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Idiopathic Lesions and Visual Deficits in the American Lobster (Homarus americanus) from Longs Island Sound, NY

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Idiopathic Lesions and Visual Deficits in the American Lobster (*Homarus americanus)* from Long Island Sound, NY

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ABSTRACT PAGE

In 1999 a mass mortality of the American lobster (*Homarus americanus*) occurred in western Long Island Sound (WLIS). Although the etiology remains unknown, warm bottom water temperature, hypoxia, heavy metal poisoning, and pesticides have been suggested as casual factors. Subsequently, lobsters from WLIS have continued to display symptoms of morbidity that include lethargy and cloudy grey eyes, caused by idiopathic lesions. The effects of these lesions on lobster vision are unknown. We therefore used electoretinography (ERG) to document changes in visual function in lobsters obtained from WLIS, while simultaneously using histology to quantify the extent of damage. Of the lobsters collected from WLIS, seventy three percent showed damage to photoreceptors and optic nerve fibers including necrosis of the optic nerve, breakdown of the rhabdom, and hemocyte infiltration through the basement membrane into the ommatidia. Animals with more than 15% of photoreceptors exhibiting histological damage also exhibited markedly reduced responses to 10 ms flashes of a broad-spectrum white light. Specifically, the maximum voltage (Vmax) response was significantly lower and occurred at a lower light intensity as compared to responses from lobsters without idiopathic lesions. Lobsters from outside WLIS did not show such reduced changes to their vision. Lobsters from WLIS still appear to be subjected to an unknown stressor with an idiopathic etiology that is causing significant functional damage to their visual system.

IDIOPATHIC LESIONS AND VISUAL DEFICITS IN THE AMERICAN LOBSTER (*HOMARUS AMERICANUS*) FROM LONG ISLAND SOUND, NY

CHAPTER 1:

IMPETUS OF RESEARCH INVOLVING IDIOPATHIC LESIONS AND VISUAL DEFICITS IN THE AMERICAN LOBSTER (*HOMARUS AMERICANUS*)

Introduction to Lobster Mortality in Long Island Sound, NY:

In 1999, a mortality of *Homarus americanus* occurred in the Long Island Sound (LIS) with causes ranging from warm bottom water temperature, hypoxia, heavy metal poisoning, and pesticide introduction. This resulted in a massive economic loss to the fishery in LIS: 70% of lobster fishers reported a 100% economic loss, with the remaining fishers reporting anywhere from a 30-90% loss (Connecticut DEP, 1999).

Long-term population trends in the lobster fishery in LIS have been monitored intermittently from 1976 to 2003 with fisheries-dependent data collected from 1996-2003 (Howell et al., 2005). The western portion of LIS, often termed "The Narrows", was the main site for lobstering. This western portion held 50-60% percent of the catch during the years that data were collected. These data included catch, discard, and water temperature (Howell et al., 2005). A mark-recapture program was used as an indicator to distance migrated from capture site. Only a small percentage of lobsters (1-9%) migrated outside of a 10-20km radius from their original capture site. Prior to the mass mortality in 1999, recruitment of lobsters entering the fishery was strong. However, after 1999 all of the stock (larval, juvenile, and adult) including egg-bearing female lobsters declined. A negative relation was drawn between water temperature and numbers of egg-bearing female lobsters. Months with the highest observed temperatures often saw temperatures in excess of 20 °C. These months had the highest observed lobster mortalities.

Oceanographic conditions in LIS may have acted as a major catalyst for the lobster mortality in 1999. Bottom waters within deeper sections of the LIS typically vary with season (Wilson and Swanson, 2005) and bottom topography has some control

over mixing and stratified conditions in LIS. For example, a cold pool exists in the interior of the LIS basin and typically can be sustained throughout spring and summer, helping to stratify the water column. The perimeter of the basin is tidally mixed and plays an important part on how bottom waters in LIS respond to local surface heat flux. Typically the waters in the western LIS (WLIS) are warmer (~5 degrees Celsius) than those in the eastern portions of LIS. For example, in 1999, WLIS had higher than normal water temperatures, potentially caused by oceanographic conditions within WLIS.

Warming waters and anthropogenically induced eutrophication both have been shown to act as a catalyst contributing to hypoxic bottom waters in field and laboratory settings (Cuomo et al., 2005; Draxler et al., 2005). Sediments leach hydrogen sulfide and ammonium into the water column under hypoxic conditions. The mortality event occurred during the fall of 1999 and continued into the spring of 2000, a critical period within the lobster fishery. The lobster mortality in 1999 coincided with hypoxia and the increase of hydrogen sulfide and ammonium in bottom waters (Cuomo et al., 2005).

Hypoxia, hydrogen sulfide, and ammonium are physiologic stressors to benthic organisms. Eutrophication and warming waters contribute to phytoplankton blooms. The subsequent sedimentation of phytoplankton into the benthos can induce hypoxia in the benthos (<5cm from bottom). As oxygen was depleted in bottom waters in WLIS, heterotrophic benthic microbes began to seek other sources of electron acceptors (i.e. sulfide, ammonia, manganese, etc) (Robohm et al., 2005). In laboratory experiments simulating environmental conditions in WLIS, similar results were found when lobsters were subjected to hypoxia and increased concentrations of sulfide and ammonium

compared to results from field studies (Draxler et al., 2005). Warm water temperature and hypoxia were lethal to half of the lobsters in experimental groups within 5 days; the addition of sulfide and ammonium reduced survival time to 3.3 days. This suggests that phytoplankton bloom and benthic sedimentation can cause secondary changes to the benthic environment causing physiologic stress to *H. americanus*.

Increased water temperatures caused changes in the blood chemistry and phagocytic properties of the hemocytes of *H. americanus* (Dove et al., 2005). In conditions that simulated the increase in bottom water temperature in LIS, lobsters showed acidosis, increased levels of free chlorine in the hemolymph (hyperchloremia), and increased serum proteins (hyperproteinemia). The phagocytic activity normally displayed in hemocytes decreased after 14 days and remained low until the end of the treatment. Once these lobsters were returned to normotensive conditions, their hemolymph chemistry returned to normal. Increased water temperatures can therefore cause undue physiologic stress to *H. americanus*. Though lobsters are capable of living within a temperature range of 10-20 °C, they prefer temperatures below 20 °C. Therefore, prolonged exposure to warmer than normal water temperature, may cause negative changes in the blood chemistry of the lobster (Dove et al., 2005). As a lobster is considered poikilothermic, temperature can act to minimize the functional niche by defining limits to habitat based on temperature.

The combination of rapidly warming waters and hypoxia may also cause immune system stress in *H. americanus*. Heavy metals like manganese can be a proxy for hypoxia causing immuno-suppression (Draxler et al., 2005). Manganese can accumulate in hemolymph and in nervous tissues of lobsters (Baden and Neil, 1998).

Apparently, manganese did not compete with copper and hemocyanin to bind oxygen molecules, however it accumulated in nervous tissues and caused a reduced escape response (i.e. tail flip).

Immuno-compromised animals are more susceptible to opportunistic pathogens. In Robohm et al. (2005), lobsters inoculated with *Aerococcus viridinans* were exposed to increased temperature and hypoxia and increased concentrations of sulfide and ammonium alone and in combination. All of the treatments increased the susceptibility of lobsters to microbial infection. For example, though sulfide and ammonium concentration in experimental conditions lowered the $LC_{50,}$ hypoxia in conjunction with *A. viridinans* accelerated mortality even further.

Opportunistic bacterial infection can be caused by increased abundance and bacterial load in immuno-compromised lobsters. However, bacterial diversity and abundance in LIS were no different than normal (Mullen et al., 2004) nor were any trace elements, PAHs, PCBs, or pesticides found in excess in the hepatopancreas and skeletal muscle tissue during the 1999 mortality. Parasitic amoebae, characteristic of the genus *Paramoeba,* were discovered in hemocoelomic viscera, nerves, and ganglia of the infected lobsters. They were also found in the retina, integumental glands, and muscular interstitium of the eyestalks. However the severity of the pathology of paramoeba was not correlated with the intensity of amoebic infection in the optic nerves (Shields, 2002 in Robohm, 2005). It should be noted that both moribund and dead lobsters were diagnosed with paramoeba infections (De Guise et al., 2005).

Within the spectrum of susceptibility to infection, the immune system of *H. americanus* is its first line of defense against pathogens. Lobsters contain different populations of hemocytes that can be differentiated by size and complexity of cellular structure. For example, circulating hemocytes do not normally proliferate until they are needed. This suggests that they are terminally differentiated once they leave the hematopoietic tissue. Phagocytosis is an important immune effecter in lobsters (Factor et al., 2005). Typically, phagocytosis is performed by semi-granulocytes (Paterson and Stewert, 1974) or fixed phagocytes in the hepatopancreas. The latter are attached to the outer wall at the terminal ends of arterioles and are constantly bathed in hemolymph. In this location, they can efficiently remove foreign particles as hemolymph circulates through the hepatopancreas. Due to the size of the hepatopancreas in relation to other organs of the digestive system, phagocytosis may play a large role in the removal of foreign bodies from hemolymph. Phagocytosis rate can be a good proxy for the health of the immune system of *H. americanus,* in that comparisons can be drawn between lobsters exposed to various environmental and human induced stresses. Physiological stress may impede the immune system's ability to clear bacterial loads in the blood.

Other efforts have been put forth to identify pollutants that may have contributed to the massive mortality. Pesticides and heavy metals can be acutely toxic to larval and juvenile lobsters. For example, the toxicity of pyrethrins can be toxic in concentrations as low as parts per trillion (Zulkosky et al., 2005). At the time of the lobster mortality in LIS, pesticides were deployed to combat mosquitoes in an effort to contain outbreaks of West Nile Virus. Pesticides can be lethal to American lobsters. Specifically organophosphates can bioaccumulate in larval lobsters (Burridge et al., 1999; Burridge

and Kaya 1997), as well as affect adult lobsters (De Guise et al., 2004). The effects can be quantified at the microgram level. In the wild, pesticides are typically below detection limits (parts per trillion) for all but the most sensitive tools. Therefore, sub-lethal effects of these pollutants may occur. Field studies have been undertaken in the LIS to measure concentrations of the pesticides resmethrin, methoprene, and malathion in surface waters (Zulkosky et al., 2005). Stage I and II larvae were acutely sensitive to all three pesticides, though resmethrin was most lethal. Pesticide levels were in the nanogram per liter concentration in WLIS, but were rarely at toxic concentrations even immediately after spraying events. Pesticide levels were at measureable levels at approximately 50% locations sampled by Zulkosky et al. (2005). These results are attributed to differential transport of the active pesticide components and degradation within surface waters.

Though it has been shown experimentally that many commercially available pesticides can cause detrimental effects to lobster physiology, no concrete evidence can directly link pesticides to the lobster mortality in LIS. Instead, researchers are suggesting that multiple stressors (i.e. pesticides, poor water quality, parasitic infection, etc.) additively were responsible for the 1999 lobster mortality in WLIS (Pearce and Balcom, 2005).

Introduction and Background to Lobster Vision:

Invertebrate vision is diverse and each visual system has a specific mechanism for image production. Two major classes of invertebrate eyes exist: the simple eye and the compound eye. Simple eyes have one common image-forming unit (i.e. cornea, lens). Compound eyes are comprised of multiple image-forming structures (i.e. ommatidium, rhabdom) (Nilsson, 1990). *Homarus americanus* has a reflecting superposition compound eye (Chronin, 1986; Meyer-Rochow, 2001). Superposition eyes are typically found in animals in dimly lit environments (Warrant, 1999). These eyes are devoid of screening pigments found in apposition compound eyes. More specifically, superposition eyes have a "clear-zone" that maximizes light absorption by allowing light from a specific source to be collected by multiple ommatidia and focused and reflected onto a single photoreceptor (Gaten, 1998; Warrant and Nilsson, 2006).

The compound eye is comprised of a corneal surface layer, beneath which multiple ommatidia are situated. Each ommatidium is a unit with multiple parts. In a superposition eye these parts are the crystalline cone and corneagenous cells (crystalline cone cells), screening pigments, and rhabdom (Fig. 8). The crystalline cone and screening pigments both act as light guides, in that they help to accurately focus light onto the rhabdom and thus excite or inhibit photoreceptors. The unique feature in the superposition eye is the apparent "clear zone", which maximizes light input to the photoreceptors (Gaten, 1998). Nerves travel from photoreceptors in the rhabdom to the central nervous system. The neuronal circuitry is arranged to organize and integrate information sent from the photoreceptors to the central nervous system so that salient

images can be formed. The "clear-zone" in the reflecting superposition eye of *H. americanus* allows multiple rhabdoms (i.e. multiple photoreceptors) to collect light information. However, both horizontal and vertical control of photoreceptor excitation and inhibition by the neuronal circuitry allow for a clear image to be produced in the vision processing center of the central nervous system. These neuronal control circuits include bi-polar cells and ganglion cells that help to organize information to the central nervous system (Fig. 7) (Delcolmyn, 1996).

Importance of the Proposed Research:

In 1999, a mortality of *Homarus americanus* occurred in the Long Island Sound (LIS). The cause of this massive mortality was classified as a systemic inflammatory disease that primarily affected the nervous system of the lobster, including the ventral nerve chord, brain, and neurosecretory portions of the eyes. The rhabdom and optic nerve showed lesions and alterations to normal morphology. The lesions were comprised of rhabdom alterations including clumping of photopigment, and degradation of optic nerve fibers (Maniscalco and Shields, 2006).

The degradation of the visual system of lobsters poses an interesting question as to the likely cause of the lobster mortality in the Long Island Sound, NY especially in WLIS. As mentioned previously, multiple stressors have been indicated as no single cause was identified. Potential contributing stressors include eutrophication, unseasonably warm water, hypoxia, pesticides, opportunistic paramoeba infections, and increased concentrations of sulfides, ammonia, and manganese.

Objectives:

With approximately 50% of lobsters within WLIS suffering some form of blindness (Maniscalco and Shields, 2006), it is important to understand how vision in lobsters from WLIS is affected and the extent of the damage to their eyes. The goal of this research is to understand how potential environmental stressors affect the visual system of *H. americanus*. The objectives are to quantify the extent of damage to the visual system using electrophysiological and histological techniques. Lobsters are chemosensory and mechanosensory specialists. Though vision may not play a major role in mating and food finding, it may aid in agonistic interaction between sexes, predator avoidance and obstacle avoidance (Snyder et al., 1992). Without accurate vision, precise and efficient escape movement and proper and timely response to the environment may compromise individual lobsters that have impaired vision.

CHAPTER 2:

IDIOPATHIC LESIONS AND VISUAL DEFICITS IN THE AMERICAN LOBSTER (*HOMARUS AMERICANUS*) FROM LONG ISLAND SOUND, NY

Abstract:

In 1999 a mass mortality of the American lobster (*Homarus americanus*) occurred in western Long Island Sound (WLIS). Although the etiology remains unknown, warm bottom water temperature, hypoxia, heavy metal poisoning, and pesticides have been suggested as casual factors. Subsequently, lobsters from WLIS have continued to display symptoms of morbidity that include lethargy and cloudy grey eyes, which contain idiopathic lesions. The effect of these lesions on lobster vision is unknown. We therefore used electoretinography (ERG) to document changes in visual function in lobsters obtained from WLIS, while simultaneously using histology to quantify the extent of damage. Of lobsters from WLIS, seventy three percent showed damage to photoreceptors and optic nerve fibers including necrosis of the optic nerve, breakdown of the rhabdom, and hemocyte infiltration through the basement membrane into the ommatidia. Animals with more than 15% of the photoreceptors exhibiting histological damage also exhibited markedly reduced responses to 10 ms flashes of a broad-spectrum white light. Specifically, the maximum voltage (Vmax) response was significantly lower and occurred at a lower light intensity as compared to responses from lobsters without idiopathic lesions. Lobsters from outside WLIS did not show such reduced changes to their vision. Lobsters from WLIS still appear to be subjected to an unknown stressor with an idiopathic etiology that is causing significant functional damage to their visual system.

Keywords:

electroretinogram, ERG, eye, V-log I, vision, blindness

Introduction:

The American lobster, *Homarus americanus*, forms the basis of an important commercial fishery on the east coast of the United States; in 2006 landings were 42,000 metric tons and were valued at $395 million (National Marine Fisheries Service Annual Landings Query). Historically, 16% of the total lobster landings were caught in Long Island Sound (LIS); within LIS, 50-60% percent of that catch was taken in western Long Island Sound (WLIS) (Fig. 1) (Howell et al. 2005). In the fall of 1999, a mass mortality of lobsters occurred in WLIS, which drastically reduced landings. By 2006 the LIS fishery contributed only 5% of the total landings on the east coast of the United States. The etiology of the mortality remains unknown but elevated bottom water temperature, hypoxia, heavy metal poisoning, increased pesticide use, and an outbreak of the facultative parasite *Neoparamoeba* sp. all have been implicated as the causal agents (Maniscalco and Shields 2006; Mullen et al. 2004; Pearce and Balcom 2005). Lobsters suffering mortality exhibited inflammation, cellular infiltrates in the central and peripheral nervous system, immune system depression (Deguise 2005, Factor et al. 2005), and alteration to the architecture of the compound eye (Mullen et al. 2004; Maniscalco and Shields 2006). Moribund lobsters were found to harbor infections of a *Neoparamoeba* spp., associated with hemocyte infiltration in nerves and ganglia (Mullen et al. 2004), though no direct correlation has been found between the mass mortality event and infections with *Neoparamoeba* sp.

Subsequent to the 1999 mortality event in the WLIS, surviving lobsters continued to display lesions of varying intensities within the compound eye. Surveys conducted in 2001 and 2004 showed eye stalk lesion prevalence to be 56% (Maniscalco

and Shields 2006). Afflicted lobsters exhibited clumping of eye pigment, damage to the basement membrane of the ommatidia, and necrosis of the ommatidia and optic nerve fibers. In extreme cases, the basement membrane was disrupted and hemocytes had infiltrated into the ommatidia region, with a loss of the general internal structure and morphology of the eye (Maniscalco and Shields 2006). The idiopathic lesions in the eyes of lobsters are of unknown etiology. More importantly, it is unknown whether lesions affect vision. As in other crustaceans (Cronin 1986; Warrant and Nillson 2006), lobsters have a reflecting superposition eye that is highly sensitive to light (Barnes and Goldsmith 1977; Warrant 1999). In this type of compound eye, individual ommatidia collect light and output from individual photoreceptors is integrated into an image in the brain. Any change to the compound eye due to damage may cause adverse effects in how effective the eye collects sensory information (Nillson and Lindström 1983; Loew 1976).

Vision is not the primary sensing mechanism for *H. americanus*; primarily they rely on their chemosensory and mechanosensory organs to perform complex behaviors including mating (Johnson and Atema 2005), foraging (Breithaupt et al. 1999), and social interactions (Breithaupt and Atema 2000). Although considered supplemental, vision may serve as a tool in obstacle avoidance (Snyder et al. 1992) and in capturing prey items (Shelton et al. 1985). However, adult Norway lobsters (*Nephrops norvegicus*) exhibit no change in survival and growth, even with light-induced damage to their superposition eyes (Chapman et al. 2000). In combination with other sensory mechanisms, however, vision is a powerful tool to acquire information from the external environment; any change to vision (i.e. damage) can drastically affect how

organisms collect sensory information from the environment (Meyer-Rochow 1994). The degradation of the visual system may be related to the lobster mortality event in WLIS observed in 1999. Although the events may be unrelated, it serves as additional evidence that these lobsters were under significant environmental stress.

Our objective was to document changes in the visual response of lobsters from WLIS with idiopathic lesions by comparing their responses to those caught outside WLIS and therefore without such lesions. We used electroretinography (ERG) to measure changes in the gross retinal response and standard histological methods to assess the extent and intensity of idiopathic lesions in the eyes of the American lobster. These procedures allowed correlations to be drawn between the retinal response and the extent of histological damage from idiopathic lesions.

Materials and Methods

Lobsters were collected from Western Long Island Sound (WLIS), as well as Rhode Island (RI), and Virginia (VA) between 2006 and 2007 using standard pot gear. All lobsters were shipped to the Virginia Institute of Marine Science and maintained in a 500-liter recirculating tank chilled to 15 °C for a minimum of two weeks before use in an experiment. Holding conditions included a 12:12 hour light-to-dark cycle for all lobsters in the study. During this period, they were fed squid (*Loligo sp.) ad libitum.* In total, 20 lobsters were processed: 14 lobsters from WLIS, five from VA, and one from RI.

ERG experiments were performed without prior knowledge of the condition of the eyes to eliminate any bias in assessing damage. Lobsters were placed in a custom-

designed Plexiglas chamber, restrained on a small platform, and allowed to dark adapt for 30 minutes.

The chamber was supplied with chilled (15 °C), aerated seawater and the water level was maintained approximately 1 cm below the eyes. The eye to be tested during the ERG experiment was immobilized at the base of the eye stalk using quick-setting glue. Platinum wire needle electrodes (Grass Technologies, Warwick, RI, USA) were inserted into the eye stalk and the dorsal musculature in between the pleomeres of the abdomen. Responses were amplified 10,000 x and filtered at 200 Hz low-pass and 3 Hz high-pass using a DAM-50 amplifier (Tucker-Davis Technologies, Gainsville, FL, USA) and further conditioned using a Humbug® active electronic filter to remove 60 Hz noise (Quest Scientific, North Vancouver, BC, Canada). To minimize ambient electrical noise, the chamber holding the lobster and amplifier were placed in a Faraday cage grounded to the seawater using a stainless steel bolt. Data digitization (2 KHz sampling rate) and stimulus presentation were controlled using Bio-Sig software (Tucker-Davis Technologies, Gainesville, FL, USA).

ERG responses were recorded to 10 ms flashes of broad spectrum white light of increasing light intensities (I, in log candela m^{-2}). The light source (model SL2420, Advanced Illumination, Rochester, VT) consisted of 20 white light-emitting diodes (LEDs) which created a 3.5 cm uniform field. Light intensity was controlled using an intensity controller (model CS410, Advanced Illumination, Rochester, VT), which in turn was controlled by the voltage output of a digital-to-analog output associated with a programmable attenuator (model PA5 Tucker-Davis Technologies, Gainesville, FL, USA), which was controlled by Bio-Sig software (TDT Inc, Gainesville, FL). The range

of light output was expanded through the use of neutral density filters (Kodak Optical Products, Rochester, NY). Light intensities ranged from 3.16×10^{1} cd cm^{-2} to 6.31×10^{3} cd cm^{-2}. To assess visual function, responses to increasing light intensities (V-log I response curves) were constructed using ERG responses to a 10 ms duration light flash, which increased in 0.1 log unit steps from light intensities producing no measureable response to intensities producing a maximum response. Light stimuli were presented every five seconds. An average was created using five ERG responses at each light intensity. Data were analyzed as either a raw voltage or normalized as a fraction of the maximum observed response for each experiment.

Following the ERG experiments, lobsters were euthanized with an overdose of cold, saturated potassium chloride injected into the ventral nerve cord. Lobsters were then assessed for any signs of external damage. The eyes were then removed at the base of the eyestalk, placed in Bouins fixative for 48 hours, and moved into 70% ethanol for storage. Eyes were subsequently decalcified overnight using a sodium-citrate-EDTA decalcifying solution (Luna 1968), cut in half with a razor blade, then dehydrated, cleared, and infiltrated with paraffin wax. Tissues were embedded in paraffin wax blocks, cut in 5-6 micron sections, and stained with Mayer's hemotoxylin and eosin (Luna 1968).

Sections were examined using a compound light microscope and photographed with an attached digital camera (Nikon DM1200, Nikon Inc., Melville, NY). Areas of specific interest included the ommatidia and rhabdoms, basement membrane of the ommatidia, optic nerve fibers, and connective tissues near the lamina ganglionaris. One eye from each lobster was selected for further analysis. Specifically, the basement

membrane between the ommatidia and the optic nerve fibers was measured using a calibrated optical micrometer at 10x magnification, where 1 optical unit (OU) on the micrometer equaled 98.9μm. The basement membrane measurement was used as a proxy for the effective surface area available to collect sensory information. Each OU was assessed and any apparent damage was documented. Data were presented as a percentage of healthy tissue from the total basement membrane measurement for each eye. Lobsters were classified as affected if more than 15% of the total measured surface area had some form of histological damage.

Paired histological and physiological data were analyzed using Minitab 15 (Minitab Inc., State College, PA, USA), Microsoft Excel 2003 (Microsoft Corporation, Redmund, WA, USA) and Systat 12 (Systat Software, San Jose, CA, USA). Linear regressions were used to quantify the relationship between the extent of damage viewed histologically and from the ERG data. A repeated measures ANOVA was used to examine differences in V-log I responses between healthy and affected lobsters. T-tests were used to compare voltage data between healthy and affected lobsters. Values with p-values less than 0.05 were considered statistically significant.

Results

The histological damage in the compound eye was quantified in 14 lobsters from western Long Island Sound, NY (WLIS). All lobsters were in good physical condition with no apparent exterior damage to the carapace or eyes. The average (± SEM) measurement of basement membrane was 35.2 (± 2.35) mm, with 4.0 (± 0.08) ommatidia mm^{-1}.

The histopathology of the eyes of lobsters from WLIS we observed was similar to that reported by Maniscalco and Shields (2006). Briefly, cellular change caused by lesions included changes to the cellular structure of the eye with necrosis of the optic nerves, but damage was not noticeable histologically beyond the lamina ganglionaris. Histopathologic changes including necrosis were most apparent in the ommatidia, rhabdom, basement membrane of the ommatidia, and optic nerve fibers anterior to the lamina ganglionaris. Pigment in the ommatidia lost its characteristic organization and uniform displacement (Fig. 2a). Pigment were often clumped and displaced from their normal location surrounding the optic nerves (Fig. 2b). The rhabdom lost its linear spindle shape (Fig. 3a) and the basement membrane often appeared ragged and degraded (Fig. 3b). The basement membrane, normally a membrane between the rhabdom and optic nerve fibers (Fig. 3a), was frequently disrupted or separated from the ommatidia. This separation allowed hemocytes to infiltrate through the basement membrane into the rhabdom (Fig. 3b). In virtually all affected eyes, the optic nerve fibers were degraded to differing degrees, ranging from localized damage of a few fibers to extensive damage encompassing all of the optic nerve fibers. Normal healthy nerve fibers had a linear and fibrous organization in well-organized nerve tracts (Fig. 3a). In extreme cases optic nerve fibers lost their organized, linear appearance and were necrotic, replaced with vascular tissue, or lost completely (Fig. 3b).

Idiopathic eye lesions were found in 10 of 14 animals from the WLIS. Lesions occurred in the ommatidia region, basement membrane, and optic nerve fibers and were essentially identical to those previously reported by Maniscalco and Shields (2006). Four lobsters from within WLIS, and five lobsters from VA and RI, had either no or

only minimal eye damage that was not representative of the idiopathic lesions observed from within WLIS.

Physiological data were collected from 10 affected and 9 unaffected lobsters. ERG responses to light flashes increased with increasing light intensity (Fig. 4) in all animals. Difference in mean ERG responses between healthy and affected lobsters became significantly divergent at 2.1 log I light intensity units using a repeated measures ANOVA ($p < 0.05$). Animals with healthy eyes responded to a greater range of light intensities and had a larger maximum voltage response (Vmax) to the brightest light intensity presented during the experiment. To reduce individual variation, data were normalized to the Vmax for each experimental trial. Lobsters with eye lesions reached 100% of their Vmax (i.e. normalized voltage response) at a significantly lower light intensity compared to animals with healthy eyes (Fig. 5). Three lobsters from the WLIS had 100% damage and showed no appreciable ERG responses at all light intensities detectable above the ambient electrical noise of the system.

Healthy lobsters (< 15% damage) had a significantly higher average Vmax (485 ± 15 uV; range: 198 uV - 1083 uV) than lobsters with affected eyes (79 ± 27 uV; range: 14 uV - 278 uV) (t-test, $p < 0.05$). One afflicted lobster had an unusually large response (278 uV maximum response, shown by the filled circle in Fig 6), though only 32% of the ommatidia in its eye was deemed healthy. All other responses from afflicted lobsters were below 155 uV (Fig. 6). For lobsters with affected eyes, the fraction of undamaged ommatidia was not significantly correlated with the physiological response data ($y = 0.2134x + 71.244$, $R^2 = 0.0063$, S = 89.288) (Fig. 6).

Discussion

The retinal response (ERG) of lobsters with greater than 15% damage was reduced both in maximal response (Vmax) at the brightest light intensity and percent maximum response over the V-log I response curve. Crustaceans with a loss of some visual function would not be blind; however, their vision would be greatly reduced (Meyer-Rochow 2001). Superposition compound eyes act as light collectors and integrate an image over the entire working area of photoreceptors (Cronin 1986; Cronin and Marshall 2001; Wald 1968). The advantage of a superposition eye is its sensitivity at lower light intensities, albeit at the expense of speed of vision (Warrant 1999; Warrant et al. 1996; Warrant and Nilsson 2006). Damage to the eye could cause a loss in sensitivity. Therefore, if a lobster loses any portion of its photoreceptors, it will not only lose light collecting organs, but also some of the neural architecture necessary for integrating images. In extreme cases (i.e. 100% damage) lobsters would be considered entirely blind as there would be no working photoreceptors to collect sensory information or optic nerves to process and integrate information from photoreceptors.

A reduced Vmax may indicate that a reduced number of photoreceptors successfully collected sensory information and that small amounts of damage can cause a drastic reduction in how effectively photoreceptors collected sensory information. Interestingly, results indicated that lobsters with eye lesions also reached Vmax at lower light intensities compared to lobsters without lesions. Fewer functioning photoreceptors likely photosaturate at lower light intensities and would therefore be unable to collect sensory information at brighter light intensities. The results from this study indicate a combination of a reduced number of functioning photoreceptors explained by

differences in the Vmax response for raw and normalized ERG data. Our research, therefore, confirms findings from Maniscalco and Shields (2006) that lesions cause drastic changes to vision in *H. americanus*, moreover that the environmental conditions resulting in these lesions still exist in WLIS.

We conclude that light-induced damage was not a factor causing lesions and reduced visual sensitivity in *H. americanus* from WLIS for the following two reasons. First, light-induced damage has not been reported in *H. americanus* (Goldsmith and Bruno 1973) as it has in Norway lobster (*Nephrops norvegicus*) (Loew 1976; Meyer-Rochow 1994). Second, all the lobsters used in these experiments were exposed to light intensities (3.16×10^{1} cd cm^{-2} to 6.31×10^{3} cd cm^{-2}) in an effort to simulate biologically relevant conditions encountered throughout the home range of *H. americanus*. Holding conditions also included a 12:12 hour light-to-dark cycle for all lobsters in the study. Control lobsters, therefore, experienced the same light levels as lobsters from WLIS, and the former showed no evidence of damage to their eyes.

Based on their size, we estimate that lobsters used in this study were approximately five years old. Although carapace length of lobsters is only an approximation (Hartnoll 2001; Hughes and Matthiessen 1962), all animals met minimum and maximum market requirements. Therefore, we are confident that no lobsters in this study were present during the original 1999 mortality event in the WLIS and the lesions we observed were not associated with the original mortality in 1999, rather the lesions discovered in this study were the result of a stressor still present in WLIS.

Similar lesions have been reported in another crustacean, the Australian prawn *Penaeus monodon* (Callinan et al. 2003; Smith 2000), but were linked to a viral infection related to Australian gill-associated virus (GAV). GAV typically caused lethargy and shell discoloration along with retinopathy including necrosis of the eye and optic nerves. Histologically, severe cell degradation and necrosis of axons and surrounding sheaths was evident; severe retinopathy was also evident with edema, cellular debris, and hemocyte infiltrated in the ommatidia. Though these lesions and eye damage from this study are similar, lobsters from WLIS showed no lethargy or shell discoloration and were generally in good health prior to the start of any experiments; few lobsters from WLIS showed cloudy grey eyes (Magel and Shields, personal observation) and subsequent analysis showed that these eyes had extreme damage.

Conclusions from the initial reports in 1999 suggest that hypoxia and pesticides were major factors in the 1999 mortality of *H. americanus* in WLIS (Pearce and Balcom 2005). Hypoxic events can leech heavy metals (i.e. Mn^{2+} and CO^{2+}) from pore waters and, therefore, have been suggested as a proxy for exposure to hypoxic water that may affect lobsters, especially those within WLIS (Draxler et al. 2005). Manganese (Mn^{2+}) is readily found in tissues of *H. americanus* and *N. norvegicus,* and due to elimination kinetics is often an indicator of hypoxia. Manganese (Mn^{2+}) has been known to affect chemosensory organs of *N. norvegicus* and their ability to locate food (Krång and Rosenqvist 2006), but it is unknown whether Mn^{2+} affects other sensory mechanisms in *N. Norvegicus* or *H. americanus*. Further, manganese has been shown to accumulate in the nerves of *N. norvegicus* exposed to hypoxia (Baden and Neil 1998). In high concentrations, manganese and other heavy metals may affect

escape behavior in *N. norvegicus* (Baden and Neil 1998). Though *H. americanus* examined in this study were not tested for high concentrations of Mn^{2+} it is a viable hypothesis that heavy metals like manganese may affect the sensory mechanisms of *H. americanus,* albeit its role in the degradation of the optic nerves remains to be determined.

During the initial 1999 mortality, pesticides were used to control mosquitoes to thwart outbreaks of West Nile Virus (Pearce and Balcom 2005). Pesticides have been linked to increased stress and mortality in lobsters (Burridge and Haya 1997; Burridge et al. 1999; Walker et al. 2005). Specifically, methoprene has an effect on juvenile hormone agonists and also in cuticle production and post-molt shell quality in adult lobsters (Walker et al. 2005). It has been shown to accumulate in the hepatopancreas, gonad, epithelial tissue, and nerve tissue in the eye stalk (Walker et al. 2005). Pesticides are volatile and may occur below detection limits (De Guise et al. 2004); no appreciable build up of methoprene or other pesticides was discovered in WLIS after the lobster mortality of 1999 (Zulkosky et al. 2005); other research indicates sublethal affects on lobsters (Walker et al. 2005). Though biochemical analysis was not performed to confirm any accumulation of pesticides in this research, pesticides cannot be ruled out as a potential cause of damage and lesions in the eyes of *H. americanus*.

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Figure Legends:

Fig 1
A map of the Long Island Sound depicting the Western (WLIS), Central (CLIS), and Eastern (ELIS) portions of the sound. All animals from within the Long Island Sound were collected from WLIS. Figure originally provided by Colleen Giannini, Connecticut Department of Environmental Protection and adapted from Maniscalco and Shields (2006).

Fig. 2
Distal portion of a healthy (a) and affected (b) eye stalk of *Homarus americanus*. Note that affected eye stalks lose general structure and often exhibit clumped pigment in the ommatidia (OM), rhabdom (RB) and basement membrane (BM) breakdown, and necrosis of optic nerve fibers (ON). Scale = 500 μm

Fig. 3
Magnified image of healthy (a) and damaged (b) rhabdom in the eye *Homarus americanus*. Healthy rhabdoms have a distinct spindle shape and form a connection to optic nerve fibers through the basement membrane. Damaged rhabdom becomes ragged and loses its spindle shape. Optic nerve fibers become necrotic. Basement membrane is ruptured allowing hemocyte infiltration into the photoreceptor space. Scale = 100 μm

Fig. 4
V-Log I response curves constructed from electroretinogram (ERG) responses to increasing light intensities (I, in log candela/m^2) in *Homarus americanus*. V-log I curves were constructed using the mean value of 5 ERG responses to a 10 ms duration flash of light increasing in 0.1 log unit steps from 0.0 (no measurable response) to 3.4 (maximum response). Light stimuli were presented every five seconds. Data are presented as voltage (uV). Data points are means ± standard error. Circles indicate data from healthy lobsters; triangles indicate data from affected lobsters.

Fig. 5
V Log I curves constructed from electroretinogram (ERG) responses to increasing light intensities (I, in log candela/m^2) in *Homarus americanus*. V-log I curves were constructed using the mean value of 5 ERG responses to a 10 ms duration flash of light increasing in 0.1 log unit steps from 0.0 (no measurable response) to 3.4 (maximum response). Light stimuli were presented every five seconds. Data presented were normalized as a fraction of the maximum observed response for each experiment. Each data point is a mean ± standard error. Circles indicate data from healthy lobsters; triangles indicate data from affected lobsters.

Fig. 6
Data correlating maximum voltage response (uV) to the percentage of healthy eye tissue (%) in *Homarus americanus*. Regression analysis shows no difference between healthy animals within (open triangle) and outside (closed triangle) Long Island Sound, NY. There is also no relationship between Vmax and the percentage of healthy tissue in lobsters with eye lesions (circles). $y = 0.2134x + 71.244$, $R^2 = 0.0063$, S = 89.288).

Fig. 7
Diagram of a compound eye. In order starting most distal from the optic nerve is the cornea, corneagenous cells (crystalline cone cells), rhabdom, and basal retinula cells. Basal retinula cells and retinula cell axons connect into the optic nerve and ultimately the central nervous system of *Homarus americanus.* In the area demarked as crystalline tract, that this area is devoid of pigment and thus is the "clear zone" of the reflecting superposition compound eye.

Fig. 8
Distal portion of a healthy eye stalk of *Homarus americanus*. Healthy rhabdoms have a distinct spindle shape and form a connection to optic nerve fibers through the basement membrane. Image took First place in the 2008 VIMS Scientific Photo Contest.

Fig 1

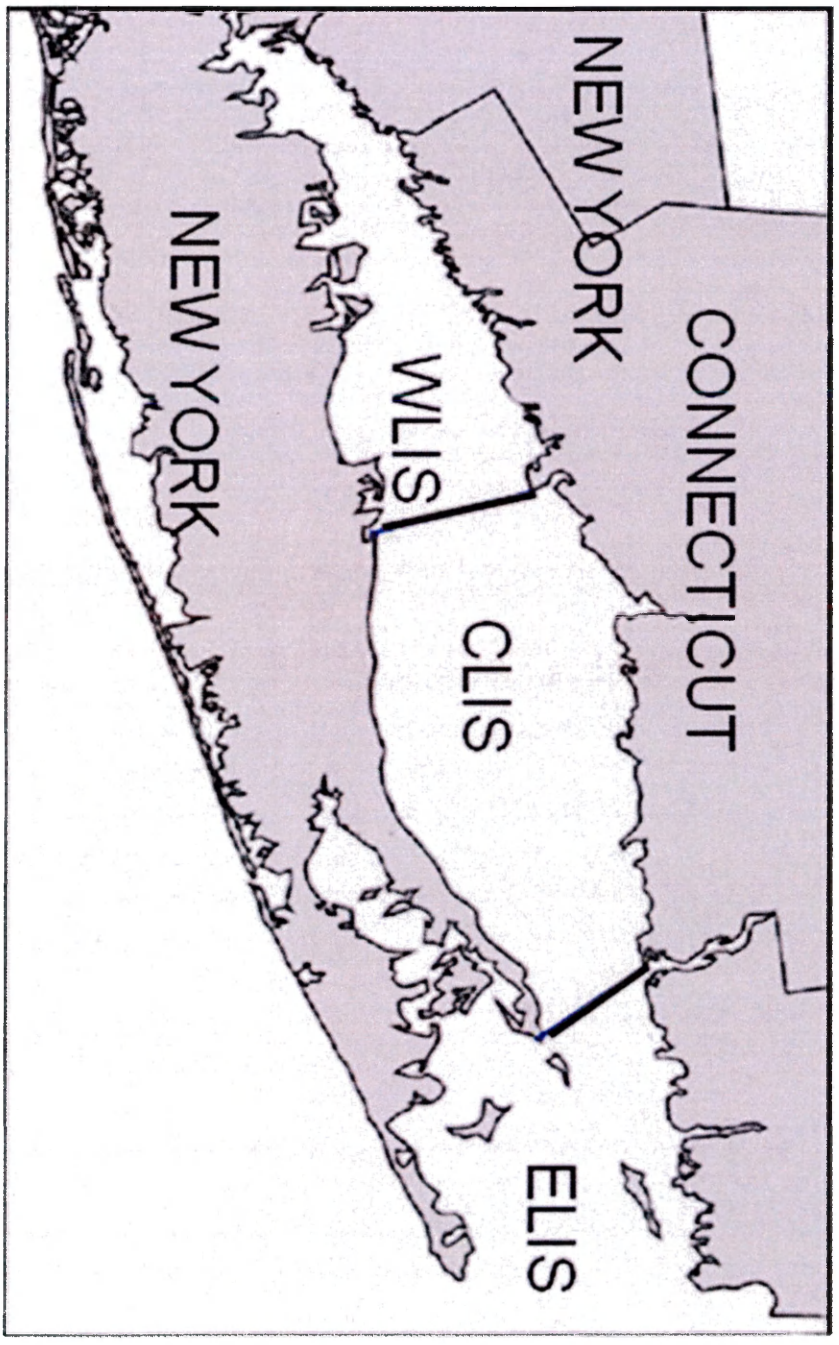


Fig 2a

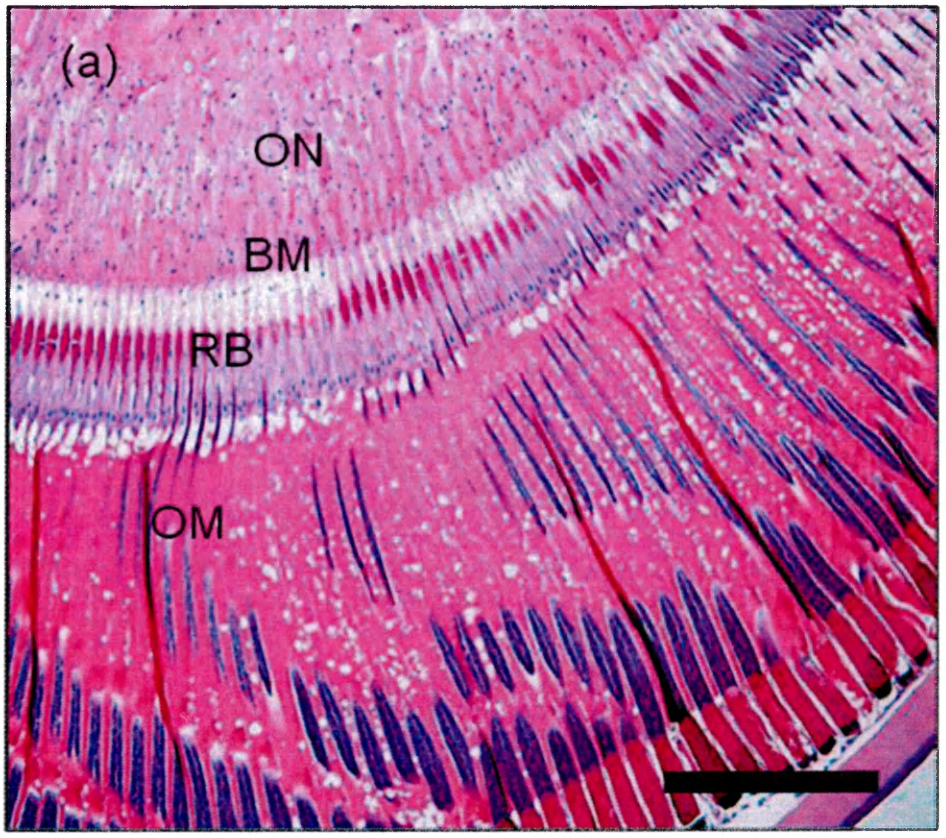


Fig 2b

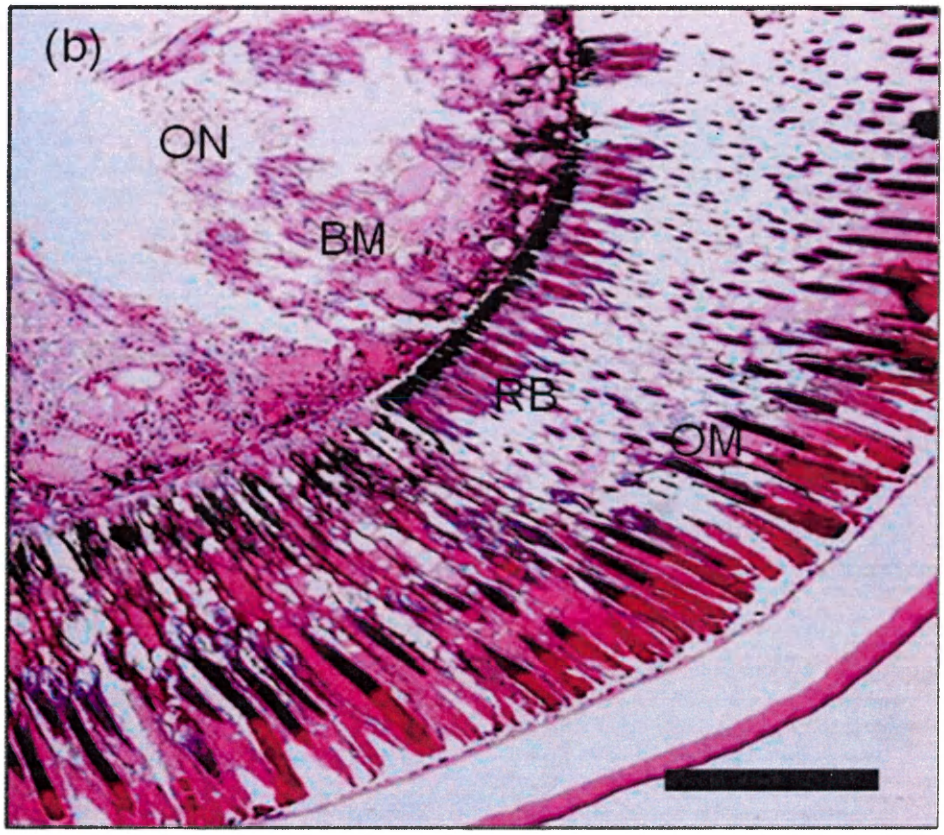
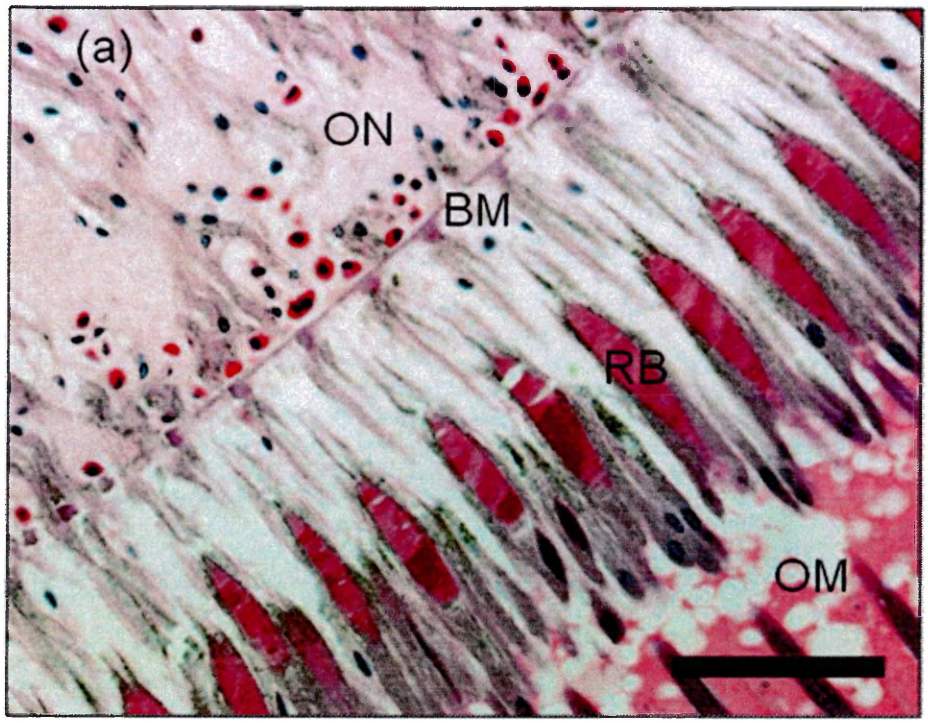


Fig 3a



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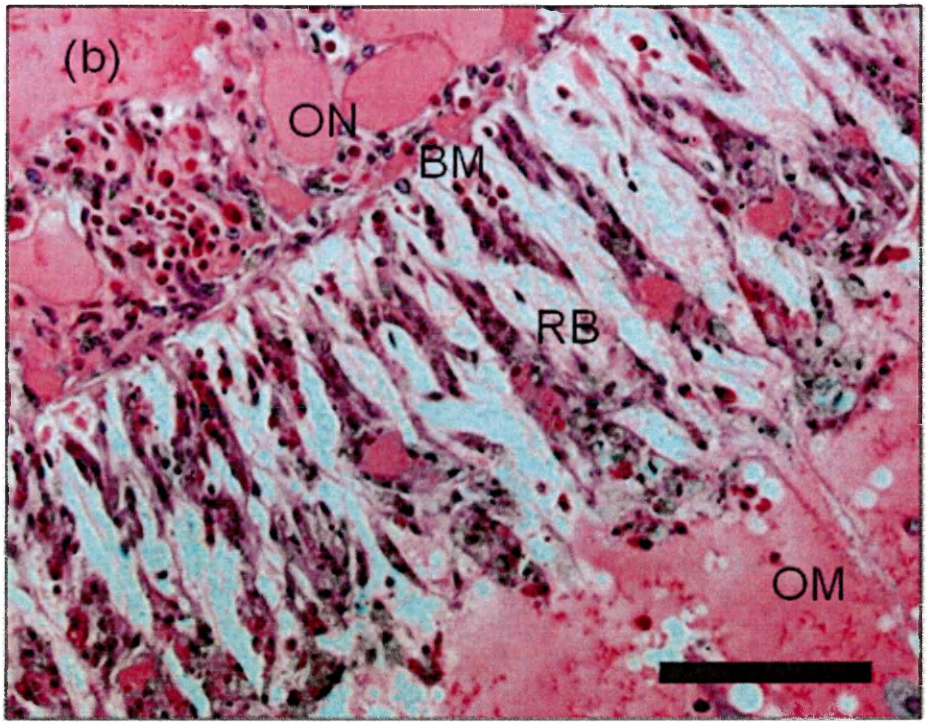


Fig 4

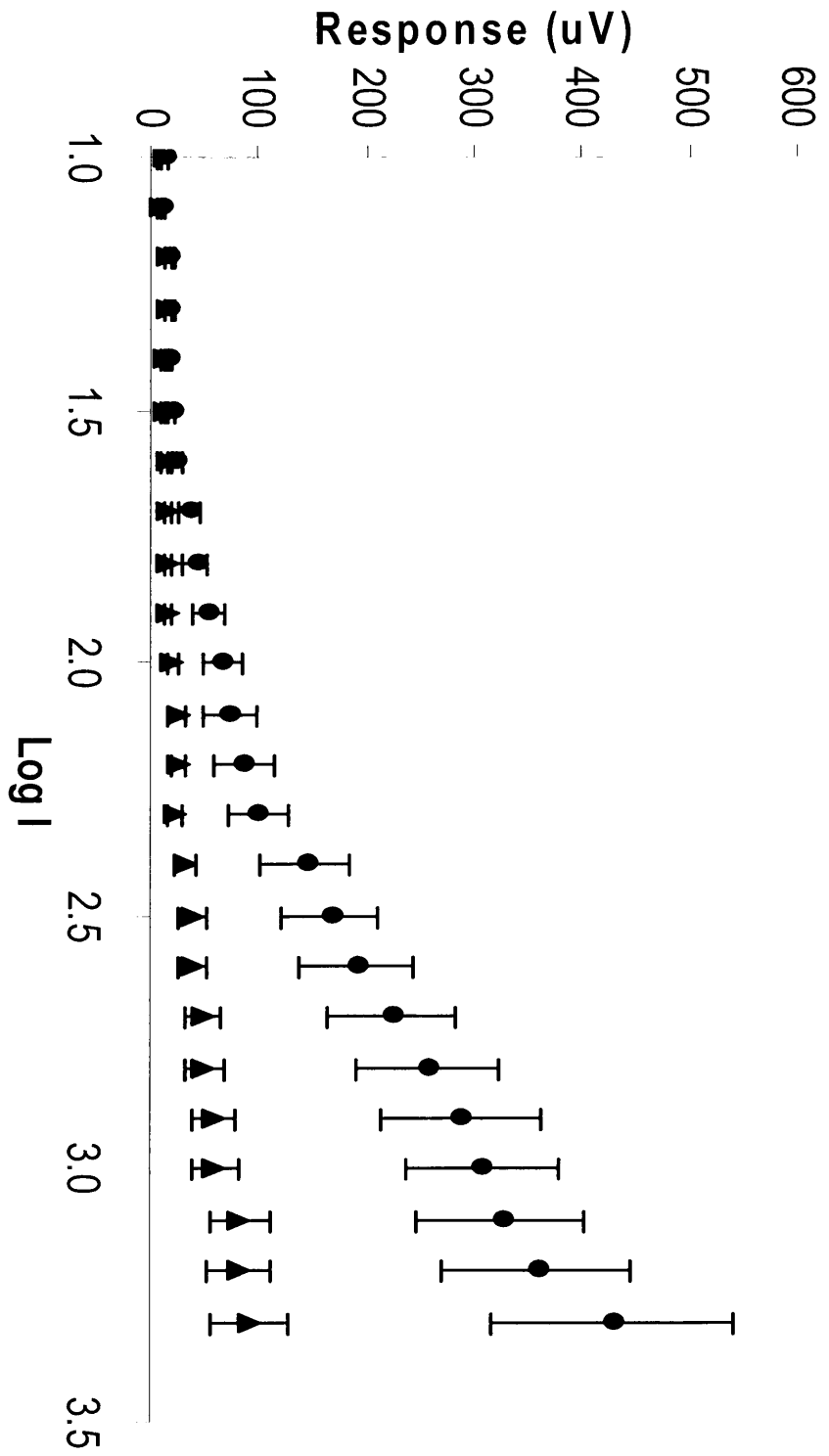


Fig 5

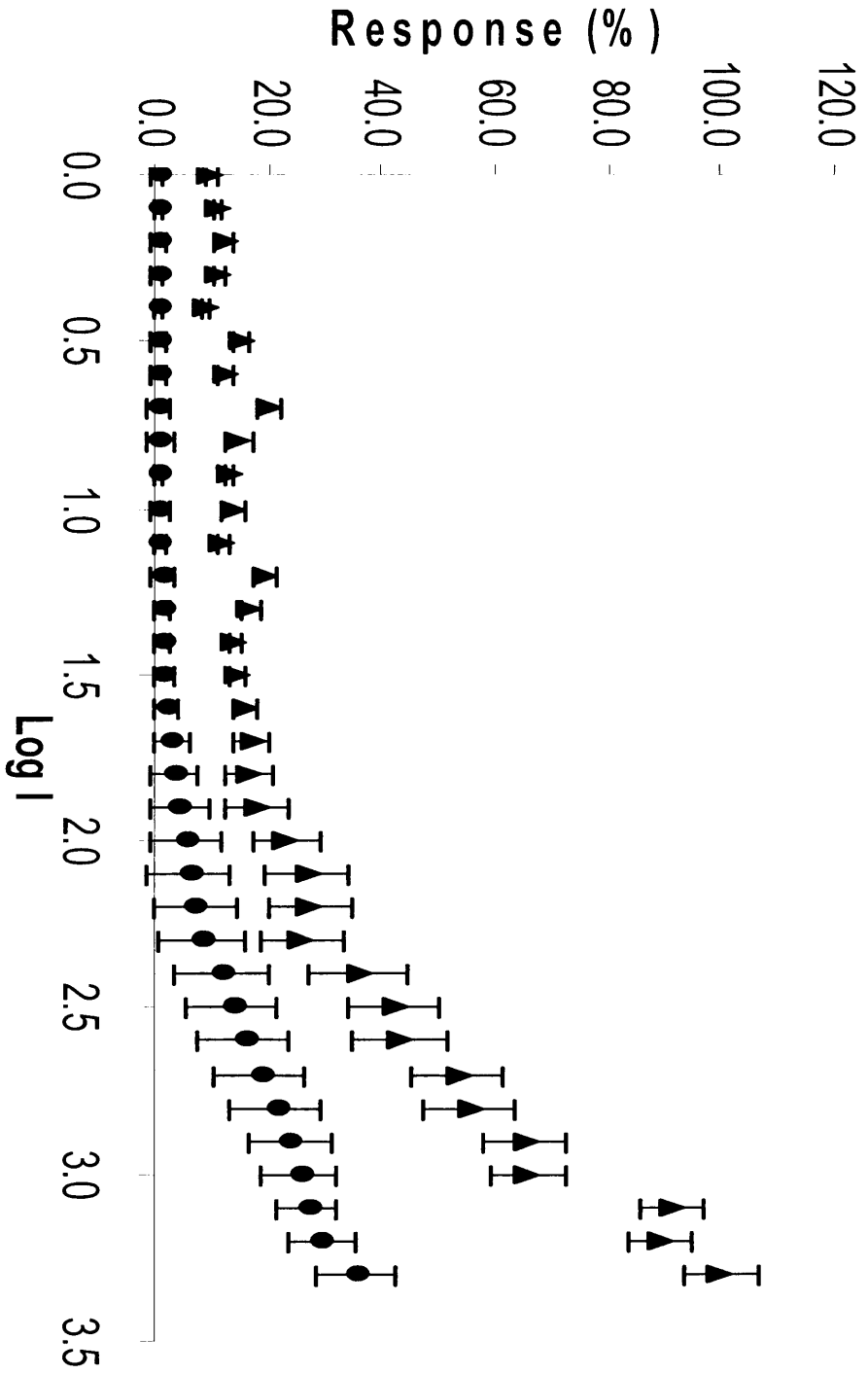

Response (%)
0.0
20.0
40.0
60.0
80.0
100.0
120.0
Log I
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

Fig 6

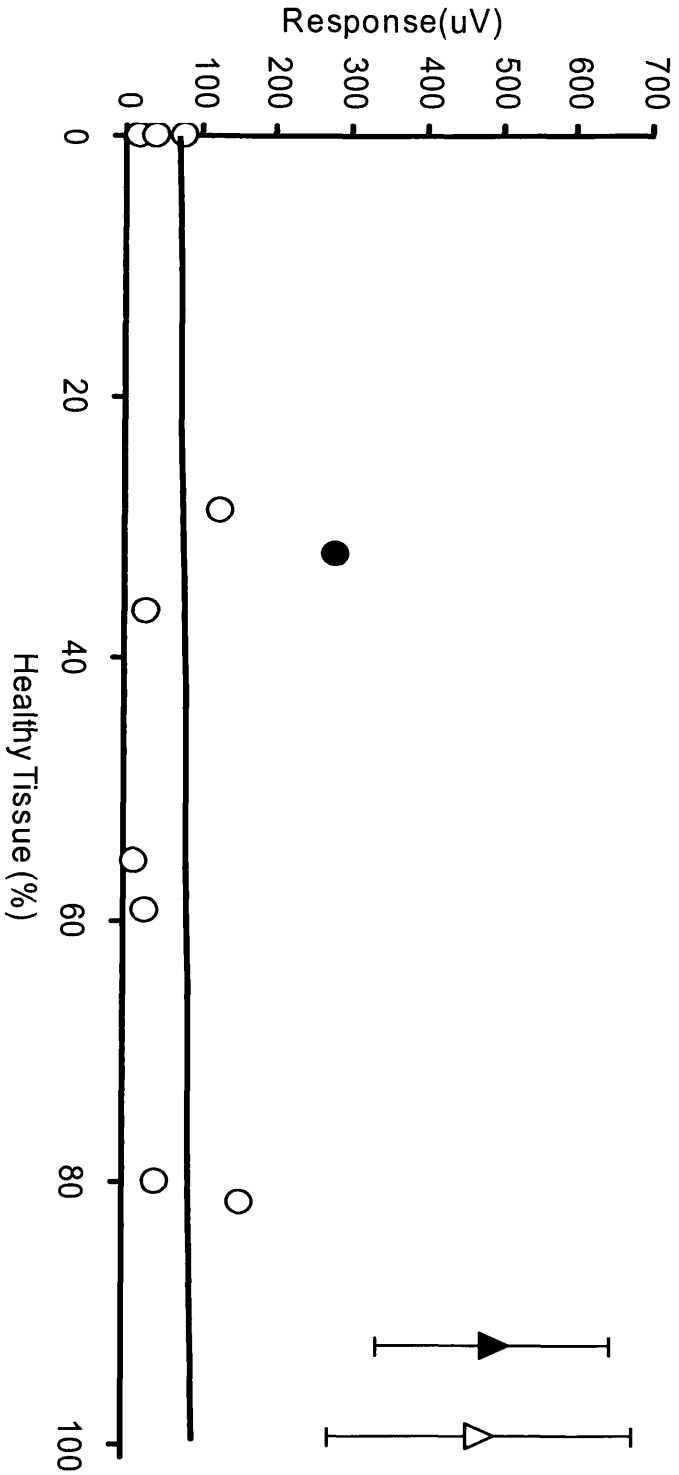

Response(uV)
0
100
200
300
400
500
600
700
Healthy Tissue (%)
0
20
40
60
80
100

Fig 7

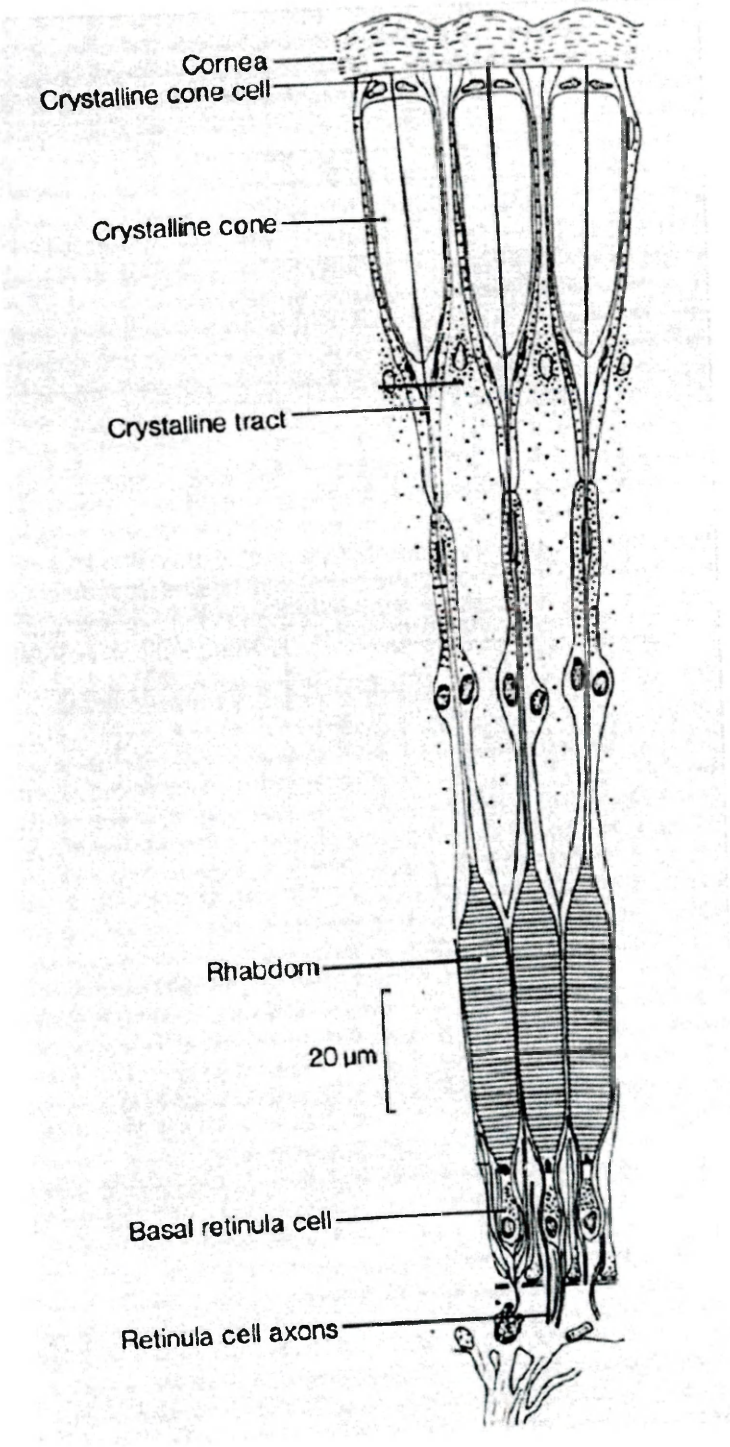
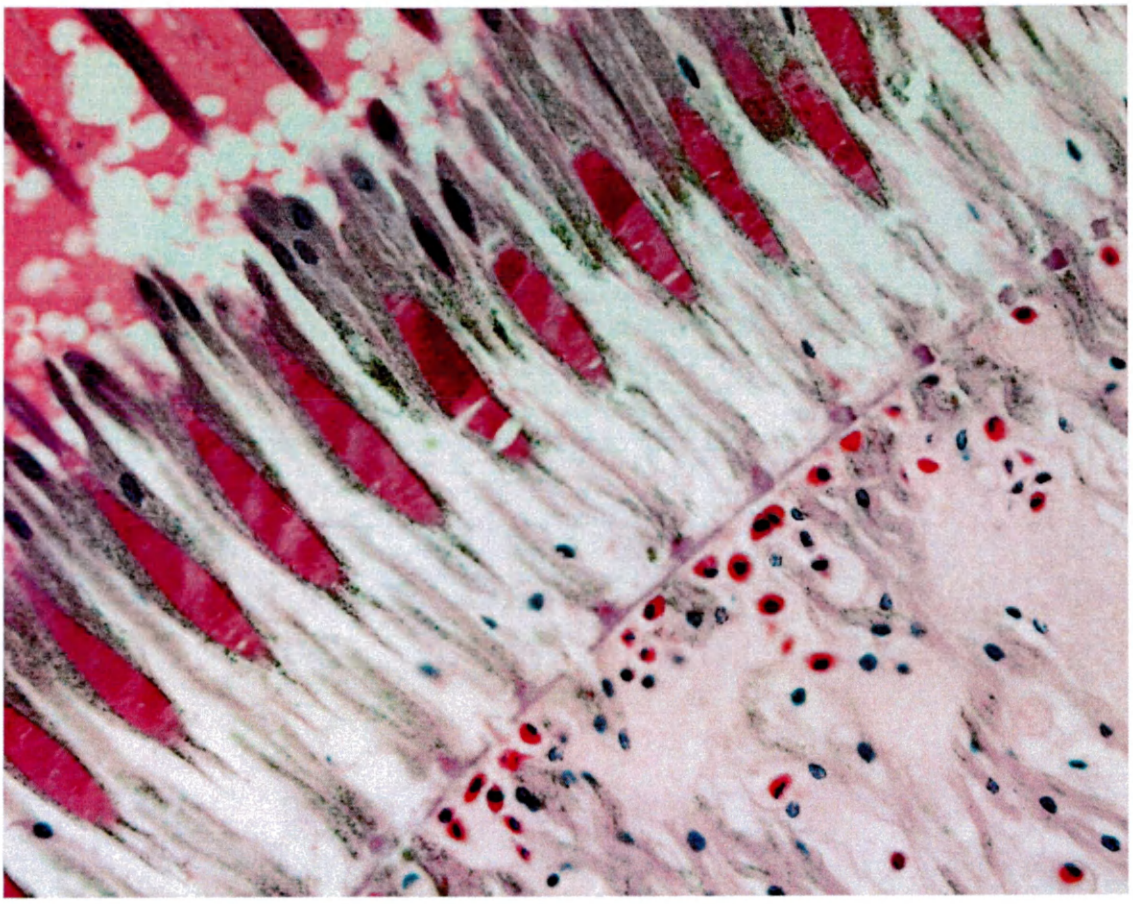

Cornea
Crystalline cone cell
Crystalline cone
Crystalline tract
Rhabdom
20 µm
Basal retinula cell
Retinula cell axons

Fig. 8



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