADAPTATIONS TO THE ENVIRONMENT

In water the loss of intensity of light is, in part, a function of the depth through which light passes. This loss of intensity is not constant for all wavelengths of light but differs according to wavelength. The predominant color inherent in water also varies from one aquatic type to another. The contrast between the darkness at great depths and the high intensity light at the surface is apparent but there also exists a continuum of color and intensity gradients at various depths in between. The presence or absence of organic matter in part determines the absorption characteristics of the water. Clear fresh- and marine-water in which little organic matter is present absorbs short and long wavelength light to a greater extent than it does light of intermediate wavelengths. Also, with increases in depth beyond 25 m red and violet light are absent and blue becomes the predominant color (i.e., it is least absorbed). In temperate zone lakes which are much less

clear due to the build-up of dissolved organic matter and the presence of yellow and green phytoplankton, light of all wavelengths is absorbed to a greater extent. Short wavelengths (violet and blue) are most strongly absorbed and the predominant colors are yellow and green (540-560 nm) In marshes, swamps and 'blackwater' rivers, with the addition of tannins, legnins, and other products of complete plant decomposition, light of all wavelengths is absorbed beyond a depth of 3 meters with the exception of long wavelengths (i.e., >600 nm) which are maximally transmitted.

A correlation can be found between the representation of light in a given environment and the particular visual capacities of those organisms inhabiting that environment. A brief survey of the spectral sensitivities of representative fish species inhabiting diverse marine and freshwater environments will be made. The aim will be to provide insight to the particular visual capacities of Gnathonemus petersii.

1/ Spectral Sensitivities of Representative Fish Species

The λ max values between groups of fish can differ either due to the visual pigments of different species not being identical (e.g., two broad categories of visual

pigment already discussed are those based on either retinene 1 (rhodopsins) or retinene 2 (porphyropsins)) or due to variance in the particular retinene/opsin combination. Clarke (1936) first suggested that visual sensitivity might conform to the spectral distribution of available light in a particular habitat. Marine fishes live in a distinctly blue photic environment. Denton and Warran (1956, 1957), Munz (1957, 1958), and Wald, Brown and Brown (1957) demonstrated that the λ max of the visual pigments extracted from deep-sea fish is in the range from 478-490 nm. They concluded that these fish probably have become adapted to the available light in their unique environments.

Levine and MacNichol (1980), surveyed various light zones and the photoreceptors capacities of different fish species inhabiting these zones. They determined four groups of freshwater fish and correlated their color sensitivities with the photic environment of their habitats. Three visual pigments maximally sensitive to violet, blue-green and yellow-green were found to exist in surface dwellers such as the guppy. A second group including the cichlids were also found to possess three visual pigments with sensitivities shifted to longer wavelengths. The bluegill is representative of a third group of fish which inhabit surface 'blackwater' at dawn and dusk. These species possess few blue sensitive cells and are maximally sensitive to green and red light.

Finally the fourth group includes those fish such as catfishes which inhabit low intensity light environments characterized by red-shifted background space light. These species possess rods and cones which are middle and long wavelength absorbing. No short wavelength receptors exist.

Levine and MacNichol (1980) proposed that due to photic conditions in deep water,in which ambient light is confined to a narrow spectral band, selection should have favored visual systems which maximize sensitivity rather than color discrimination. As was mentioned previously, water at various depths and of different qualities exhibits an inherent color (i.e., background space light); those wavelengths not absorbed by the particulate matter in the water. A major visual task for the organism is to discriminate an object (e.g., another fish) from this background space light. A deepwater fish would have the highest possible visual sensitivity if its visual pigment had a peak absorption probability matched to the color of the background space light. Levine and MacNichol (1980) surveyed the absorption spectra of various fish species living at different depths and compared their λ max values with the spectral distribution of the available light in their respective habitats. They found a strong correlation for those fishes living at greater depths. However, for coastal marine and freshwater species the fit was not as good.

Temperate zone fishes inhabiting shallow saltwater were found to be most sensitive to blue-green light (500-510 nm) with yellow local space light (525-550 nm). Freshwater species living at intermediate and deeper depths have a λ max at or below 540 nm while the background light is reddish-orange (600 nm.). Lythgoe (1966), suggested that the observed differences between the λ max values of fish species not inhabiting deep saltwater environments and the characteristic wavelengths of the ambient light might serve to maximize contrast.

The above constraints are operative for those organisms which possess only a single visual pigment class. A clear adaptive advantage exists in those species possessing more than one visual pigment each with a unique λ max value. The result would be increased opportunities for contrast. McFarland and Munz (1975) proposed that a two-pigment visual system has a major advantage over a one-pigment system in that it makes possible discrimination of both bright and dark objects against the background space light. Levine and MacNichol (1980) conclude that the initial advantage of cone cells were probably not related to color vision but derived instead from their better adaptation to bright light. Additionally, the inclusion within the retina of photoreceptors other than the rods, having their own unique λ max, would allow visual contrast across a broad spectral band.

The above survey attests to the intimate relationship between the visual sensitivities of an organism and the available illumination in its habitat. In bright surface waters a full exploitation of visual stimuli (including color) is achieved through the presence of multiple visual pigments each maximally sensitive to a unique wavelength. At greater depths where most of the ambient light is absorbed and perhaps only light of a single narrow spectrum is transmitted (e.g., >600 nm) few species possess more than one visual pigment maximally sensitive to the primary wavelength in that spectrum.

2/ Gnathonemus petersii

The fish species under investigation in the present study is Gnathonemus petersii, a teleost species within the family Mormyridae which inhabits fresh 'blackwater' lakes of central Africa (Gosse, 1963 and Poll, 1933). Being a freshwater teleost it is assumed that its rod pigment is a porphyropsin based on 3-dihydroretinal (vitamin A_2) with a λ max greater than 500 nm. Such a shift in peak absorbance to wavelengths longer than 500nm has been shown to be of adaptive significance due to the prevalence of such light in freshwater environments. Due to the presence of dissolved organic matter and other products of total plant decomposition such as tannins and

legnins in 'blackwater,' the hypothesis that the λ max for this particular species is shifted beyond 500 nm is further suggested. The work of McEwan (1938, see following section) on the retinas of related species in the same family demonstrates that the retinas are of the bundle type with many rods encircling a single cone. No fovea is apparent. This would seem to suggest that acuity is poor and a color sense may only be rudimentary or non-existent. The presence of a tapetum lucidum containing guanine would be adaptive due to the poor transmission of all wavelengths in murky-water environments. The sensitivity of the visual system of G.petersii might, in effect, be heightened when tested under clear water conditions as in the present study.

Based on information regarding the ecology and family's retinal morphology, it could be postulated that this species possesses a rather limited color sense, if at all, due to 1) the turbid conditions of its aqueous niche which would filter out much of the visible light at the lower end of the visible spectrum and 2) the ratio of rods to cones which according to McEwan (1938) is approximately eight to one and 3) that it is a nocturnal species. According to Lythgoe (1966) and McFarland and Munz (1975) a single visual pigment can be adaptive for maximizing contrast if this is combined with the possession of a limited number of cones in the retina with a different λ max from the rods.

3/ Retinal Characteristics of the Family Mormyridae

McEwan (1938) has compiled the most extensive data on retinal characteristics of the Mormyridae to date. Histology was done on two species: Petrocephalus stuhlmanni and Marcusenius macrolepidotus. Both species are native to freshwater streams of Africa.

The pigment epithelium contains both brown and white pigment. The brown pigment which is the normal retinal pigment melanin occurs in small granules and is not plentiful. The white pigment, most probably guanine, is plentiful occurring in all pigment cells. Brown pigment migration or concentration in various parts of the retina as a function of changes in light intensity or adaptation duration did not occur. The most characteristic feature of the mormyrid retina is the arrangement of the photoreceptors into bundles. A bundle-type retina is not unique to these fish but occurs in other families as well, for example, the Hiodontidae and Elopidae (Ali and Anctil, 1976), which include species inhabiting turbid waters. Each bundle in the mormyrid retina is composed of some eight slender rods around a single cone, numerous bundles are grouped and ensheathed in a layer of pigment epithelial cells. Extensive vascularization by tiny capillaries between bundles supplies vital nutrients.

The mormyrid retina is classified as being thin, between 200μ and 230μ, below the teleost average of 247μ. Ganglion cells are not abundant and are scattered and irregularly arranged. The largest constituent of the retina comprises the pigment epithelium which constitutes 63-73% of the thickness of the retina. The inner part of each pigment cell forms a large wedge-shaped projection which together with similar such projections from adjacent pigment cells forms a sheath surrounding a bundle of rod and cone groupings. The effect of a division of photoreceptors into bundles, according to McEwan, seems to be that of limiting the extent to which pigment and visual elements intermingle. It is believed that such an arrangement would be adaptive in those species inhabiting turbid waters since the absorption of light by the pigment will not be as efficient as in a retina in which no bundle arrangement occurs (McEwan, 1938). She concludes that the eyes of these species most probably are incapable of adaptation to bright light and they should possess a poor sense of sight, as evidenced by Teyssedre and Moller (1982).

1.3] EXPERIMENTAL METHODS

Techniques for studying the visual capacities of non-human animals have been derived, in large part, from those used to assess other aspects of animal behavior.

In general, a stimulus is presented to the organism, such as a monochromatic light of a certain intensity, and a particular response either elicited or emitted by the organism is taken as an indice of detection or discrimination. The relationship between stimulus and response can either be unlearned or learned. Unlearned responses are sometimes referred to as reflexive or species-typical. Such responses are readily elicited by an appropriate stimulus, and do not require reinforcement for maintenance, however they may be subject to habituation. Learned responses, in contrast, are acquired through training and are either classically or operantly conditioned. Classical conditioning hinges upon a pre-existing, unconditioned relationship between a particular stimulus (e.g., shock) and a reflexive response such as fear. The conditioned learning is built upon this 'pre-existing' relationship in that a neutral stimulus (e.g., monochromatic light) repeatedly paired with the unconditioned stimulus (shock) comes to elicit the unconditioned response (withdrawal). Alternatively, operant conditioning emphasizes the relationship between the organism's making a response and the consequences of its responding; specifically reinforcement or punishment. Here, the organism learns to respond (e.g., a fish swimming through a hoop) to a particular stimulus in order that reinforcement be delivered or a noxious stimulus be escaped or avoided.

The primary advantage of conditioning techniques is that they can be molded to meet the requirements of the particular problem, whereas, with unlearned responses the experimenter must design testing within a very limited confine demanded by the exacting stimulus conditions which elicit the specific reflex.

Differential responding, in terms of responding to stimulus presence and not to stimulus absence or responding to the favored stimulus and not to the unfavored stimulus becomes, in animal research, the conditioned equivalent of the verbal report. The conditioning paradigm is tailored to promote good stimulus control such that an accurate appraisal of the organism's sensory resolving powers can be assessed.

The following is a limited survey of research designs employing either unlearned or learned responses in the assessment of absolute spectral sensitivity. Under the unlearned response category is included the optomotor response, the preferred motor response, and initial mention of a startle response employed in the present study. Under the learned response category is included classically conditioned heartrate and swimming activity responses and responses operantly conditioned via single-stimulus and forced-choice techniques.

1/ Unlearned Responses

Optomotor Response

Cronley-Dillon and Muntz (1965) employed the optomotor response, the tendency of many animals to visually track a moving object in the field of view, to assess photopic spectral sensitivities in the goldfish (*Carassius auratus*) and the tadpole of the clawed toad (*Xenopus laevis*). The apparatus used was the optokinetic drum, a cylindrical glass container filled with water and enclosed in a second cylinder which was lined with white paper onto which was projected the visual stimulus: alternating black and white stripes. The stripes were made to rotate at an angular velocity of 19.50^{o}/sec. The light source was provided by a 500 W projector lamp and spectral bands were derived from nine interference filters. Intensity was controlled by neutral density filters in increments of 0.2 log units. Absolute thresholds were determined per wavelength and at any given intensity ten trials were given. A particular intensity was considered above threshold when the optomotor response was observed in 80% of the trials. Subjects were light-adapted with a 100 W tungsten bulb for 2 hrs prior to testing. Background light was

provided by a 60 W tungsten bulb projected onto the inner walls of the external cylinder.

The results of the goldfish experiments showed two absorption maxima: one in the region of 615-630 nm and another in the region of 520-535 nm. The first peak was due to cone activity and the second to rod activity. In addition, there was a slight inflection occurring around 460-470 nm. Further experiments were performed to tease out these separate functions. Decreasing the background illumination resulted in a higher peak in the region of 530 nm and gave evidence for the suggestion of rod activity under photopic conditions.

The results of the clawed toad tadpole experiments differed from those of the goldfish most notably in the effect of different levels of background illumination on the shape of the spectral sensitivity curve. For the tadpole, greatest sensitivity in the red region remained constant under both low and high background illumination. Sensitivity was weakest in the blue and blue-green regions.

Some factors should be mentioned as proposed by Jacobs (1981) regarding problems which may result in using the optomotor response: a) getting the animal to attend to the stimulus pattern especially with animals that are untamed or "ill-at-ease", b) determining the precise width of the stripes and their rate of movement which must be set according to the dimensions to which

the particular organism is sensitive, and c) accommodation, for if the subject should fixate beyond or in front of the stimulus pattern the result may be the total absence of any optomotor response.

Preferred Motor Response

Muntz (1962) demonstrated that in frogs (Rana temporaria) a positive phototactic response could be elicited such that subjects preferentially jumped toward lights of predominately short wavelength composition. The apparatus employed was a box with two illuminated windows side by side on one wall. The subjects were placed in front of the windows midway between both, and the window to which the subject jumped was recorded. If after 30 sec no response occurred the subject was prodded. In the first experiment responding to monochromatic lights of equal intensity (via Wratten filters) but different wavelengths was assessed. The average rate the frog jumped to a window of a particular wavelength was recorded. Results showed a strong positive phototactic response with blue light most often chosen over any other wavelength. In a second experiment, employing the same apparatus, blue light was paired with an additive mixture of blue and green and green light alone. Results confirmed a preference for blue light over green light with the

additive mixture of blue and green intermediate in effect. In addition, an illuminated window was consistently chosen over a non-illuminated window.

In another experiment employing a different apparatus the intensity of the monochromatic stimulus was manipulated. In this study a box with a single white disc on one wall was used. The disc was illuminated by the light stimuli. The frog was placed in the box on a given trial at right angle to the disc either facing left or right. Three possible responses could be made: 1) the frog turned and moved towards disc, 2) the frog moved away from disc, 3) the frog moved directly forward at 90^{o} from disc. The results showed a greater number of movements toward the disc when it was illuminated with blue light than with green light. The response frequency to a green light was consistantly low and intensity differences in the light did not seem to be related to response frequency. Even when the intensity of the blue light was decreased to 80% of initial intensity, a greater number of responses were still made to blue than to green light of any intensity.

The authors concluded that these experiments demonstrated a primitive sort of color vision present in this species in which blue light can be distinguished from all other colors independently of both intensity and saturation.

The results obtained using the preferred motor

response will be seen to differ strikingly from those reported by Cronley-Dillon and Muntz (1965) in which the optomotor response was used as the indice of visual functioning in the same species. In their study, it will be recalled, the stimulus which elicited highest responding was in the red region in contrast to a strong preference to blue light as reported by Muntz (1962). These incongruent findings have been explained by suggesting that the neural centers involved in controlling the optomotor response may receive different retinal information from those centers involved in discriminative responses (Jacobs, 1981). Such findings demonstrate the intimate relationship between the methodology employed and results.

Startle Response

Gnathonemus petersii is a species of weakly electric fish belonging to the family Mormyridae. These fish, together with the neotropical gymnotoids, possess a unique electrosensory system and ability to generate weak electric impulses. Lissmann (1958), suggested that these weak discharges were part of a sensory system based on electrical interactions between the fish and its surroundings.

The ability of these fish to 'react' to changes in the peripheral environment with changes in an ongoing

electric organ discharge (EOD) train has been noted. Numerous studies have equated changes in the electrical field surrounding the fish to precise changes in the EOD (see Szabo and Fessard, 1965; Viancour, 1979; and Bastian, 1981 for a review). For example, in the mormyrid fish, G. (Brienomyrus) niger, an analysis of the discharge pattern of individual animals in response to artificial electrical stimuli showed orderly relationships between stimulus and response patterns (Moller and Bauer, 1973).

Alterations in EOD pattern and especially rate resulting from abrupt changes in the visual field of the organism were first reported by Serrier (1974). Specifically, a simple movement such as a wave of the hand across the aquarium wall was sufficient to provoke an acceleration in the EOD rate. Our experiments have demonstrated such instantaneous accelerations in EODs to brief presentations of monochromatic light to dark-adapted G. petersii. Such immediate and reflexive responding to transient visual stimuli is unlearned and highly consistent (relative to stimulus parameters), as such, it lends itself to measurement of absolute spectral sensitivities in these fish.

2/ Learned Responses

Classical Conditioning

1. Conditioned Heartrate

Beauchamp and Rowe (1977) employed a conditioned heartrate technique to measure spectral sensitivity in goldfish (Carassius auratus). A classical conditioning paradigm was used in which a noxious unconditioned stimulus, shock delivered to the tail, was repeatedly paired with a neutral stimulus, light of some wavelength and luminance (the conditioned stimulus). The pairings of UCS and CS continued until the unconditioned emotional response, a change in heartrate, normally elicited by the UCS was regularly elicited by the CS. Between 3-25 pairings of light and shock were required.

Subjects were placed in plastic restrainers and positioned such that the left eye of the fish was at the center of and 5.5 cm away from, a projection screen onto which the monochromatic spot stimuli were shown. Two groups of fish were run under similar conditions except that in one group the fish were immobilized with curare in order to maintain the eyes in a stable position. Subjects were given a series of trials in which both wavelength and luminance varied, also, dummy trials were interspersed in which no stimulus was presented to insure that subjects were responding to light stimuli and not to

extraneous stimuli such as shutter openings or programming apparatus noise. Threshold was designated as that neutral density value in the stimulus beam when the first of a series of positive responses were obtained (UCRs). If no response was elicited by a particular stimulus, shock did not follow and the intertrial interval was shorter than the normal ITI which varied between 40 and 120 sec.

The results demonstrated highest sensitivity to short wavelength stimuli. Results coincided for both groups excepting that the curarized fish had higher absolute sensitivities which was interpreted as 'superior learning ability.'

2. Conditioned Swimming Activity

The photopic spectral sensitivity of the rudd (Scardinius erythrophthalmus) was measured by Northmore and Muntz (1974) using a classical conditioning paradigm in which shock (UCS) was paired with monochromatic moving and stationary bars and diffuse light stimuli of varying wavelengths and intensities (CS). Shock presentation occupied the last 0.5 sec of CS presentation. Swimming activity was monitored throughout the experiment via a thermistor which was embedded in the base of the experimental tank. The unconditioned response to shock was increased swimming activity. Initial training paired shock with a range of high intensity stimuli with an

inter-trial interval of between 1-2 min. During threshold determination prefixed sequences of shock-paired stimuli were interspersed with test trials in which test stimuli were unpaired with shock. Test stimuli which were suprathreshold elicited the conditioned response of increased swimming activity. The results showed high sensitivity at long wavelengths with the moving bar stimulus and a peak sensitivity near 630 nm. Sensitivity was greatest for both moving and stationary bars when the width of the stimulus was between 3^{o} and 5^{o}. With diffuse light stimuli the absolute sensitivity was greatly decreased and pronounced insensitivity was evidenced in the region of 600 nm.

Operant Conditioning

1. Single Stimulus Operant

Tavolga (1976), assessed spectral sensitivity in the goldfish (Carassius auratus) using an avoidance conditioning technique. The CRT of a black and white television was used to deliver diffuse, broad band light stimuli. By a combination of brightness adjustment of the CRT, control of the number of scan lines, and neutral density filters a dynamic range of greater than four log units was achieved. The total light falling on the test box (irradiance) was calculated to be 2.25μW/cm. The

test tank was basically an aquatic shuttle box with a center hurdle the width of the tank over which the fish could swim. A trial sequence began with the onset of light, followed after 10 sec (the CS-US interval) by electric shock (10 V, 60 Hz) delivered through stainless-steel electrodes placed at the long ends of the tank. The form of the electric shock was a 10 msec pulse delivered at the rate of one pulse/sec until the fish crossed the hurdle. Upon crossing, the fish broke an infra-red beam which automatically terminated shock delivery and turned off the light stimulus. A variable intertrial interval (30-120 sec) followed. If the subject did not cross the hurdle during the CS-US interval but only after shock was delivered it was considered an 'escape' response. An 'avoidance' response was one in which the subject did respond during the CS-US interval. The level of reliability of light detection was set and achieved at 80% avoidance over a session of 25 trials. An 'up-down' psychophysical technique was employed such that after each avoidance the light level was lowered for the next response while after each escape the light level was raised. Threshold was determined at the 50% point on the psychometric graph (i.e., midway between the level of an avoidance trial and the next escape level).

2. Forced-choice Technique

Using a forced two-choice operant conditioning paradigm, Muntz and Cronley-Dillon (1966) studied the ability of goldfish (Carassius auratus) to discriminate between different color pairs. Initially, the fish were pretrained to swim to food troughs at the end of the experimental tank. Two troughs were present each attached to one side of the central partition of a discrimination screen. Once the fish were feeding from the troughs regularly training continued using a single trough placed randomly over trials on either side of the discrimination screen. The color stimuli used in the study were 10 x 10 cm metal panels painted on one side with either blue, green, or red. During discrimination training two differently colored panels were placed side by side in front of the discrimination screen with a food trough placed centrally in front of each panel. The fish were trained to make simultaneous discriminations between the colors of the panels with brightness made irrelevant by using multiple panels of the same color but each of a different luminance. Six groups of subjects were trained to discriminate between a single color pair; two groups with blue and green, two groups with green and red, and two groups with blue and red. Additionally, each group of two differed as to which pair-color served as S+. The

position of S+ on either side of the screen partition was randomly sequenced. If the subject chose the correct color it was reinforced with a single food pellet. A pebble was positioned in the trough situated in front of S-. Training continued until the subject reached a criterion of 90% correct choices.

The results showed that all groups reached the 90% criterion of correctly responding to S+ although certain groups attained criterion sooner than others. In general, the red-blue discrimination was mastered in less time than either the blue-green or the red-green. The fish experienced the greatest difficulty learning the blue-green discrimination. Also, the results suggest a color preference for blue since the blue-green discrimination was learned significantly faster when blue was the positive stimulus than when green was positive. These results confirmed that color vision in the goldfish is at least trichromatic since green could be discriminated from both blue and red.

Yager (1967) used conditioned discrimination procedures to investigate spectral sensitivity in C. auratus. Fish were maintained in a light-adapted state by means of a 40 W incandescent bulb and second smaller bulb, about 1/17th the intensity of the first, attached to the underside of the tank cover. While both stayed on for at least 30 sec prior to each trial, the smaller bulb was illuminated at all times except during illumination

of the discriminative stimulus light. A two-lever choice paradigm with discrete trials was used with an initiating response (operating a black bakelite target) required to turn on one of the two stimulus lights and extinguish the adapting lights. Correct responses of operating the illuminated target resulted in presentation of a food pellet. Wavelength and light intensity changes were controlled by manipulating the filter and wedge settings by hand between blocks of trials. A 150 W tungsten coil filament bulb provided a collimated beam of light which passed through the translucent rectangular targets with narrow interference filters to serve as the visual stimuli. Energy levels were varied in steps of approximately .25 log units with from four to six different energy levels covering the entire response range. A criterion of 75% correct responses was used for each of twelve spectral frequencies: 401, 453, 484, 510, 535, 554, 582, 598, 625, 654, 690, and 755 nm in a modified method of constant stimuli. Results indicated that this species is sensitive to light ranging from 401-755 nm and suggestions of secondary maxima at 535 or 554 and 625 or 653 nm.

1.4] GENERATION OF THE PRESENT STUDY

1/ Startle response as behavioral indice to determine spectral sensitivity

The present study investigated spectral sensitivity in the weakly electric fish, Gnathonemus petersii using the fish's sudden and transient acceleration in EOD (startle response) as the indice of detection of the light stimuli.

Serrier (1974) first noted the mormyrid startle response to optical stimuli. Meyer (1982) investigated the behavioral response of weakly electric fish to complex impedances. Electric fish detect the complex impedance of objects in their near field environment. Meyer (1982) used the startle response (first termed novelty response by Szabo and Fessard, 1965) as the basis for determining whether or not a novel stimulus had been detected by a fish. He found that an object introduced into the water in close proximity to the fish elicited large and sustained increases in rate of EOD. He concluded that mormyrids examine feedback from individual EODs to determine the presence of novel objects in their environment.

The results from such investigations as Meyer (1982) prompted the employment of the startle response as a means to assess spectral sensitivity in the present

study. Initial pilot work demonstrated the fish's reflexive acceleration in EOD rate to light stimuli of varying wavelengths and intensities. The experimental paradigm is a non-intrusive psychophysical technique which lends itself well to an assessment of spectral sensitivity in these fish.

EXPERIMENT #1. Psychophysics: Assessment of Spectral Sensitivity under Dark-Adapted and Light-Adapted Conditions

[2] METHODS

2A. DARK-ADAPTED CONDITION

2A.1] SUBJECTS

Four naive African weakly electric fish, Gnathonemus petersii, ranging in size from 10 to 12 cm (standard length) were used in this experiment. When not being tested, the fish were housed communally in a 50 liter aquarium measuring W 21 x H 40 x L 60 cm. The water in the home aquarium was maintained at 23 $\pm$ 2^{o}C, and the water conductivity at 180 $\pm$ 30 μS/cm. The Light-Dark cycle was set at LD = 12:12 hrs with the light phase running from 0900 to 2100 h (40 W GE soft-white bulb suspended 1 m above the opaque-covered aquarium). The home aquarium was housed in a light-tight laboratory cubicle. The fish were fed live tubifex worms weekly. To distinguish among individual fish the anal and dorsal fins were clipped. This procedure had no effect on the fish's discharge activity.

2A.2] APPARATUS

1/ Experimental Aquarium

The light-proof testing cubicle measured W 3 x H 4.5 x L 4 m. The experimental tank was a 20 liter aquarium measuring W 21.3 x H 26.3 x L 35.3 cm. Clear tap water free of particulate matter was used. Water conditions were maintained, as closely as possible, at home tank levels. To exclude visual and electric disturbances the external sides of the tank were covered with aluminum foil. The circular stimulus display window (5 cm in diameter) was cut out of the aluminum foil in the center of one of the narrow sides of the aquarium, 3.8 cm from the base. It was covered with a layer of translucent glass-coated plastic so as to provide a diffusing surface for the light. The water level in the tank was maintained at the 3/4 mark. A submerged corner filter was running at all times. The electric heater was disconnected during testing to prevent any electrical noise from affecting the subject or recording equipment.

2/ Restraining Shelter Tube

The fish were restrained in a clear plastic shelter tube measuring L 15 x D 3.5 cm secured to a clay dish

(H 3.5 cm) situated at the bottom of the experimental tank, and with one end of the tube kept flush with the observation hole. The opposite end of the shelter was covered with cheesecloth to prevent the fish from swimming out.

3/ Recording Electrodes

Affixed to the top surface and at either end of the shelter tube were Ag/AgCl electrodes used to monitor the fish's electric organ discharges (EODs). The EODs were amplified using a stimulus amplifier (Differential AC amp gain=1000 set at 0.3KHZ Hi cut and 3HZ Lo cut) and recorded on one channel of a dual-channel cassette recorder (Sony, Model TC-152SD).

4/ Event Marker

The occurrence of a stimulus was recorded on the second channel in the form of a switch closure produced by the shutter drive and a timer in combination with a 1.5 V battery. Since the event marker (switch closure) was not always represented by a single pulse on channel 2 the first EODs prior to and following the stimulus marker, which exceeded in duration 50 msec, were not considered in the determination of pre- and post-stimulus means.

5/ Spectral Stimuli Presentation

Spectral stimuli were produced by a Bausch and Lomb monochrometer (model # 60CD) situated 33.8 cm from the experimental tank with the beam focused so as to illuminate the display window. The distance between the sidewall of the aquarium and the eye of the fish was approximately 2-3 cm. The bandwidth at half-amplitude was 20 nm (Figure 1). There was no secondary spectrum (i.e., re-entry) when using long wavelengths. A Uniblitz shutter (model EE5L4A2T5) was fastened to the lens of the monochrometer in order to control stimulus duration and was driven using a Uniblitz shutter drive (model 100-2B) and a Hunter timer (model 100100-C) set for 0.5 sec. The shutter was activated by the experimenter using a manual switch. Stimulus intensity was fixed at 110 V by a Variac (model W10MT3W) variable transformer and attenuated by Wratten neutral density filters (1, 2, 3, 0.1, 0.2, 0.3, and 0.6 log).

Table 1 lists the irradiance values in $\mu W/cm^2$ of the eight unattenuated wavelength stimuli as measured with a photometer (UDT model 40A). The probe of the photometer was placed in the path of the light beam and flush with the inside wall of the empty test aquarium (i.e., at the stimulus display window).

Figure Caption

Figure 1. Spectral bandwidth of the monochrometer showing half amplitude (50%) spread = 20 nm for a 700 nm light.

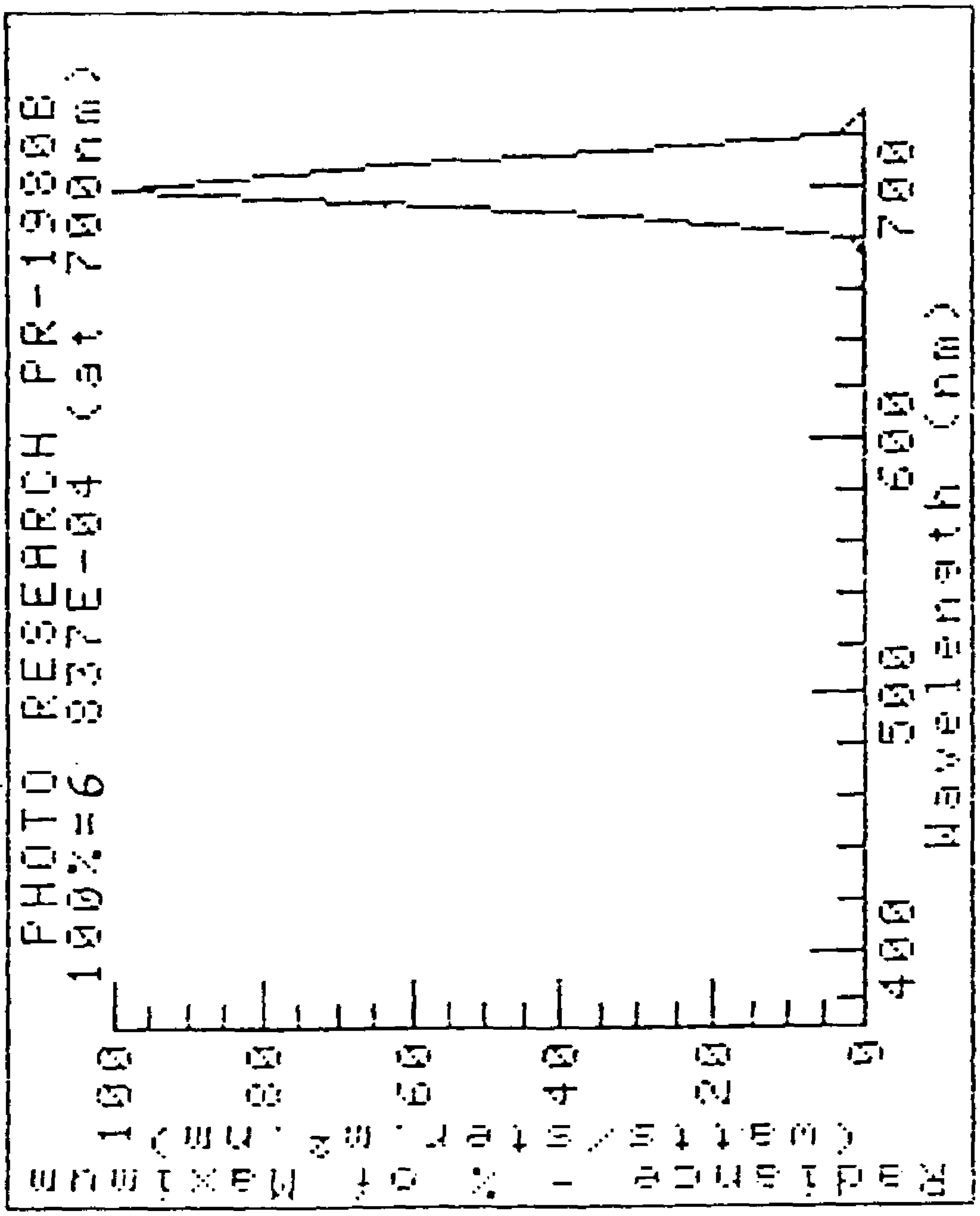


PHOTO RESEARCH PR-1980B
100%=6.837E-04 (at 700nm)
Wavelength (nm)
400
500
600
700
Radiance - % of Maximum (watts/sr.m2.nm)
100
80
60
40
20
0

Table 1

Irradiance values ($\mu W/cm^2$) of the eight unattenuated wavelengths

Wavelength (nm)	Irradiance in μ W/cm^2
425	0.62
475	1.80
525	2.95
575	4.58
625	5.50
675	5.42
725	4.48
800	1.10

2A.3] PROCEDURE

1/ Fish restraint

The four fish were tested during the light period between 1200-1700 hours. An attempt was made to keep all extraneous mechanical and electrical noise to a minimum throughout the entire session. The fish was placed in the experimental tank and inside the shelter tube tail-first such that the head was facing the display window at all times with the eyes approximately 1.5 cm from the display window. The fish was then dark-adapted 30 min prior to actual testing and all testing occurred in the darkened state.

2/ Nature of the startle response

Electric organ discharges (EODs) are ongoing discrete events. Waveform and duration of individual pulses do not vary and are species-typical. The inter-EOD time interval, hereafter referred to simply as EOD interval, which separates two pulses can vary considerably. This interval length variability in the length of the interval is what gives an individual fish its characteristic baseline EOD activity or 'signature'

(Moller, Serrier, and Bowling in prep.). When undisturbed, the fish's sequence of pulse intervals is composed of periods of variable low frequency and periods of stable high frequency EOD activity. This EOD activity is modifiable through both internal and external stimuli and serve in electric communication and electro-location (Moller, 1980; Hopkins, 1986). Stimulation derived from internal and external sources can alter ongoing baseline activity, for example in an accelerated fashion, 'silence' or patterned Sequence of Pulse Intervals (SPIs). The startle response (Serrier, 1974), is a sudden, transient acceleration in EOD rate produced by external stimulation (optical, electrical or mechanical). The time during which EOD activity remains accelerated in response to external stimuli depends on several variables including the fish's physical condition and its prior exposure to stimulus events.

Acceleration of EOD rate is directly analogous to decreases in inter-EOD interval durations. Under the present experimental conditions, it was observed both by listening and by computer analysis of the fish's tape-recorded EOD activity that a decrease in interval duration following stimulus-onset occurred within the first or second interval and extended, in the majority of trials to the fifth or sixth interval. On trials in which the fish responded with the largest acceleration, up to 12 intervals could be affected.

The experimenter initiated each trial at a time when the fish appeared to be discharging at rate of about 3-5Hz, an average daytime activity baseline rate for this species. The introduction of an above threshold stimulus during such a period interrupts this ongoing activity decreasing the time between pulses, the interval durations.

The EOD activity is a manifestation of an ongoing system, responsive to internal and external stimulation, and differs among fish. Post-stimulus activity was evaluated relative to pre-stimulus activity. Changes in the average post-stimulus inter-response time relative to pre-stimulus averages were the basic data used in evaluating the fishes' response to the stimuli.

3/ Stimulus presentation and recording of response

A trial was initiated when the fish was discharging at a low rate of about 3-5 Hz. Prior to the actual experiment, the intensity at which a startle response was obtained on approximately 50% of the trials for each wavelength and for each fish was determined. The intensities used ranged from 0.4 log units below the estimated absolute threshold for the particular wavelength in the dark-adapted state through 0.4 log units above this value, in 0.2 log unit steps giving five

intensity values. A test session consisted of eight trials in which eight different wavelengths were presented, at one of five intensities. Twenty such sessions were given. Each wavelength was presented 20 times, four times at each of the five intensities. The eight wavelengths were ordered according to a modified balanced square design with the first two rows of the 8 x 8 square repeated (rows 9 and 10). Every fish received every row of the balanced square twice. The starting points within the square differed for each fish (i.e., 1st, 5th, 6th, and 10th rows). Four sequences of five rows: A, B, C, and D respectively, were used. The sequencing for each fish was as follows: fish #1=A,C,B,D; fish #2=D,B,C,A; fish #3=B,D,A,C; and fish #4=C,A,D,B. The five intensity values per wavelength were randomly assigned for the first five rows (1 - 5), and again, separately for the second five rows (6 - 10) (Table 2).

During a trial, the stimulus was delivered to the fish for 0.5 sec. The EOD activity of the fish 5 sec prior to stimulus presentation through 5 sec following stimulus offset was recorded on tape and monitored with a loud speaker. An intertrial interval (ITI) followed, the duration of which alternated over trials among 3, 5, and 7 minutes. During the ITI, the stimulus was set for the wavelength and intensity for the next trial.

To insure that the fish responded to the stimulus

Table 2

Balanced square design for stimulus (λ) order

(Dark-adapted condition)

	Row	Wavelength in nm. (Log unit attenuation values)							
A	1	425(3.1)	475(3.3)	800(6.0)	525(4.0)	725(2.8)	575(3.4)	675(3.8)	625(3.8)
	2	475(2.9)	525(4.4)	425(2.9)	575(4.2)	800(6.0)	625(3.2)	725(2.4)	675(3.6)
	3	525(4.2)	575(3.6)	475(3.1)	625(4.0)	425(2.5)	675(3.2)	800(6.0)	725(2.6)
	4	575(4.0)	625(3.4)	525(4.6)	675(3.4)	475(2.7)	725(2.2)	425(2.7)	800(6.0)
B	5	625(3.6)	675(3.0)	575(3.8)	725(2.0)	525(3.8)	800(6.0)	475(3.5)	425(2.3)
C	6	675(3.2)	725(2.6)	625(4.0)	800(6.0)	575(3.6)	425(2.5)	525(4.2)	475(3.1)
	7	725(2.4)	800(6.0)	675(3.6)	425(2.9)	625(3.2)	475(2.9)	575(4.2)	525(4.4)
	8	800(6.0)	425(3.1)	725(2.8)	475(3.3)	675(3.8)	525(4.0)	625(3.8)	575(3.4)
	9	425(2.3)	475(3.5)	800(6.0)	525(3.8)	725(2.0)	575(3.8)	675(3.0)	625(3.6)
D	10	475(2.7)	525(4.6)	425(2.7)	575(4.0)	800(6.0)	625(3.4)	725(2.2)	675(3.4)

Note. A sequence consists of five adjacent rows and the direction of each sequence is indicated by the arrows.

and not to any extraneous variables, such as shutter opening or tape recorder operation, each session included an 800 nm stimulus at 6 log attenuation of maximum intensity. Pilot studies have shown that the fish do not exhibit the startle response to this stimulus.

2B. LIGHT-ADAPTED CONDITION

2B.1] SUBJECTS

Four naive African weakly electric fish, Gnathonemus petersii were used in this experiment. Maintenance of the subjects was the same as for Experiment #1A.

2B.2] APPARATUS

1/ Adapting Light

The light-proof testing cubicle and experimental tank was the same as used in Experiment #1A. An adapting light was housed in a cardboard fixture (W 21.25 x H 45 x L 36.25 cm) fitted firmly atop the test tank. It was lined with aluminum foil on all sides and perforated at various points along the sides approximately 6 cm from

the bottom to provide ventilation. The area around these perforation points (approx. 2 cm^2) inside the fixture was painted black to minimize loss of light. Additionally, a slit (W 1 x L 10 cm) was cut at the top of the fixture for ventilation, the area around which (approx. 10 cm^2) inside the fixture was painted black. As a further prevention of light loss, black cardboard slats (W 5 x L 13cm) were secured at one end on the outside surface of the fixture wherever there were ventilation holes. The adapting light fixture housed two 7.5 W tungsten light bulbs which were suspended 5 cm from the top of the fixture. A cardboard sheet (W 21.3 x H 0.6 x L 36.2 cm) was used to mount a plate of heat absorbing glass (W 15 x L 15 cm) and two Kodak 78A filters (W 7.5 x L 10 cm) for the purpose of achieving a color temperature of approximately 5000^{o}K. The entire adapting light fixture rested atop the test aquarium upon a thin sheet of opaque white plastic which over-extended the dimensions of the tank and acted as a light diffuser. The illuminance from the adapting light as measured at the water level inside the empty experimental chamber was 1.42 foot candles.

The experimental shelter tube was the same as used in Experiment #1A.

Spectral stimuli were produced in the same way as in Experiment #1A.

2B.3] PROCEDURE

Pilot studies were performed to determine the approximate threshold intensities for each of the seven wavelengths as done in Experiment #1A. A control stimulus (800 nm light at 6 log attenuation of maximum intensity) was used in each session as in Experiment #1A. The fish were tested during the same period of the Light-Dark cycle as in Experiment #1A. After placing the fish in the experimental tank it was light-adapted 30 min prior to actual testing. During a session the adapting light remained on continuously and ambient light in the cubicle was low. EOD activity was amplified, monitored and recorded on tape as in Experiment #1A.

Presentation of light stimuli and number of trials was the same as in Experiment #1A.

The eight wavelengths were ordered according to the modified balanced square design shown in Table 3 and every fish received every row of stimuli twice.

ITI durations were the same as in Experiment #1A.

2C. DATA ANALYSIS (Experiments #1A and B)

2C.1] COMPUTER ANALYSIS OF DATA

The temporal patterning of tape recorded EODs was analyzed using an Apple II+ computer and a scanning

Table 3.

Balanced square design for stimulus (λ) order

(Light-adapted condition)

	Row	Wavelength in nm. (Log unit attenuation values)							
A	1	425(0.8)	475(1.0)	800(6.0)	525(0.9)	725(0.8)	575(0.4)	675(1.0)	625(0.8)
	2	475(0.6)	525(1.3)	425(0.6)	575(1.2)	800(6.0)	625(0.2)	725(0.4)	675(0.8)
	3	525(1.1)	575(0.6)	475(0.8)	625(1.0)	425(0.2)	675(0.4)	800(6.0)	725(0.6)
	4	575(1.0)	625(0.4)	525(1.5)	675(0.6)	475(0.4)	725(0.2)	425(0.4)	800(6.0)
B	5	625(0.6)	675(0.2)	575(0.8)	725(0.0)	525(0.7)	800(6.0)	475(1.2)	425(0.0)
C	6	675(0.4)	725(0.6)	625(1.0)	800(6.0)	575(0.6)	425(0.2)	525(1.1)	475(0.8)
	7	725(0.4)	800(6.0)	675(0.8)	425(0.6)	625(0.2)	475(0.6)	575(1.2)	525(1.1)
	8	800(6.0)	425(0.8)	725(0.8)	475(1.0)	675(1.0)	525(0.9)	625(0.8)	575(0.4)
	9	425(0.0)	475(1.2)	800(6.0)	525(0.7)	725(0.0)	575(0.8)	675(0.2)	625(0.6)
D	10	475(0.4)	525(1.5)	425(0.4)	575(1.0)	800(6.0)	625(0.4)	725(0.2)	675(0.6)

Note. A sequence consists of five adjacent rows and the direction of each sequence is indicated by the arrows.

program developed by Jacques Serrier of the CNRS laboratory in Gif-sur-Yvette, France. The presence or absence of changes in the fish's EOD activity in response to a stimulus was assessed in terms of inter-EOD interval durations (in msec). Figure 2 shows exerpts from a real-time print-out of a fish's EOD activity during 2 trials from an actual experimental session. The event marker (switch closure) is represented by a small cluster of points (each less than 50 mHz) appearing in near proximity to the X-axis in this figure. During a single trial, both switch closure (i.e., stimulus onset) and switch release were recorded on the magnetic tape and both may be seen in the figure. Pre-stimulus EOD activity (represented by points prior to the first event marker of a trial) may be seen to be unaccelerated and of a typical pattern for this species. Trial "A" in Figure 2 shows an acceleration in EOD (i.e., inter-EOD intervals of briefer duration) as a result of onset of a stimulus detected by the fish. Trial "B" shows no acceleration in EOD as a result of onset of a stimulus not detected by the fish. These inter-EOD intervals (represented by each point's magnitude on the Y-axis in this figure) were transformed into digital form and used to analyze EOD activity per trial.

Figure Caption

Figure 2. EOD activity (interval duration in msec) for two trials: trial A = EOD acceleration to stimulus onset, trial B = no EOD acceleration to stimulus onset. Stimulus onset is identified by intervals of very brief duration (< 50 Hz).

Note. Low EOD values which occur simultaneously with stimulus onset and offset are artefacts due to electrical pick-up from the shutter controllers.

EOD Interval Duration (ms)
600
400
200
0
on
off
A
on
off
B
Time: 5s

1/ Determination of number of intervals to be analyzed

In order to compare the fish's EOD activity prior to and following stimulus onset, the appropriate number of inter-EOD intervals to use for calculation of mean pre- and post-stimulus interval durations had to be determined. This number had to be sensitive to small as well as large changes in single interval durations. A sample of the data, representative of all stimuli at each attenuation level, obtained under the dark-adapted condition was tested. Means were calculated based on one, three, five and 10 pre- and post-stimulus intervals. A comparison of pre- and post-stimulus EOD activity was performed in two ways: 1) the mean post-stimulus activity was expressed as a percentage of the mean pre-stimulus activity, and 2) mean post-stimulus activity was expressed as an absolute difference from mean pre-stimulus activity.

Mean percentage data

The relative decrease in mean interval duration following stimulation, as compared to the pre-stimulus mean interval duration was greatest when five intervals were averaged. These results are presented in Figure 3a. For the five-interval set the average of all post-stimulus sample means amounted to 52% of the pre-stimulus

average. The other three sets of intervals (1, 3, and 10) showed smaller changes in magnitude.

The startle response is a transient response, manifesting itself in a short-lived train of post-stimulus intervals. These results indicate that as the number of intervals used increases beyond five, the magnitude of the response measure is reduced. The most pronounced effect in the startle response is limited to an evaluation of five post-stimulus intervals. Beyond the fifth interval, the discharge rate approaches average baseline. With the inclusion of more than ten intervals the post- to pre-stimulus percentage ratio would approach 100%.

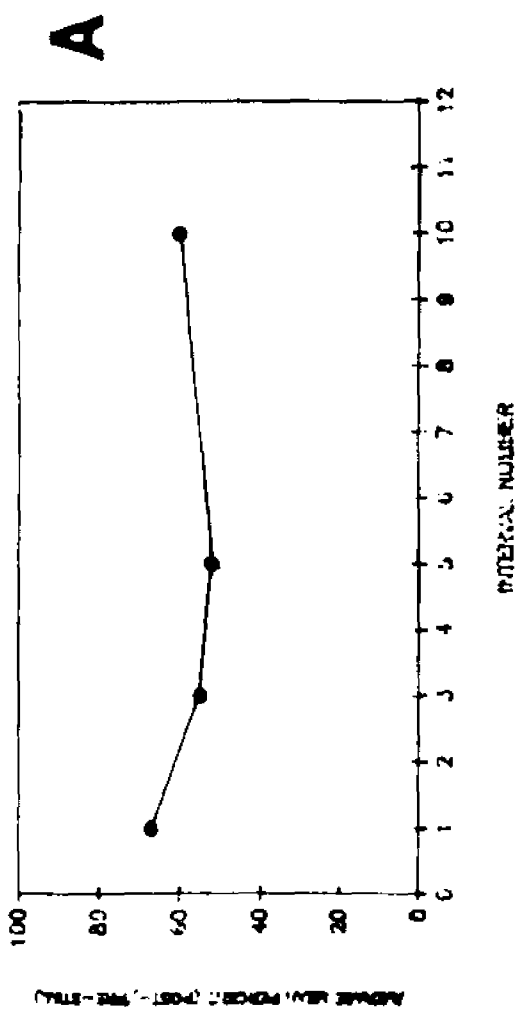
Mean absolute difference data

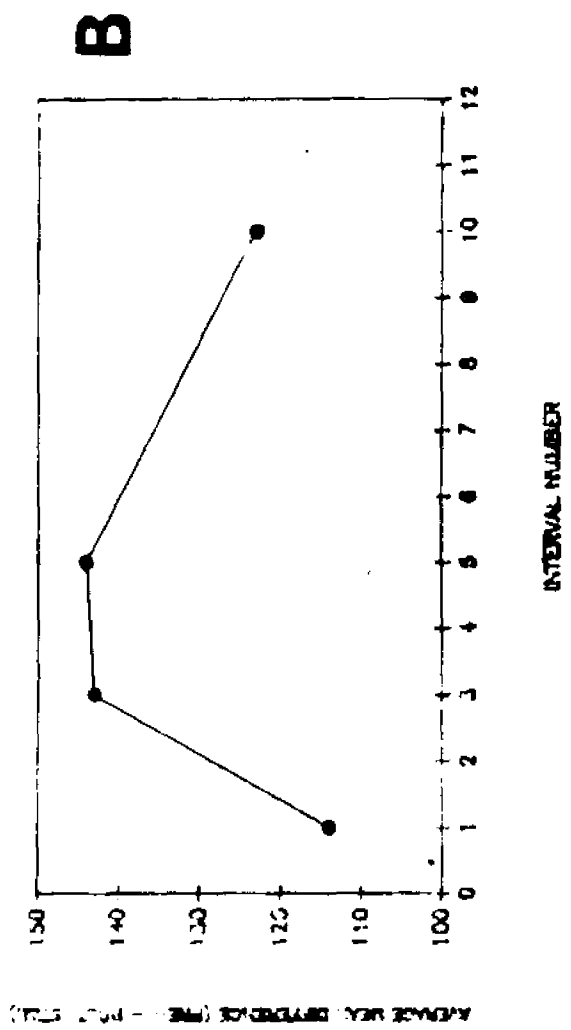
The average difference between pre- and post-stimulus interval duration means as a function of interval set is presented in Figure 3b. The results concur with those obtained using mean percentages. The greatest was obtained when mean differences were derived from five pre- and post-stimulus intervals. The results using three intervals were nearly identical. The mean differences decreased when sets of one or ten intervals were compared.

This analysis again indicates that the set of intervals in which the startle response is best absorbed

Figure Caption

Figures 3a and b. Average mean percent as a function of the number of pre- and post-stimulus intervals (1,3,5, and 10) used in calculation (3a). Average mean difference as a function of the number of pre- and post-stimulus intervals used in calculation (3b).


A
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12
INTERVAL NUMBER


B
150
140
130
120
110
100
0 1 2 3 4 5 6 7 8 9 10 11 12
INTERVAL NUMBER

is five. Therefore, this set of intervals was used to determine spectral sensitivity.

2/ Determination of spectral sensitivity

For each trial the data were expressed as the relative change in EOD (post- / pre-stimulus averages of five intervals). This will be referred to as percent response. The four trials for each fish at each wavelength and each attenuation level were averaged. These data were plotted as mean percent response against log intensity expressed in $\mu W/cm^2$. The data were plotted separately for individual fish for each wavelength (the separate wavelength functions contained five points corresponding to percent response for each of the five attenuation levels).

A percent response value of 75 was used to determine the absolute limen for each wavelength. Combined data for the four fish under the dark-adapted condition yielded separate wavelength functions for which the slopes were comparable. However, the slopes of the percent response curves for individual fish in both conditions of light adaptation differed substantially. Therefore, for these curves, the threshold values for each fish at each wavelength were determined by sliding

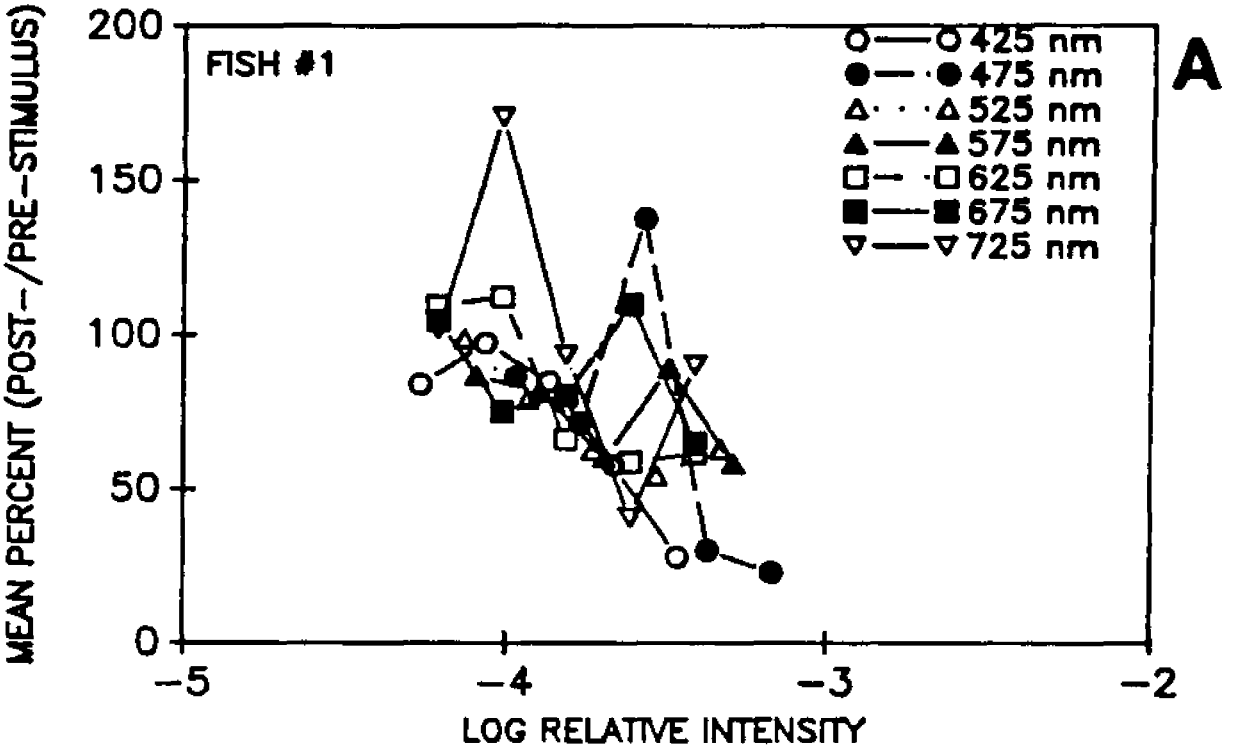
the individual wavelength functions horizontally to visually superimpose them onto the function for the 525 nm light. This was done also for the combined data.

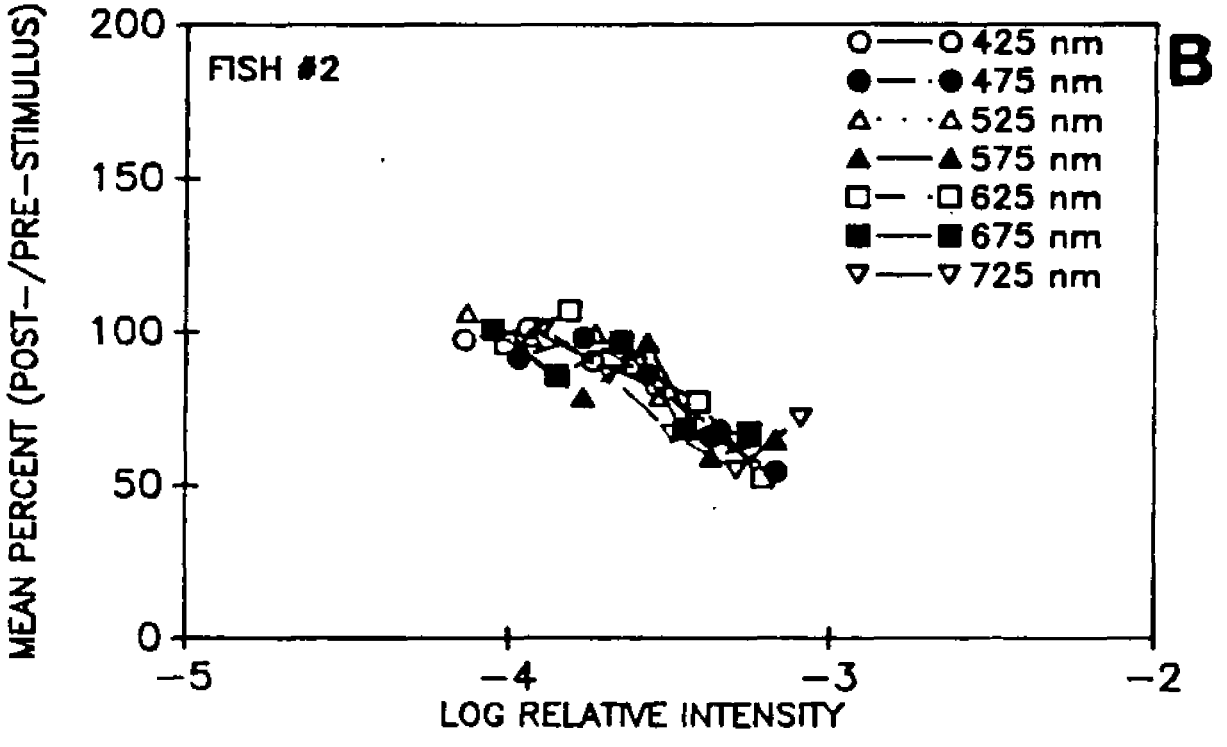
Shifted mean percent response values per wavelength as a function of log relative intensity for individual fish in two conditions of light adaptation are presented in Figures 4a, b, c and d, and 5a, b, c, and d. These curves demonstrate an inverse relationship between mean percent response and log relative intensity. As stimulus intensity decreased percent response (post-/pre-stimulus interval) approached 100%. As such, it can be concluded that the startle response is a graded response in these fish. This relationship is more rigid in dark-adapted fish, reflecting the light-adapted fish's increased variability in EOD activity under light conditions.

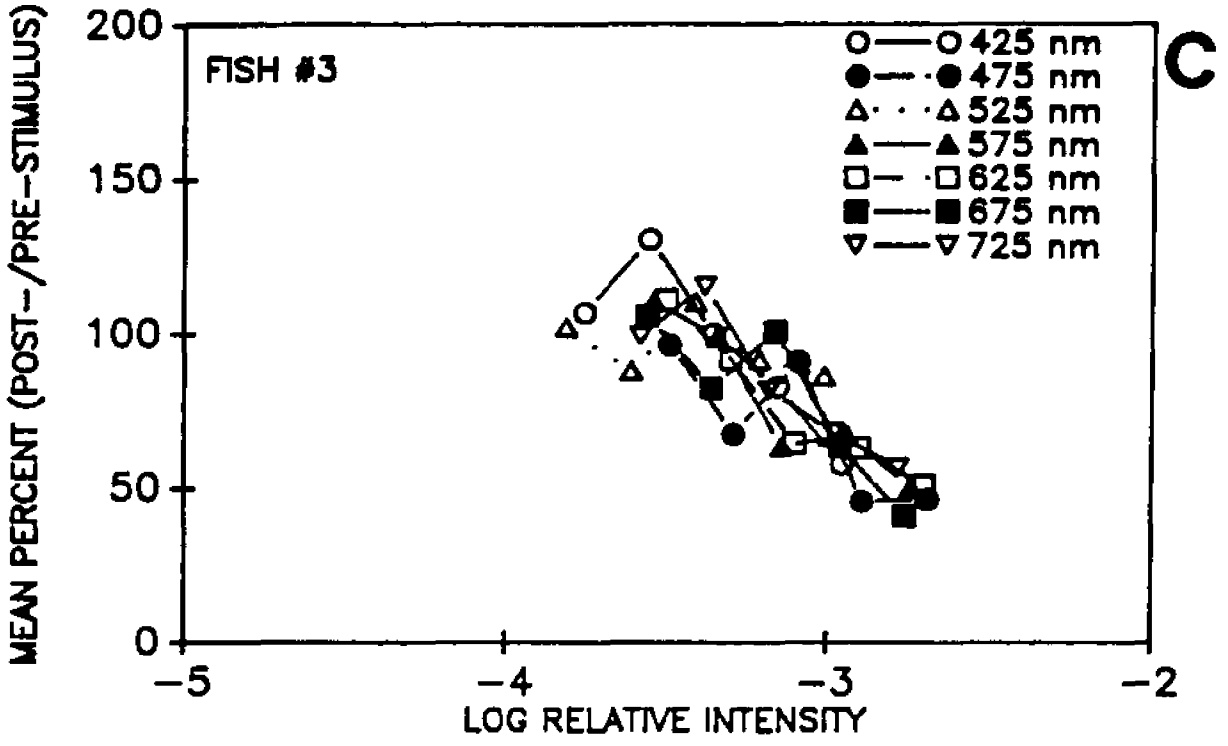
The shift in log relative intensity required in order that each wavelength's percent response curve could be superimposed onto that of the 525 nm light was used to estimate log relative spectral sensitivity (in energy units) for individual fish in both conditions of light adaptation. Averages of individual fish sensitivities per wavelength were used to generate log relative spectral sensitivity (in energy units) curves for the groups.

Figure Caption

Figures 4a, b, c, and d. Mean percent response as a function of intensity of stimulus light for dark-adapted fish.


FISH #1
425 nm
475 nm
525 nm
575 nm
625 nm
675 nm
725 nm
A
MEAN PERCENT (POST-/PRE-STIMULUS)
200
150
100
50
0
-5
-4
-3
-2
LOG RELATIVE INTENSITY


FISH #2
425 nm
475 nm
525 nm
575 nm
625 nm
675 nm
725 nm
B
MEAN PERCENT (POST-/PRE-STIMULUS)
200
150
100
50
0
-5
-4
-3
-2
LOG RELATIVE INTENSITY


FISH #3
C
425 nm
475 nm
525 nm
575 nm
625 nm
675 nm
725 nm
MEAN PERCENT (POST-/PRE-STIMULUS)
200
150
100
50
0
−5
−4
−3
−2
LOG RELATIVE INTENSITY

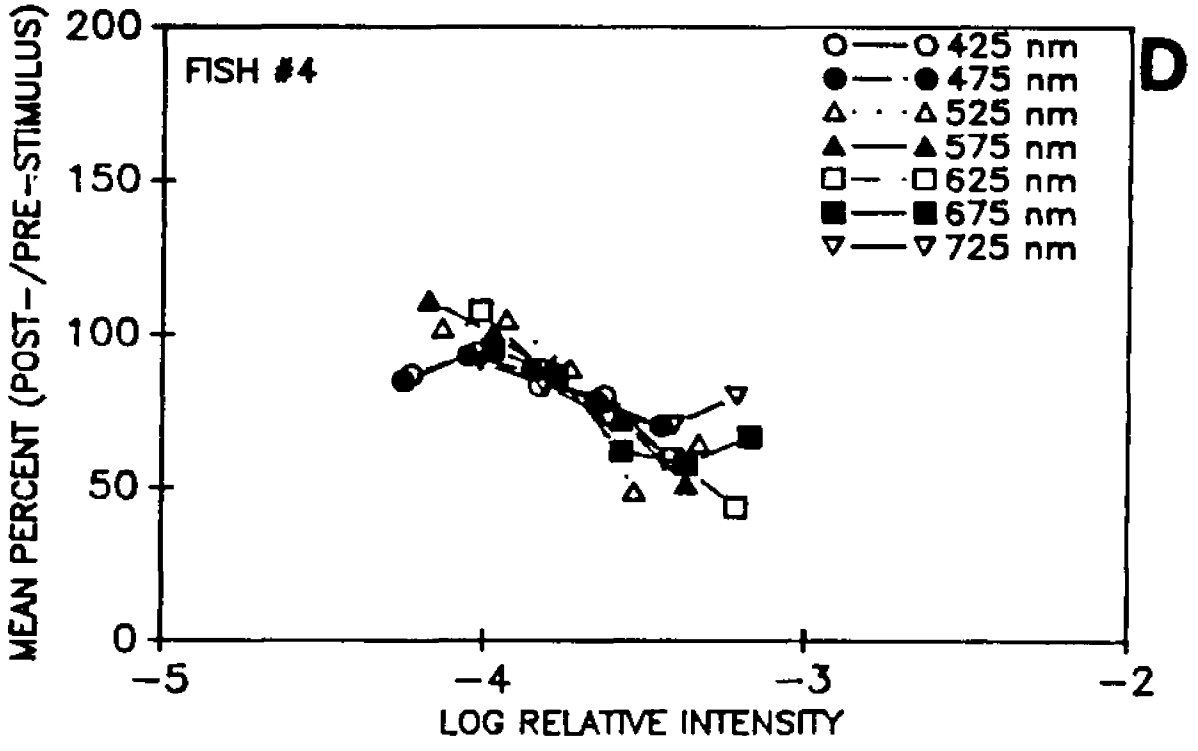
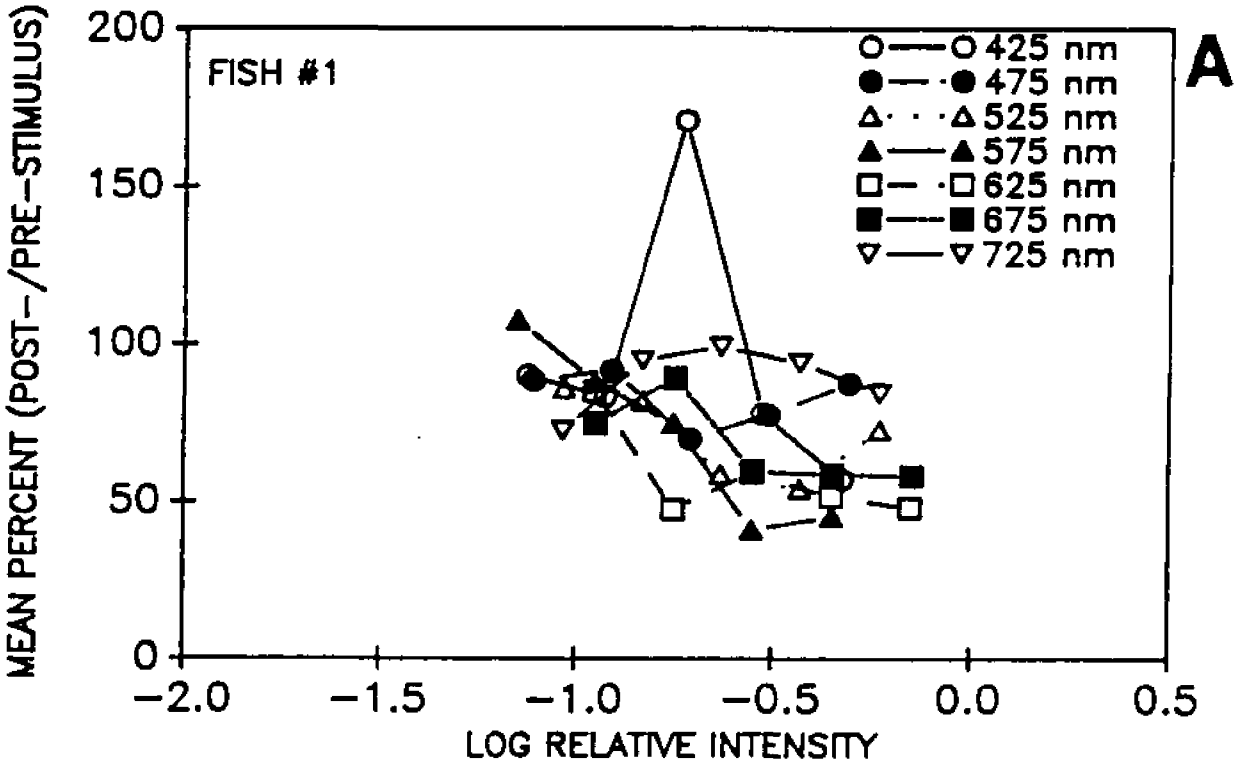
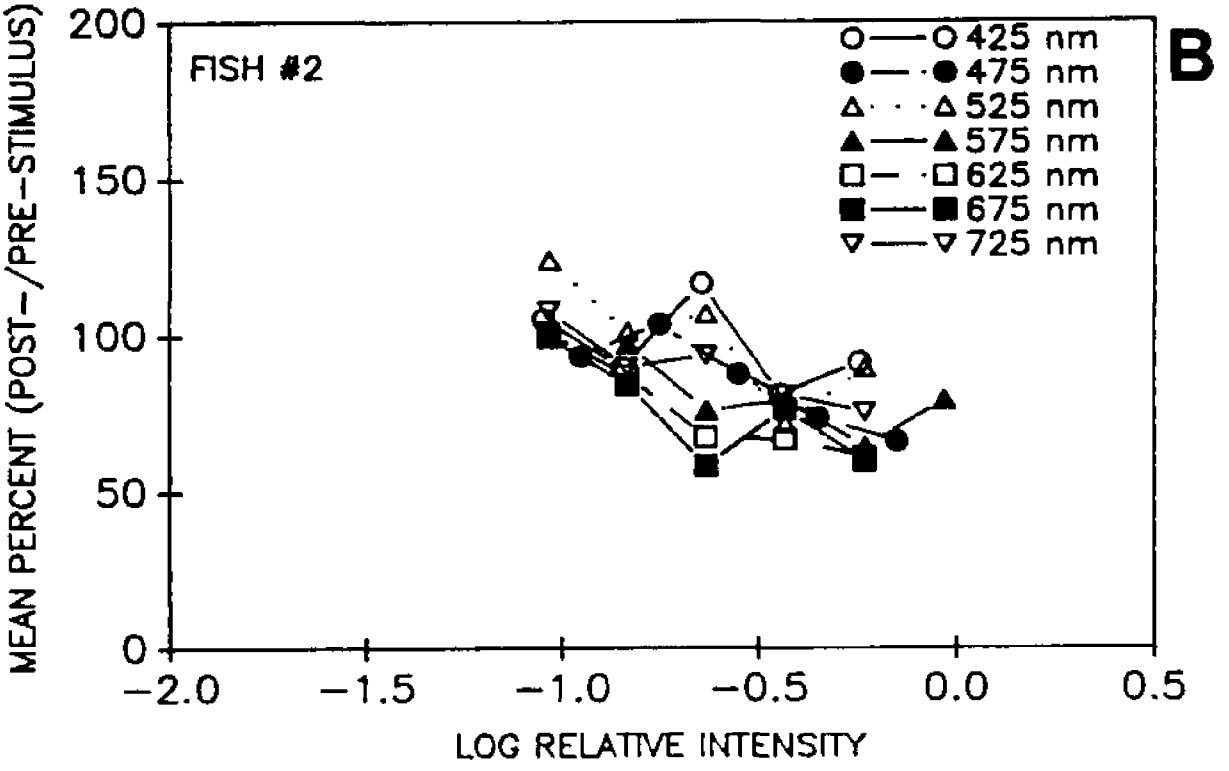
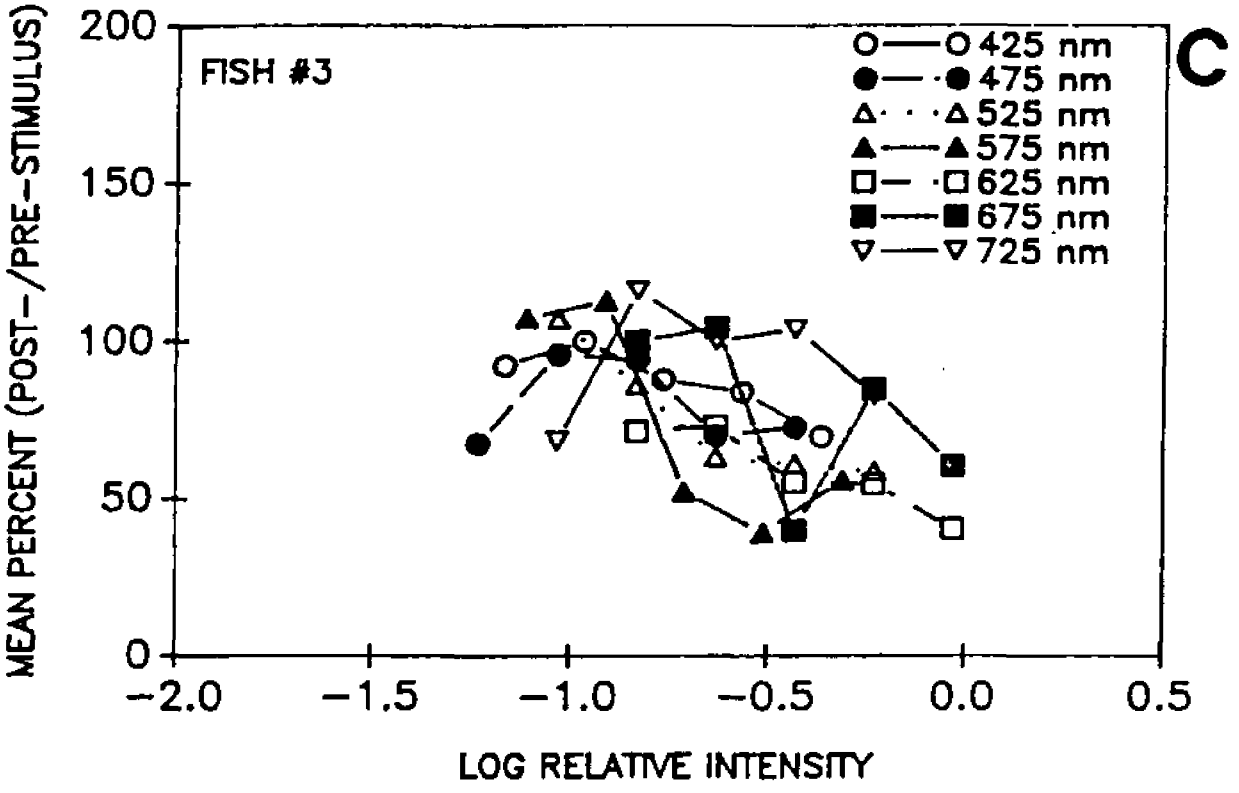

FISH #4
D
425 nm
475 nm
525 nm
575 nm
625 nm
675 nm
725 nm
MEAN PERCENT (POST-/PRE-STIMULUS)
200
150
100
50
0
−5
−4
−3
−2
LOG RELATIVE INTENSITY

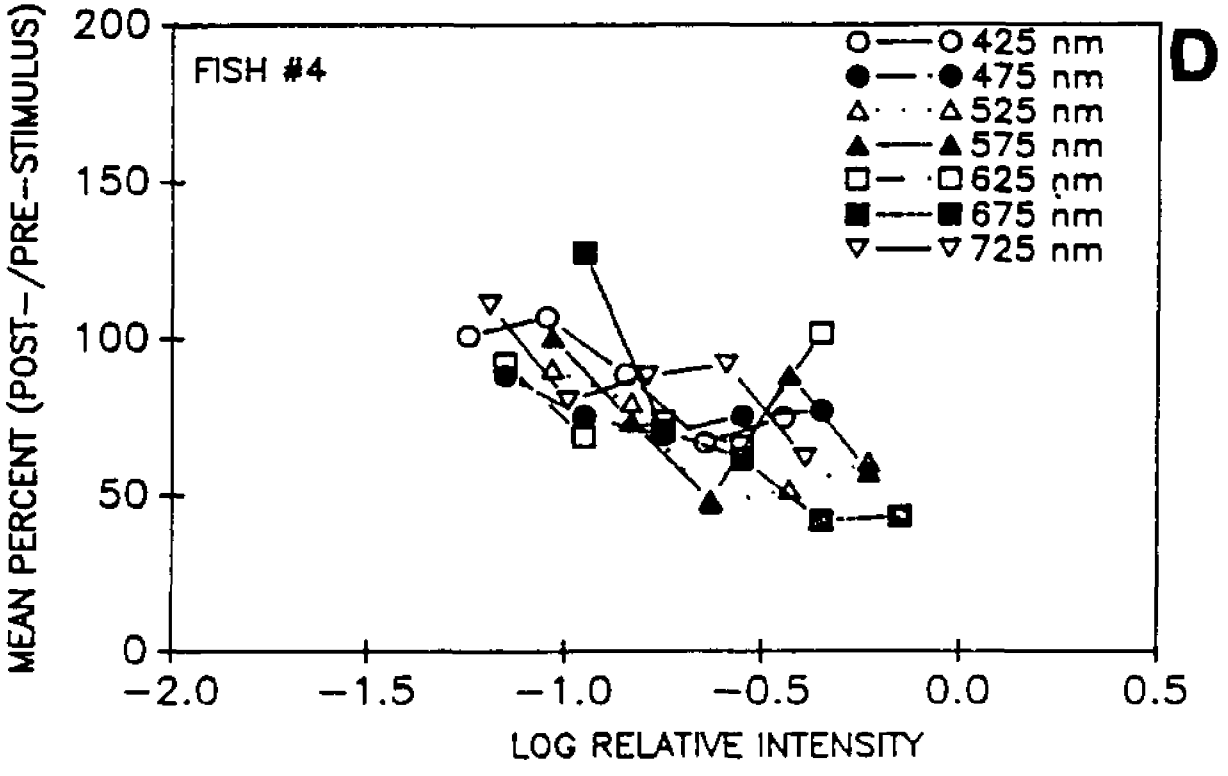
Figure Caption

Figures 5a, b, c, and d. Mean percent response as a function of intensity of stimulus light for light-adapted fish.


FISH #1
A
425 nm
475 nm
525 nm
575 nm
625 nm
675 nm
725 nm
MEAN PERCENT (POST-/PRE-STIMULUS)
200
150
100
50
0
−2.0
−1.5
−1.0
−0.5
0.0
0.5
LOG RELATIVE INTENSITY


FISH #2
B
425 nm
475 nm
525 nm
575 nm
625 nm
675 nm
725 nm
MEAN PERCENT (POST-/PRE-STIMULUS)
200
150
100
50
0
−2.0
−1.5
−1.0
−0.5
0.0
0.5
LOG RELATIVE INTENSITY


C
FISH #3
425 nm
475 nm
525 nm
575 nm
625 nm
675 nm
725 nm
MEAN PERCENT (POST-/PRE-STIMULUS)
200
150
100
50
0
−2.0
−1.5
−1.0
−0.5
0.0
0.5
LOG RELATIVE INTENSITY


D
FISH #4
425 nm
475 nm
525 nm
575 nm
625 nm
675 nm
725 nm
MEAN PERCENT (POST-/PRE-STIMULUS)
200
150
100
50
0
−2.0
−1.5
−1.0
−0.5
0.0
0.5
LOG RELATIVE INTENSITY

3/ Statistical analysis of the data

Analysis of Variance

1. 1-way ANOVAs.

One-way ANOVAs for repeated measures were performed on the data from Experiment #1A to determine whether there were any effects of serial order. The data for a single wavelength and intensity were analyzed across sessions. Tukey post hoc comparisons of significant mean differences ($p<0.05$) were performed.

2. 3-way ANOVA.

A three-way ANOVA with repeated measures for factors B (wavelength) and C (intensity) was performed on the entire data set (Experiments #1 and #2) to determine any main effects for 1) light-adaptation condition, 2) wavelength, 3) intensity, and interaction effects. Tukey post hoc comparisons of significant mean differences ($p<0.05$) were performed.

2C.2] AUDITORY JUDGMENT DATA

As a second means of examining the fish's response to the test stimuli the experimenter listened to the changes in ongoing rate of EOD activity at stimulus

onset. Since it was EOD rate and not inter-EOD interval duration which could be evaluated, only three types of responses could be detected in this way: 1) no response (no observed change in EOD rate), 2) response of small magnitude (slight change in EOD rate) and 3) response of large magnitude (substantial increase in EOD rate). These classification-type data (yes/no responses) were also used to generate spectral sensitivity curves.

On-site observation and data collection were useful for these reasons: 1) observation of daily fluctuations in the fish's EOD baseline activity could be made providing informative data about physiological state, and 2) spectral sensitivity data based on on-site judgement could be compared with those derived from computer analysis of tape recorded EOD activity.

The absolute limen per wavelength was identified as the lowest intensity level at which the fish responded in two of the four presentations with an audible acceleration in EOD. These separate wavelength absolute limen were plotted together to derive spectral sensitivity functions (see figures 8 and 11). The highest absolute limen per wavelengths shared by two out of four fish were used to generate group spectral sensitivity functions.

[3] RESULTS

The following section presents spectral sensitivity results obtained from dark-adapted and light-adapted fish.

3.1] SPECTRAL SENSITIVITY RESULTS (COMPUTER-ANALYZED)

3.1A. RESULTS FOR DARK-ADAPTED FISH

1/ Spectral sensitivity

Figure 6 presents log relative sensitivity as a function of wavelength for the individual fish and the group average. The data for the four fish are quite similar and mirror the group average. Peak sensitivity occurred to the 525 nm light and sensitivity decreased progressively through the short and long wavelengths.

Log relative spectral sensitivity as determined from the group average is presented again in Figure 7 with Bridges' nomogram (1972) for a porphyropsin pigment (corrected from quantal to energy units) superimposed for a best fit of the points. The greatest deviation between the present data and those of Bridges' occurs at the long wavelengths. Sensitivity remains relatively strong for

Figure Caption

Figure 6. Log relative spectral sensitivity as a function of wavelength for dark-adapted fish.

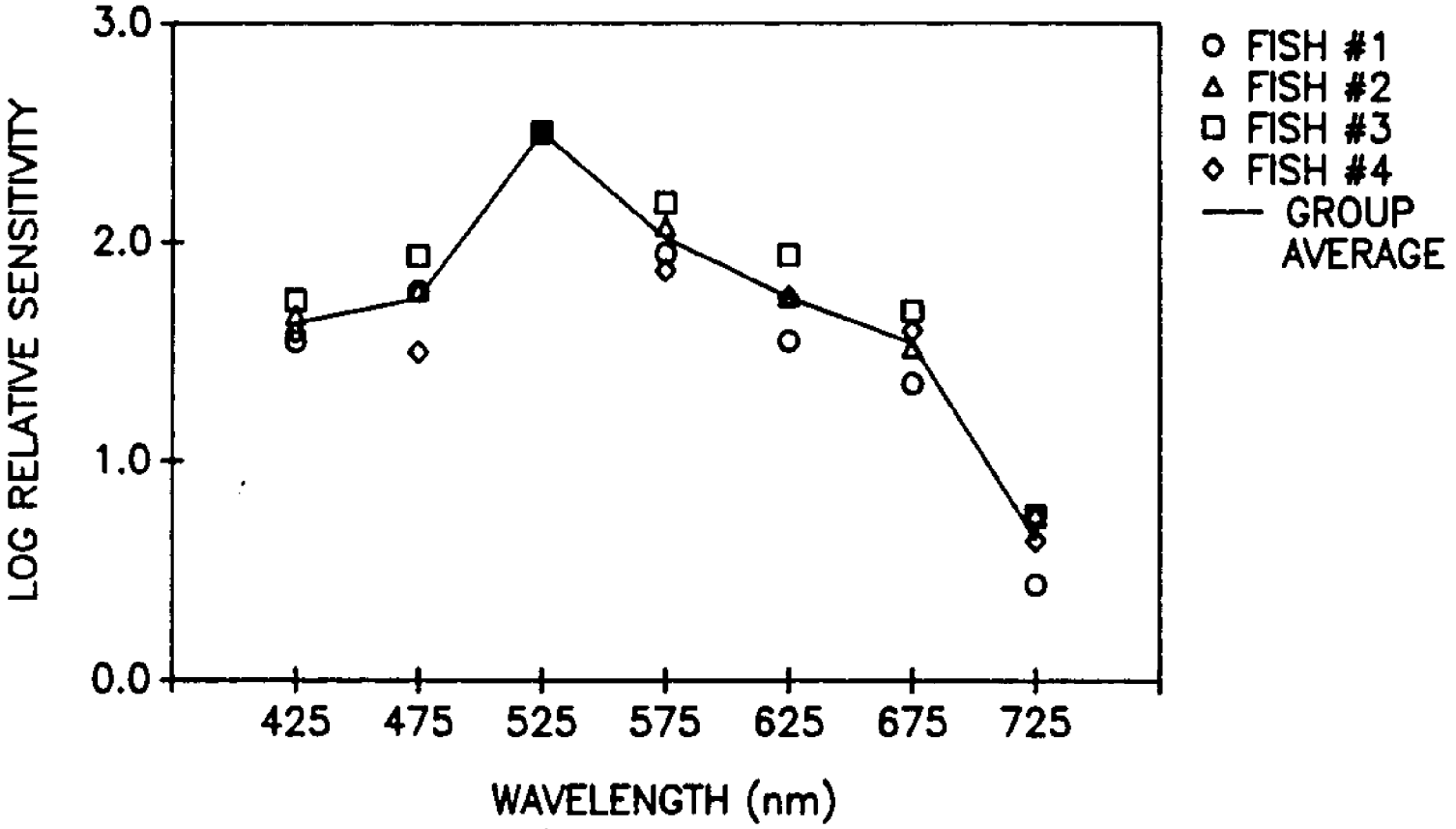
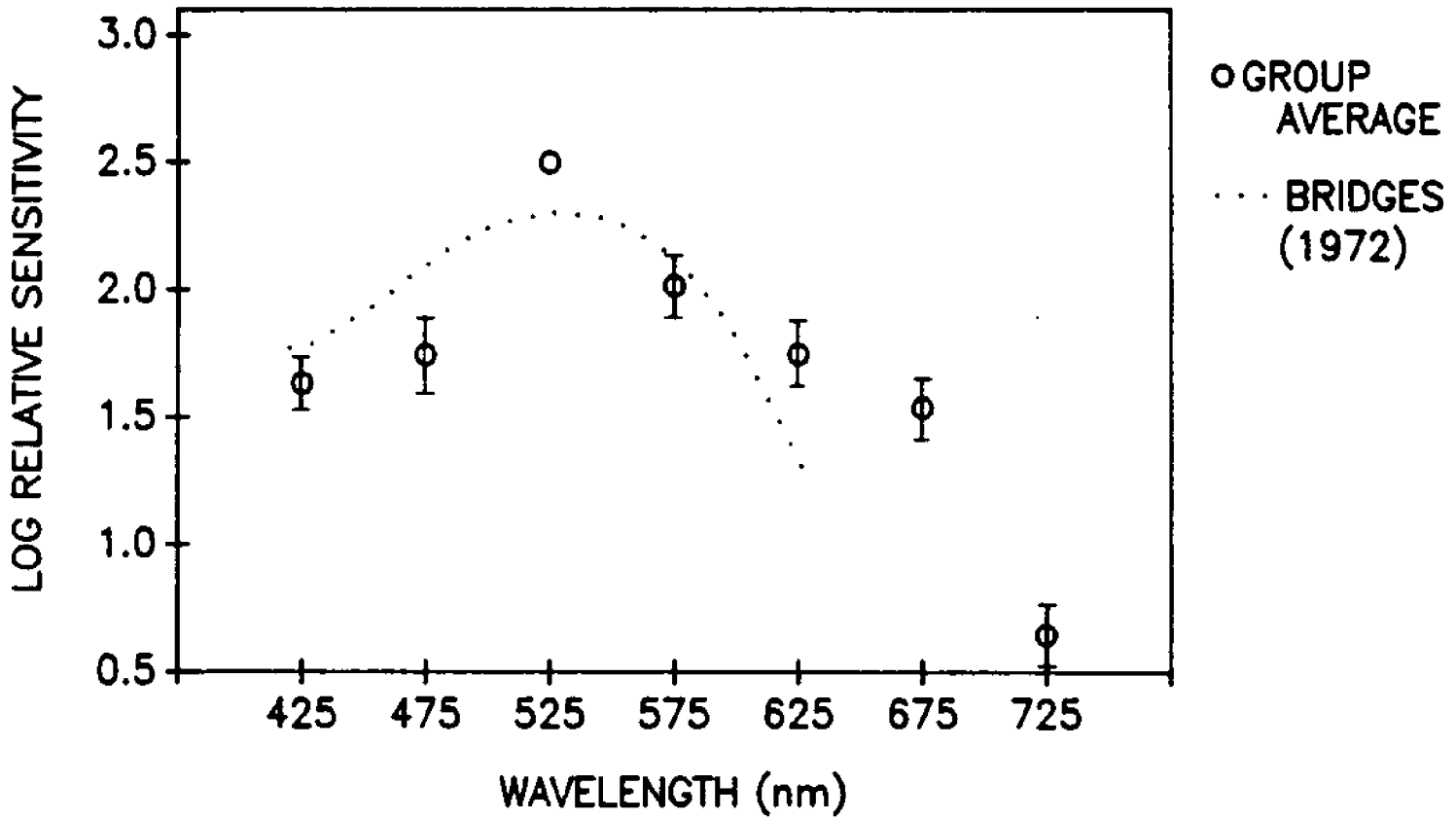

3.0
2.0
1.0
0.0
LOG RELATIVE SENSITIVITY
425
475
525
575
625
675
725
WAVELENGTH (nm)
FISH #1
FISH #2
FISH #3
FISH #4
GROUP AVERAGE

Figure Caption

Figure 7. Log relative spectral sensitivity as a function of wavelength for dark-adapted fish, Bridges' (1972) nomogram for a 525 nm photopigment is superimposed onto the group function for a best fit of the points.

Note. Error bars = SEMs.


LOG RELATIVE SENSITIVITY
3.0
2.5
2.0
1.5
1.0
0.5
425
475
525
575
625
675
725
WAVELENGTH (nm)
GROUP AVERAGE
BRIDGES (1972)

G. petersii at these wavelengths as indicated by the broad shoulder into the long wavelengths (beyond 575 nm and including the 725 nm light).

Results for the control stimulus (800 nm light at 6 log attenuation) showed that the fish were not responsive to this stimulus. The average percent response for all 800 nm trials (grouped data for four fish) was greater than average baseline (i.e., >100%).

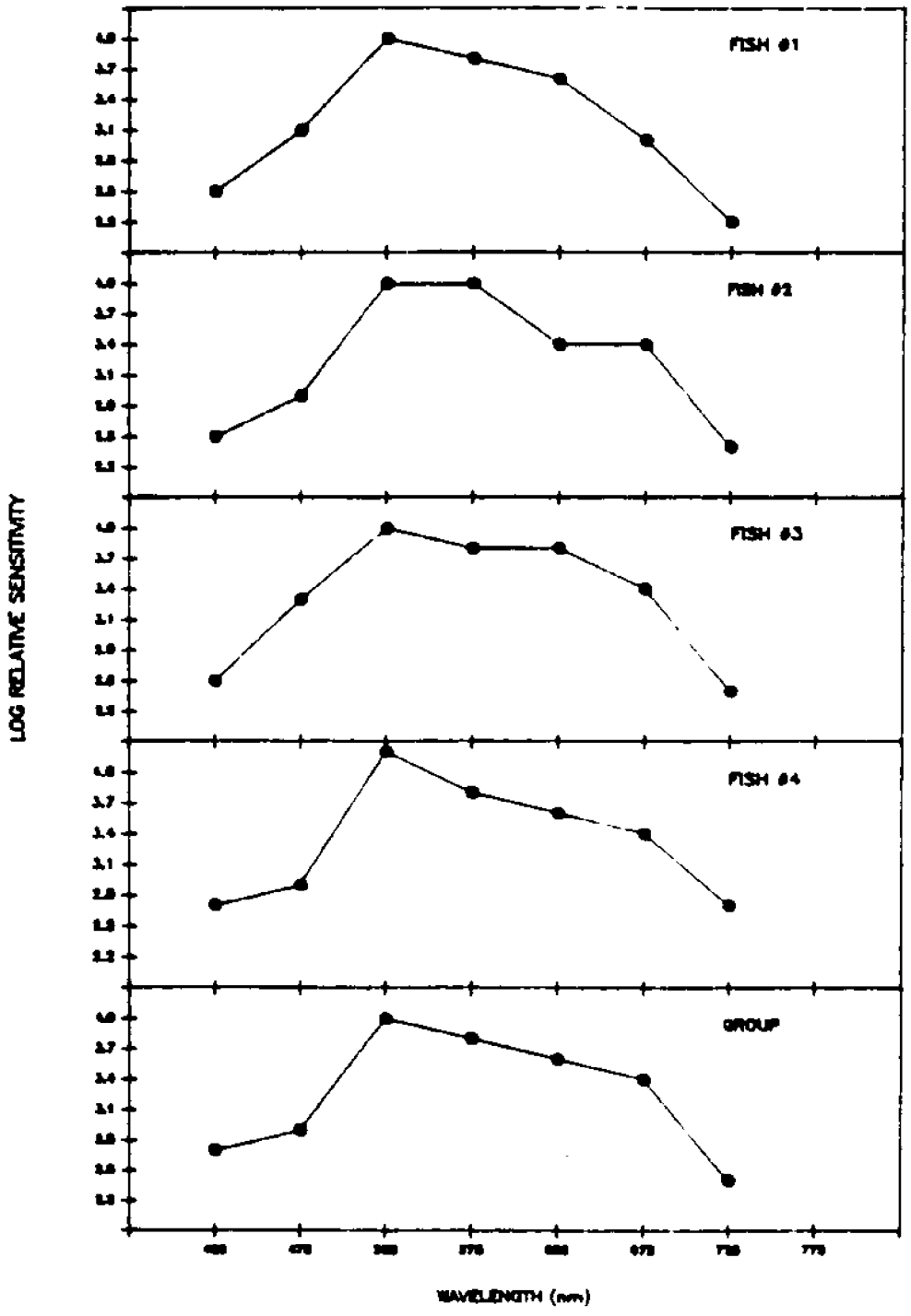
The spectral sensitivity functions derived from auditory judgment data are presented in Figure 8. The functions for individual dark-adapted fish show peak sensitivity occurring to the 525 nm light with sensitivity falling off at wavelengths shorter and longer than λ max. All fish were responsive to the extreme long wavelengths (675 and 725 nm) and, in general, the functions demonstrate a broad shoulder of relatively acute sensitivity through the long wavelengths. Two of the individual fish were more sensitive to a 425 nm light than to a 475 nm light. The group function is also shown in Figure 8 and can be seen to be a good representative of the individual fish curves.

2/ Statistical analysis

Results of the one-way ANOVAs for repeated measures for a single wavelength and intensity across sessions for

Figure Caption

Figure 8. Log relative spectral sensitivity as a function of wavelength for dark-adapted fish: individual and group functions derived from auditory judgment data.


FISH #1
FISH #2
FISH #3
FISH #4
GROUP
LOG RELATIVE SENSITIVITY
WAVELENGTH (nm)

dark-adapted fish are presented in Appendix A: Table A-1.

Of the 35 analyses, two demonstrated significant results: 1) 525 nm at the third attenuation level and 2) 575 nm at the third attenuation level. Tukey post hoc comparison of means showed that for the 525 nm light, the mean of session #1 significantly differed from all other session means (Q .05 (df=3,9)= 3.949; MSE=38.7) and for the 575 nm light, the mean of session #1 significantly differed from that of session #4 (Q .05 (df=3,9)= 3.949; MSE=55.9).

With only two of 35 analyses reporting significant results, it may by concluded that mean EOD interval durations did not significantly vary for repeated presentations of the same stimulus over sessions.

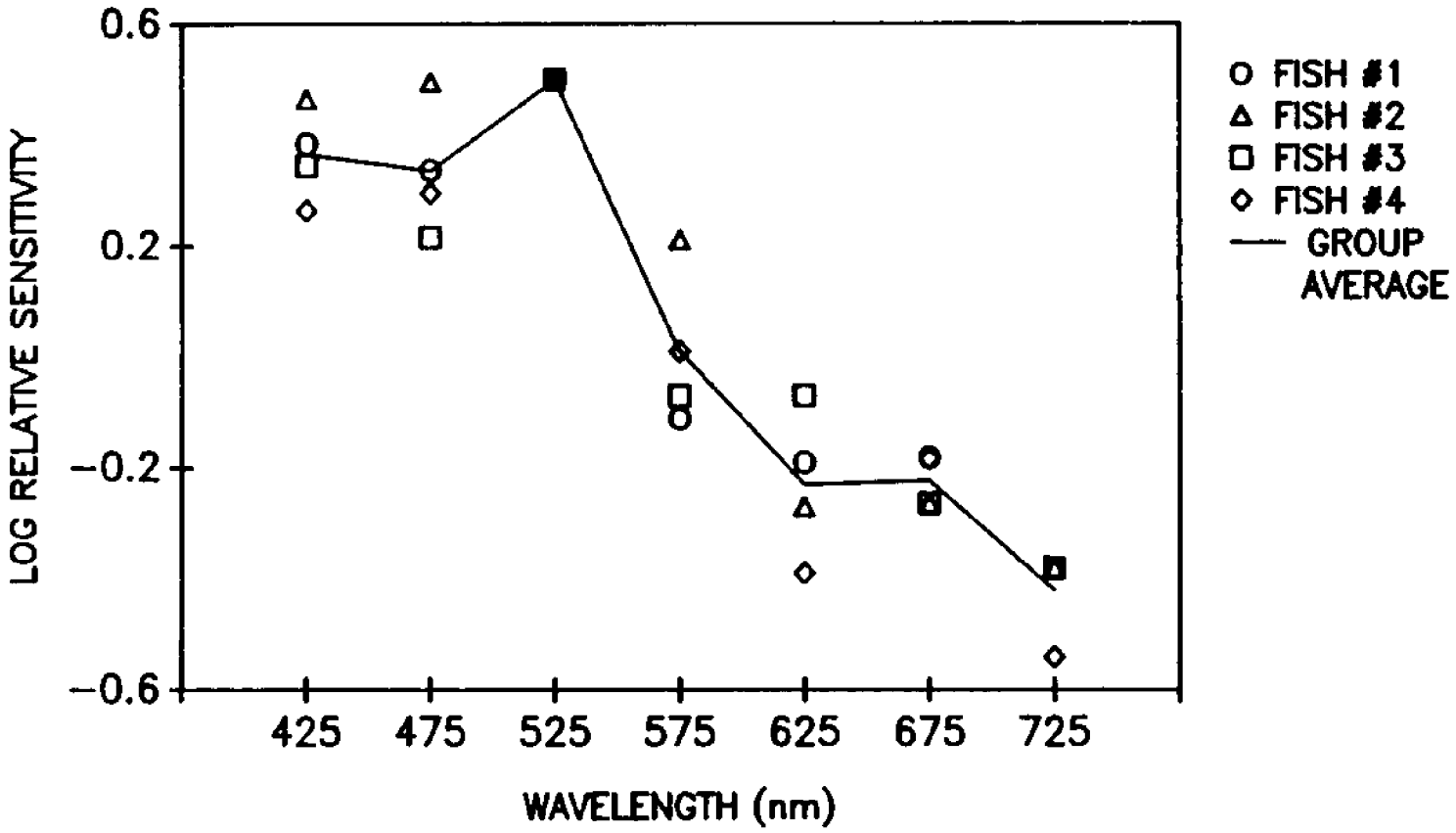
3.1B. RESULTS FOR LIGHT-ADAPTED FISH

1/ Spectral sensitivity

The group average log relative sensitivity function and individual fish spectral sensitivity data are presented in Figure 9. The group average function shows peak sensitivity to a 525 nm light, the λ max for the dark-adapted condition. Also, a broad shoulder

Figure Caption

Figure 9. Log relative spectral sensitivity as a function of wavelength for light-adapted fish.


0.6
0.2
−0.2
−0.6
LOG RELATIVE SENSITIVITY
425
475
525
575
625
675
725
WAVELENGTH (nm)
FISH #1
FISH #2
FISH #3
FISH #4
GROUP AVERAGE

showing sensitivity to long wavelength light is again evident in the data for light-adapted fish and there is evidence of a secondary peak in the region of 625-675 nm. The decrease in sensitivity to wavelengths shorter than λ max is less pronounced than for wavelengths longer than λ max.

Individual fish data are comparable, however the data for some wavelengths exhibit large variability among points (e.g., 625 nm). All fish were most sensitive to the 525 nm light although Fish #2's function is quite flat from λ max through the short wavelengths. Secondary peaks at the long wavelengths are apparent.

Figure 10 shows the group average log relative sensitivity function and Bridges' nomogram (1972) superimposed arbitrarily for a best fit of the points. The group average function deviates considerably from the nomogram with G. petersii demonstrating greater sensitivity to both short and long wavelengths.

Auditory judgment data functions are presented in Figure 11 and are comparable in terms of λ max occurring at 525 nm for all fish as well as the relative spectral sensitivity among wavelengths. Two of the fish (#2 and #3) did not reach criterion responding to the 425 and 725 nm lights. Most individuals were more sensitive to a 675 nm light than to a 625 nm light, again suggesting

Figure Caption

Figure 10. Log relative spectral sensitivity as a function of wavelength for light-adapted fish, Bridges' (1972) nomogram for a 525 nm photopigment is superimposed onto the group function for a best fit (i.e., by eye) of the points.

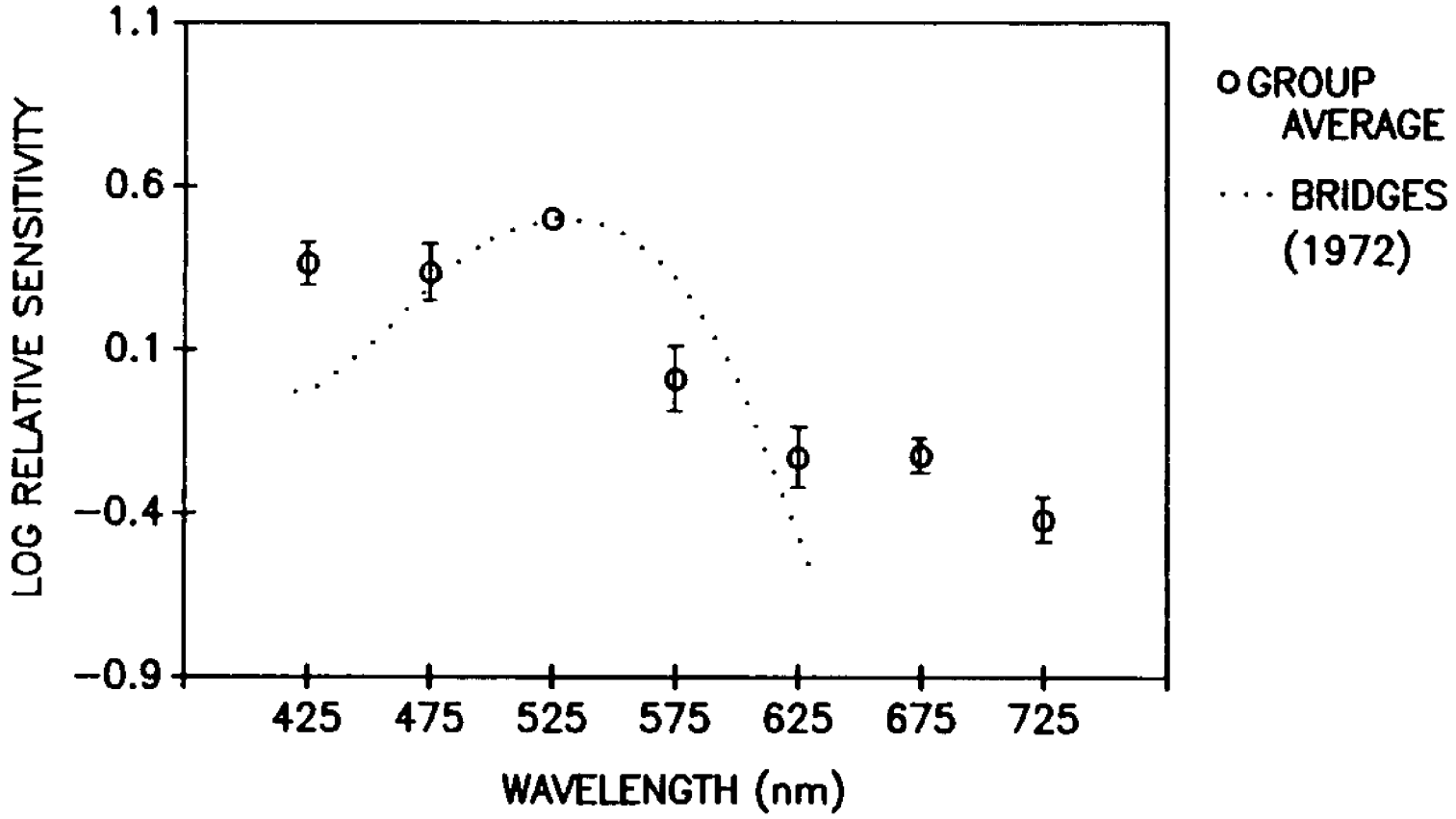
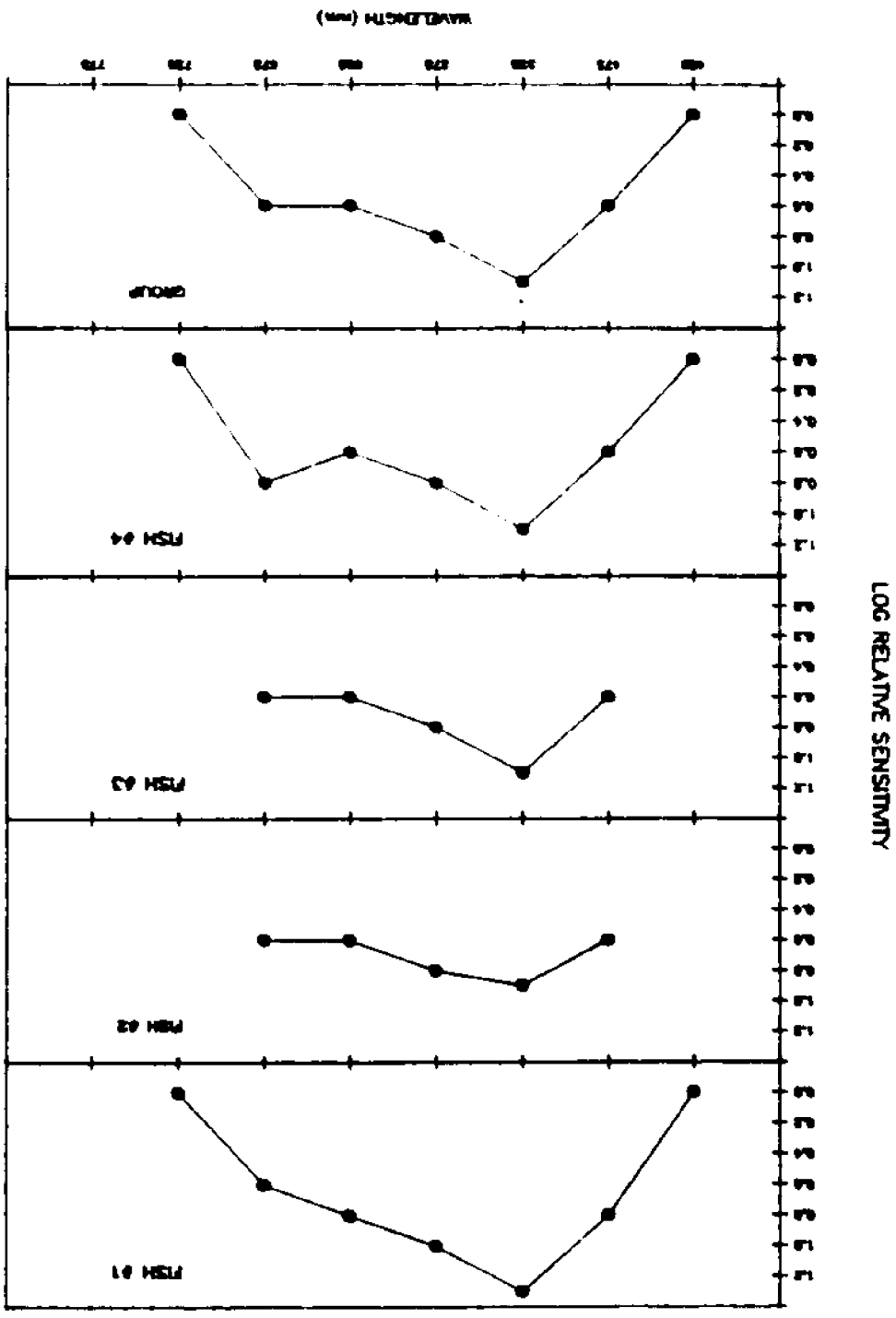

1.1
0.6
0.1
−0.4
−0.9
LOG RELATIVE SENSITIVITY
425
475
525
575
625
675
725
WAVELENGTH (nm)
GROUP AVERAGE
BRIDGES (1972)

Figure Caption

Figure 11. Log relative spectral sensitivity as a function of wavelength for light-adapted fish: individual and group functions derived from auditory judgment data.


WAVELENGTH (nm)
GROUP
FISH 64
FISH 63
FISH 62
FISH 61
LOG RELATIVE SENSITIVITY

the possibility of a secondary peak at this wavelength. These observations are generally well-reflected in the group curve also presented in Figure 11.

Results for the control stimulus (800 nm light at 6 log attenuation from maximum) were similar to results from the dark-adapted condition. The fish's post-stimulus EOD activity (average group data for all 800 nm trials) was always equal to or larger than average baseline (100%).

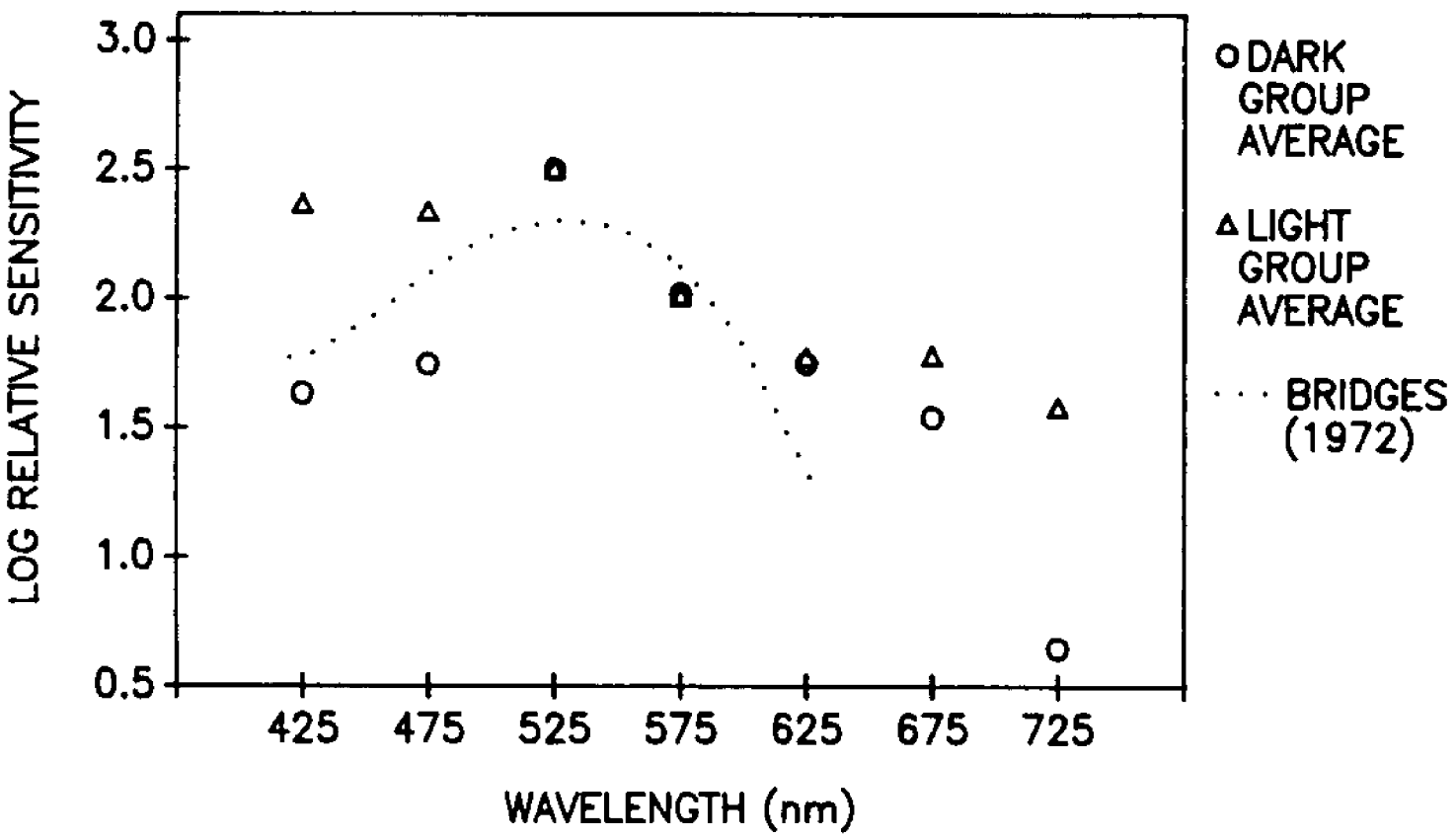
3.1C. COMPARISON OF DARK- AND LIGHT-ADAPTED SPECTRAL SENSITIVITY CURVES

1/ Spectral sensitivity

Log relative spectral sensitivity functions for both conditions of light adaptation for group fish averages are presented together in Figure 12 along with Bridges' nomogram (1972). Dark- and light-adapted functions differ in shape. The curve obtained in the light-adapted condition shows less pronounced relative sensitivity differences among wavelengths and a secondary peak at the long wavelengths. The data for both conditions of light adaptation do show clearly that these fish are most sensitive to a 525 nm light.

Figure Caption

Figure 12. Log relative spectral sensitivity functions for dark- and light-adapted fish and Bridges' (1972) nomogram for a 525 nm photopigment.


LOG RELATIVE SENSITIVITY
3.0
2.5
2.0
1.5
1.0
0.5
425
475
525
575
625
675
725
WAVELENGTH (nm)
DARK GROUP AVERAGE
LIGHT GROUP AVERAGE
BRIDGES (1972)

2/ Statistical analysis

The summary table for the 3-way ANOVA for repeated measures for factors 1) light adaptation condition, 2) wavelength, and 3) intensity is presented in Appendix B: Table B-1.

It can be seen that the effect of light adaptation (Factor A) alone was not significant. The effect of wavelength (Factor B) alone was significant. Wavelength (B) and light adaptation condition (A) did interact significantly (Factor AB) such that the light adaptation condition did have different effects for different wavelengths. The effect of stimulus intensity (Factor C) alone was highly significant. The intensity factor interacted significantly with both light adaptation condition (Factor AC) and wavelength (Factor BC). Finally, the effect of the levels of each factor differed at different levels of the other two factors such that a significant three-way interaction (Factor ABC) was found.

Due to the significant interaction effect among all three factors, simple main effects will not be discussed further.

Tukey post hoc comparisons of means were performed on the three significant interactions: AB, AC, BC. Additionally, the means for each interaction were graphed. These interactions are discussed separately

below.

Light Adaptation Condition x Wavelength (AB) Interaction.

Figure 13 presents mean interval durations as a function of wavelength for two conditions of light adaptation and across five attentuation levels. The functions interact at both the short and long wavelengths such that the means for the 425 nm, 475 nm, and 725 nm lights for the light-adapted fish are greater than those of the dark-adapted fish. Tukey post hoc comparisons of mean pairs per wavelength showed that only for the 625 nm wavelength did the means of the two different light adaptation conditions differ ($Q_{.05}$ (df=6,180)= 4.1, MSE=11.4).

Light Adaptation Condition x Attenuation Level (AC) Interaction.

Figure 14 presents mean interval duration as a function of light adaptation condition for five attenuation levels and across seven wavelengths. First, a clear graded effect for intensity is evident with mean interval duration increasing as a function of attenuation level in an orderly fashion (save AL = 3, light-adapted condition). The interaction effect is evident for attenuation levels 3,4, and 5, with the mean interval duration for AL = 3 surpassing in value those of AL = 4 and

5 in the light-adapted condition. Tukey post hoc comparisons of means showed that the means for the two light adaptation conditions significantly differ at AL = 1 and 3 (Q .05 (df=4,120)=3.68, MSE=10.7)

Wavelength x Attenuation Level (BC) Interaction.

Figure 15 presents mean interval duration as a function of attenuation level for seven wavelengths across two conditions of light adaptation. These seven fucntions can be seen to interact at numerous levels of both factors but most apparently not at middle levels of attenuation. Tukey post hoc comparisons of means showed the following significant mean differences: 425 nm x 575 nm (AL=3), 425 nm x 625 nm (AL=3), 425 nm x 675 nm (AL=3), 475 nm x 625 nm (AL=3) (Q .05 (df=24,720)=5.01, MSE=28).

Figure 16 presents mean interval duration as a function of wavelength for five attenuation levels and two conditions of light adaptation (ABC interaction). Due to the inordinate number of functions involved, the interaction is considered non-interpretable for practical purposes.

Figure Caption

Figure 13. Light adaptation condition x wavelength (AB) interaction.

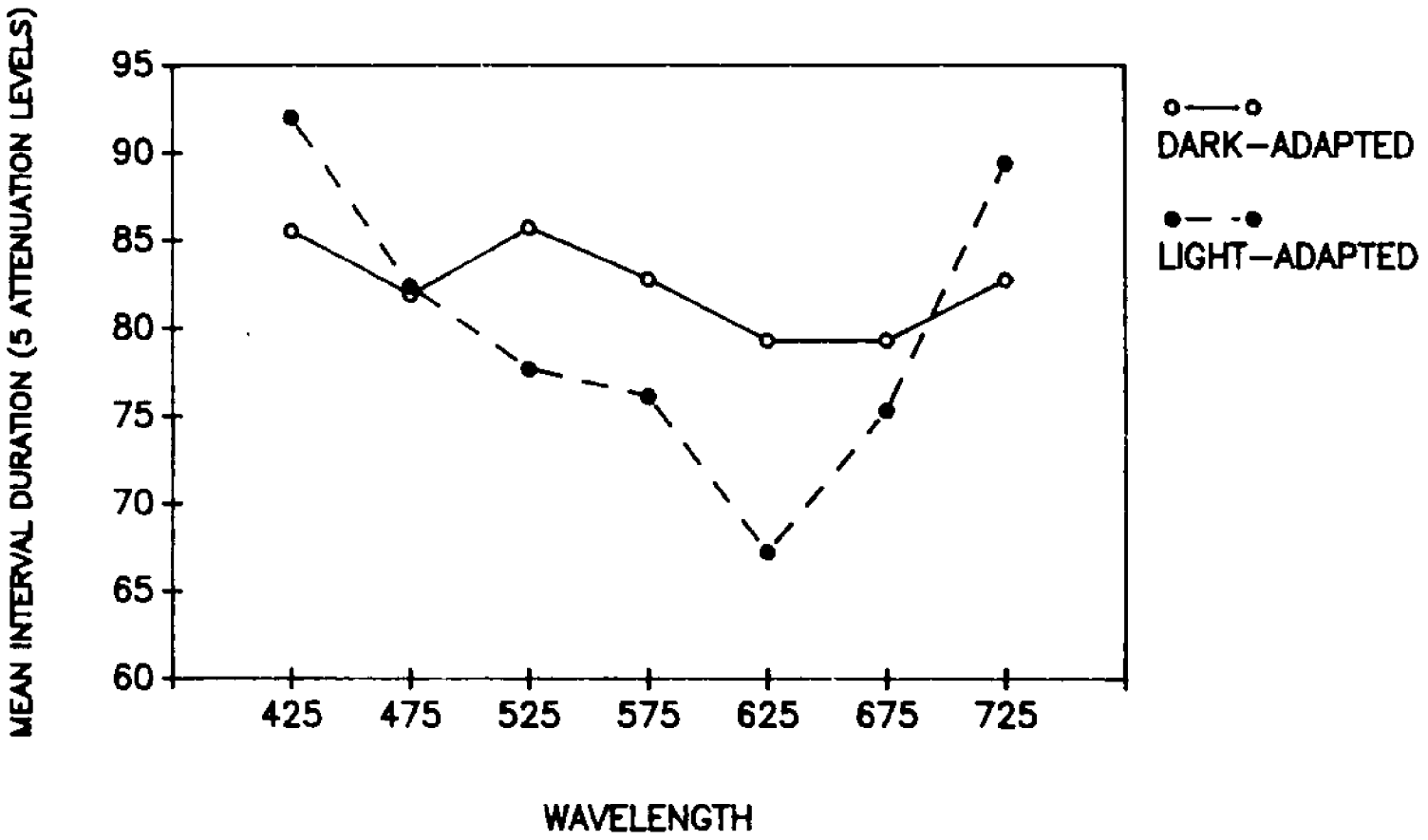

MEAN INTERVAL DURATION (5 ATTENUATION LEVELS)
95
90
85
80
75
70
65
60
425
475
525
575
625
675
725
WAVELENGTH
DARK–ADAPTED
LIGHT–ADAPTED

Figure Caption

Figure 14. Light adaptation condition x attenuation level (AC) interaction.

Note. AL = attenuation level.

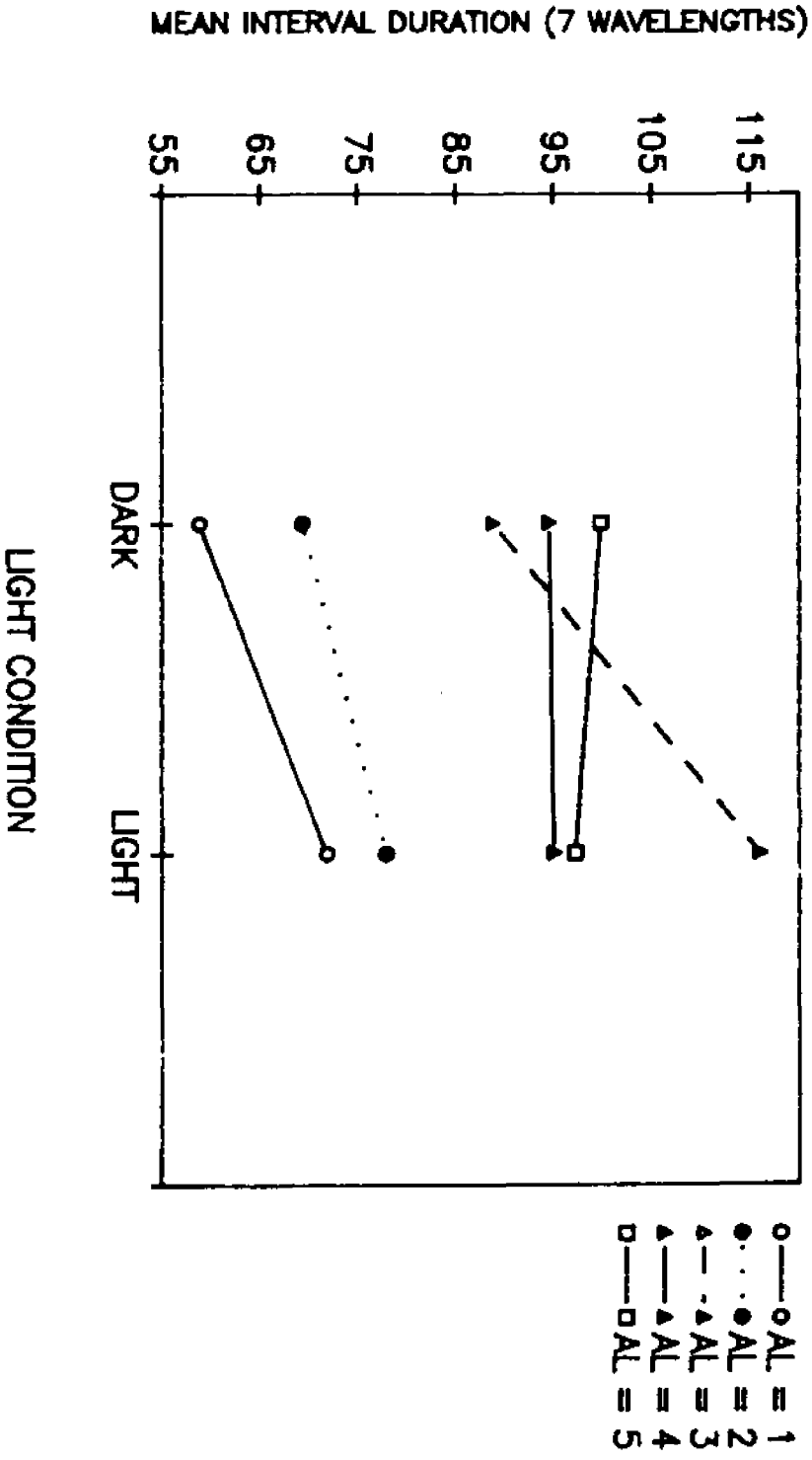

MEAN INTERVAL DURATION (7 WAVELENGTHS)
55
65
75
85
95
105
115
DARK
LIGHT
LIGHT CONDITION
AL = 1
AL = 2
AL = 3
AL = 4
AL = 5

Figure Caption

Figure 15. Wavelength x attenuation level (BC) interaction.

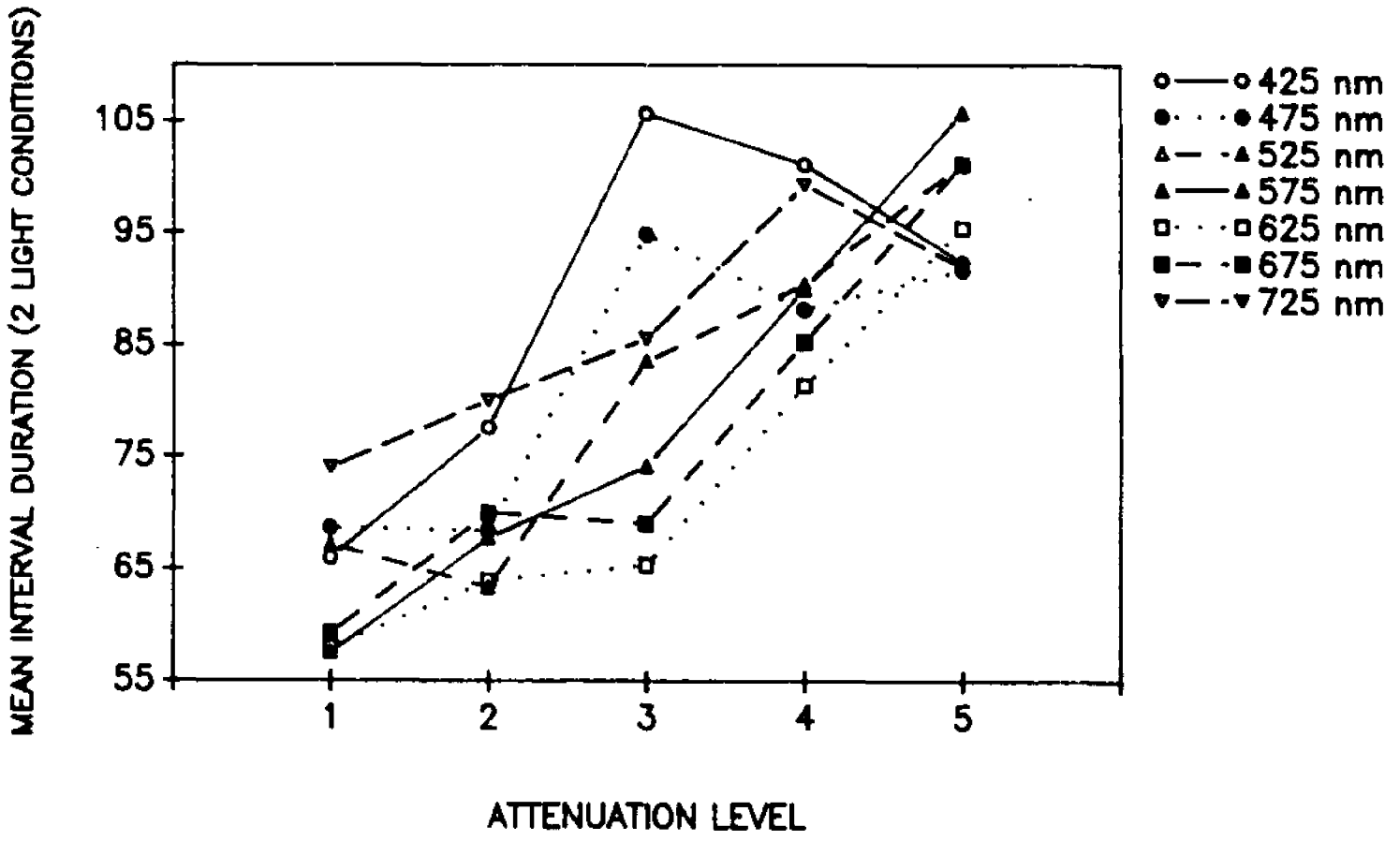
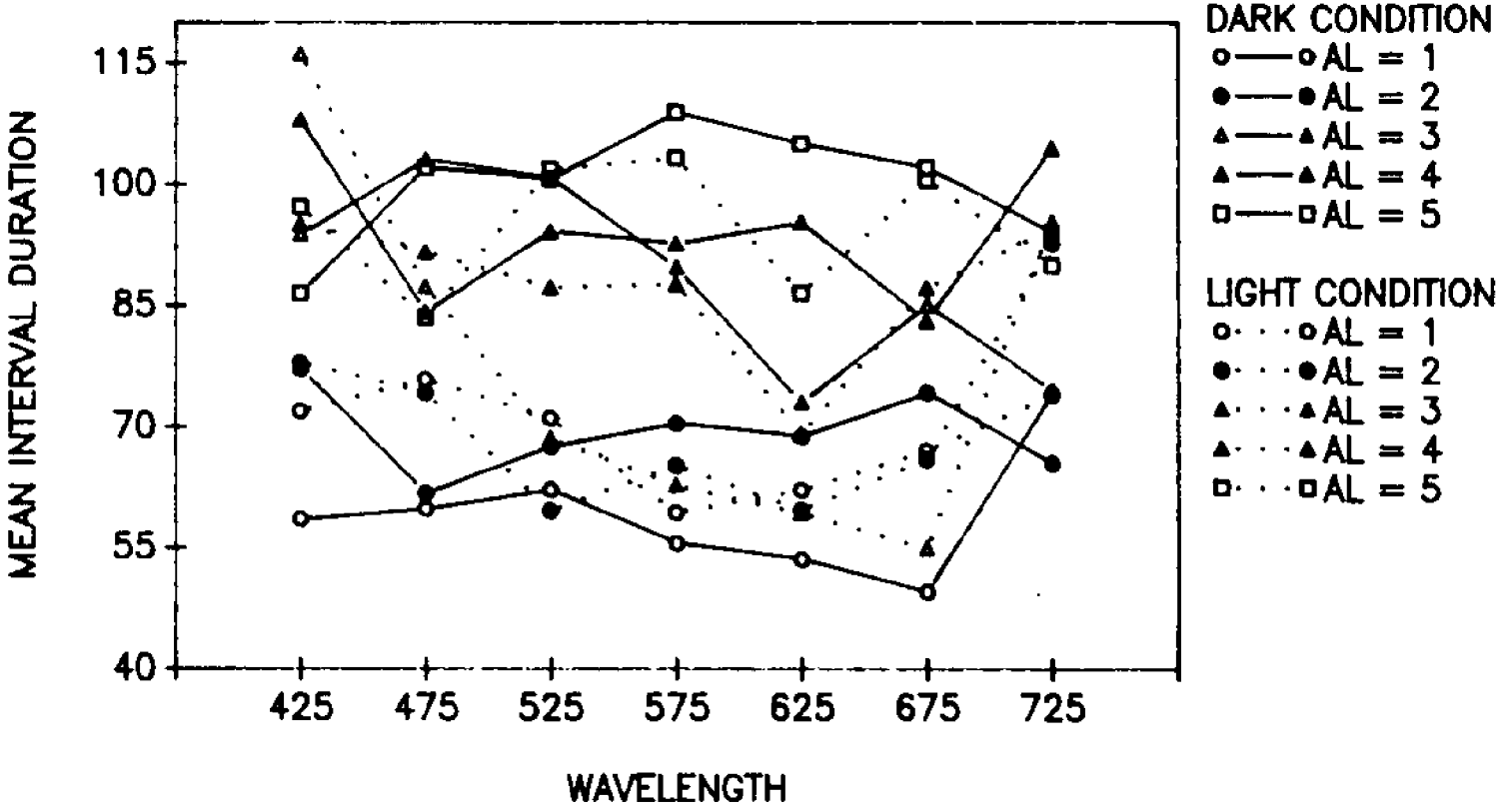

MEAN INTERVAL DURATION (2 LIGHT CONDITIONS)
105
95
85
75
65
55
1
2
3
4
5
ATTENUATION LEVEL
425 nm
475 nm
525 nm
575 nm
625 nm
675 nm
725 nm

Figure Caption

Figure 16. Light adaptation condition x wavelength x attenuation level (ABC) interaction.

Note. AL = attenuation level.


MEAN INTERVAL DURATION
115
100
85
70
55
40
425
475
525
575
625
675
725
WAVELENGTH
DARK CONDITION
AL = 1
AL = 2
AL = 3
AL = 4
AL = 5
LIGHT CONDITION
AL = 1
AL = 2
AL = 3
AL = 4
AL = 5

EXPERIMENT #2. Histology

4] METHODS

To follow up McEwan's (1938) findings and suggestions regarding the mormyrid visual system histological studies were performed on the subject of this study.

4.1] EXCISION OF THE EYEBALLS

A single light-adapted G. petersii was sacrificed by severing the spinal cord. A dissecting microscope was used to remove the eyeballs from the orbits, being careful to keep intact a small portion (i.e., approximately 1-2 mm) of the optic nerve attached at the rear of the eyeball.

4.2] PERFUSION, FIXING AND EMBEDDING OF THE RETINA

The method for perfusion and fixing of the retinas was Heidenhain's Susa and Picric Acid (LaBossiere and Glickstein, 1976). A solution was made of 500 cc of mercuric chloride (38 gm) saturated in 0.6% sodium chloride (3 gm); 20 gm trichloracetic acid; 40 cc acetic

acid; 200 cc Formaldehyde; 300 cc distilled water; 5 gm picric acid. The eyes were enucleated and then placed in the fixative. They remained in the fixative solution for ten hours after which the cornea, lens, and vitreous were removed in 70% ethyl alcohol.

Following perfusion and fixing, the retinas were embedded via the Alcohol method (LaBossiere and Glickstein, 1976) with the following change in procedure: methyl salicylate was used instead of toluene.

4.3] SECTIONING AND MOUNTING

Following embedding in paraffin the retinas were sectioned and mounted. The paraffin block was trimmed to make sides parallel and then cut on a rotary microtome. The sectioned ribbons (5μ in thickness) were placed on the surface of a water bath (a few degrees lower than melting point of paraffin), shiny side down. As sections spread out they were placed onto albumen-coated rubbed slides with dissecting needles. The sections were then blotted with double layer filter paper to remove excess water and thoroughly dried, first on a warming plate (45^{o}C) for 1-2 hours, then in an oven overnight at the same temperature.

4.4] STAINING

Following sectioning and mounting the method for Cason's Mallory Heidenhain (LaBossiere and Glickstein, 1976) was used for staining.

4.5] INSPECTION

Following staining, the retina was examined primarily for the purpose of identifying photoreceptor grouping (i.e., bundle-type retina) and epithelial pigment abundance and location about the photoreceptors. Examination of the sections took place at the level of light microscopy only using an Olympus BH-2 photomicroscope. Following examination, preferred sections were photographed (Kodachrome 64 color film) at two levels of magnification: 1) 40X and 2) 63X (oil immersion objective).

5] RESULTS

At the level of light microscopy the retina of G. petersii was seen to be of the bundle-type, as described by McEwan (1938) for G. macrolepidotus and P. stuhlmanni, with photoreceptors arranged in groups of some 15-30 elements (Figures 17 and 18). As first described by

McEwan (1938), for related species, the bundles are broadest near the external limiting membrane and viewed in cross-section appear irregularly circular, square-shaped or pentagonal. The bundles narrow and are finely tapered at their distal extremities. The photoreceptors are slender filaments which are tightly packed within the bundle. There was no evidence for the presence of cones at these levels of magnification. Rod nuclei are abundant and were located inside the external limiting membrane. Retinal layers could be identified and appear similar in number to those of other teleosts.

Brown pigment (most probably melanin) was not plentiful and was in greatest abundance from the distal extremities of the bundles midway to their proximal sections (Figure 18). Since the fish was sacrificed in the light-adapted state it is therefore apparent that little or no migration of epithelial pigment occurs when the eye is exposed to moderate levels of light. Pigment epithelial cell prolongations can be seen to surround individual bundles. According to McEwan (1938) wedge-shaped segments from four or five pigment cells form a sheath around a bundle of photoreceptors.

Figure Caption

Figure 17. Photomicrograph of a cross-section of the retina of *G. petersii* showing bundle arrangement of photoreceptors.

20 μ

Figure Caption

Figure 18. Photomicrograph of a longitudinal section of the retina of G. petersii showing bundle arrangement of the photoreceptors and pigment localization.

20 μ

[6] DISCUSSION

The following summarizes results and conclusions:

1. G. petersii are most sensitive to light of about 525 nm (λ max) in both the dark- and the light-adapted states.

2. There is evidence that the retina of G. petersii contains an additional visual pigment which maximally absorbs light of about 625 nm.

3. Sensitivity is greatly reduced in the light-adapted state and in bright light the effect of photoreceptor adaptation renders the visual system inoperable. A lack of epithelial pigment migration and the presence of reflecting material in the retina (McEwan, 1938) may also contribute to the decrement in sensitivity.

4. The photoreceptors are grouped into bundles. Whether each bundle operates as a macroreceptor to further insure the capture of light quanta in a dim-light environment or for signal pooling is not known.

The following discussion attempts to substantiate and further elucidate these conclusions through both an evaluation in light of previous research on spectral sensitivity in other teleost species and a more theoretical analysis of the adaptive fit of the visual system of G. petersii to its particular environmental

niche.

6.1] SPECTRAL SENSITIVITY RESULTS: Dark-Adapted Condition

1/ Present results compared to other findings

Relative Spectral Sensitivity

The dark-adapted spectral sensitivity functions for the four fish showed a peak (λ max) at 525 nm indicating that the fish were maximally sensitive to this wavelength. Bridges (1972) reported a nomogram for λ max= 522nm for the goldfish (Carassius auratus) under conditions of dark-adaptation. This function shows a peak at 522 nm with a slight negative acceleration to shorter wavelengths and a more pronounced negative acceleration to wavelengths longer than 522 nm. The dark-adapted spectral sensitivity of G. petersii exhibits some striking differences from that reported by Bridges (1972) for the goldfish, a freshwater cyprinid: 1) greater sensitivity to λ max (525 nm) for G. petersii, and 2) sensitivity remains relatively acute into the long wavelengths for G. petersii.

Tavolga and Jacobs (1971) investigated the scotopic threshold of Tilapia heudelotti using a shuttle-box avoidance procedure. They reported peak sensitivity at λ

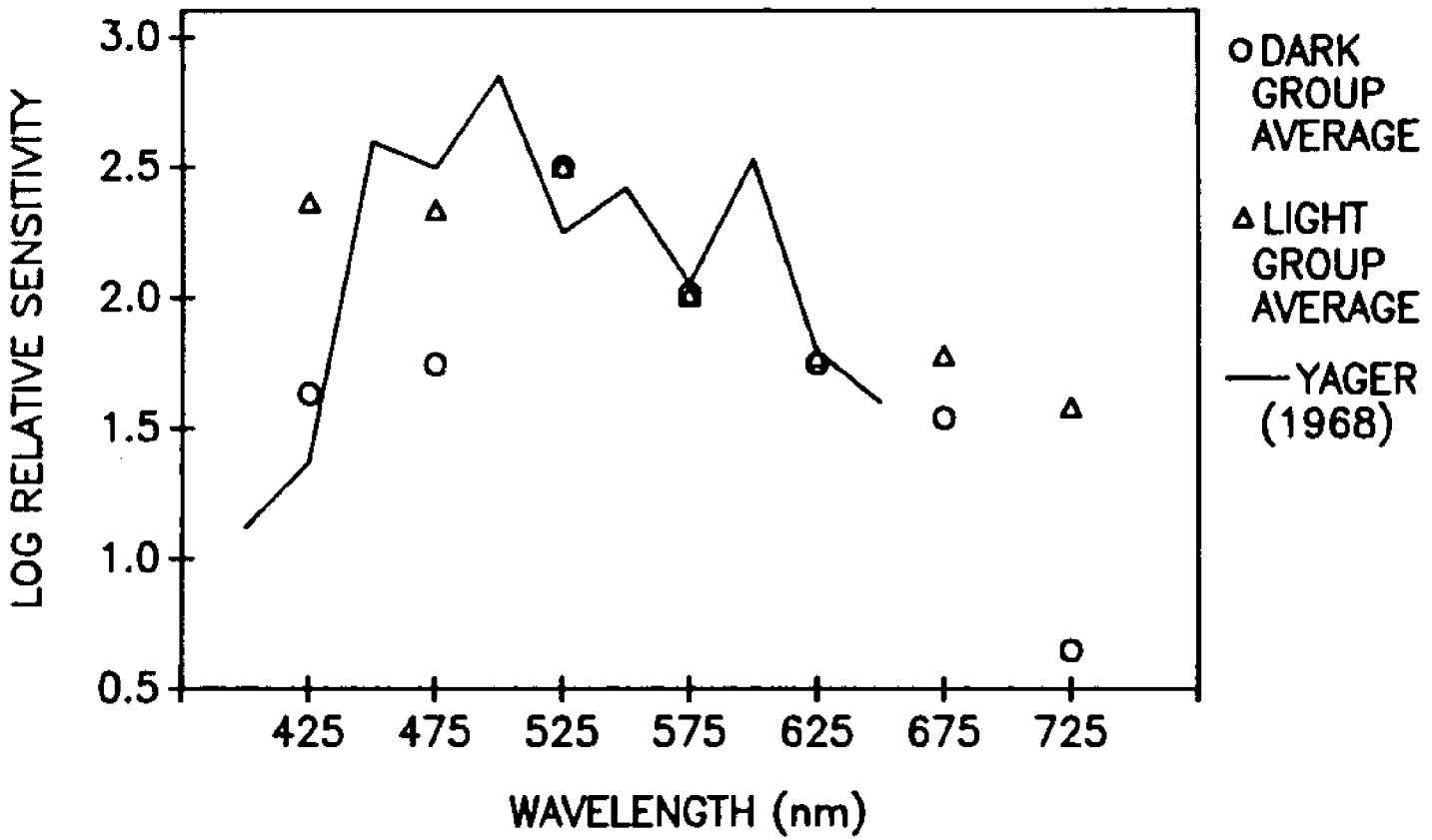
max = 525 nm with a pronounced negative acceleration to both short and long wavelengths. This spectral sensitivity function is representative of that of G. petersii. Both species appear to be most sensitive to a 525 nm light indicating the presence of the pigment porphyropsin in the rods.

The present findings are also comparable to results reported by Yager (1968). He employed a two-lever choice behavioral procedure to ascertain spectral sensitivity in the goldfish under dark-adapted conditions. He reported peak sensitivity at about 500 nm and sensitivity remained acute into the long wavelengths. For comparison purposes, the spectral sensitivity curves obtained for G. petersii under two conditions of light adaptation and Yager's (1968) spectral sensitivity curve are presented in Figure 21.

The present data show relative spectral sensitivity functions for two conditions of light adaptation which are quite comparable at the middle wavelengths to the function reported by Yager (1968). The three functions can be seen to differ, first, at the short wavelengths, with spectral sensitivity remaining relatively acute for G. petersii in the light-adapted condition and falling off more abruptly in the dark-adapted condition as compared with Yager's findings.

Figure Caption

Figure 19. Log relative spectral sensitivity functions for dark- and light-adapted fish and Yager's (1968) spectral sensitivity function for the goldfish (*Carassius auratus*).


LOG RELATIVE SENSITIVITY
3.0
2.5
2.0
1.5
1.0
0.5
425
475
525
575
625
675
725
WAVELENGTH (nm)
DARK GROUP AVERAGE
LIGHT GROUP AVERAGE
YAGER (1968)

Sensitivity to wavelengths longer than > max remained relatively acute and G. petersii demonstrated sensitivity to extreme long wavelengths (675 nm and 725 nm) not predicted by Bridges' (1972) nomogram. Yager (1968) reported sensitivity to a 650 nm light in the goldfish (Carassius auratus) and justified the differences between his findings and those of Dartnall's nomogram for a porphyropsin pigment (1953) by proposing that under dark-adapted conditions there was a significant contribution from cone systems effecting the higher sensitivities achieved with his behavioral procedure.

Inter-wavelength-stimulus distance.

The question whether 525 nm is a true λ max for G. petersii is to be considered. In the present study, a 50 nm spread between stimuli existed such that the nearest wavelengths about 525 nm were a 475 nm and a 575 nm light. Most fish with porphyropsinoid visual pigment exhibit peak sensitivity at wavelengths longer than 510 nm which partially detracts from the need to test wavelengths below this value and above 475 nm. Dartnall and Lythgoe (1965) surveyed over 40 different teleost species for the λ max of their visual pigments. Although a predominance of species with rhodopsin were classified, some species with porphyropsin were included in the survey. The findings suggested that the λ max positions

were not equivalent. For the freshwater fish, these spectral positions were found to be: 512 nm, 524 nm, and 534 nm. In addition, approximately twice as many species exhibited a 524 nm λ max compared to the combined number of species exhibiting the other two pigment classes.

Daylength and short-wavelength sensitivity.

In Experiment #1A, fish were dark-adapted for 30 min prior to testing in order to obtain the scotopic spectral sensitivity function. The fish were tested during approximately the same four hours (i.e., 1300-1700 h) each day. Other researchers (e.g., Muntz and Northmore, 1973) have reported that daylength affects the scotopic receptors. They found considerable variation in terms of relative sensitivity to different wavelengths between individuals as a function of the number of hours of daylength experienced. This variability was especially pronounced at short wavelengths, and sensitivity to short wavelength light increased with longer daylength. Although all G. petersii in the present study experienced the same constant L:D cycle throughout the study, it is possible that the decreased sensitivity to short wavelength light was, in part, a function of the number of hours of light the fish experienced. It is also possible that the testing of scotopic thresholds at different periods throughout the L:D cycle as well as under varying amounts of daylight hours could produce

more varied results for G. petersii. Muntz and Northmore (1973) attributed the variability in sensitivity to short wavelengths exhibited by fish exposed to different periods of daylength to the activity of two different pigments, one based on retinol (λ max = 507 nm) and the other on 3-dehydroretinol (λ max = 535 nm), both found in the retina of their subjects (Scardinius erythropthalmus). With increasing daylength, they observed a greater contribution from the retinol-based pigment. Since it would appear that the retina of G. petersii possesses multiple visual pigments, special care is warranted as to methodological conditions when testing scotopic as well as photopic thresholds.

6.2] SPECTRAL SENSITIVITY RESULTS: Light-adapted condition

1/ Present results compared to other findings

Relative spectral sensitivity

In Experiment #1B, fish were light-adapted for 30 min prior to testing using a 15 W incandescent lightbulb. The adapting light remained on throughout testing. All fish showed peak sensitivity to a 525 nm light. Relative spectral sensitivity differences among wavelengths were less pronounced than those obtained in the dark-adapted condition. In addition, the function for the light-

adapted fish indicated a secondary peak in sensitivity in the long wavelengths. Cronley-Dillon and Muntz (1965) tested goldfish photopic sensitivity using the optomotor response. Their results showed that the spectral sensitivity curve contained two maxima, one peak occurred in the region of 615-630 nm which the authors attributed to cone activity. The other peak occurred in the region of 520-535 nm and was due to rod activity under photopic conditions. Silver (1974) assessed photopic spectral sensitivity in the neon tetra (Paracheirodon innesi) using a dorsal light tilt reaction; neon tetras and many other teleosts tilt their bodies so that the dorsal part is exposed to a light source. Using the method of forced choice, it was discovered that these freshwater teleosts were most sensitive to a 535 nm stimulus, the λ max for this species. The photopic spectral sensitivity of the neon tetra is complex and the author concluded that more than one component (pigment) could account for some of the inter-subject variability. No photochemical information about any visual pigments of neon tetras exists, but in a related species, the bleeding-heart tetra (Hyphes sobrycon sp.) pigments of λ max 503 nm and 527 nm were extracted. Thus it is possible that the neon tetra's retina is one containing mixed pigments (vitamins A_1/A_2).

Adapting light intensity and photoreceptor adaptation.

In the present study, the adapting light chosen was a 15 W incandescent bulb. In pilot studies, other adapting light intensities were experimented with. Two of these other intensities did not elicit a startle reaction to stimulus lights of maximum intensity. These were 200 W and 100 W bulbs. In addition, when a 40 W adapting lightbulb was used the results showed responding at 475 nm, 525 nm, and 575 nm only, with greatest sensitivity to the 525 nm light. No response was obtained to the shorter and longer wavelengths. The notion of functional blindness (see Teyssedre and Moller, 1982) is to be considered here. The probable effect of a lack of retinomotor activity, with little or no migration of retinal epithelial pigment about the photoreceptors (McEwan, 1938), may be a lowered threshold for photoreceptor adaptation rendering the photoreceptors incapable of detecting light stimuli with background illuminations that are less intense than generally used. This effect has been referred to as functional blindness indicating the temporary state of non-responsiveness of the photoreceptors. Again, the environmental niche to which G. petersii is adapted is one of dim light. The morphological adaptations of its retina which afford greater sensitivity to the minimal ambient light available in its niche render the system

inoperable under bright-light conditions. Knowledge of the natural environment of this species and its unique adaptations to that environment provides information pertinent to methodological questions concerning the assessment of its visual system. In this instance, factors such as a lowered threshold for photoreceptor adaptation, informed the need for an adapting light of low intensity. In other instances (e.g., Tavolga and Jacobs, 1971), attempts to mimic known environmental conditions (e.g., use of overhead illumination to mimic field conditions) helped to ensure more valid results of the operating capacities of the particular organism. Due to the constraints of G. petersii's visual system a low intensity adapting light was necessitated.

6.3] MULTIPLE VISUAL PIGMENTS

1/ Comparison of dark- and light-adapted spectral sensitivity curves

From the spectral sensitivity results of Experiments #1A and #1B it is possible to answer the question of whether G. petersii's retina possesses only a single visual pigment or multiple pigments. Evidence for a second pigment class in the retina of G. petersii was manifested by the results of the present study. The finding that a 725 nm light was responded to by the fish

when considerably attenuated (by 2.4 log units) further attests to this species' acute sensitivity throughout the long-wavelength spectrum.

The fish were found to be most sensitive to a 525 nm light in both conditions of light adaptation. This would indicate that the rod activity contributed most to the spectral sensitivity for these fish in both conditions. However, in the light-adapted state, relative spectral sensitivity differences among wavelengths were less pronounced than those obtained in the dark-adapted state while the relative positions of the wavelengths remained the same. The downward shifted spectral sensitivity curve for the light-adapted fish suggests that in this condition the visual system was less sensitive.

The data from Experiments #1A and 1B cannot be considered definitive as regards a precise characterization of the secondary visual piment. It would appear that the second visual pigment absorbs maximally light in the range of 600-675. Three out of four fish demonstrated behavioral evidence for peak sensitivity occurring at 675 nm. However, there is no behavioral evidence for any freshwater fish pigment absorbing maximally light of 675 nm. Most probably the peak for the second visual pigment in G. petersii occurs at 625 nm. Sirovich and Abramov (1977) contend that behavioral evidence for photopigments maximally absorbing at wavelengths longer than those

predicted by standard nomograms (derived from spectrophotometry) is the result of response interaction effects of various receptor types (e.g., rod and cone) and labelled such photopigments "pseudo-pigments."

The adapting light used was of minimal intensity. It would be logical to assume that the startle response would not have been extinguished entirely under strong adapting light illumination (100 W and 200 W) were there a larger ratio of cones to rods in the visual system of G. petersii. Bell (1982) found that in the cichlid fish Hemichromis bimaculatus high sensitivities to the blue and green end of the visible spectrum persisted despite strong light adaptation. They explained this as implication for cone dominance in the retina of this fish. They found a ten-fold sensitivity change with light adaptation and a shift of 20 nm in the blue and red maxima. Yager (1968) reported a downward shift in sensitivity of the magnitude of approximately 2 log units under light-adapted conditions. In his experiment, goldfish were light-adapted using monochromatic red and blue adapting lights. The extent to which the decrease in sensitivity was due to photopic regeneration, photomechanical effects or structural factors could not be determined.

6.4] VISUAL ECOLOGY AND SELECTIVE ADAPTATION

One of the aims of this study is that of understanding the visual capacities (i.e., spectral sensitivity and retinal anatomical adaptations) in light of the unique niche to which G. petersii has adapted. There are numerous factors which can be correlated with what is now understood about its visual system.

1/ Adaptation of the mormyrid retina to the light environment of its niche

The ambient light state of the aqueous environment of G. petersii and its behavioral ecology provide relevant and informative clues by which its visual capacities may be better understood. The loss of light intensity and the spectral absorption in murky water, such as that of African 'blackwater' lakes, is profound with nearly all light absorbed within a depth of three meters. Lythgoe (1979) explains that there are two ways the eyes can increase the number of light photons captured. First, anatomically, the eye can be designed to maximize the brightness of the image and to permit the absorption by photopigment of the greatest number of photons. Second, the area of image sampled may be increased and/or the sampling time may be lengthened. Both adaptations physiologically permit more photon hits

to be included in each sample. The mormyrid retina is exceptionally well adapted to a dim-light environment. The anatomy of its retina includes the arrangement of photoreceptors into bundles. This finding confirms similar results reported by McEwan (1938) for related species of the family Mormyridae. Each bundle functions as a macroreceptor with individual receptors interacting synaptically. With such an arrangement less light quanta may be required to stimulate the system. Additional adaptations which further insure the maximal number of quanta to be captured are the absence of epithelial photopigment migration around receptor processes and the presence of reflecting material (i.e., guanine) within the bundles. The first of these adaptations limits the absorption of quanta by epithelial pigment; quanta which did not become absorbed by photoreceptors while passing through the retina. The second adaptation permits additional opportunities for quanta to be absorbed by photoreceptors through reflection back into the interior of the bundle. Lythgoe (1979) further contended that visual systems capable of color vision possess adaptations for sampling and comparing different wavelengths which necessitate a reduction in the number of photon hits in each sampling. He concluded that it is significant that color vision is rare, or perhaps nonexistent, in animals active at very low light intensities. The findings of the present study

which suggested that, although the retina of G. petersii contains multiple visual pigment classes, the number of cone cells are relatively few compared to rods and would probably be insufficient to allow for color vision. This corroborates Lythgoe's conclusion and demonstrates an adaptation which maximizes one function of a system, scotopic sensitivity, at the expense of another function, color vision, due to their mutual exclusivity.

Mormyrids are nocturnal species most active from dusk through dawn (Moller, Serrier, Belbenoit and Push, 1979). During this time, many species migrate from river inlets, where they pass the daylight hours, to the inlets' openings where they feed. Munz and McFarland (1979) illustrated that predation exercises a selective pressure on scotopic vision especially during the periods of twilight and sunrise. Within thirty minutes after sunset, the light level falls from what is adequate for photopic vision to values that require scotopic vision. Most bony fish exhibit a 'quiet period' of about 20 min at this time, during which activity remains at a minimum. This serves as a means to decrease probability of predation as a function of limited vision. These authors view this behavior as adaptive as it allows time for the eyes to adjust to the diminished light intensity through processes such as photomechanical movements and neural adaptation requiring up to one hour for completion. The adaptive advantage G. petersii

garners, given the absence of photomechanical movements within its retina, is evident: dark adaptation may occur in a briefer period than for other bony fish. The increased sensitivity to dim light that a bundle-type retina affords, and the increased probability of photon absorption through a reflecting material such as guanine, maximizes the efficacy of the scotopic system in a crepuscular/nocturnal potential prey species

It is not certain whether sensitivity is enhanced due to the shift in spectral sensitivity of the rods that results from the rhodopsin to porphyropsin change. Munz and McFarland (1977) claim that the porphyropsins of many freshwater fishes are generally more sensitive to green light than the rhodopsins of marine animals. Their measurements in freshwater lakes containing abundant chlorophyll, which promotes a 'greenish' color as opposed to the blue of marine environments, showed that at increasing depths the twilight spectrum is shifted to longer wavelengths. The λ max of G. petersii has been estimated to be at about 525 nm, a common λ max value for freshwater fish. This species increased sensitivity to longer wavelength light can be viewed as having adaptive value given the spectral characteristics of the ambient light in its niche.

2/ Contrast sensitivity and offset visual pigments

Natural bodies of water scatter light much more intensively than air (Easter, 1974). The veiling luminance that results is called the background space light (BSL). The effect of the BSL is a reduction in visual contrast due to the equal addition of light to rays which emanated from dark and light surfaces. The inherent color or BSL of different aquatic environments varies (see Introduction: Vision in fish). Freshwater lakes exhibit a BSL which ranges from greenish to the orange-brown color of 'blackwater' lakes. Levine and MacNichol (1980) have proposed that contrast sensitivity may be maximized by having visual pigments be matched to the BSL (see Introduction: Vision in fish). Further, in retinas possessing more than a single visual pigment, contrast sensitivity is increased by having different visual pigments maximally absorb quanta of different wavelengths, including one matched to the BSL. G. petersii seems to possess a rod visual pigment which maximally absorbs light of about 525 nm, however, the BSL of its aqueous niche maximally transmits light of approximately 600 nm. The apparent mismatch between the λ max of G. petersii and the BSL is typical of most freshwater fish. The greatest shift into the red of a rod pigment that has currently been investigated is a porphyropsin pigment with λ max approximately equal to

543 nm (Dartnall and Lythgoe, 1965). Lythgoe (1979) suggests that a visual pigment of λ max, which is offset from the wavelength of maximum water transparency, would have an advantage in the detection of bright objects against the water background. He further asserts that visual pigments which mediate night vision cannot absorb at longer wavelengths, which would match the BSL, because of the need to avoid infrared noise emanating from the animal's own body tissue. Interestingly, results of the spectral sensitivity of G. petersii revealed acute sensitivity well into the red area of the visual spectrum. Such a mechanism could represent an evolutionary adaptation to better match the absorption characteristics of the photopigment and the BSL. Alternatively, it could be viewed as contamination of the effect of heightened contrast sensitivity derived from an offset pigment.

Easter (1974) suggested that the problem of light scatter underwater and reduced contrast sensitivity has been responsible for the long wavelength dominance and spectral opponent mechanisms of photopic aquatic retinas. He further suggested that multiple pigment vision first evolved for this reason and secondarily was adapted to color vision. The spectral sensitivity curves obtained under dark- and light-adapted conditions for G. petersii suggest that a rod pigment and a second visual pigment, probably a cone pigment, are present in the retina.

Lythgoe (1979) explains that which evolutionary path an organ system takes, for example, increasingly more complex eyes capable of good acuity and wavelength discrimination or regressed eyes, depends on whether the animal already possesses non-visual senses that can be developed to serve similar functions. He further contends that there comes a quit point at which visual conditions are so poor that the animal abandons vision as a primary sense. These animals frequently have the ability to develop other senses (e.g., echolocation and electroreception). Often, fishes which are electrosensitive live in silt-laden water and are nocturnal and have poorly developed or regressed eyes. Turbid water conditions reduce both visual contrast and the animal's ability to discriminate those contrasts present. The same conditions are further compounded by a nocturnal lifestyle. Lissman (1958) pointed out that weakly electric fish living in turbid water are active mainly at night. He proposed that by this strategy they avoid predators whose normally good vision would be practically useless in dark, turbid waters. An evolutionary strategy such as this is adaptive in that the effect is the avoidance of capture by predators, however, the conditions for vision which such a strategy imposes are greatly impoverished. The physical dimensions of the eyes of G. petersii are regressed and locomotion, object location, and conspecific

intercommunication are highly correlated with the functioning of the electrosensory system. Without its highly developed electric sense the adaptive success of G. petersii undoubtedly would have been compromised. As has been demonstrated by the results of the present study, the visual system of G. petersii is still quite functional in spite of the constraints imposed by its environment and behavioral ecology. The system is primarily a scotopic one and the costs of an ability to discriminate between wavelengths would probably outweigh the advantages. As Levine and MacNichol (1980) have succinctly explained it:

> "For a hue-sensitive retina to function properly it must be bathed in enough light over a broad band of wavelengths to stimulate substantial signals from all types of cones. Any photo-receptor cells that do not emit signals leave 'holes' in the visual image. Holes degrade the image, and so any cone pigments in the retina that do not contribute frequently to the visual image are actually dysfunctional. A fish that is active only at night or at twilight, or one that lives in the deep sea, seldom encounters enough light to stimulate cone cells at all. For such a fish....the price of survival in the dark is the loss of sensitivity to color" (Levine and MacNichol, 1980, p. 149).

6.5] STARTLE RESPONSES

1/ What is a startle response?

Eaton and Hackett (1984) described the 'tailflip' startle response of bony and cartilagenous fish as well

as amphibians to a simple tap on the side of the aquarium wall. This response of brief latency and duration is the outcome of stimulation of the Mauthner cells. Mauthner cells reportedly receive input from the principal trigeminal nucleus, the cerebellum, and the tectum (Bartelmez, 1915). Acoustic, lateral line, visual, or electrical sitmulation of Mauthner cells causes successive excitation of the contralateral spinal motoneurons, proceeding from rostral to caudal (Eaton, Bombardieri, and Meyer, 1977).

Eaton and Hackett (1984) explained the adaptive value of such a response in terms of its being an effective escape movement that enables the animal to avoid sudden attacks by predators. Startle responses can be viewed as initiators or triggers of many behaviors, rather than commands for a single behavior (Eaton and Hackett, 1984). This view is an extension of the concept of modulation of escape responses. Serrier (1974) first described the immediate reaction (i.e., acceleration in rate of EOD) in G. petersii to a wave of the hand across the wall of the aquarium. Startle responses may trigger the electric sensory system to orient to various aspects of the environment. Amplitude characteristic of the orienting response (i.e., acceleration in rate of EOD) may be only partially under stimulus control (e.g., stimulus intensity) and a product of numerous other factors some of which have been cited above.

Well-defined startle responses have been identified in many animals. In mammals, intense acoustic stimulation is effective in eliciting startle. Other things being equal, more intense stimuli produce larger responses (Eaton, 1984). The mammalian startle response is highly graded in amplitude. Changes in startle amplitude is the major measure of startle plasticity in mammals. Ekman, Friesen, and Simons (1985) studied the human startle reaction to a gunshot. They found uniformity in the response across subjects in terms of the latency (within 200 ms) and duration (< 1 s) as well as which facial, neck and trunk movements were involved. The authors made a tentative conclusion that the startle reaction in humans is qualitatively and quantitatively different from human emotional responses such as surprise, happiness, and fear and would moreso qualify as a reflex. They contended that the startle reaction is not cognitively mediated and attempts to suppress the reaction failed due to the very brief latency between the eliciting stimulus and the response. However, the investigators did not manipulate stimulus parameters (e.g., loudness) of the gunshot and no evidence for graded responding was obtainable from their study.

Startle responses and escape responses are generally graded (Eaton and Hackett, 1984). The giant fiber systems of earthworms and crayfish and other low vertebrates demonstrate variability in their responses

under different circumstances. Gradations in behaviors may result from a change in frequency of activity in a command neuron or because motor systems activated by command systems receive other inputs, and their excitability can thereby be affected (Eaton and Hackett, 1984). In the crayfish, tailflips evoked by giant fibers are completely inhibited when the animal is restrained by its carapace. Evidence suggests that graded responding results from inhibition of motoneurons. In the present study the variations in the fish's responses to monochromatic stimuli of varying intensities showed an orderly incremental effect with percent response values (post- /pre-stimulus) increasing as a function of decreasing stimulus intensity. As a graded response, the EOD startle response of G. petersii is a useful behavioral indice for the study of visual, and other sensory capacities.

6.6] INTERSENSORY INTEGRATION

Intersensory facilitation or inhibition implies that stimulation in one modality affects responding in another modality (e.g., visual and electrosensory). Maier and Schneirla (1935) suggested that this type of intersensory effect need not be dependent on a direct relationship between the modalities involved but could be mediated by

the effects of stimulation in one modality on arousal and on the consequences of shifts on arousal or responsiveness in the other modality.

1/ Optic tectum as locus of visual and electrosensory integration

In weakly electric fish, the torus semicircularis of the midbrain is a primary relay center for electrosensory processing (Heiligenberg, 1986). A major target of the torus efferents is the optic tectum, which would implicate the tectum in the processing of electrosensory information. Bullock and Heiligenberg (1986) contend that weakly electric fish may provide interesting examples of integrative mechanisms used in multimodal sensory processing due to their well-developed electrosensory system and functional visual system. Gymnotid and mormyrid species have evolved independently and are only distantly related. Their electrosensory systems are examples of parallel development and homologous mechanosensory systems effecting similar behavior (Finger, Bell, and Carr, 1986). The following discussion presents results of research done on gymnotid fish. Extending these findings to a discussion of the mormyrids does not presuppose fact, but serves only to formulate hypotheses regarding

mormyrid behavior.

The visual system of weakly electric fish is limited; their eyes are regressed and they are nocturnal in the majority. Bastian (1982) reported that the optic tectum of gymnotid fish processes electrosensory information and that light stimuli not only evoke responses in most tectal cells, but also strongly affect the electrosensory responses. Movement is a necessary component of effective stimuli for these cells. Single unit recordings in deep cells of the interior medial quadrant of the optic tectum of Apteronotus albifrons, a South American gymnotid, demonstrated a contingency between the direction of movement of either visual or electrical stimuli and the responses of these cells. Evoked activity in units recorded in the superficial layers of the tectum to stationary light flashes, such as those used in the present experiments, has also been demonstrated. Most cells received input from either the visual or the electrosensory modality. Of particular importance was the finding that for most of the cells studied, the receptive fields for vision and electroreception were in register. Carr and Maler (1986) reported that the somatotopically ordered pisciculus in the torus is in spatial register with the retinotopic map in the tectum, such that electrosensory and visual input from a specific portion of the fish's environment is referenced in the same column in the tectum. The

majority of tectal efferents project to motor neurons and/or serve as descending inputs to the electrosensory system. Bastian (1986) suggested that these multimodal neurons could function as final common pathways through which different sensory modalities could have access to the same motor or premotor pathways.

Bastian (1982) demonstrated that electrosensory responses were similar for stimuli that were presented both in a 'headward' and 'tailward' fashion. However, visual responses were directionally sensitive with tailward movement being more effective. When both electrosensory and visual stimuli were presented simultaneously in a tailward movement, the shape and magnitude of the response was nearly what would be expected assuming that the two response types simply sum. When stimuli were presented in a headward direction the addition of light caused a slight reduction in the response.

2/ Adaptive value of visual and electrosensory integration

The electrosensory system exhibits a large degree of plasticity especially in pulse-type species of which G. petersii is one. For example, the presentation of a novel stimulus leads to a near immediate acceleration in the rate of EOD (i.e., the startle response) (Toerring

and Moller, 1984). Dye and Meyer (1986) proposed that the possible value of the startle response is that of improving the temporal resolution of electroreception. Improved temporal resolution would facilitate electrolocation and maximize the orienting capacity of the system. Bastian (1986) highlighted the vulnerability of the electrosensory modality to interference and jamming (see his article for a review of these terms). He explained that the visual system remains an important sensory modality in weakly electric fish since the tectal cells are not prevented from responding to visual stimuli at times when eletrosensory stimulation is obscured by noise. A major adaptive value of multimodal neurons could be to insure the detectability of a stimulus when the signal carried in one or another modality is weak. In the present study, the fish repeatedly responded with an acceleration in EOD rate to light stimuli which were at or above threshold. That the response persisted is curious because upon orienting electrically (i.e., acceleration in rate of EOD) in response to light stimulation, the fish invariably detected no change in its electrical field. Such persistent responding in the absence of feedback has a reflexive quality, especially given the very short latency and the resistance to habituation. Whether EOD acceleration to light stimuli is viewed as purely reflexive or if central control processing is hypothesized, the primacy of the

electrosensory system as a field-orienting system, 'mustered' into action via the detection of ambient stimuli including light, is evident.

6.7] FUTURE RESEARCH

Two broad areas of future research involve further investigation of the visual capacities of G. petersii and other weakly electric species including an assessment of wavelength discrimination, and employment of the present study's methodology to assess sensory capacities of other systems.

1/ Visual sensory system

Although the results of the current experiments and a knowledge of the behavioral ecology and retinal morphology of G. petersii inform the tentative conclusion that this species does not possess a color sense, a simple wavelength discrimination study could be undertaken and prove valuable in further elucidating this issue. Previous pilot studies in our laboratory (Ciali, unpublished) determined that G. petersii could be trained to swim through a plastic hoop to escape and avoid a noxious stimulus (i.e., bright light). Training was asymptotic within two sessions of 20 trials each. This paradigm could be used in the assessment of wavelength

discrimination. For example, fish could be trained to discriminate between pairs of lights of different wavelengths with only one of the pair serving as the discriminative stimulus. A response in the presence of S+ would be followed by light termination while a response to S- could be followed by an additional aversive stimulus such as shock. Brightness could be made irrelevant by varying the intensity of each light in the pair in a random fashion. Since light of low intensity (5W) was found to be an effective stimulus in pilot studies an entire range of intensities per wavelength could be used.

Lythgoe (1979) reported that regularly spaced patterns of light and dark bars are common in fishes. He proposed that it may not be by chance that these patterns are present due to the light scattering property of water. G. petersii possesses two stripes which extend from the base of the dorsal fin to the base of the anal fins. These stripes are yellow-green in color (although they can change under different conditions of ambient light) against the body's dark brown color. The stripes may play an important role in conspecific recognition and it is noteworthy that they nearly match the hue of the species' λ max value. Research on the effect of gratings of different spatial frequency and color on gross behavior as well as on a more molecular level (e.g., single-unit recording) might be performed.

2/ Other sensory systems (e.g., auditory)

The methodology employed in the present study (i.e., the startle response as an indice of detection) can be used to assess sensory capacities of other systems. At the present time, pilot research is being conducted in our lab on the fishes' ability to hear sounds. A similar paradigm to the one used in the present study is being employed. At present, it has already been determined that the fish will respond to a sound at threshold with an acceleration in EOD (i.e., startle response). Further investigation will involve determination of relative thresholds. Bastian (1986) has reported that acoustic information is also processed in the tectum and trimodal cells for electric, auditory, and visual systems. Future research could assess the interaction of the three sensory systems and such research would necessitate a thorough and comprehensive understanding of each separate system.

APPENDIX A

Table A-1. 1-Way ANOVA Summary Tables for Seven Wavelengths at each of Five Attenuation Levels

	SOURCE	SS	DF	MS	F	p
425 nm AL = 1	SUBJ	648.97	3	216.33	.409	
	A	1460.45	3	486.82	.921	>.05
	RESID	4757.93	9	528.66		
425 nm AL = 2	SUBJ	288.633	3	96.21	.094	
	A	2406.38	3	802.13	.785	>.05
	RESID	9192.98	9	1021.44		
425 nm AL = 3	SUBJ	1053.41	3	351.14	.497	
	A	2539.7	3	846.57	1.197	>.05
	RESID	6363.81	9	707.09		
425 nm AL = 4	SUBJ	3021.2	3	1007.07	1.315	
	A	7237.45	3	2412.48	3.151	>.05
	RESID	6890.67	9	765.63		
425 nm AL = 5	SUBJ	1195.5	3	398.5	1.052	
	A	2751.58	3	917.19	2.423	>.05
	RESID	3406.97	9	378.55		

Table A-1 cont'd.

	SOURCE	SS	DF	MS	F	p
475 nm AL = 1	SUBJ	1436.42	3	478.81	.886	
	A	277.56	3	92.52	.171	>.05
	RESID	4861.93	9	540.21		
475 nm AL = 2	SUBJ	2173.27	3	724.42	.713	
	A	3090.39	3	1030.13	1.015	>.05
	RESID	9137.9	9	1015.32		
475 nm AL = 3	SUBJ	1727.77	3	575.92	.747	
	A	797.67	3	265.89	.345	>.05
	RESID	6941.91	9	771.32		
475 nm AL = 4	SUBJ	2260.98	3	753.66	.73	
	A	1522.45	3	507.48	.492	>.05
	RESID	9285.73	9	1031.75		
475 nm AL = 5	SUBJ	2061.98	3	687.33	.587	
	A	8046.34	3	2682.11	2.29	>.05
	RESID	10542.1	9	1171.35		

Table A-1 cont'd.

	SOURCE	SS	DF	MS	F	p
525 nm AL = 1	SUBJ	2587.22	3	862.41	.923	
	A	2173.22	3	724.41	.776	>.05
	RESID	8405.52	9	933.95		
525 nm AL = 2	SUBJ	5494.98	3	1831.66	7.744	
	A	1417.96	3	472.65	1.998	>.05
	RESID	2128.58	9	236.51		
525 nm AL = 3	SUBJ	5794.25	3	1931.42	7.526	
	A	10379.8	3	3459.92	13.483	<.01
	RESID	2309.59	9	256.62		
525 nm AL = 4	SUBJ	596.98	3	198.99	.425	
	A	2504.08	3	834.69	1.784	>.05
	RESID	4211.56	9	467.95		
525 nm AL = 5	SUBJ	72.20	3	24.07	.052	
	A	1980.8	3	660.27	1.435	>.05
	RESID	4140.22	9	460.03		

Table A-1 cont'd.

	SOURCE	SS	DF	MS	F	p
575 nm	SUBJ	541.05	3	180.35	.618	
AL = 1	A	333.25	3	111.08	.38	>.05
	RESID	2625.18	9	291.67		
575 nm	SUBJ	1656.88	3	552.29	1.065	
AL = 2	A	1227.52	3	409.17	.789	>.05
	RESID	4665.5	9	518.39		
575 nm	SUBJ	3070.38	3	1023.46	1.489	
AL = 3	A	9548.21	3	3182.74	4.629	<.05
	RESID	6188.02	9	687.56		
575 nm	SUBJ	1423.38	3	474.46	.504	
AL = 4	A	4347.64	3	1449.21	1.54	>.05
	RESID	8467.75	9	940.86		
575 nm	SUBJ	1077.91	3	359.3	1.518	
AL = 5	A	569.38	3	189.79	.802	>.05
	RESID	2130.03	9	236.67		

Table A-1 cont'd.

	SOURCE	SS	DF	MS	F	p
625 nm AL = 1	SUBJ	4584.86	3	1528.29	2.854	
	A	1047.69	3	349.23	.652	>.05
	RESID	4819.51	9	535.5		
625 nm AL = 2	SUBJ	1004.18	3	334.73	.382	
	A	313.69	3	104.56	.119	>.05
	RESID	7876.41	9	875.16		
625 nm AL = 3	SUBJ	3177.9	3	1059.3	1.376	
	A	1507.59	3	502.53	.653	>.05
	RESID	6927.79	9	769.75		
625 nm AL = 4	SUBJ	914.48	3	304.83	.514	
	A	1833.44	3	611.15	1.03	>.05
	RESID	5338.84	9	593.21		
625 nm AL = 5	SUBJ	604.89	3	201.63	1.281	
	A	142.36	3	47.45	.301	>.05
	RESID	1416.3	9	157.37		

Table A-1 cont'd.

	SOURCE	SS	DF	MS	F	p
675 nm AL = 1	SUBJ	1324.89	3	441.63	.335	
	A	9344.01	3	3114.67	2.361	>.05
	RESID	11869.2	9	1318.8		
675 nm AL = 2	SUBJ	920.4	3	306.8	.171	
	A	201.63	3	67.21	.038	>.05
	RESID	16121.2	9	1791.24		
675 nm AL = 3	SUBJ	4844.08	3	1614.69	.987	
	A	172.88	3	57.63	.035	>.05
	RESID	14720.0	9	1635.55		
675 nm AL = 4	SUBJ	177.81	3	59.27	.078	
	A	1496.86	3	498.95	.66	>.05
	RESID	6799.23	9	755.47		
675 nm AL = 5	SUBJ	252.3	3	84.1	.225	
	A	190.89	3	63.63	.170	>.05
	RESID	3362.61	9	373.62		

Table A-1 cont'd.

	SOURCE	SS	DF	MS	F	p
725 nm AL = 1	SUBJ	3797.56	3	1265.85	.548	
	A	11378.7	3	3792.9	1.643	>.05
	RESID	20781.4	9	2309.04		
725 nm AL = 2	SUBJ	720.73	3	240.25	.817	
	A	2876.46	3	958.82	3.262	>.05
	RESID	2645.13	9	293.9		
725 nm AL = 3	SUBJ	1450.45	3	484.48	.427	
	A	3674.27	3	1224.76	1.082	>.05
	RESID	10185.4	9	1131.71		
725 nm AL = 4	SUBJ	7657.25	3	2552.42	1.473	
	A	957.09	3	319.03	.184	>.05
	RESID	15599.2	9	1733.25		
725 nm AL = 5	SUBJ	1224.34	3	408.12	.897	
	A	183.98	3	61.33	.135	>.05
	RESID	4092.97	9	454.77		

Note. A = Sessions. AL = Attenuation Level.

APPENDIX B

Table B-1. 3-Way ANOVA Summary Table for Factors: Light Adaptation Condition (A), Wavelength (B), and Intensity (C) and Interactions

SOURCE	SS	DF	MS	F	p
BETWEEN SUBJ.					
A	1632.5	1	1632.5	1.077	>.05
SWG	45492.5	30	1516.42		
WITHIN SUBJ.					
B	25018.5	6	4169.75	4.423	<.01
AB	12838.0	6	2139.67	2.269	<.05
B X SWG	169700.0	180	942.76		
C	174807.0	4	43701.6	53.016	<.001
AC	14614.0	4	3653.5	4.432	<.01
C X SWG	98917.0	120	824.31		
BC	41754.0	24	1739.75	1.973	<.01
ABC	35276.5	24	1469.85	1.667	<.05
BC X SWG	634945.0	720	881.87		

Note. SWG = Subjects Within Groups.

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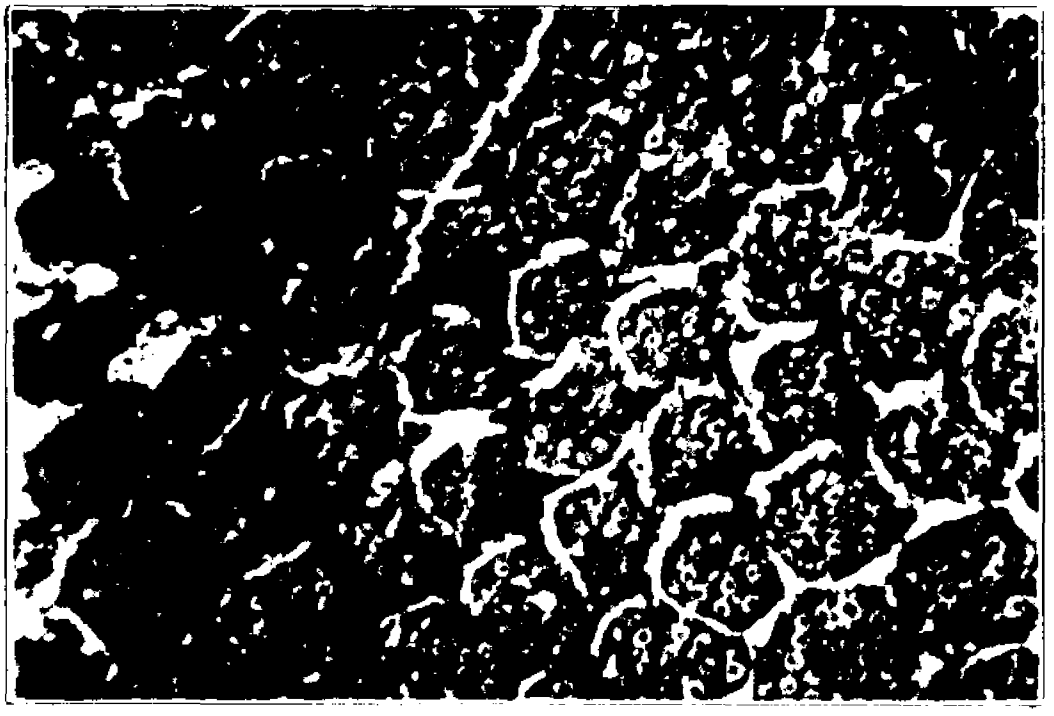

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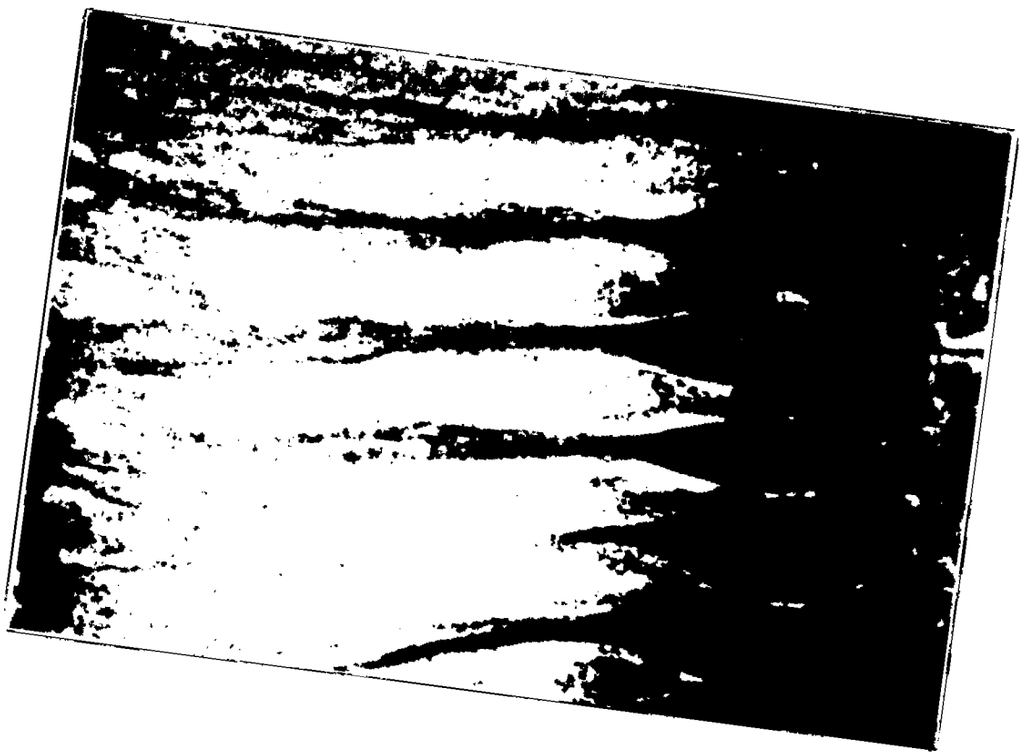

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