

**VIRGINIA RECREATIONAL FISHING DEVELOPMENT FUND
SUMMARY PROJECT APPLICATION***



NAME AND ADDRESS OF APPLICANT: Virginia Institute of Marine Science P.O. Box 1346 Gloucester Point, VA 23062	PROJECT LEADER (name, phone, e-mail): Andrij Z. Horodysky (andrij@vims.edu) (804) 684-7522 Dr. Richard Brill (rbrill@vims.edu) (804) 684-7875
PRIORITY AREA OF CONCERN: Research: continuing project	PROJECT LOCATION: Virginia Institute of Marine Science
DESCRIPTIVE TITLE OF PROJECT: Visual function in Chesapeake Bay sport and prey fishes: summer flounder, bluefish, cobia, and Atlantic menhaden	
PROJECT SUMMARY: Building on our successes with research funded by RFAB grant RF 05-14 in 2005 and year one of RF06-08, we will continue to assess the color vision, visual acuity (i.e., the ability to differentiate separate objects at a distance), and speed of vision of important sport and prey fishes in Chesapeake Bay: summer flounder (<i>Paralichthys dentatus</i>), bluefish (<i>Pomotomus saltatrix</i>), cobia (<i>Rachycentron canadum</i>), and Atlantic menhaden (<i>Brevoortia tyrannus</i>) using state-of-the-art electroretinographic (ERG) and retinal topography techniques.	
EXPECTED BENEFITS: Understanding the visual abilities of Chesapeake Bay fishes is of great importance to researchers and recreational fishermen. Competing predatory species likely have different visual abilities, and thus different prey detection capacities. Our investigations should thus reveal important mechanisms driving predatory-prey interactions, including the competitive advantages of some species over others under specific visual conditions. This two year proposal builds on research funded by RF 05-14, extending this framework to three recreationally important target species (summer flounder, cobia, bluefish), and a major prey species (menhaden). This proposal, together with data from RF 05-14, will provide a network of vision data for competing predators (bluefish, striped bass, weakfish) and major predator-prey complexes (bluefish, striped bass, weakfish vs. menhaden, spot, and croaker) and will greatly improve scientific understanding of the potential role of vision in regulating predator-prey dynamics in Chesapeake Bay.	
COSTS: Continuing Project: Year 2 of 2 VMRC Funding: \$ 50,289 Recipient Funding: \$ 7,933 Total Costs: \$ 58,222 Detailed budget must be included with proposal.	

Updated 6/1/05

*This form alone does not constitute a complete application, see application instructions or contact Sonya Davis at 757-247-8155 or sonya.davis@mrc.virginia.gov : Due dates are June 15 (Jul. – Nov. Cycle) and December 15 (Jan. – May Cycle)

Proposal submission to

THE RECREATIONAL FISHING ADVISORY BOARD
VIRGINIA MARINE RESOURCES COMMISSOIN

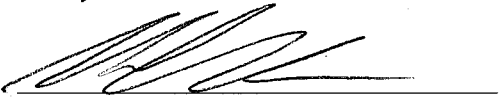
By

THE VIRGINIA INSTITUTE OF MARINE SCIENCE
COLLEGE OF WILLIAM AND MARY


**Visual function in Chesapeake Bay sport and prey fishes: summer flounder, bluefish, cobia,
and Atlantic menhaden**



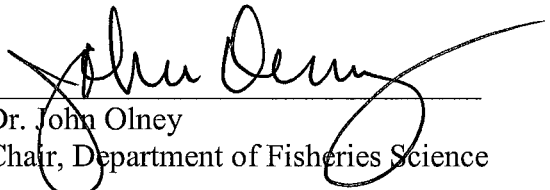
Andriy Z. Horodysky
Co-Principal Investigator



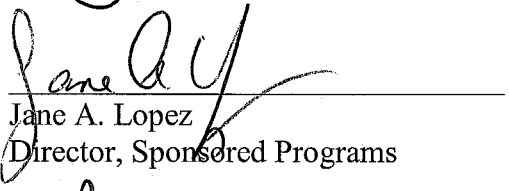
Dr. Richard W. Brill
Co-Principal Investigator



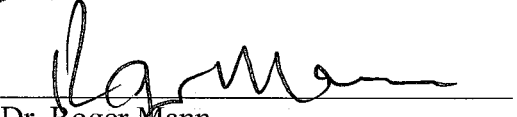
Dr. Robert J. Latour
Co-Principle Investigator



Dr. John Olney
Chair, Department of Fisheries Science



Jane A. Lopez
Director, Sponsored Programs



Dr. Roger Mann
Director of Research and Advisory Services

December 15, 2006

Background/Need

General analyses of body shape and structure suggest that vision is an important mechanism affecting predation success of fishes. Color vision, visual acuity, and speed of vision are important adaptations in fishes as they affect the recognition of mates and fellow conspecifics (Guthrie and Muntz, 1993; Kynard et al., 2002), the avoidance of predators (Poling and Fuiman, 1999), and the location and capture of prey (Browman et al., 1994). Predation influences the structure and dynamics of aquatic communities, but little is known about how estuarine and coastal predators such as summer flounder, bluefish and cobia, and prey fishes such as Atlantic menhaden use visual cues to feed and avoid predation because a complete description of visual function in these fishes is lacking.

We propose, therefore, to use state-of-the-art electroretinographic (ERG) and retinal topography techniques to assess the color vision, visual acuity (i.e., the ability to differentiate separate objects at a distance), and speed of vision of several important sport and prey fishes in Chesapeake Bay: summer flounder (*Paralichthys dentatus*), bluefish (*Pomatomus saltatrix*), cobia (*Rachycentron canadum*), and Atlantic menhaden (*Brevoortia tyrannus*). Data from this proposed research, obtained using the same methods as data supported by RF 05-14 obtained by the authors in 2005 (striped bass, weakfish, spotted seatrout, Atlantic croaker, spot, and red drum), will form a network of vision data for competing predators (bluefish, striped bass, weakfish) and major predator-prey complexes (bluefish, striped bass, weakfish vs. menhaden, spot, and croaker).

Color Vision of Fishes

The eyes of fishes are similar to those of other vertebrates. Light enters the eye through a transparent cornea, a spherical lens focuses the image on the retina, cells within the retina translate the arriving photons to neural activity, and the optic nerve carries visual signals to the brain. Fish retinas generally contain the two main types of photoreceptor (sensory) cells, rods and cones. Rods, which are sensitive to low light, provide vision at night and at great depth, whereas cones are responsible for color and spatial vision in bright light.

Fishes' visual function is generally well-matched to their habitat (Guthrie and Muntz, 1993). For example, shallow water marine fishes, such as striped bass, weakfish, and spotted seatrout, have up to three photoreceptor pigments within their cone cells to cover the wide spectrum of light penetrating the upper surfaces of the water column. As a result, these fishes have the ability to distinguish colors and levels of brightness (Figure 1). In contrast, fishes occupying dim light conditions, such as on the bay bottom, generally have only one or two pigments most sensitive to blue and green wavelengths, because these wavelengths penetrate water the deepest (Helfman et al., 1997; Warrant et al., 1999). Fishes differ greatly in visual acuity and speed of vision. Species that occupy the clear pelagic environment generally have both high visual acuity and high speed of vision, whereas those occupying dim light environments (or those primarily active at night) have a slow speed of vision to integrate visual signals over longer time periods.

The visual abilities of Chesapeake Bay sportfishes have not been described in detail to date. Previous research (RF 05-14) has shown that striped bass, weakfish, spotted seatrout, Atlantic croaker, spot, and red drum have differing abilities in both bright and low light conditions. Species whose visual capabilities well match their specific environments may outcompete those whose visual function does not for access to prey. In addition, species are likely to differ in their abilities to modify their visual function during the day/night cycle.

Electrophysiology and Fish Vision

A suite of electrophysiological and microscopic techniques can be applied to describe the visual function in invertebrate and vertebrate species (McMahon and Barlow, 1992; Warrant, 1999). We intend to apply two fairly straightforward electrophysiological techniques to describe color vision and speed of vision of these fishes: electroretinographic (ERG) spectral sensitivity (SS) and flicker fusion frequency (FFF). Electroretinographic techniques measure the light-induced changes of electrical activity of the light-sensitive rod and cone cells within the retina. ERG is, therefore, a very useful tool for assessing color sensitivity and speed of vision (Figure 2 - Makhankov et al., 2004). We also propose to investigate the spatial distribution of photoreceptors and ganglion cells within the retina (retinal topography). The distribution of these cells within the retina can provide highly detailed information on the relative importance of vision and regions of enhanced vision relative to the body (Figure 3). Additionally, retinal topography investigations will also provide data on the visual acuity (ability to resolve small objects from a distance) for the study species.

Expected results/Benefits

Benefit of Fish Vision Experiments to Fisheries Management

Understanding the importance of vision in predator-prey interactions holds important implications for testing community-level trophic interactions and foraging models. The visual capabilities of fishes to discriminate and select prey, based on cues such as size and color, are central to estimating prey encounter probabilities required for predator-prey interactions models (Walton et al, 1997). This is especially important when considering the interactions of predatory species that feed primarily during the day in brightly lit surface waters with those that feed primarily at night or at depth (i.e., species which are more effective predators at low-light conditions). For example, striped bass, bluefish, and weakfish appear to compete for fish prey populations (Hartman and Brandt, 1995), yet research supported by RF 05-14 demonstrated that striped bass and weakfish have very different color sensitivities and capacities for effective vision in dim light, and ultimately resulting in different prey detection capacities. An evaluation of the visual abilities of Chesapeake Bay's other recreationally-important predatory fishes (summer flounder, cobia, and bluefish) and their prey (Atlantic menhaden) will reveal important mechanisms that potentially drive the predatory or competitive interactions under different visual conditions (Vogel and Beauchamp, 1999). Together with data supported by RF 05-14 obtained in 2005 (visual function of striped bass, weakfish, spotted seatrout, Atlantic croaker, spot, and red drum), this research will form a network of vision data for both competing predators (bluefish, striped bass, weakfish) and major predator-prey complexes (bluefish, striped bass, weakfish vs. menhaden, spot, and croaker). Combined, this data will greatly improve scientific understanding of the potential role of vision in regulating predator-prey dynamics of the dominant recreationally important fishes and their prey in Chesapeake Bay. Moreover, by constructing equations relating the combined effects of light and turbidity on predator reaction distances, the prey detection capabilities of piscivores can be modeled as a function of depth and time in natural environments (Vogel and Beauchamp, 1999).

Benefit of Fish Vision Experiments to Recreational Fishermen

Visual acuity and sportfish color vision bear important implications for artificial lure design and selection by recreational fishermen. Research into the color vision of six estuarine species, including striped bass, weakfish, spotted seatrout, and red drum (RF 05-14) shows that these fishes have fast vision (to resolve high speed prey like menhaden) and up to three visual pigments (similar to humans). These fishes, therefore, see colors ranging from purple through red, although there are strong species-specific differences (Fig 1.). In contrast, marine fishes that feed at great depth or that

forage at night (such as black sea bass and swordfish), have only one visual pigment and very slow vision. As a result, they see well under dim light conditions, but detect light only within the blue-green range (Singarajah and Harosi, 1992; K. Fritsches, pers. comm.).

The most effective lure choices should present animals with colors they see or that effectively contrast against surrounding space light. A study conducted on largemouth bass suggests that this species can readily discriminate colors – animals showed a higher sensitivity to red wavelengths than blue (Kawamura and Kishimoto, 2002). In addition, these authors also showed that the largemouth bass eye is well adapted to discriminate differences in prey shape (visual acuity) and movement (speed of vision), leading to the conclusion that color, shape, and the proper movement of fishing lures resembling their prey are important to potentially increase largemouth bass catches. The retinas of striped bass respond far better to red wavelengths than those of weakfish, spotted seatrout, and red drum during daylight hours; however, none of the species appear to respond to red wavelengths at night. Green wavelengths, including the popular lure color chartreuse, are readily distinguished by all of the above estuarine species.

Approach

Brief methodology:

We will assess color vision, speed of vision, and visual acuity in summer flounder, bluefish, cobia, and Atlantic menhaden. Experiments will be conducted on six to ten animals of each species under bright-light and dim-light conditions to simulate vision during different periods of the day/night and at depth. Each trial will be completed in roughly six hours. Subjects will be anesthetized and suspended with foam rubber and cloth straps (leaving only the eye exposed) in a plexiglass box filled with seawater. A hose delivering oxygenated filtered sea water will be placed in the subject's mouth to ensure adequate ventilation.

A schematic summary of the electroretinographic experiments of fish color vision and speed of vision is presented in Figure 2. During ERG experiments, electrodes will be placed on or just under the skin immediately adjacent to the cornea to measure retinal response to light stimuli. Flashes of light of various frequencies (i.e., colors) and amplitudes (i.e., brightness) will be presented via a custom designed computer-controlled system. Responses will be recorded by the same computer system, which we will not need to purchase – it is already available at VIMS.

We also propose to investigate the spatial distribution of photoreceptors and ganglion cells within the retina (retinal topography) of study species. The distribution of these cells within the retina can provide highly detailed information on the relative importance of vision and regions of enhanced vision relative to the body (Collin and Pettigrew, 1988a; Collin and Pettigrew, 1988b; Figure 3). Additionally, retinal topography investigations will also provide data on the visual acuity (ability to resolve small objects from a distance) for the study species. Retinas are removed from studied species, dissected, and placed in paraformaldehyde-glutaraldehyde preservative. Once fixed, retinas are flat mounted on slides and overlaid with a grid. Photoreceptor cells and ganglion cells are counted manually for each grid, and the resulting distribution of cell counts is mapped for the entire retina.

Location

All summer flounder, bluefish, cobia, and Atlantic menhaden will be obtained live from Chesapeake Bay via the VIMS ChesMMA survey, by cast net (menhaden), hook and line, and via contacts with recreational anglers. Animals will be transported to holding facilities at the VIMS Gloucester Point

or Eastern Shore (Wachapreague) campuses. All experiments will take place at the VIMS Gloucester Point or Eastern Shore Laboratories and will follow protocols approved by the College of William and Mary Institutional Animal Care and Use Committee.

Accomplishments to date

To date, we have obtained spectral sensitivity (color vision) and flicker fusion frequency (speed of vision) data from four flounder (Fig. 4), three cobia, and three menhaden. Whenever possible, day and night recordings were completed for both spectral sensitivity and flicker fusion frequency experiments. After electrophysiological experiments, eye dissections were performed on these fishes. Retinas were removed from both eyes of each specimen and preserved in paraformaldehyde/gluteraldehyde fixative.

Our results thus far indicate interesting species-specific differences in the spectral sensitivity (color vision) and dynamic range (dim-to-bright light range of the retina). Like weakfish, menhaden respond well to the shorter UV stimuli, though menhaden have faster vision and the steepest and smallest dynamic range of any studied species. Bright light overwhelms the menhaden retina, presumably because this species is has very sensitive rod retinal photoreceptor cells. Other findings include that like striped bass, cobia have extremely fast vision (50-60 Hz), but a far more limited color range (no responses at red wavelengths for cobia). Flounder appear to have broad dynamic range (light sensitivity), and very good green-sensitive vision (range: blue through yellow/orange). All species can discriminate green (including chartreuse) – in many cases, the green/yellow border is seen extremely well, which may explain the generally good performance of chartreuse-colored baits.

We are making the preliminary results of this study available to the Virginia Angling community by presenting at local fishing organization meetings. We welcome any such invitations to present results at meetings of local fishing organizations.

Estimated cost

This proposal covers the second and final year of this continuing project. We expect the cost of this study to be \$49,976 for the requested second year. We have independently obtained the major electronic and specialized computer equipment subcomponents required for color vision/speed of vision research from several sources (nearly \$60,000). Accordingly, we will not need to ask the RFAB for funds to obtain this expensive equipment. Animal collection, husbandry, and extensive visual experimentation will be the primary focus. For the second half of year two, we will place a greater influence on outreach via presentations at additional fishing clubs and the development of a color Adobe PDF brochure demonstrating the color vision (similar to Fig. 1 and 4) and retinal topography (similar to Fig. 3) of the species in RF 05-14 and this proposal.

Requested funds would cover:

- (1) the salary costs of a VIMS graduate student to conduct this work,
- (2) the summer salary costs of an hourly intern to assist with animal collection and maintenance,
- (3) a research supply/expenditure budget of \$5,500 which would cover the purchase and maintenance of pumps, tanks, filters, temperature control, and feed for animal holding requirements, as well as anesthetics, retinal preservatives, and required disposable laboratory supplies (electrodes, etc.),

(4) a travel budget of \$3,000 to cover collection and transportation of fishes from local sources to the VIMS animal holding facilities and travel support for presentations at meetings,

(5) a vessels budget of \$500 for collection of fishes,

(6) VIMS Facilities & Administrative Costs at the VMRC reduced rate of 25% (the standard institutional rate is 47.45%). VIMS will provide the difference of the reduced rate versus the institutional rate as match funds.

Literature cited

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Figure 1. Schematic representation of the spectral sensitivity and dynamic range responses of a striped bass (*Morone saxatilis*), spotted seatrout (*Cynoscion nebulosus*) weakfish (*Cynoscion regalis*), to different wavelengths of light (colors) (A. Horodysky, unpubl. data. Research funded by RF 05-14). Images by J. Tomelleri and D. Peebles.

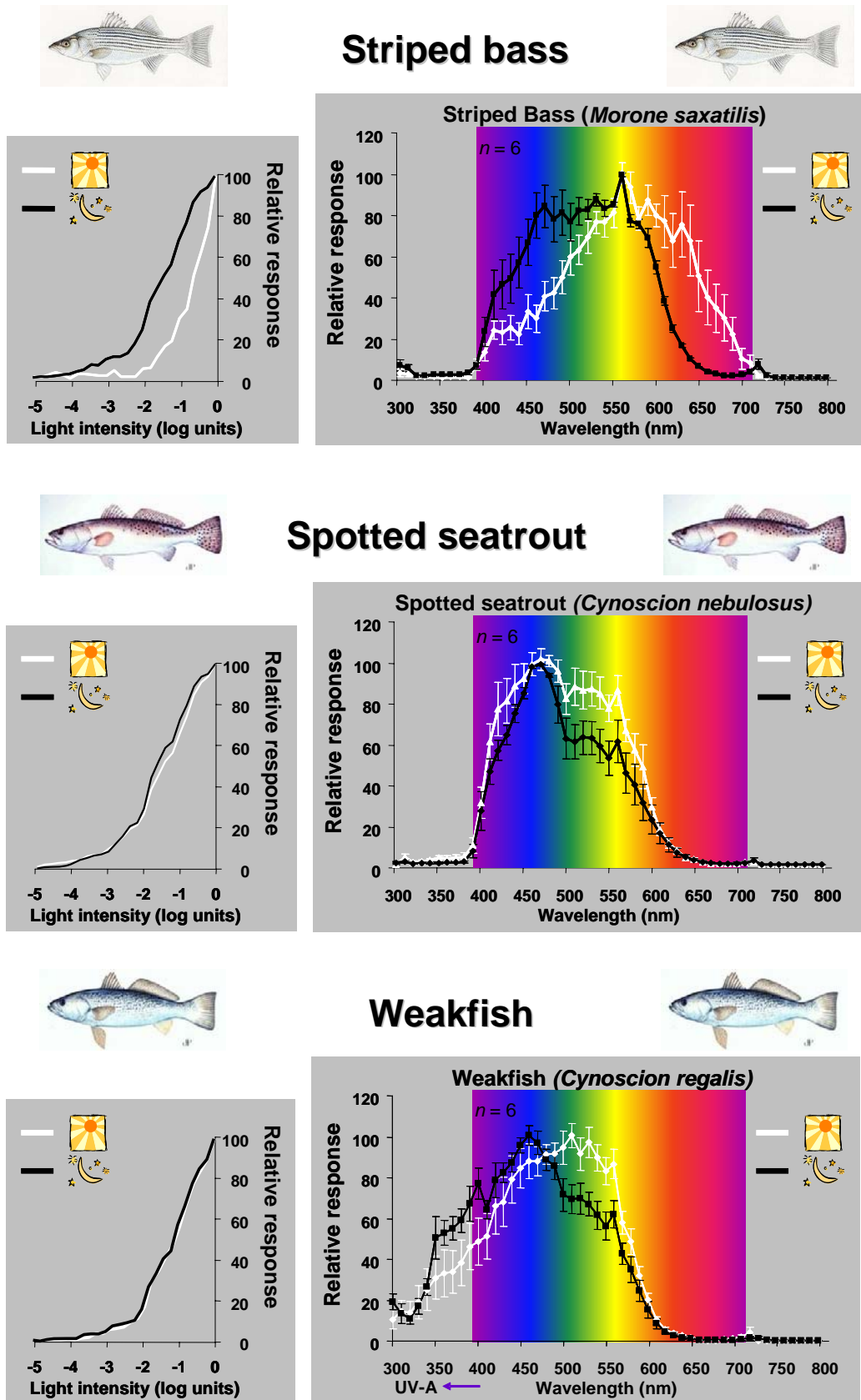


Figure 2. Schematic diagram showing a typical electroretinography (ERG) arrangement for fish color vision and speed of vision experiments. Subjects are anaesthetized and provided with flowing seawater to allow respiration. Electrodes are placed in the skin above the optic nerve to measure electric potentials in response to light stimuli. Flashes of light of various frequencies and amplitudes, as well as various colors will be presented to the subject by a custom designed computer-controlled system. The light will be passed through a filter and focused onto a mechanical shutter head before reaching the subject's eye. Light-induced electrical potentials will pass from the eye to the electrodes and through the positive outputs of differential amplifiers before being recorded by the computer system. We will not need to purchase to such an experimental system, because we have recently obtained this technology at VIMS.

Methods: ERG

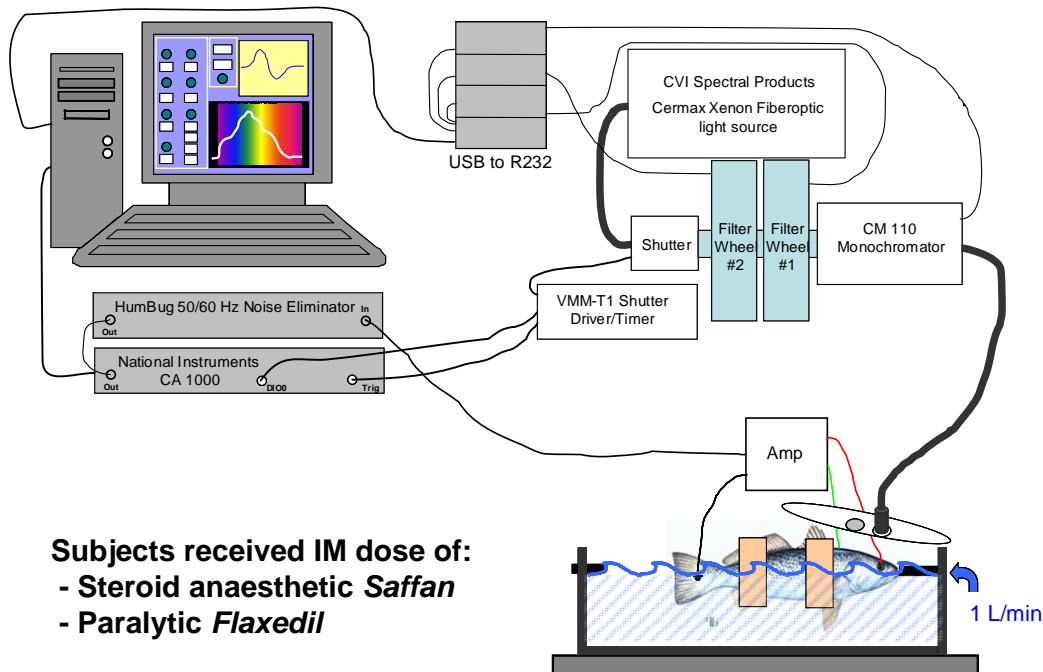


Figure 3. Retinal topography map of a flat-mounted retina of a shovelnose ray *Aptychotrema rostrata* using isodensity contours to show retinal ganglion cell distributions. (A) A visual streak (region of high cell density) in the dorsal retina of the shovelnose ray *A. rostrata*. Dark green indicates regions of highest cell density, black circle indicates the optic nerve head. Slits are cut into the retina to assist wholemounting procedure. For orientation N=nasal, V=ventral, scale=1mm. (B) Cone projection depicting the corresponding regions of the visual field best sampled by the ray's visual system. Adapted from Litherland, 2001.

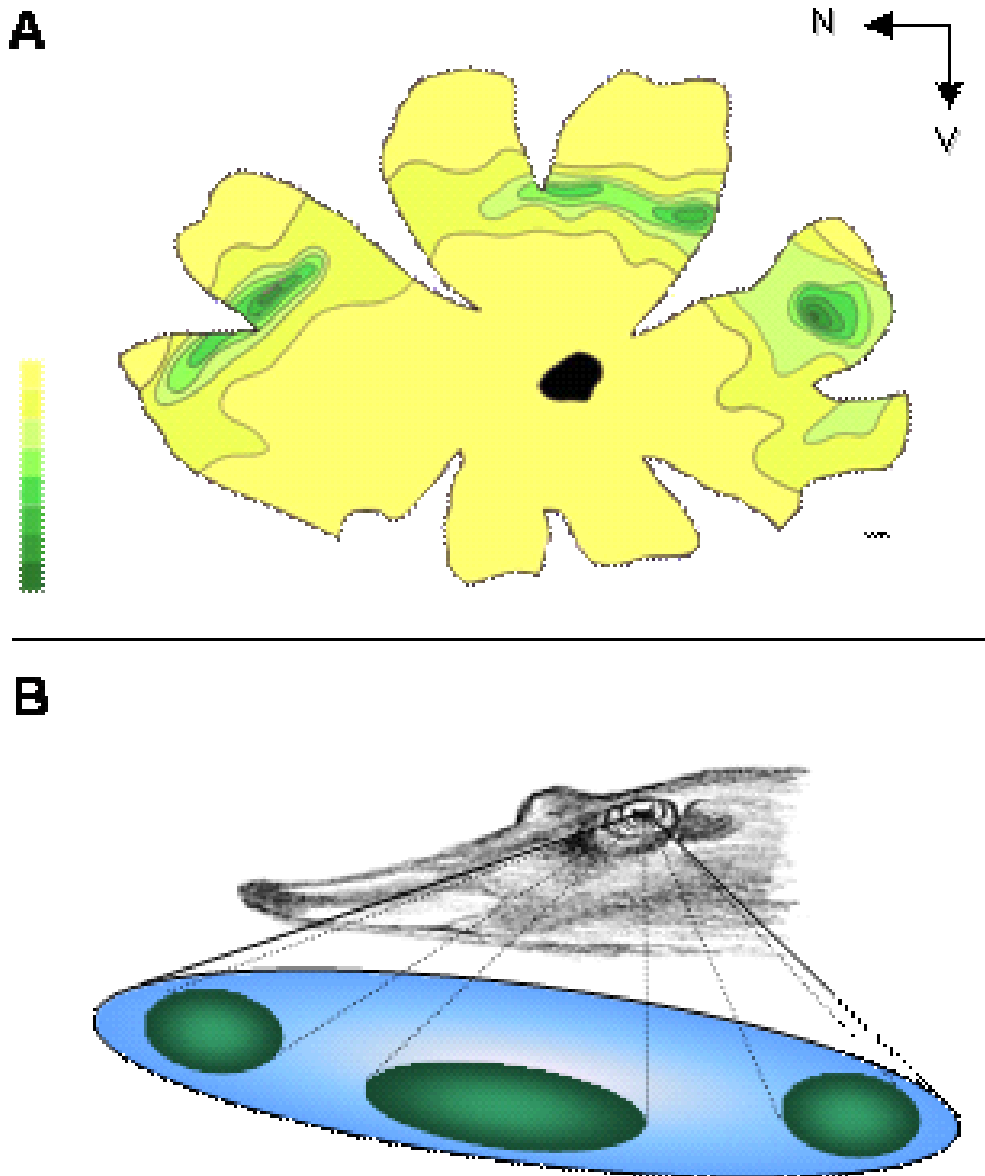
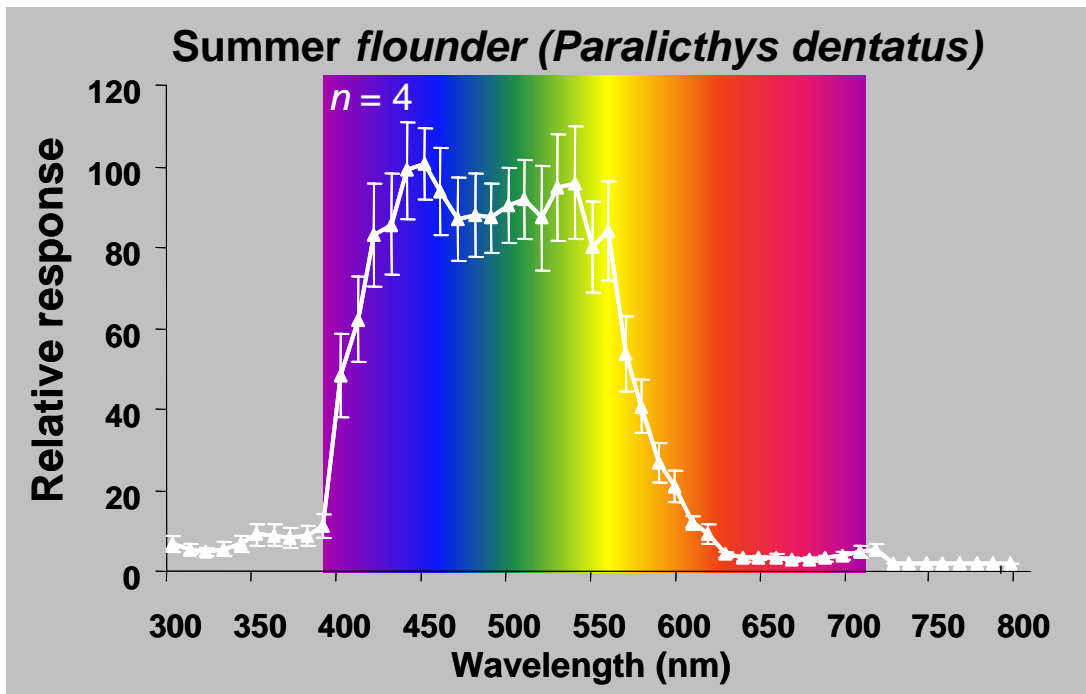


Figure 4. Schematic representation of the daytime spectral sensitivity responses of several summer flounder (*Paralichthys dentatus*) to different wavelengths of light (colors) (A. Horodysky, unpubl. data. Research funded by RF 06-08).



**VISUAL FUNCTION IN CHESAPEAKE BAY SPORT AND PREY FISHES: SUMMER
FLOUNDER, BLUEFISH, COBIA, AND ATLANTIC MENHADEN**

ANTICIPATED BUDGET – Horodysky, Brill, and Latour

	MRFAB	VIMS	Total
<u>Personnel</u>			
R. Brill (1.0 month)	0		0
R. Latour (1.25 month)	7,428		7,428
A. Horodysky, Res Asst	19,574		19,574
Summer hourly help	2,000		2,000
Fringe, 30% salaries	2,229		2,229
<u>Supplies</u>			
Animal holding & maintenance; filters; food, anesthetics, electrodes, batteries	5,500		5,500
<u>Travel</u>			
Field sites for sample collection, presentations at meetings	3,000		3,000
<u>Vessel Rental</u>			
Rental & fuel	500		500
Facilities & Administrative Costs*	10,058*	7,933	17,991
Total	50,289	7,933	58,222

Facilities & Administrative Costs:

The VIMS institutionally approved rate is 45%, however, F&A costs for VMRC requests are limited to 25%. The remaining costs are contributed as part of VIMS match for this project

Dr. Richard Brill will contribute 1 month of his time to the project, but is not requesting any funding since he is supported by NOAA/NMFS.