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Eye Pathologies Found in Several Decapod Crustaceans

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EYE PATHOLOGIES FOUND IN SEVERAL DECAPOD CRUSTACEANS

CHAPTER 1: HISTOPATHOLOGY OF IDIOPATHIC LESIONS IN THE EYES OF *HOMARUS AMERICANUS* FROM LONG ISLAND SOUND

ABSTRACT

In 1999, a mortality event struck lobsters in western Long Island Sound (WLIS). A number of possible causes were identified, including a pathogenic amoeba. Concurrent with the mortality event was the spraying of the region with various pesticides, including malathion, methoprene, sumithrin, and permethrin, to combat the spread of West Nile Virus. While studying the after effects of the initial mortality in WLIS in September 2001, it was noted that moribund lobsters from the Sound had "cloudy" eyes.

The present study examined the eyestalks of 23 lobsters collected from WLIS in 2001, 10 lobsters collected from WLIS in 2004, 20 lobsters collected from central LIS (CLIS) in 2004, and 10 lobsters collected from a lobster facility in Nova Scotia, Canada as a control. Idiopathic lesions were identified in the ommatidia and optic nerve fibers running proximal to the ommatidia in 29 (56%) of the lobsters from LIS. No lobsters from Canada were found to have lesions. Lesions were categorized as either moderate or severe. Moderate lesions had altered rhabdoms, clumped retinal pigment, and altered optic nerve fibers. Severe lesions were marked by either absent rhabdoms, clumped pigment in both the ommatidial region and in the optic nerve region, and optic nerve fibers that had been completely destroyed and were replaced by vascular tissue. Given the damage to the optic nerve and ommatidia, these lesions resulted in blind spots in the lobsters' visual field.

The presence of eye lesions was widespread. There was no significant difference in the severity of lesions between lobsters collected in 2004 from western Long Island Sound (WLIS) and central Long Island Sound (CLIS). These data indicate that the disease agent is present throughout a large portion of the Sound, and that the lobsters were probably continually exposed or exposed at around the same time. The eyes were divided into ventral, central, and dorsal regions to examine the spatial extent of the lesions. Idiopathic lesions occurred primarily in the central and ventral regions of the eye, and with much less frequency in the dorsal portion. The dorsal portion was more likely to be damaged when more than 50% of the total area of the eye was damaged, indicating that the lesions first occurred in the ventral or central regions and progressed to the dorsal region over time. In addition, damage to the dorsal area tended to occur only when the severity of lesions was high, indicating a spatially progressive pattern to the lesion development. That is, lesions began in the ventral or central regions of the eye and, as they became more severe, extended into the dorsal region.

The cause of the lesions in lobster eyes is unknown, although the lesions were similar to those described in a shrimp, *Penaeus monodon*, with a viral infection. However, there was no evidence of a viral or microbial infection in the lobsters from the present study. Finally, using light microscopy, chemical agents cannot be ruled out as the cause of the eye lesions, as several of the chemicals used in New York and Connecticut during the West Nile Virus scare are known to damage nerves or nerve function.

INTRODUCTION

The American lobster *Homarus americanus* is an important commercial species in the United States. Landings of *H. americanus* in the United States for 2002 were recorded at 82.3 million pounds with an economic value of $293.3 million (National Marine Fisheries Service 2003) and worldwide landings are valued at over $750 million (National Marine Fisheries Service Annual Landings Query, Fisheries and Oceans Canada: State of Canada's Fishery 2002 Fact Sheet). The *H. americanus* fishery accounts for over 90% of the value of commercial landings for Long Island Sound. Prior to 1999, the *H. americanus* fishery for New York in Long Island Sound was the third largest in the country, with annual landings of 8.5 million pounds; the Connecticut LIS fishery brought in 2.5-3.7 million pounds. For 1998, the combined landings for New York and Connecticut were valued at over $40 million (Connecticut Department of Environmental Protection 2000).

In fall 1999, a mortality of *Homarus americanus* occurred in western Long Island Sound (WLIS). Surveys of local lobster fishers taken after the mortality event found that sporadic mortalities occurred in 1998, and to a lesser degree in 1997, but that the 1999 event was severe and widespread (Connecticut DEP 1999). The same surveys also reported that there had been delayed molting for *H. americanus* in the Sound in 1999—the fall molt did not occur until mid December rather than in September-October when it normally occurs there (Connecticut DEP 1999).

Symptoms in affected lobsters were reported as lethargy and a pink discoloration to the ventral surface of the abdomen. The disease was classified as a systemic inflammatory disease that primarily affected the nervous system of the lobster; there was damage to areas such as the ventral nerve cord, brain, and neurosecretory portions of the eye. More specifically, pathology associated with infection included discoloration of hemolymph and muscle, granulocytopenia, granulomas in connective tissues, hypertrophy and necrosis in nerve tissues, and altered hemolymph clotting ability (French 2000, Russell et al. 2000). Further study by Mullen et al. (2004) found hemocytic infiltrates in nerves and ganglia of infected lobsters, as well as in the retina and in tegumental and muscular interstitium of eyestalks. A parasitic protozoan was found throughout the hemocytic infiltrates. The same parasite was found in neural tissue, within the cytoplasm of neurons, and between nerve fibers of the ventral nerve cord, antenna, and eye, even when no hemocytic infiltration was observed. The parasite also occurred in the tegumental glands and nerves of the antennae and eyestalks, where degenerate epithelium and nerves, respectively, were present in hemocytic infiltrates. The parasite was also found with less frequency in foci of hemocytic infiltration outside of the nervous system (Mullen et al. 2004).

A pathogenic amoeba, identified as *Paramoeba* sp., was diagnosed as the disease agent from WLIS (Connecticut DEP 1999, Russell et al. 2000, Mullen et al. 2004). Mullen and Frasca (2002) attempted to characterize the amoeba responsible for the disease condition. The organism has a secondary nucleus that differentially stains using the Feulgen technique. Further analysis using TEM confirmed the presence of the

nucleus-like organelle, or Nebenkörper—a feature of the genus *Paramoeba* Schaudinn, 1896.

In an effort to identify possible chemical causes for the lobster die-off, the Department of Environmental Protection Water Bureau and the Marine Fisheries Office (Connecticut) conducted water quality analyses for 30 sites in western Long Island Sound. They tested for 66 volatile organic compounds, 137 semi-volatile organics, 30 organochlorine pesticides, 18 chlorinated herbicides, and toxic algae, and found no abnormal levels for any of the compounds (Connecticut DEP 1999). However, the lobster mortality coincided with spraying of malathion, an organophosphate pesticide, and other pesticides (sumithrin, permethrin, and methoprene) to combat the spread of West Nile Virus in the areas surrounding the sound. DeGuise et al. (2004) conducted experiments to discern whether or not malathion could have played a role in the lobster mortality. Although phagocytosis by hemocytes—a cellular defense mechanism—was suppressed in lobsters experimentally exposed to malathion, the concentration of malathion was unknown in Long Island Sound during the lobster mortality; however, low levels of the pesticide (<5 ppb) caused significant suppression of phagocytosis (DeGuise et al. 2004). Mullen et al. (2004) note that lobster mortalities were reported in fall of 1998, before pesticides were being used to control West Nile Virus. Toxicological testing did not find measurable amounts of target pesticides—such as malathion, methoprene, and resmethrin--in experimentally exposed lobster tissue (Mullen et al. 2004), but these are biologically active compounds with potentially short half lives in living tissue (DeGuise et al. 2004).

The lobster mortalities in Long Island Sound had severe consequences for local lobster fishers. Approximately 70% of the lobster fishers reported a 100% loss of income, while the remaining fishers reported losses of 30-90% of their income (Connecticut DEP 1999). These losses occurred over 2-16 months, with an average duration of 6.8 months, driving most of the fishermen out of business (Connecticut DEP 1999).

Although *Paramoeba sp.* has been implicated as the cause of the 1999 mortality (Russell et al. 2000, Mullen et al. 2004), the official cause is still undetermined, and it is unknown whether *Paramoeba* is a primary or secondary pathogen for the mortalities, particularly because no infections have been reported since 1999. While studying the after effects of the initial mortality in Long Island Sound during September 2001, J. Shields (unpublished data) noted that moribund lobsters from the Sound had "cloudy" eyes. Preliminary observations have shown that many of the eyes from these lobsters were pathologically altered. This type of eye pathology has not been reported for blue crabs infected with *Paramoeba perniciosa* (Johnson 1977).

The goal of this research was to understand abnormalities in the eyes of American lobsters *Homarus americanus* from Long Island Sound and where possible to determine their causes. I examined the level of severity of the eye pathology as well as the spatial extent of the damage, and attempted to determine potential causes of the pathology.

Crustacean Vision

Decapod crustaceans have compound eyes, which consist of multiple units called ommatidia. Each ommatidium provides a small part of the entire image that the organism sees (Barnes et al. 1993); i.e., many smaller images create one large compound image. The resolution of the image increases as the angular separation between ommatidia decreases (Barnes et al. 1993); so the more ommatidia present in the eye, the better the resolution. However, compound eyes have low resolution compared to vertebrate eyes (Barnes et al. 1993).

In most compound eyes, each ommatidium consists of four main parts: a corneal lens, a crystalline cone, receptor (retinular) cells, and pigment cells (Barnes et al. 1993) (Figures 1 and 2). The corneal lens is the transparent, distal cap of the ommatidium (Waterman 1961). Beneath the cornea are the corneagenous cells, which secrete the corneal lens (Waterman 1961). Proximal to the corneagenous cells are the four cells that produce the lens-like crystalline cone, which itself can either be cylindrical, or a long, tapering cone (Waterman 1961). Next are the retinular cells, arranged radially around the ommatidial axis (Waterman 1961). Microvilli that project from the inner surfaces of the retinular cells form the rhabdom of each ommatidium (Barnes et al. 1993). Finally, there are the screening pigments which absorb or reflect oblique rays of light that enter through the corneal lens from an acute angle (Pearse et al. 1987).

In eyes that are adapted to bright light, where the crystalline cone is directly apposed to the rhabdom, the rhabdom is the light guide, directing the light down the eye (Pearse et al. 1987, Barnes et al. 1993). In eyes that are adapted to dim light, the

crystalline cone is at some distance from the rhabdom (Pearse et al. 1987). These are called clear zone or superposition eyes and are the type of eye found in *H. americanus* (Atema and Voigt 1995). The crystalline tract, which connects the cones and the rhabdom, acts as the light guide in clear zone eyes (Pearse et al. 1987). Light that is absorbed by the visual pigment in the rhabdom serves as a visual stimulus, generating a nerve impulse (Pearse et al. 1987). This impulse travels along the optic nerve fibers from the ommatidium to the optic centers of the central nervous system (Pearse et al.1987).

Light-adapted eyes are the least sensitive to light due to the distribution of the proximal pigment (Pearse et al. 1987). In bright light conditions, each ommatidium is isolated from the others by its screening pigment (Pearse et al. 1987). Pigment granules lie against the rhabdom within retinular cells, absorbing light and preventing the bleaching of visual pigments (Pearse et al. 1987). The proximal retinal pigment restricts the entrance of light to the axial region of the eye (Waterman 1961). The distal retinal pigment, located around the outer part of the ommatidium, including the crystalline cone, absorbs light that was scattered or refracted out of the axial dioptric system (Waterman 1961).

In dim light, there is increased sensitivity due to a rapid accumulation of visual pigment and by movements of pigments and cells (Pearse et al. 1987). In dark-adapted eyes, the proximal pigment withdraws toward or through the basilar (basement) membrane and the distal pigment retracts toward the cornea (Waterman 1961) (Figure 2). The reduced screening area allows more light to enter the eye. The dark-adapted pigment distribution leaves the sides of the crystalline tract and rhabdom exposed, improving their efficiency as light guides, and permitting escaping light to be absorbed by nearby

rhabdoms (Pearse et al. 1987). This leads to an overlap of the fields of adjacent rhabdoms, resulting in an image that is less sharp (Pearse et al. 1987).

MATERIALS AND METHODS

Collection of Animals

American lobsters *Homarus americanus* used in this study were collected from Long Island Sound in September 2001 and June-July 2004. Lobsters collected in 2001 were taken from commercial lobster pots in western Long Island Sound (WLIS), southeast of Stamford, CT (Figure 3). Overall, 31 lobsters were taken, with eyes being collected from 23 of those animals. Ten lobsters collected in 2004 were taken from commercial lobster pots in WLIS. Twenty additional lobsters were collected from commercial lobster pots in central Long Island Sound (CLIS). Lobsters collected in 2004 were shipped overnight from Connecticut to the Virginia Institute of Marine Science in Gloucester Point, Virginia. Ten additional lobsters were collected from a lobster facility in Nova Scotia, Canada, as a control, and their eyes were compared with those of lobsters from LIS.

Dissection and Fixation

Lobsters from 2001were processed for histology dockside following collection; the 2004 samples were processed upon arrival at the Virginia Institute of Marine Science. Notes were taken on the general physical appearance and behavior of lobsters prior to

dissection. Approximately 1.5 ml of whole hemolymph was extracted and frozen at -80°C for future study. Hepatopancreas, hindgut, hemopoietic tissue, heart, gill, brain, skin, and eyestalk were fixed in Bouin's solution for approximately 48 hours and then held in 70% EtOH.

Histology

Collected eyestalks were decalcified using the formic acid-sodium citrate method (Luna 1968). Following decalcification, eyestalks were cut in half using stainless steel, single edged razor blades. Eyestalks and other tissues were dehydrated, cleared, and infiltrated in paraffin using the Shandon Hypercenter XP tissue processor. Tissues were embedded in paraffin blocks using a Miles Scientific Tissue Tek embedding console. The tissues were cut into 5 µm sections using ThermoShandon blades and an Olympus Cut 4055 microtome or an American Optical 820 microtome. Slides were stained by hand using a regressive Harris' hematoxylin and eosin procedure based on Luna (1968).

Histology of the Eye

Most tissues collected were used for disease diagnosis, however, the eyestalks were examined in more detail. Areas of interest in the eyestalks were the ommatidia, basement membrane, tegumental glands, optic nerve fibers, lamina ganglionaris, the three medullar areas, sinus gland, neurosecretory cells, connective tissues, and hemal spaces (Figure 4). Tissues were examined using an Olympus BX51 compound microscope and

photographs were taken using a Nikon DXM1200 with the aid of the ACT-1 computer program (Nikon).

Damage found in the eye was rated for both severity and spatial extent. The scale for severity ranged from 0 to 2, with 0 = no damage, 1 = moderate damage, and 2 = severe damage. Spatial extent was subjectively assessed with a scale between 0 and 2, where 0 = no damage, 1 = 1-49% of the area damaged, and 2 = 50-100% damaged. The spatial extent was an estimated parameter and was based on the observation of the entire area of the ommatidial and optic nerve regions present on a slide. The total area of the eye was rated, as were ventral, central, and dorsal regions. These ratings will be referred to as the total area index (TAI), ventral area index (VAI), central area index (CAI), and dorsal area index (DAI). For each eye, both halves were rated for severity and spatial extent of damage.

Statistics

Data were analyzed with Microsoft Excel and SYSTAT (SPSS Inc.). The prevalence of the lesions in 2001 and 2004 was examined as a contingency table using Pearson's Chi Square to determine if there was a significant difference between years, as were changes in severity between years, and differences in severity between animals collected in 2004 from WLIS and CLIS. Differences in severity between two eyes from the same animal were also examined. The spatial extent of eye damage was examined in order to determine whether there was a region of the eye--central, ventral, or dorsal—where the lesions preferentially occurred. Variations in severity in the different regions

were also examined. In addition, the relationships between lobster size and lesion severity as well as lobster age and TAI were examined.

RESULTS

Idiopathic lesions in the eyes of *Homarus americanus*

The severity of the pathology was evaluated in the eyes of 52 lobsters from LIS. The idiopathic lesions occurred in the ommatidia and in their associated optic nerve fibers that run proximal to the ommatidia to the lamina ganglionaris. One lobster had to be removed from the study because it exhibited pathology that was obviously different from that described here (see below). No pathology was observed in the eyes of the 10 lobsters from Nova Scotia.

Moderate damage involved changes in structures within the ommatidia and the optic nerve; these changes were very subtle in some sections, and more pronounced in others. In moderately damaged eyes, hemocytes frequently crossed the basement membrane into the ommatidia, a condition not seen in healthy eyes (Figure 5). Hemocyte aggregations were observed in the blood vessels of the optic nerve area of the eye, distal to the lamina ganglionaris (Figure 6). One of the most obvious changes to the eye structure was the altered appearance of retinal pigments. While in healthy eyes these pigments are well dispersed, pigments in affected eyes are clumped in the ommatidial area (Figure 7). The shift in pigmentation revealed slight changes in the appearance of the retinular cell layer, most noticeable by the absence or relocation of retinular cell nuclei, and the loss of the spindle shape of the rhabdoms. (Figure 8). Occasionally the

crystalline tracts of the damaged regions appeared to be shortened, but this pathology was rare. Finally, moderately affected eyes had alterations in the structure of the optic nerve fibers proximal to the damaged ommatidial areas. These optic nerve fibers had lost their usual well ordered, straight, fibrous appearance; instead they appeared loose with ragged edges, and they became more basophilic (Figure 9). The fibers, while still present, no longer converged into well-defined tracts, but became diffuse across wider areas.

Severely damaged eyes had noticeable changes in the ommatidia and in the optic nerve region. In the eyes of some of these lobsters, hemocyte infiltration occurred into the ommatidial region, and hemocyte aggregations could be observed in the now enlarged hemal spaces of the optic nerve region. Retinal pigments were clumped on both sides of the basement membrane (Figure 10). The retinular cell layer had lost its structure, and was either filled with cellular debris or was free of any remnants of the retinular cells or their rhabdoms (Figure 11). In the ommatidial region, damage ranged from destruction of only the retinular cell layer, to complete obliteration of the ommatidia. The crystalline tracts of severely damaged lobsters became vacuolated, (Figure 12A) and occasionally the distal pigment that surrounded the crystalline cone cells of healthy and moderately damaged ommatidia was dispersed throughout the clear zone. Material that appeared to be remnants of the crystalline tract had moved proximally into the former retinular cell layer in some lobsters (Figure 11A). Perhaps the most striking pathology was the complete loss of optic nerve fibers along the damaged tracts (Figure 12). Increased presence of vascular tissue occurred in the region where the optic nerve fibers previously existed. This could either be the result of new tissue growth, or an increased hemolymph

flow causing the already existing blood vessels to enlarge into the newly vacant areas. In severely damaged eyes, no remnants of the optic nerves were visible.

In none of these cases was there apparent pathology to the other structures in the eyes, including the lamina ganglionaris, medullar areas, tegumental glands, and sinus gland.

Analysis of data from one eye vs. two eyes

For most lobsters collected in 2001, only one eye was collected from each animal. In 2004, two eyes were taken from each lobster. In one of the 2004 samples, only one eye could be included in the study because the second eye was pathologically altered by what was determined to be previous mechanical damage. The total number of lobsters from which both eyes were examined was 29 (Table 1). Of these, 18 animals had lesions in their eyes, and 72% (13/18) of the animals with lesions had them in both eyes. Five lobsters exhibited lesions in only one eye. Of the 29 lobsters examined, 24 (83% of the total) exhibited the same degree of pathology in both eyes.

When lesions were present in both eyes, they had the same severity ranking (Table 2). Therefore, examining only one eye is a good indicator of the prevalence of eye lesions, as well as the overall severity of the lesions, although it would lead to an underestimate of the prevalence. Nine lobsters had moderate lesions in both eyes, and 4 had severe lesions in both eyes. For the 5 lobsters that had lesions in only one eye, 3 of these had lesions with moderate severity, and 2 had lesions ranked as severe. In these 5 lobsters, the other eye did not have idiopathic lesions.

Prevalence and severity between years and locations

Idiopathic lesions were found in 43% of lobsters sampled in 2001 and 66% of lobsters sampled in 2004, for an overall prevalence of 56% (Table 3). Prevalence was not significantly different between years ($\chi 2=2.53$, d.f.=1, $p>0.05$).

The severity of the lesions was examined between 2001 and 2004 (Table 4). Severity varied significantly between years ($\chi 2=13.88$, d.f.=2, $p<0.001$). This was due to the presence of 13 lobsters with moderate lesions in 2004, whereas there were no moderate lesions found in lobsters collected in 2001.

Because lobsters from 2004 were taken from both WLIS and CLIS, the severity of lesions between locations was also examined (Table 5). There was no difference in severity between locations ($\chi 2=1.07$, d.f.=2, $p>0.05$). Although sample size was low, there were no apparent trends in the severity of lesions at the different locations.

Spatial extent of lesions

The spatial extent of eye damage was variable between animals (Figure 13), ranging from 1-100% of the total area of the eye examined. The spatial extent was also variable in different sections of the same eye, and this was the case for both moderately and severely damaged eyes. In some cases, lesions were present in one half of the eye, but not in the other half.

The eye was divided into ventral, central, and dorsal areas to further examine the spatial extent of the lesions (Figure 14). The ventral area index (VAI) showed a

significant difference from the total area index (TAI) ($\chi 2=10.71$, d.f.=2, $p<0.01$). Those lobsters without apparent lesions (i.e., TAI equal to zero) were excluded, leaving 29 of the initial 52 lobsters for comparison. When the TAI was high, damage was likely to occur in the ventral region, but when TAI was low, damage was only sometimes associated with the ventral region (Table 6). There were only 5 affected lobsters with no damage in the ventral region.

The central area index (CAI) showed similar results when compared with the TAI (Table 6). There was a significant difference between the indices ($\chi 2=15.65$, d.f.=2, $p<0.001$). When the TAI was high, the CAI was likely to be high as well, but when TAI was low, damage also occurred in the central region, but this damage was more likely to cover a smaller area (7/12 lobsters with CAI=1) than a larger area (4/12 lobsters with CAI=2). Only one affected lobster (of 29) had no damage to the central region.

The dorsal area index (DAI) was also significantly different from the TAI ($\chi 2=6.7$, d.f.=2, $p<0.05$), but the data are interpreted to have a different meaning. In 12 affected lobsters, no damage occurred in the dorsal region (Table 6). Furthermore, if damage did occur in the dorsal region, it was more likely to occur when TAI was 2, i.e., 50% or more of the total area was damaged. Lesions frequently occurred in the ventral and central regions of the eye, and occurred less frequently in the dorsal region. It should also be noted that the dorsal region could be observed in only 28, rather than 29, lobsters.

Severity was examined in relation to area affected. It was unrelated to TAI, VAI, CAI (Table 7), but there was a significant relationship between DAI and severity ($\chi 2=8.49$, d.f.=2, $p<0.05$). When there was no damage to the dorsal region, severity tended to be lower, and vice versa.

Relationship between eye lesions and size

The relationships between lobster size and TAI, and size and severity were examined using Pearson's Chi Square. The size of lobsters was categorized by carapace length (CL), where 1 = 68≤CL<77.5 mm, 2 = ,77.5≤CL≤80 mm and 3 =. 80≤CL≤93 mm These rankings were chosen based on sample size. There was no significant difference between size and TAI ($\chi 2=2.20$, d.f.=2, $p>0.05$). However, there was a significant difference between size and severity ($\chi 2=12.42$, d.f.=2, $p<0.01$), with larger, and therefore older, lobsters having more severe lesions than younger lobsters (Table 8).

Other eye pathologies

In many of the lobsters examined, granulomas were observed in various regions of the eye, including connective tissues, tegumental glands, medullar areas, lamina ganglionaris, and the optic nerve region (Figure 15). There was no association between the presence of granulomas and the severity of lesions in the eyes ($\chi 2 =2.77$, d.f.=2, $p>0.05$) (Table 9).

As previously noted, one of the lobsters (ID# AM078) from 2004 was eliminated from the study because it exhibited a pathology that was unlike that described from the other lobsters. As with lobsters having moderate idiopathic lesions, the eye from this animal had clumped retinal pigment, altered rhabdoms, and degenerated optic nerve fibers (Figure 16). Most of the retinal pigment was shifted into the ommatidial region; i.e., there was little to no pigment present in the optic nerve region. What distinguished

this animal from the others is that new tissue was regenerating proximal to and parallel to the basement membrane (Figure 17). This tissue was unlike any other tissue observed in the lobster eye. It consisted of long, thin fibers with elongated nuclei. There were also hemocytes in between the fibers, which could indicate diapedesis or possibly vascularization. Remnants of optic nerve fibers were present, though it was unclear if they were actually an extension of the new tissue (Figure 18). This new tissue was extensive in one eye from this lobster, and appeared to be developing in the second eye in a region of degrading retinular cells (Figure 19). It is possible that the tissue was neoplastic expansion of the basement membrane, but neoplasia is rare in Crustacea (Brock and Lightner 1990)

Additional pathology was seen in one of the eyes from a lobster (ID# AM085) used in this study. Mechanical injury had cleaved off the distal portion of the eye, resulting in a granulomatous response that lead to the walling off and melanization of the outer surface of the eye (Figure 20). New basophilic tissue unlike that in AM078 was deposited proximal to the melanized region. The ommatidia and optic nerve fibers were completely absent. There was intense hemocyte infiltration throughout the entire eyestalk, especially in the connective tissues, and what would have been the ommatidial and optic nerve areas (Figure 21). A number of granulomas were present, occurring in the lamina ganglionaris, medullar areas, connective tissues, and the former optic nerve and ommatidial regions. The lamina ganglionaris had lost its organization distally, possibly due to the large degree of edema in the tissue (Figure 21). However, the lamina ganglionaris retained much of its structure in the region proximal to the unaltered

medullar region. While the medullar areas were not altered, the connective tissues surrounding them were filled with hemocytic infiltrates (Figure 22).

General condition of lobsters

In general, the lobsters were in good physical condition prior to dissection. In 2004, two lobsters exhibited lethargy; one of these lobsters had no idiopathic lesions and the other had moderate lesions. One lobster without eye lesions was missing a claw and one lobster with severe lesions had two legs missing.

DISCUSSION

Idiopathic lesions of the eye were common in lobsters from Long Island Sound, occurring in 56% of the 52 lobsters examined. Because of the destruction of the visual receptor cells, the lesions in these lobsters can render them effectively blind. This blindness can be extensive, as 59% of the lobsters had at least half of their retinular cell layer and optic nerve fibers affected, and 2 lobsters had all of their retinular cells and optic nerve fibers destroyed. In other lobsters, the area affected was variable between the eyes and between sections of a single eye, but at least 56% exhibited impaired vision. The lesions did not evoke an all or nothing type of blindness; some lobsters retained limited vision when affected by this condition. However, because all of the ommatidia in the eye combine to form a mosaic image (Pearse et al.1987, Barnes et al. 1993), it is likely that affected lobsters experienced blind spots in their visual field. Because ommatidia are connected by interneurons, they can influence each other; due to lateral inhibition, a single ommatidium will have a greater level of response when it alone is exposed to light than when it is exposed to light as part of a group of ommatidia (Barnes et al. 1993). Therefore, lesions in the lobster ommatidia may lead to altered responsiveness in the remaining healthy portions of the eye.

No pathology was observed in the eyes of the 10 lobsters collected from Nova Scotia. This can rule out the possibility that the pathology observed in the LIS lobsters

was a result of staining or fixation artifact, because the lobsters were all fixed and stained using the same methods.

Homarus americanus may use their visual sense to aid in shelter acquisition and feeding. While some lobsters frequently change shelters, others may remain in a single shelter for up to 10 weeks (Karnofsky et al. 1989), probably because they act as central place foragers (Lawton and Lavalli 1995). American lobsters use vision as a supplement to their mostly chemosensory foraging methods (Atema and Voigt 1995, Lawton and Lavalli 1995). They often act as ambush predators, and respond only to prey that are moving rather than stationary (Hirtle and Mann 1978). Large blind spots could make this feeding technique less successful, since lobsters rely on successive stimulation of ommatidia to see moving objects (Pearse et al. 1987). However, they feed on bivalves too, and likely rely on olfaction to find sedentary prey (Hirtle and Mann 1978). In addition to shelter and feeding, lobsters also use vision and light detection to determine time of day, as they tend to be active only at night (Karnofsky et al. 1989). Light may also be important as a seasonal cue for molting and migration. However, lobsters do not use vision for mating or agonistic behaviors (Snyder et al. 1992).

A systemic exposure or etiology is indicated as the cause of the idiopathic lesions in the eyes of *H. americanus* because 72% of animals with lesions had them in both eyes (when both eyes were examined); and they had the same degree of severity in both eyes. Further, the disease agent was present throughout a large portion of the Sound, and the lobsters in WLIS and CLIS were probably simultaneously exposed to it because affected lobsters were found at the same level of severity from both regions in 2004. These data

suggest that the etiology of the lesions caused a progressive disease in lobsters with moderate lesions advancing to severe lesions over time.

The idiopathic lesions occurred primarily in the central and ventral regions of the eye, and with much less frequency in the dorsal portion. The causal agent first damages the central and ventral portions of the ommatidia before progressing to the dorsal portion. Larger and presumably older lobsters had more severe lesions supporting the hypothesis that lesions increase in severity over time. In addition, damage to the dorsal portion occurred more frequently when the severity was high, indicating a spatially-progressive pattern to lesion development. That is, lesions began in the central and ventral regions of the eye and, as they became more severe, extended into the dorsal region. When decapods molt, they add additional rows of ommatidia mainly at the dorsal eye margin (Parker 1890 and Shelton et al. 1981). Therefore, it is likely that unaffected dorsal regions are the result of new ommatidial development and that damage occurs prior to the latest instar.

Possible causes of lesions

There are a number of possible etiologies for these idiopathic lesions in the eyes of *H. americanus*, including but not limited to microbial or parasitic infection (e.g., paramoebiasis) , excretory calcinosis, light exposure, or chemical exposure.

Paramoebiasis, although implicated in the 1999 mortality event, is probably not the etiological agent for this eye pathology. Mullen et al. (2004) examined the eyes of lobsters infected with paramoebiasis, but the only pathologies they reported were

hemocytic infiltrates in the retina and in tegumental and muscular interstitia of eyestalks. The type of nerve pathology associated with paramoebiasis (Mullen et al. 2004) was not observed in the present study. Furthermore, no amoebae were seen in any tissues from lobsters of the current study, including the skin, nerve, hepatopancreas, heart, gill, antennal gland, and eyestalks. Because there was no evidence that the lobsters in this study had amoebic infections, and no mention of the lesions was made in prior studies of paramoebiasis, it is doubtful that *Paramoeba* sp. caused the lesions in *H. americanus* eyes.

Microbial agents have been reported to cause eye damage in crustaceans (Smith 2000, Callinan et al. 2003). Smith (2000) reported on suppurative inflammation (edema, hemocyte infiltration, and localized abscesses) in the eyes of the shrimp *Penaeus monodon* with vibriosis and viral infection. The abscesses, containing necrotic and pyknotic cells were found frequently in the dioptric region. There were also granulomas affecting the ommatidia, ganglia, and other internal structures of the eye (Smith 2000). Malacia, characterized by necrosis of nervous tissue, vacuolation, and vascular proliferation in the medullar ganglia was also observed.

Callinan et al. (2003) reported on a disease, peripheral neuropathy and retinopathy (PNR), that caused minor to heavy mortalities in *P. monodon* on an Australian shrimp farm. In affected shrimp, peripheral nerve axons and their sheaths were degenerated and necrotic, with apoptosis of associated glial cells, and retinular cells and their axons were degenerated and necrotic (Callinan et al. 2003). In addition, the retinular cells that were not destroyed often fused together. Edema occurred in the fasciculated zone and the lamina ganglionaris, and dilated blood vessels with hemocyte aggregations were present

in the fasciculated zone, replacing the necrotic axons (Callinan et al. 2003). In advanced cases, there was fragmentation and liquefaction of the crystalline tracts overlying the necrotic retinular cells. What the authors refer to as "retinular cell axons" are what we have called "optic nerve fibers" in the present study. Therefore, the pathology reported by Callinan et al. (2003) is very similar to the pathology in the eyes of the lobsters from LIS. The fasciculated zone is proximal to the basement membrane, where the optic nerve fibers occur in American lobsters, and the increased vascularization observed in the region by Callinan et al. (2003) is similar to what was observed in the optic nerve region of LIS lobsters. Although the reported pathologies in these two studies appear similar, it is doubtful that the lobsters in this study had viral infections. Unlike the shrimp in the other study, most lobsters in the present study were not lethargic and did not present with any signs of disease. There was no microscopic evidence of viral, bacterial, or protozoan pathogens observed in the lobsters. In fact, other tissues from the affected lobsters, including the eyestalk ganglia, showed no pathological alterations, with the exception of granuloma formation, which was not associated with the severity of the lesions in lobster eyes.

Excretory calcinosis, a disease of LIS lobsters (Dove et al. 2004), can also be ruled out as the causal agent for the eye lesions. Lobsters from the current study did not exhibit the lethargy and orange discoloration associated with excretory calcinosis, nor did they exhibit coagulopathy. Microscopically, the gills and antennal glands of affected lobsters from this study did not have the multifocal or diffuse granulomatous inflammation that is characteristic of excretory calcinosis.

Light damage, although possible, is an unlikely etiological agent for the eye lesions observed in *Homarus americanus*. To date, there are no reports of light damage in the eyes of American lobsters and Shelton et al. (1985) state that it does not occur in this species. In Norway lobsters, *Nephrops norvegicus,* with light damage, left and right eyes of the same animal were consistently damaged (Shelton et al. 1985). However, in at least 5 lobsters from the current study, one eye had lesions while the other did not. In addition, the center of the retina is always affected in Norway lobsters exhibiting light damage (Shelton et al. 1985); in the present study, the central region was not always affected and the ventral region, that most shaded from light, was affected almost as frequently as the central region. Finally, 44% of lobsters collected in LIS for the present study had no damage to their eyes and they were presumably from the same habitat as those with damage.

Chemical exposure is a possible cause of lesions in the eyes of American lobsters from LIS. Long Island Sound is not a pristine environment, and its inhabitants are exposed to a wide range of chemical compounds (Robertson et al. 1991). Biggers and Laufer (2004) reported the presence of alkylphenols in the hemolymph and tissues of *H. americanus* from LIS. These compounds have a high activity for juvenile hormone (JH) in bioassays, and may result in serious toxic and endocrine-disrupting effects in lobsters. Because JH mimics are believed to increase respiration in crustaceans, it is possible that the alkylphenols contributed to the lobster mortality in LIS by making the lobsters more susceptible to hypoxia (Biggers and Laufer 2004). However, it is unlikely that JH mimics contributed to the idiopathic lesions reported here, although they can affect the x-organ sinus-gland complex by stimulating molting. In recent years, malathion, an

organophosphate pesticide, has been used in the LIS watershed to combat the spread of West Nile Virus. Methoprene, another pesticide used in the region, was found to bioaccumulate in the eyestalks of adult lobsters, more so than in other tissues that were examined (Walker et al. in press). Although immunotoxicity studies have been done on malathion (DeGuise et al. 2004, Mullen et al. 2004) and methoprene (Walker et al. in press) exposure in lobsters, no histopathological work has been done. Therefore, chemical intoxication cannot be ruled out as an etiological agent for these idiopathic lesions in the eyes.

Lobsters from Long Island Sound have faced a number of threats in the past few years, including the paramoebiasis that likely caused the 1999 mortality event, elevated bottom water temperatures, and introduction of pesticides and other pollutants into the Sound. The idiopathic eye lesions described in this paper are no doubt indicators of the continuous exposure to stressors faced by lobsters in Long Island Sound.

TABLE 1. Frequency of idiopathic lesions in lobsters where both eyes were examined.

	Frequency
# with no lesions	11
# with lesions in one eye only	5
# with lesions in both eyes	13
Total	29

TABLE 2. Qualitative level of severity for lobsters with lesions in both eyes (A or B). Lobsters with no pathology were rated as 0, moderate pathology was rated as 1, and severe pathology was rated as 2.

		Eye A Severity		
		0	**1**	**2**
Eye B	**0**	11	0	0
Severity	**1**	0	9	0
	2	0	0	4

TABLE 3. Frequency and prevalence of lesions in lobsters from Long Island Sound collected in 2001 and 2004.

Year	No Lesions	Lesions	Total # Lobsters
2001	13 (57%)	10 (43%)	23
2004	10 (34%)	19 (66%)	29
Total	23 (44%)	29 (56%)	52

TABLE 4. Qualitative rating of severity of lesions between 2001 and 2004. Lobsters with no pathology were rated as 0, moderate pathology was rated as 1, and severe pathology was rated as 2.

	Severity		
Year	**0**	**1**	**2**
2001	13	0	10
2004	10	13	6
	$\chi 2=13.88$	d.f.=2	$p<0.001$

TABLE 5. Qualitative rating of severity of idiopathic lesions in lobsters from western Long Island Sound (WLIS) and central Long Island Sound (CLIS). Lobsters with no pathology were rated as 0, moderate pathology was rated as 1, and severe pathology was rated as 2.

	Severity		
	0	**1**	**2**
WLIS	4	5	1
CLIS	6	8	5
	$\chi 2=1.07$	d.f.=2	$p>0.05$

TABLE 6. Comparison of indices from (A) ventral (VAI), (B) central (CAI), and (C) dorsal (DAI) areas of the eye with the total area index (TAI). Only lobsters with eye lesions were assessed for extent of damage. Area index is 0 = 0%, 1 = 1-49%, and 2 = 50-100%.

A.

		TAI		
		1	**2**	
VAI	**0**	5	0	
	1	4	4	
	2	3	13	
				29

$\chi 2$=10.71 d.f.=2 p<0.01

B.

		TAI		
		1	**2**	
CAI	**0**	1	0	
	1	7	0	
	2	4	17	
				29

$\chi 2$=15.65 d.f.=2 p<0.001

C.

		TAI		
		1	**2**	
DAI	**0**	8	4	
	1	1	6	
	2	2	7	
				28

$\chi 2=6.7$ d.f.=2 $p<0.05$

TABLE 7. Comparison of indices from (A) total area (TAI), (B) ventral (VAI), (C) central (CAI), and (D) dorsal (DAI) areas of the eye with severity (SEV). Only lobsters with eye lesions were assessed for extent of damage. Area index is 0 = 0%, 1 = 1-49%, and 2 = 50-100%.

A.

		SEV	
		1	**2**
TAI	**1**	7	5
	2	6	11
	$\chi 2$=1.51	d.f.=1	p>0.05

B.

		SEV	
		1	**2**
	0	3	2
VAI	**1**	3	5
	2	7	9
	$\chi 2$=0.64	d.f.=2	p>0.05

C.

		SEV 1	SEV 2
	0	0	1
CAI	**1**	3	4
	2	10	11
$\chi 2=0.89$		d.f.=2	$p>0.05$

D.

		SEV 1	SEV 2
	0	9	3
DAI	**1**	3	4
	2	1	8
$\chi 2=8.49$		d.f.=2	$p<0.05$

TABLE 8. Relationship between carapace length of lobsters and severity of lesions. CL = carapace length of lobsters in mm. For SEV (severity), 1 = moderate lesions, 2 = severe lesions.

		SEV	
		1	**2**
SIZE	**68≤CL<77.5 mm**	6	3
	77.5≤CL≤80 mm	7	3
	80≤CL≤93 mm	0	10
	$\chi 2=12.42$	d.f.=2	$p<0.01$

TABLE 9. Relationship between presence of granulomas (GRA) and severity (SEV) of idiopathic lesions. For GRA, 0 indicates no granulomas, 1 indicates granulomas were present. For SEV, lobsters with no pathology were rated as 0, moderate pathology was rated as 1, and severe pathology was rated as 2.

		SEV		
		0	**1**	**2**
GRA	**0**	16	12	11
	1	7	1	5
		$\chi 2=2.77$	d.f.=2	$p>0.05$

FIGURE 1. Basic structure of a longitudinal section of an ommatidium of a compound eye. From Barnes et al. 1993.

1

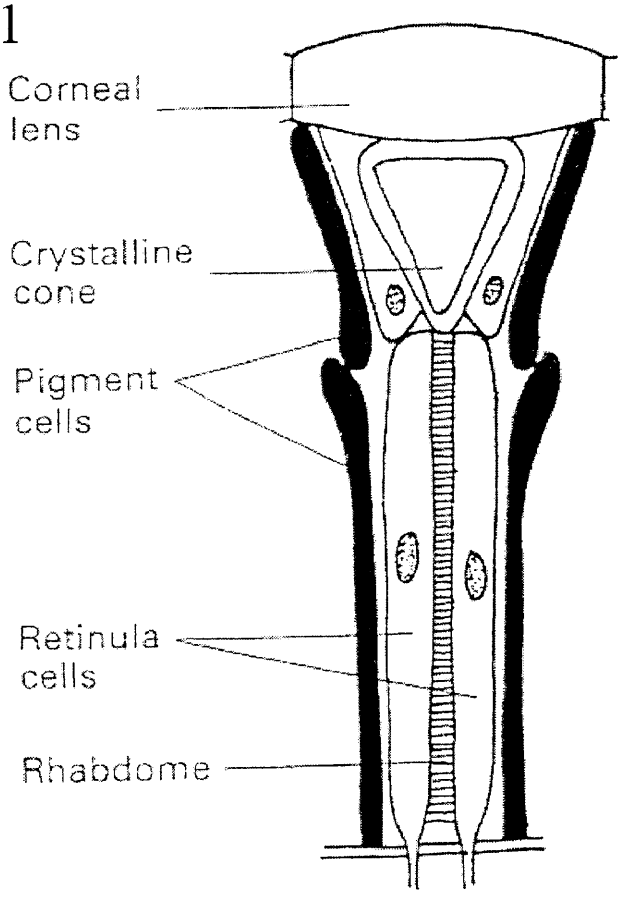

Corneal lens
Crystalline cone
Pigment cells
Retinula cells
Rhabdome

FIGURE 2. Structure of a decapod ommatidium, showing differences in light-adapted (left) and dark-adapted (right) eyes; c.o., cornea; e.c., corneagenous cells; d.p., distal pigment; c.r., crystalline cone cells; c.c., crystalline cone; c.s., crystalline cone stalk; r.n., retinular cell nucleus; r.c., retinular cell; r., rhabdom; p.p., proximal pigment; t.c., tapetal cell; b.m., basilar membrane; o.f., optic nerve fibers (proximal axons of retinular cells). From Waterman 1961.

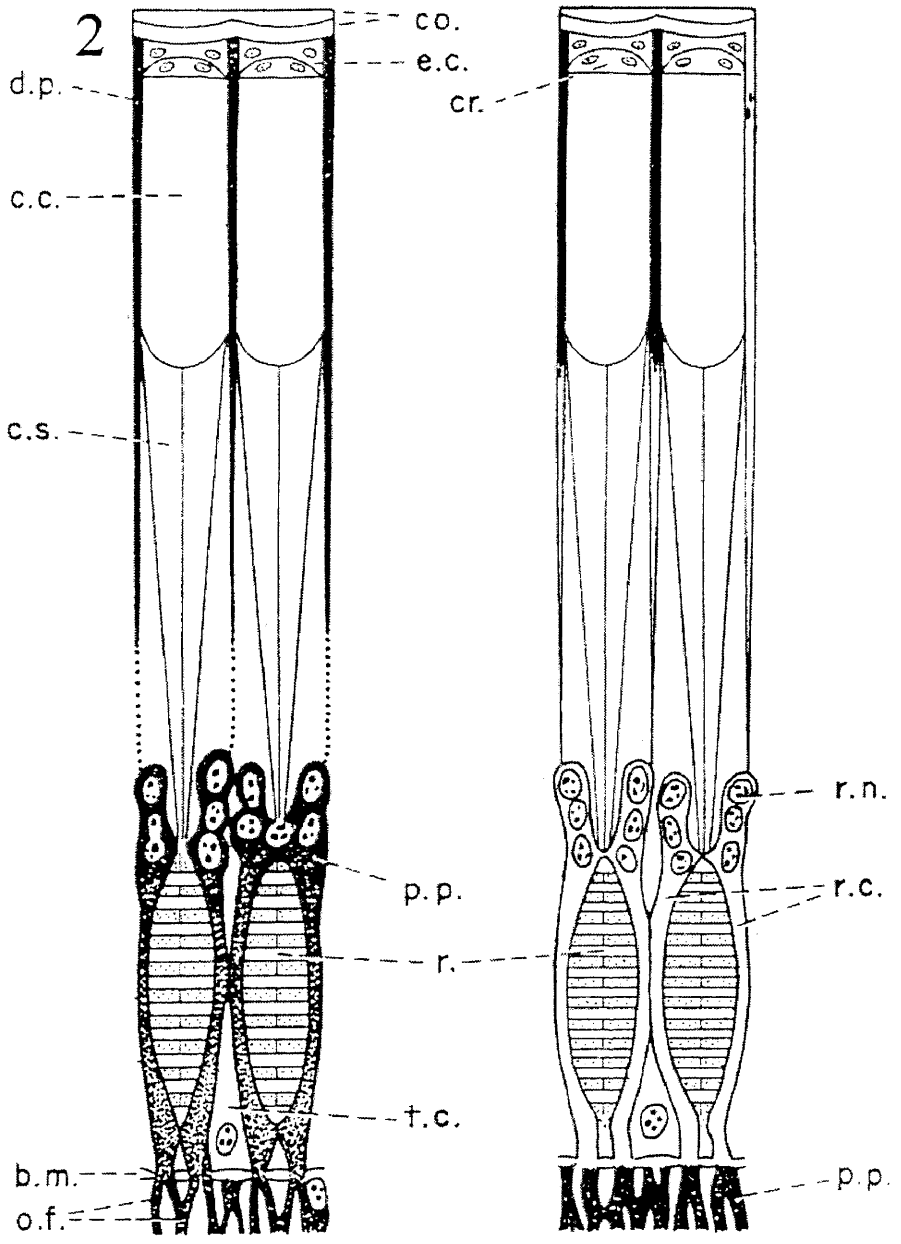

2
co.
e.c.
d.p.
cr.
c.c.
c.s.
r.n.
p.p.
r.c.
r.
t.c.
b.m.
o.f.
p.p.

FIGURE 3. Map of Long Island Sound showing approximate delineations between western (WLIS), central (CLIS), and eastern (ELIS) portions of the Sound. Figure provided by Colleen Giannini, Connecticut Department of Environmental Protection.

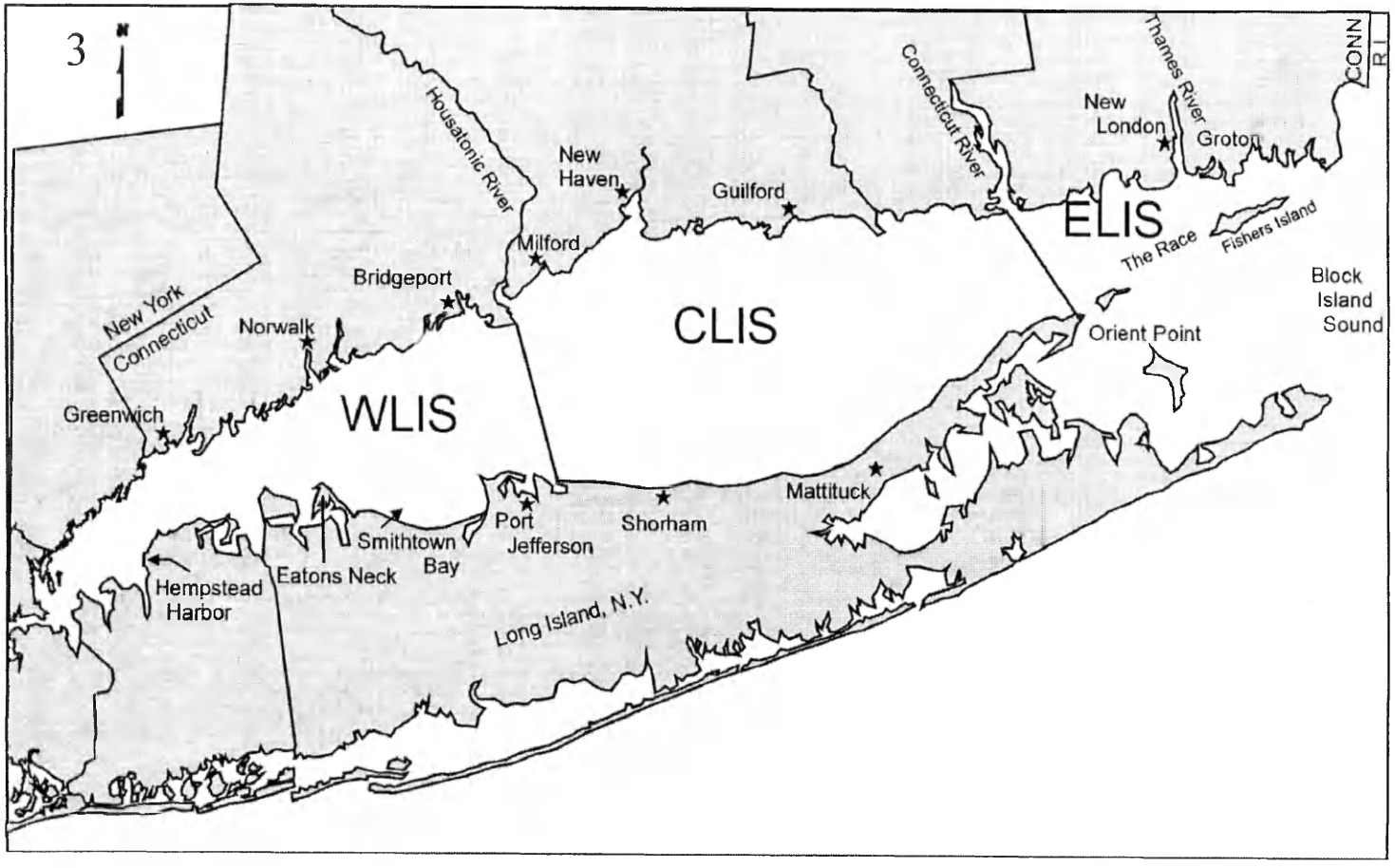

3
N
Housatonic River
Connecticut River
Thames River
CONN
RI
New Haven
Guilford
New London
Groton
ELIS
Milford
The Race
Fishers Island
Bridgeport
Block Island Sound
New York
Connecticut
Norwalk
CLIS
Orient Point
Greenwich
WLIS
Mattituck
Port Jefferson
Shorham
Smithtown Bay
Eatons Neck
Hempstead Harbor
Long Island, N.Y.

FIGURE 4. Longitudinal section of a healthy lobster eyestalk; OM, ommatidial region; BM, basement membrane; ON, optic nerve region; LG, lamina ganglionaris; ME, medulla externa; MI, medulla interna; MT, medulla terminalis; S, sinus gland; T, tegumental glands . scale = 2000 μm

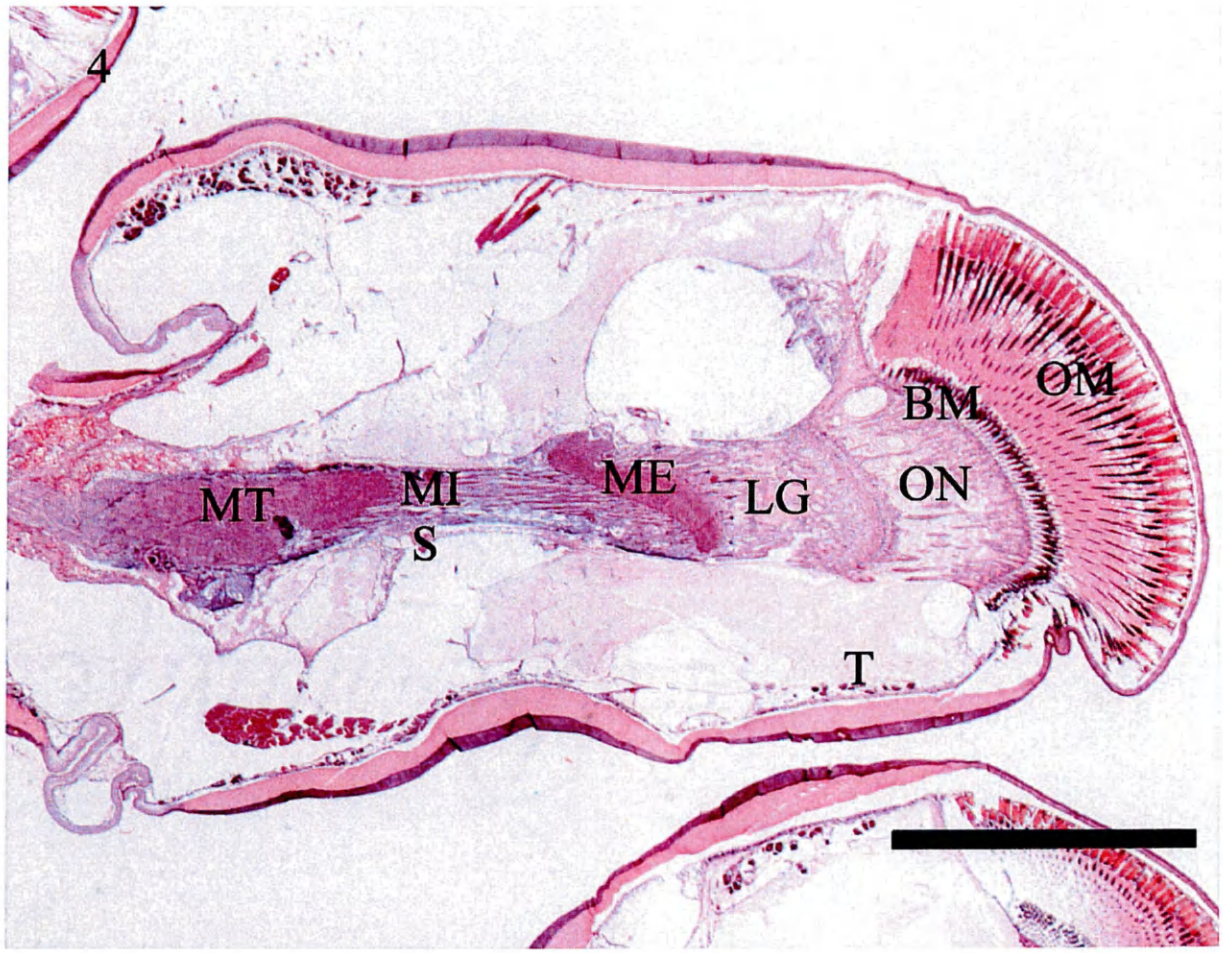

4
OM
BM
ON
LG
ME
MI
S
MT
T

FIGURE 5. Hemocyte infiltration in the ommatidia, showing altered rhabdoms (R), displacement of retinular cell nuclei (N) and invading hemocytes (H) This is an example of moderate damage given a rank of 1. scale = 50μm

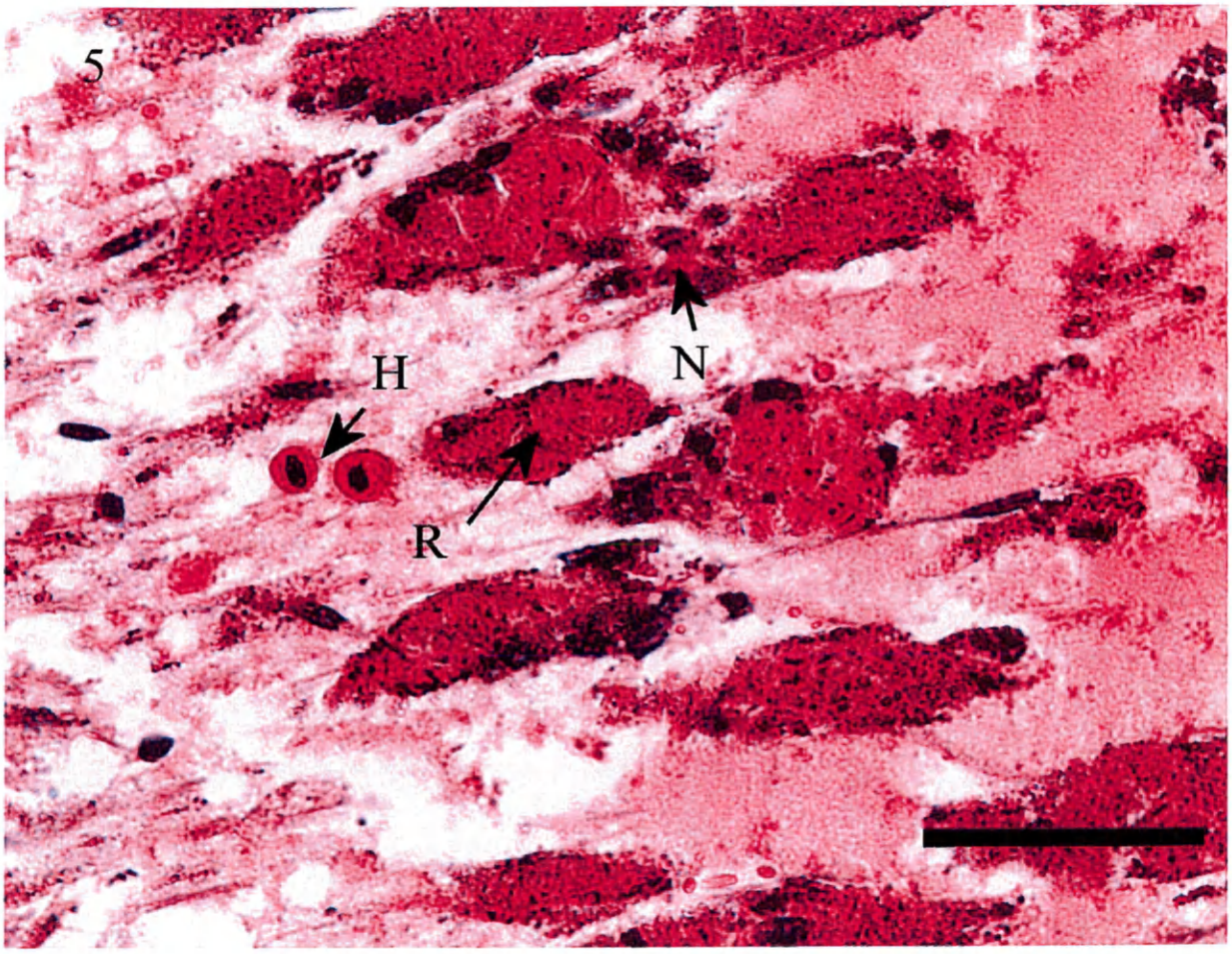
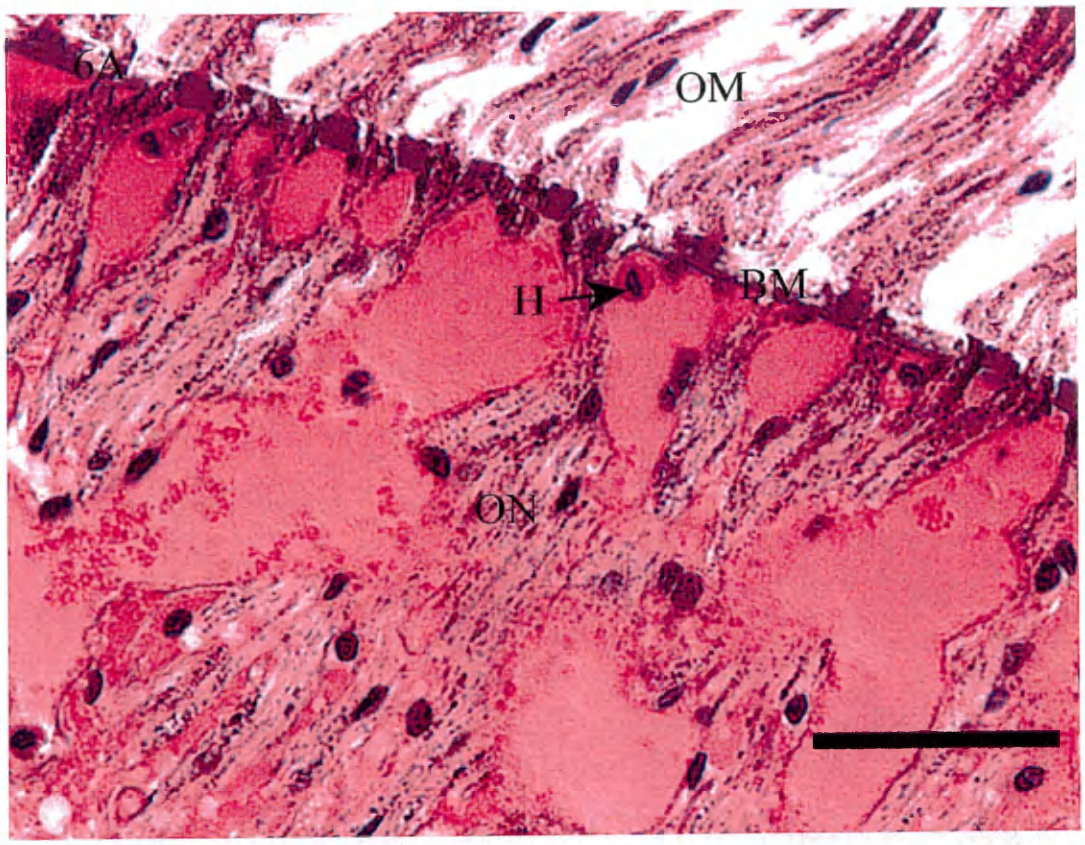

5
H
N
R

FIGURE 6. Hemocyte aggregations in hemal sinuses of the optic nerve region. (A) Healthy eye with few hemocytes in hemal sinuses of the optic nerve region. (B) Moderately damaged eye with hemocyte aggregations in the hemal sinuses of the optic nerve region; OM, ommatidial region; BM, basement membrane; ON, optic nerve region; H, hemocytes. scale = 50 μm


6A
OM
H
BM
ON

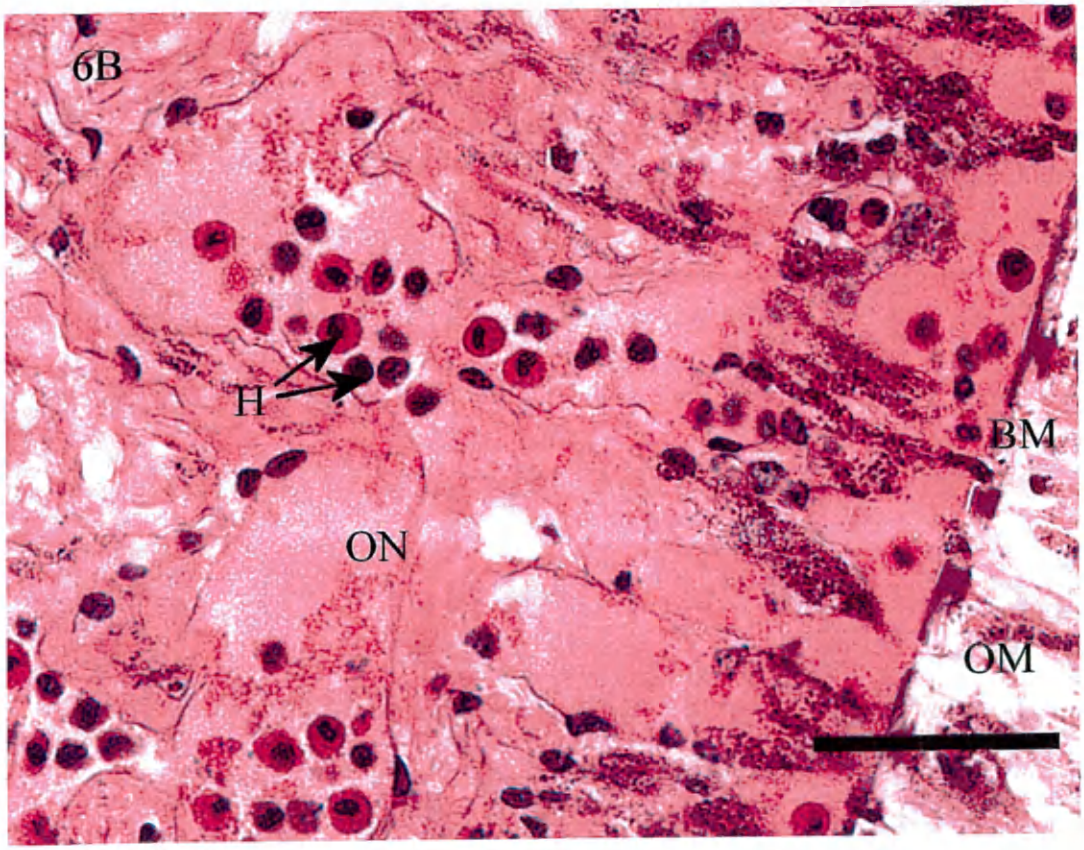

6B
H
BM
ON
OM

FIGURE 7. Clumped pigment vs. normal pigment distribution in a moderately damaged eye; C, clumped pigment; HR, healthy rhabdom; AR, altered rhabdom; BM, basement membrane; HP, healthy pigment distribution; RN, retinular cell nuclei. scale = 100 μm

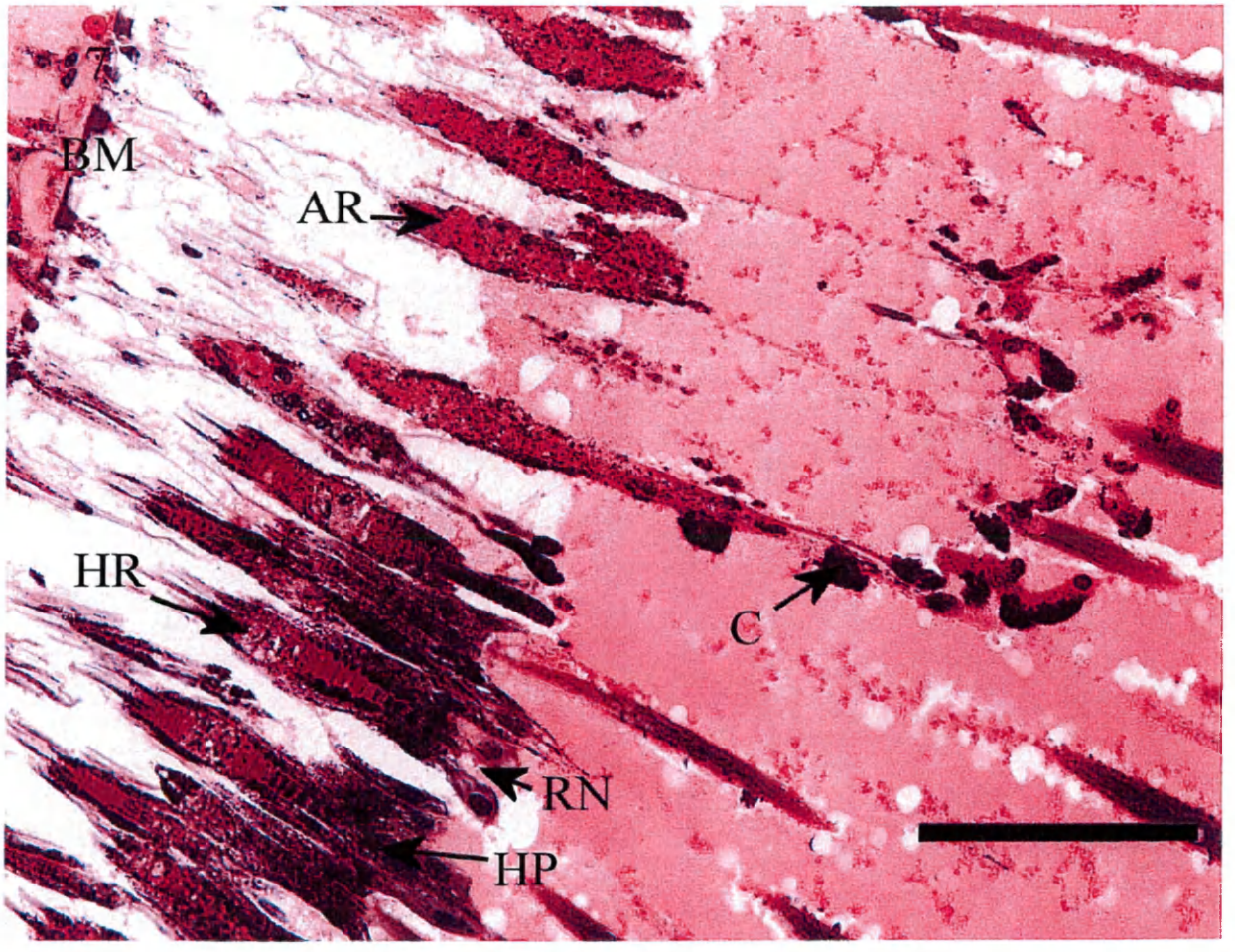
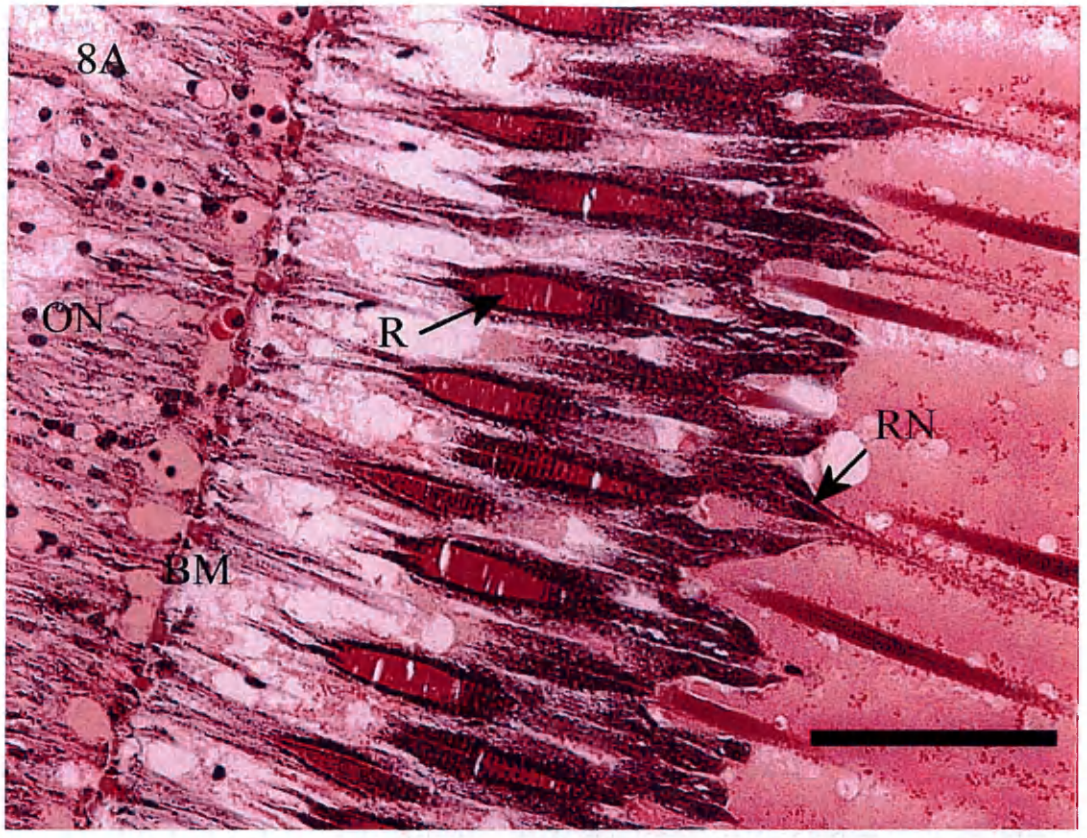

BM
AR
HR
C
RN
HP

FIGURE 8. Rhabdoms and retinular cell nuclei in (A) healthy and (B) moderately damaged lobster eyes; BM, basement membrane; R, rhabdom; RN, retinular cell nuclei; N, displaced retinular cell nuclei; ON, optic nerve region; H, hemocytes. scale = 100 μm


8A
ON
R
RN
BM

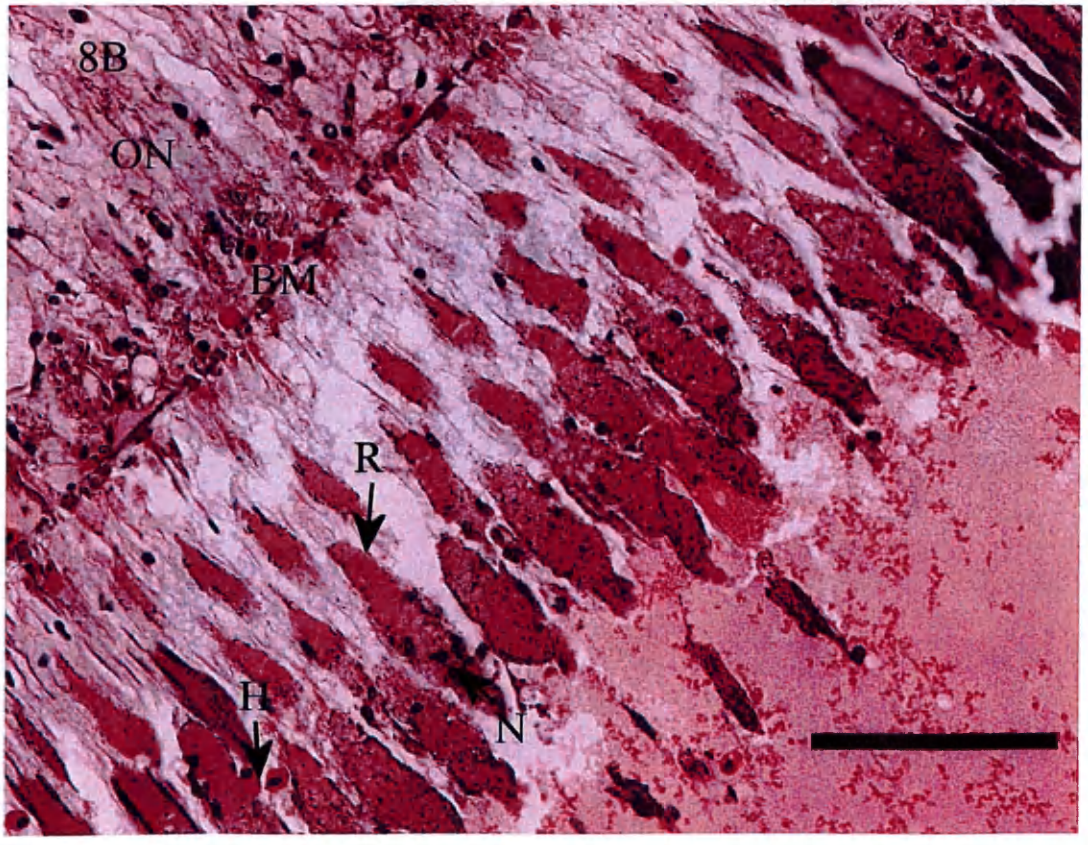
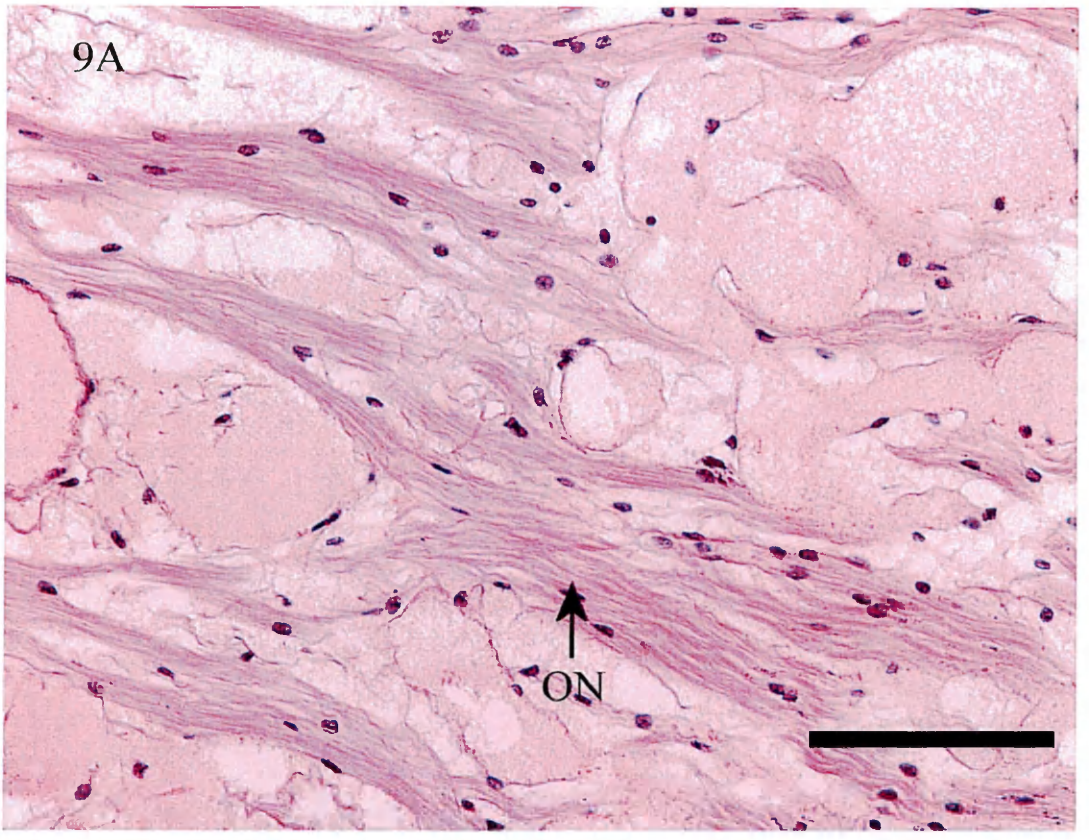

8B
ON
BM
R
H
N

FIGURE 9. Optic nerve tracts from (A) healthy and (B) moderately damaged lobster eyes; ON, optic nerve fibers; BM, basement membrane. Note increased basophilia in the moderately damaged fibers and their loss of integrity. scale = 100 μm


9A
ON

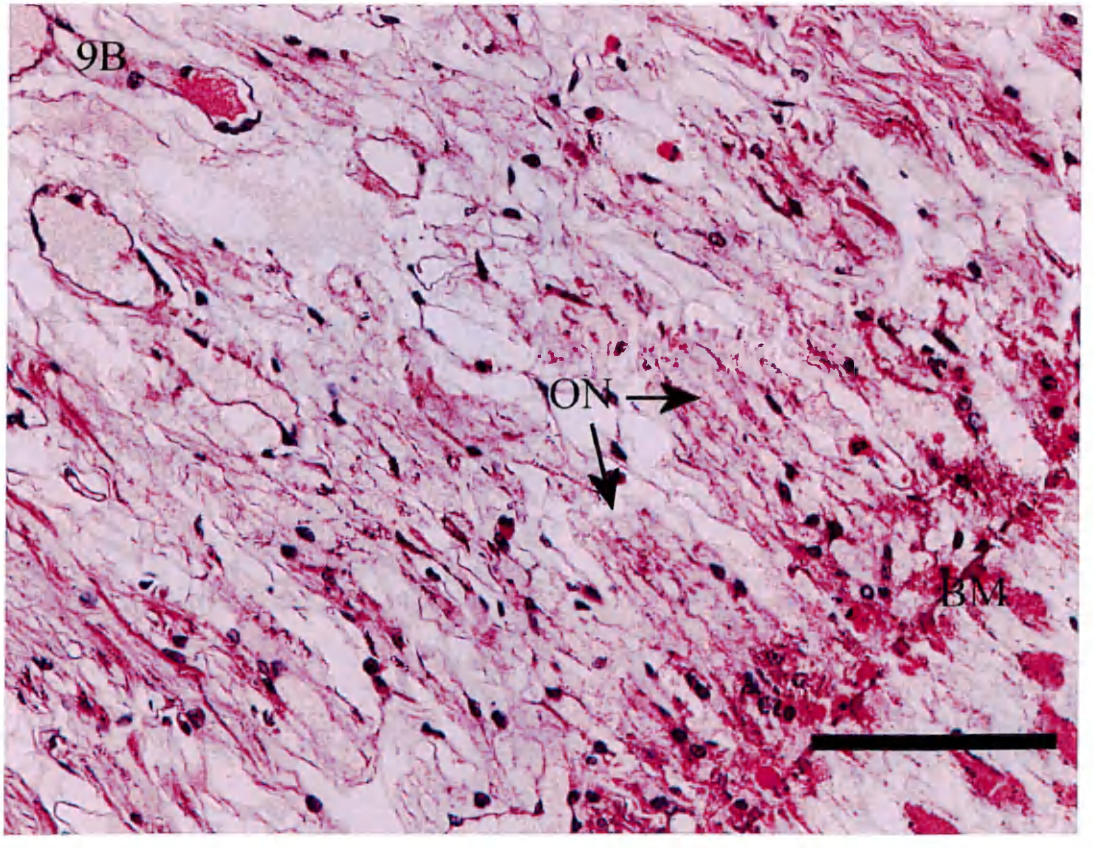

9B
ON
BM

FIGURE 10. Clumped pigment from a lobster with severe lesions. Note the loss of organized ommatidia and optic nerves; ON, optic nerve area; C, clumped pigment; BM, basement membrane; OM, ommatidial region. scale = 200 μm

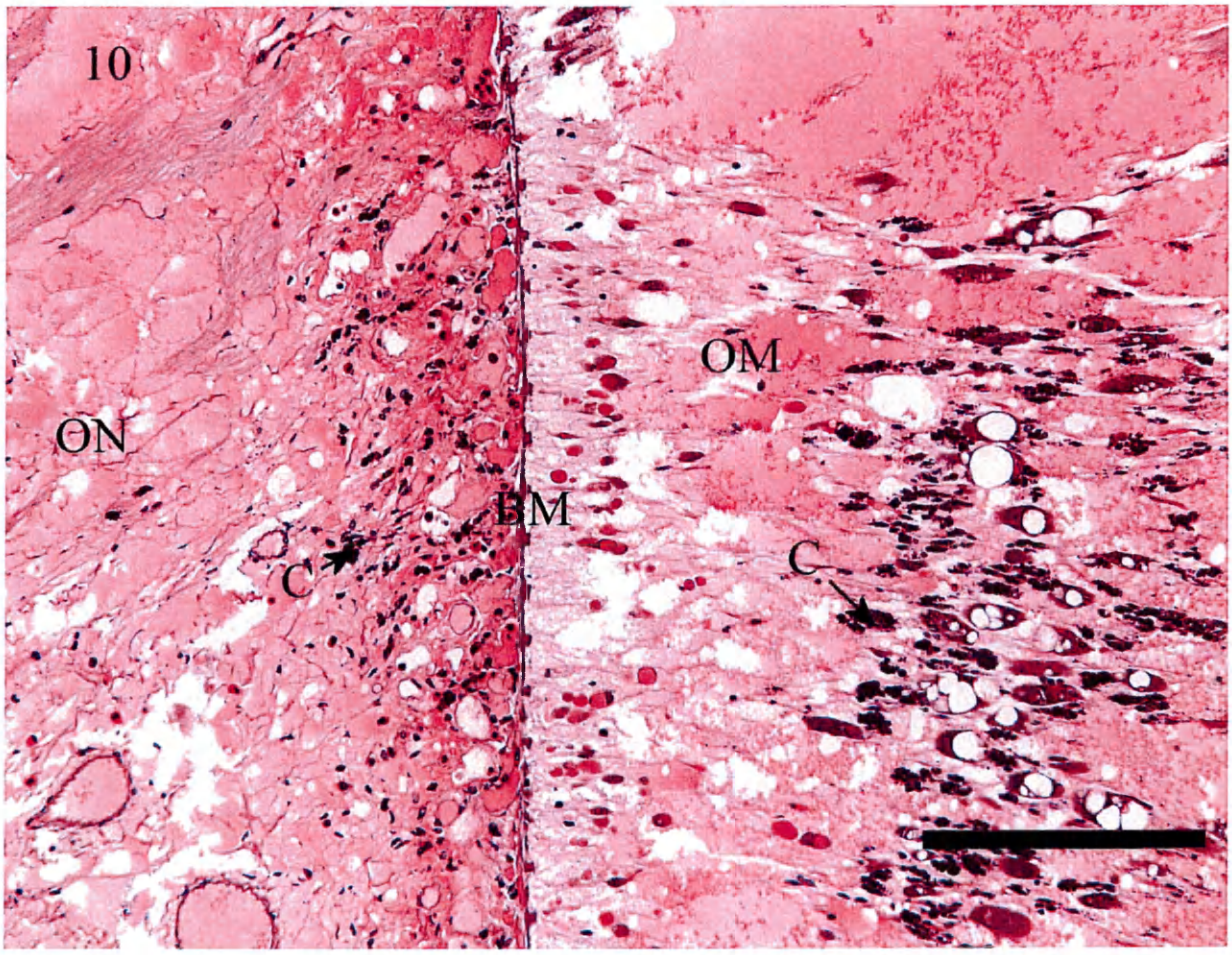
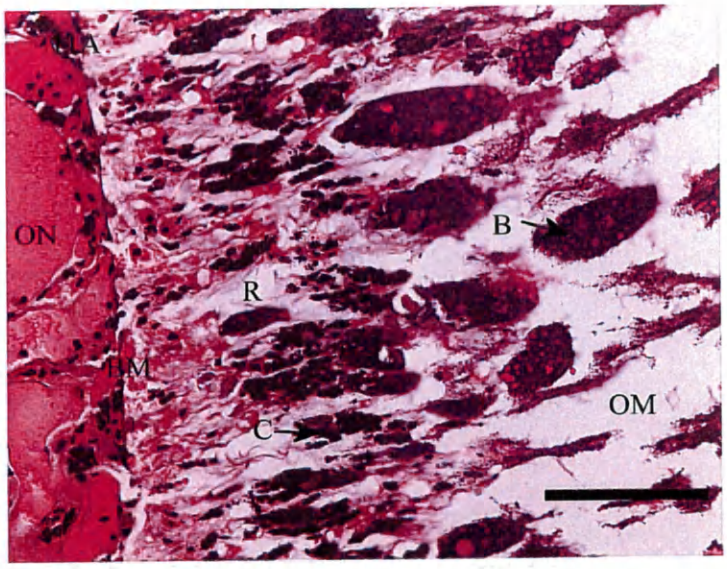
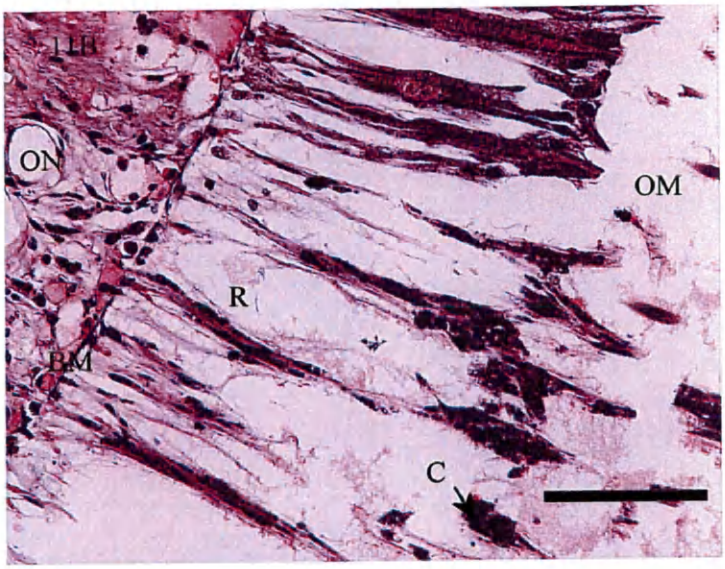

10
ON
C
BM
OM
C

FIGURE 11. Ommatidia from lobsters with severe lesions. The retinular cell layers in severely damaged eyes are either (A) filled with cellular debris and clumped pigment, or (B and C) missing most remnants of cellular structures; In (B) the pigment remained in place of the retinular cell layer, while in (C), the pigment shifted distally. Note basophilic bodies in (A) that appear to be remnants of the crystalline tract, with eosinophilic vacuoles. ON, optic nerve region; BM, basement membrane; OM, ommatidial region; R, retinular cell layer; C, clumped pigment, B, basophilic body. scale = 100 μm


LA
ON
B
R
BM
OM
C


LH
ON
OM
R
BM
C

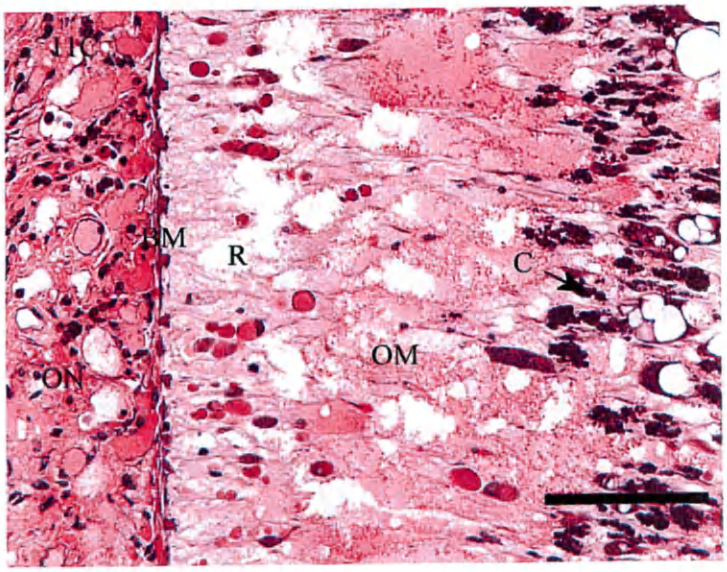
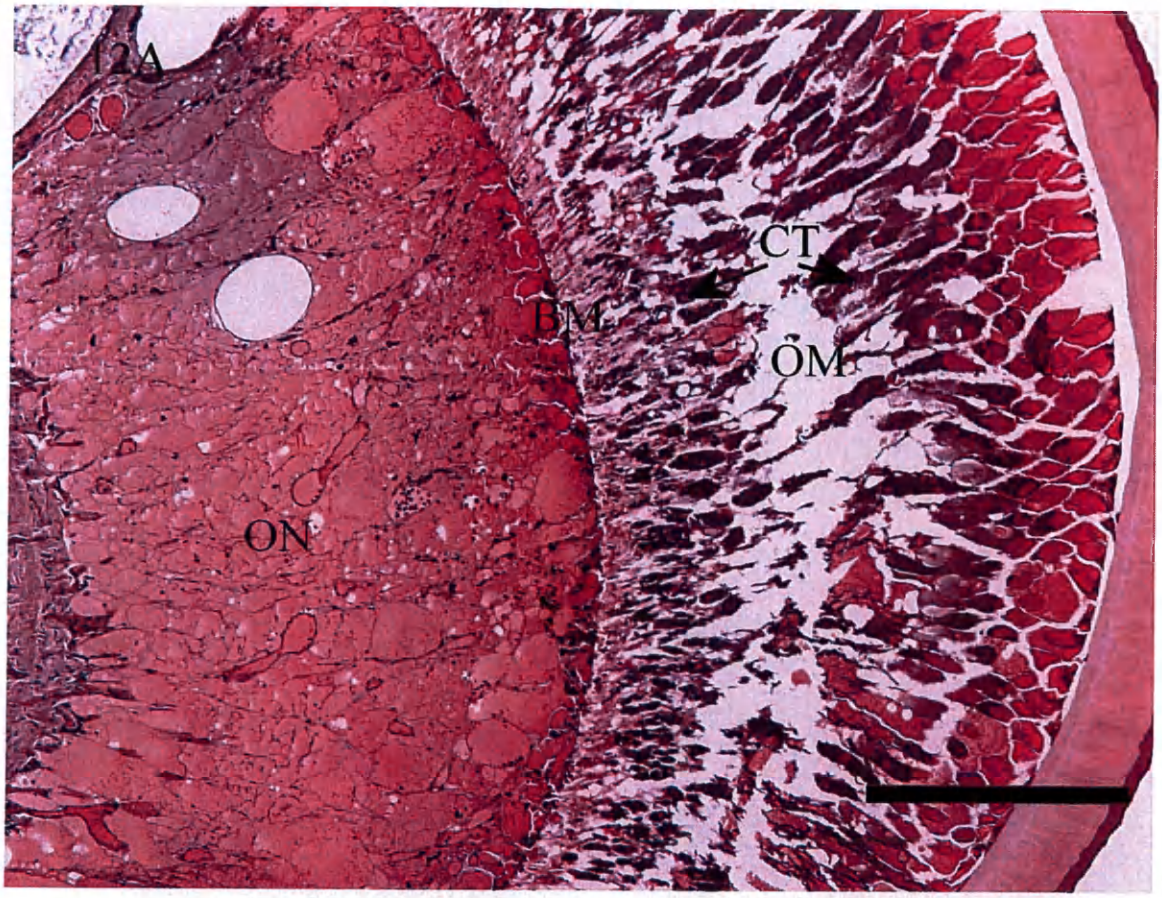

LC
BM
R
C
OM
ON

FIGURE 12. (A) Distal region of the eye from a lobster with severe lesions with 100% of ommatidia and optic nerve fibers destroyed. Note vacuolization and proximal movements of the crystalline tract. scale = 500 μm (B) Optic nerve region of a severely damaged eye. scale = 100 μm. ON, optic nerve region; BM, basement membrane; OM ommatidial region; CT, crystalline tract; H, hemocytes; C, clumped pigment; E, enlarged blood vessel


12A
CT
BM
ÖM
ON

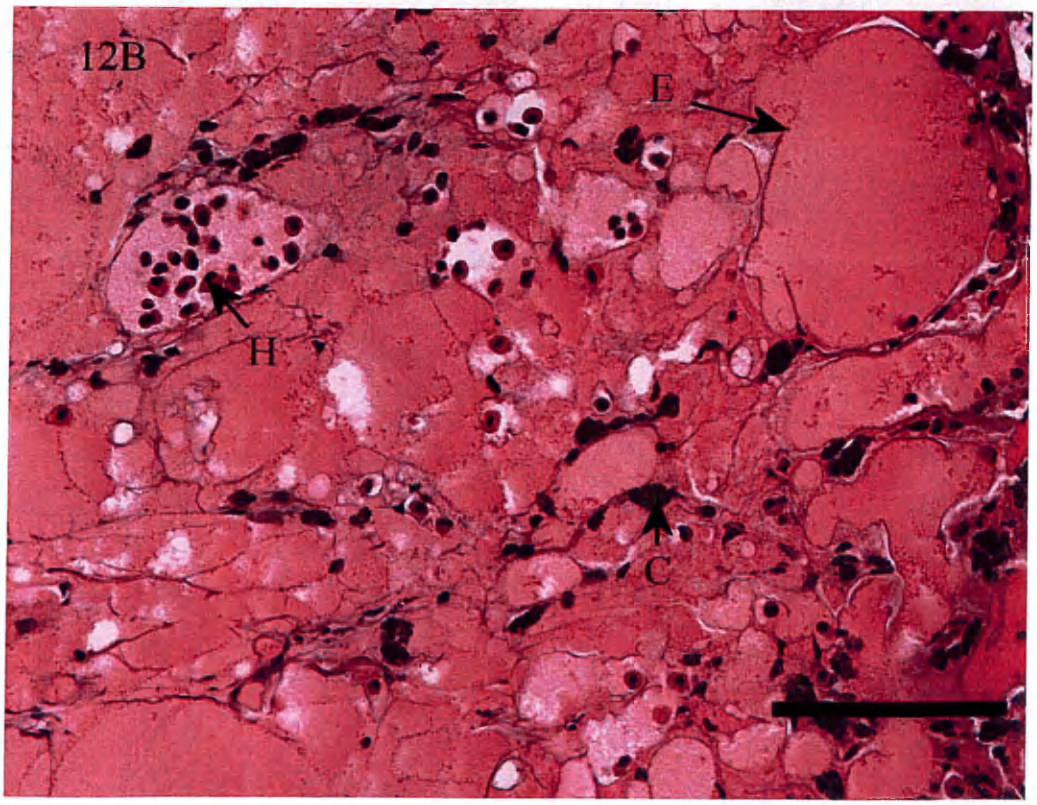
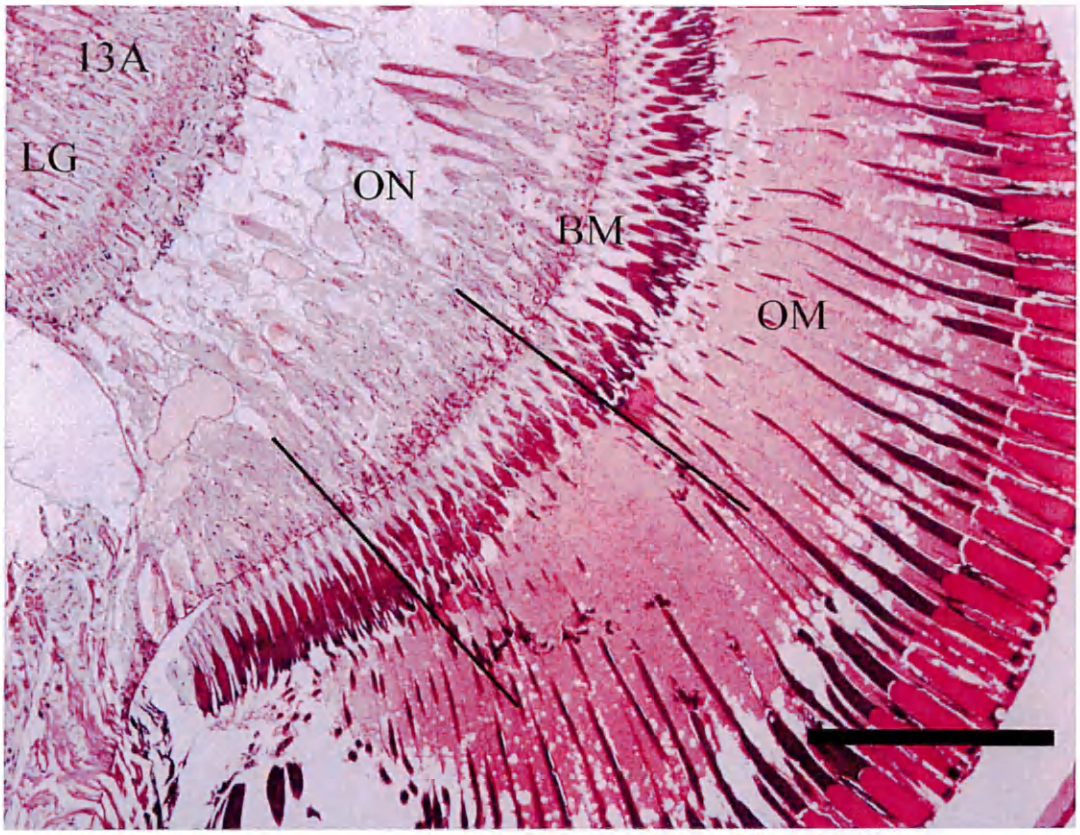

12B
E
H
C

FIGURE 13. Moderately damaged eyes with (A) 20% and (B) 50% of their ommatidia and optic nerve fibers affected. Affected areas are shown within bars; ON, optic nerve region; BM, basement membrane; OM, ommatidial region; LG, lamina ganglionaris. scale = 500 μm


13A
LG
ON
BM
OM

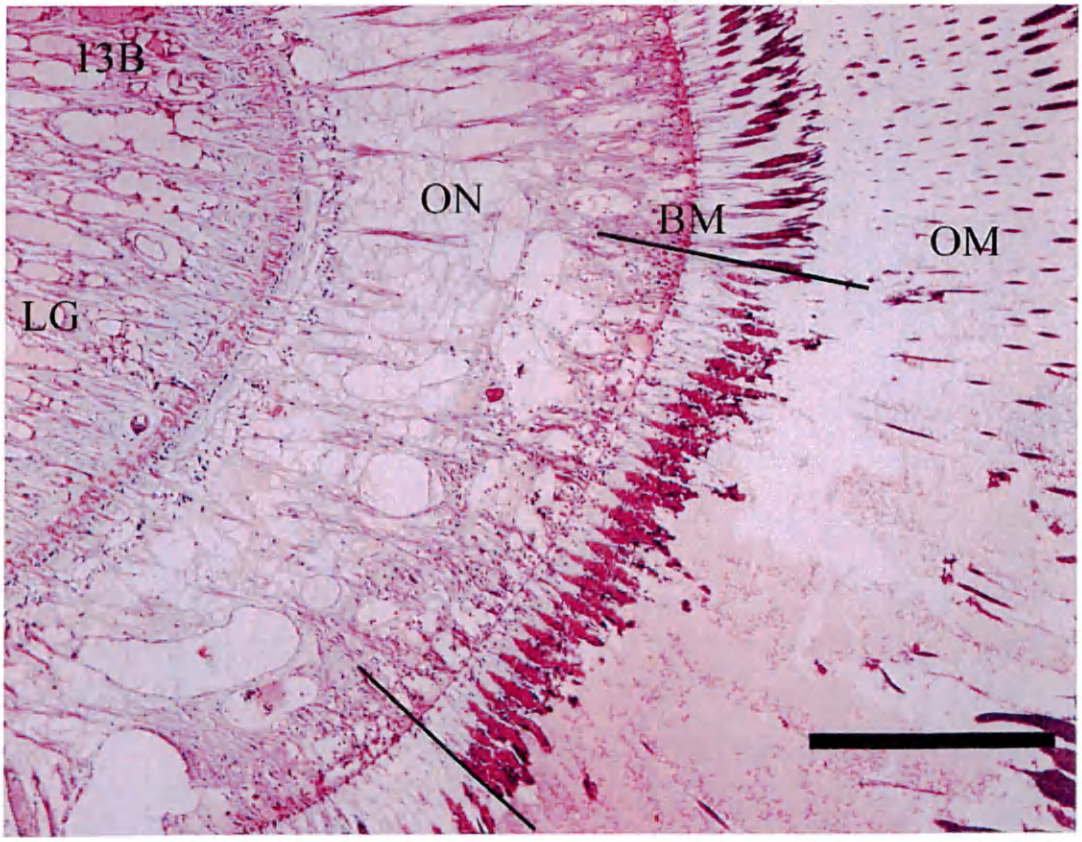

13B
ON
BM
OM
LG

FIGURE 14. Determination of ventral, central, and dorsal areas for indices. Figure indicates ventral (V), central (C), and dorsal (D) regions of the eye. The bend in the eye (arrow) was used to determine which portion was dorsal.

14

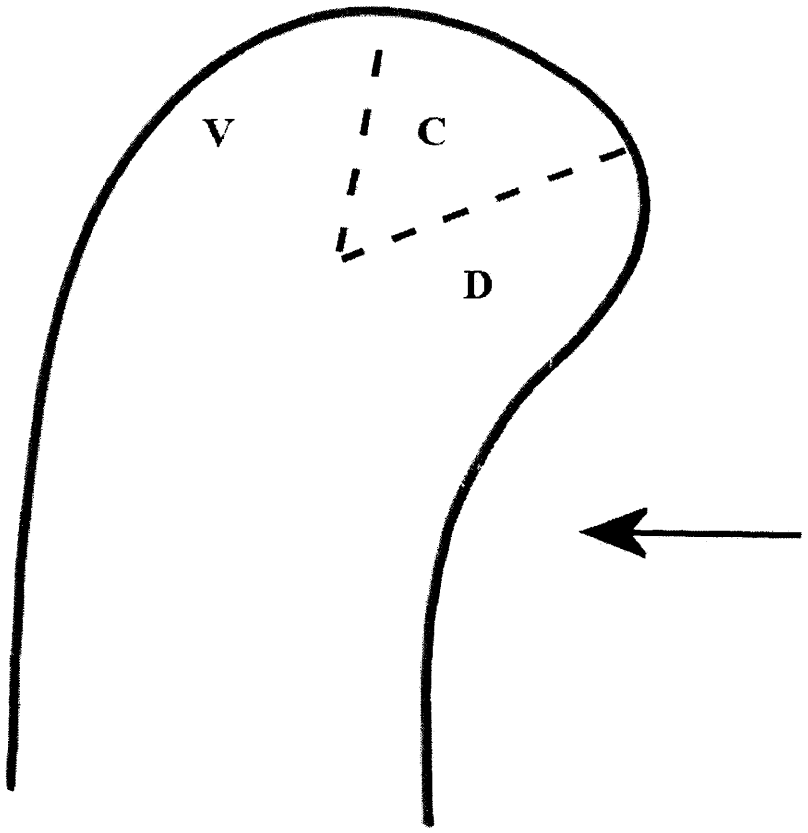
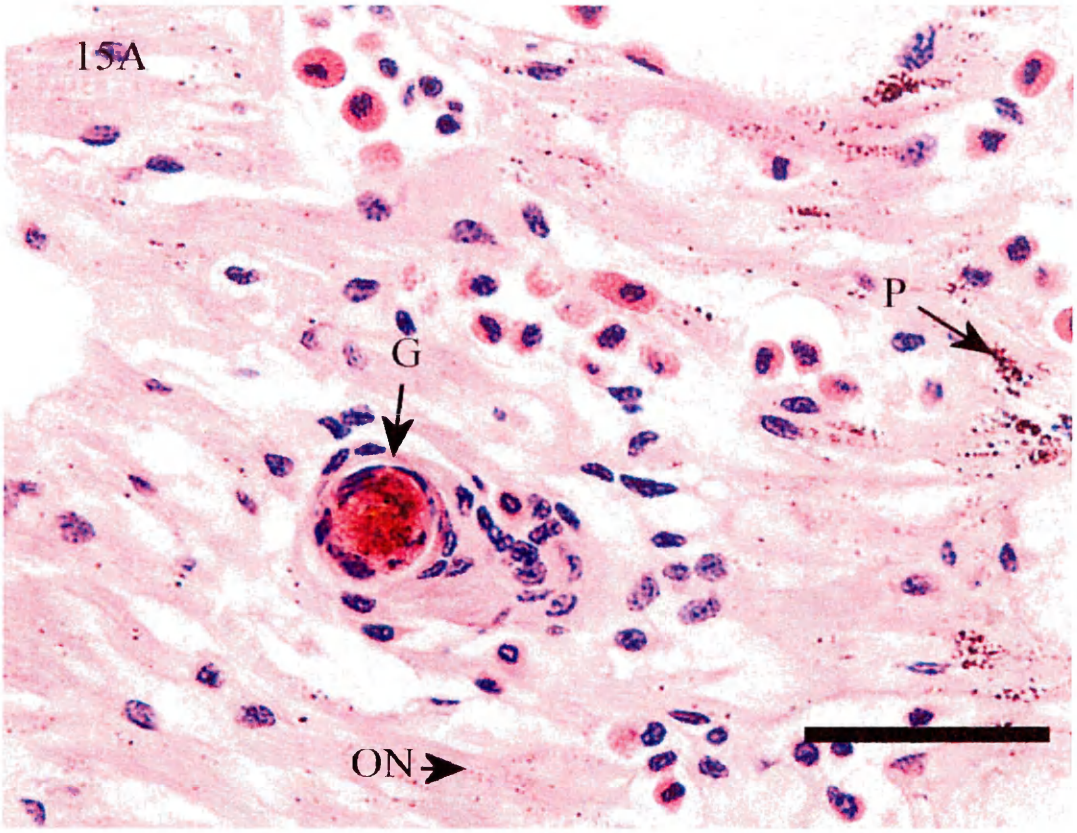


FIGURE 15. Granulomas from the eye of a lobster. (A) Granuloma in the optic nerve region. (B) Granulomas near the tegumental glands of the epidermis in the lobster eye; G, granuloma, ON, optic nerve fibers; T, tegumental gland; P, optic nerve pigments. scale = 50 μm

15A



15B

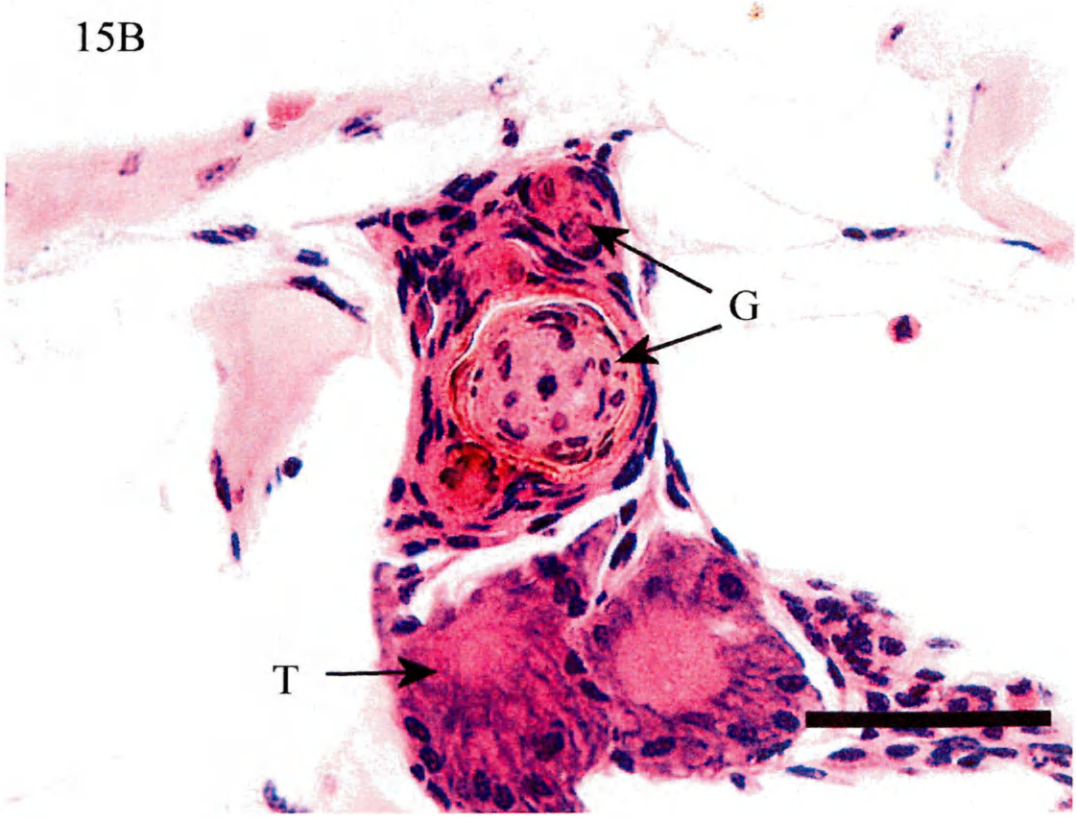


FIGURE 16. Damaged eye with extensive and atypical tissue growth proximal to the basement membrane; ON, optic nerve region; OM, ommatidial region; X, new tissue. scale = 200 μm

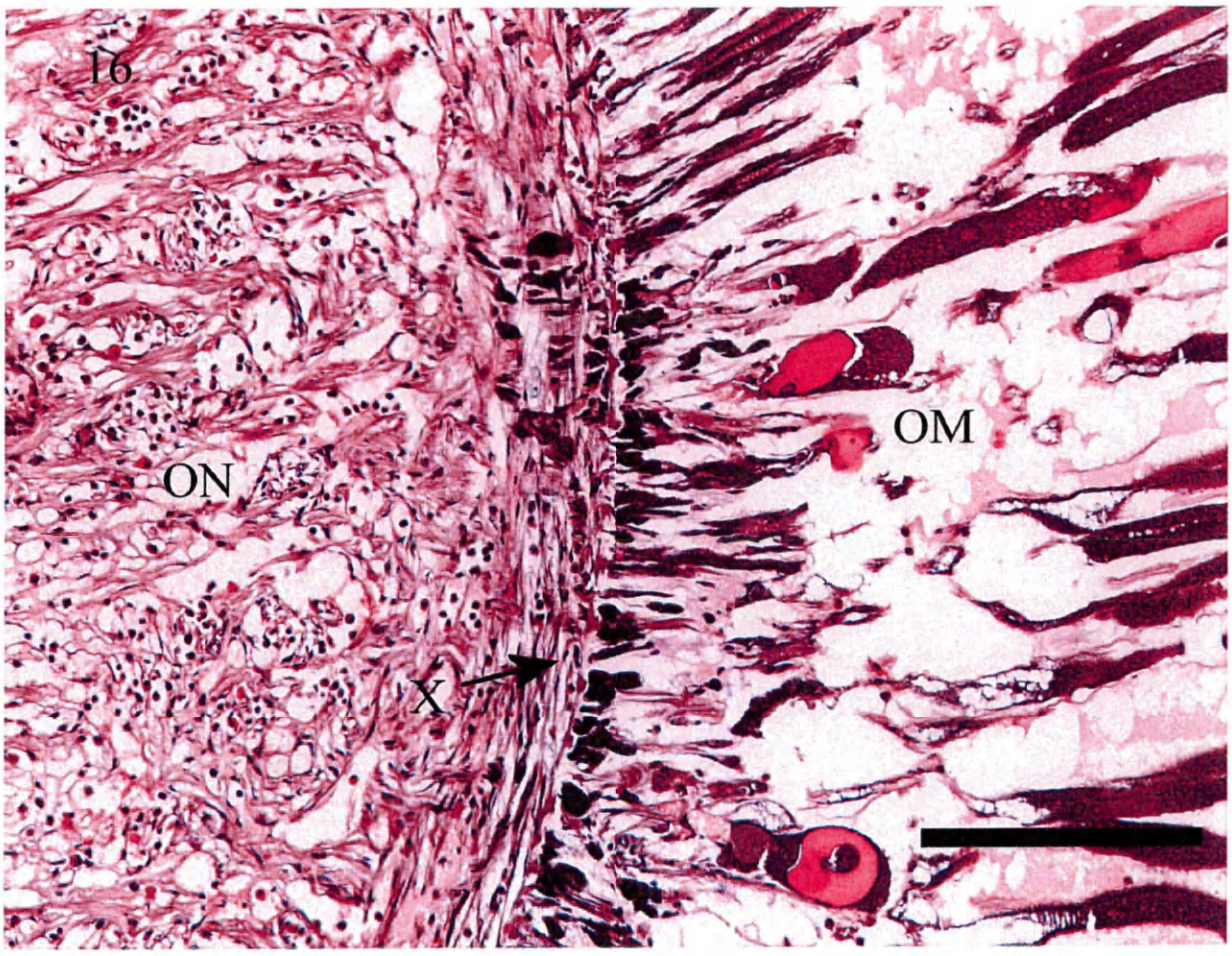

16
ON
X
OM

FIGURE 17. Higher magnification of Figure 16 showing new tissue growth parallel and proximal to the basement membrane; ON, optic nerve region; OM, ommatidial region; X, new tissue. scale = 100 μm

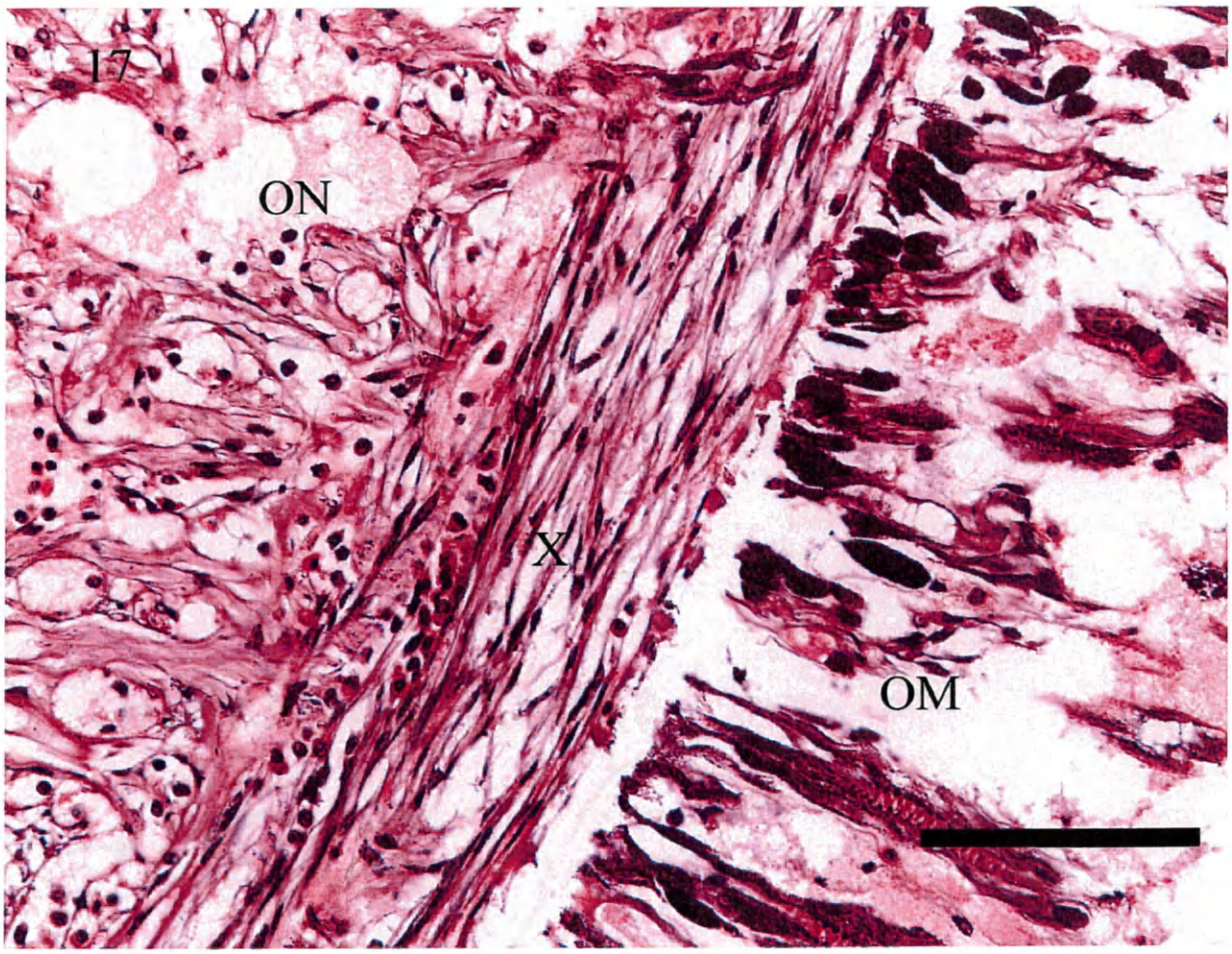

17
ON
X
OM

FIGURE 18. Altered remains of optic nerve fibers; A, altered optic nerve fibers; X, new tissue. scale = 50 μm

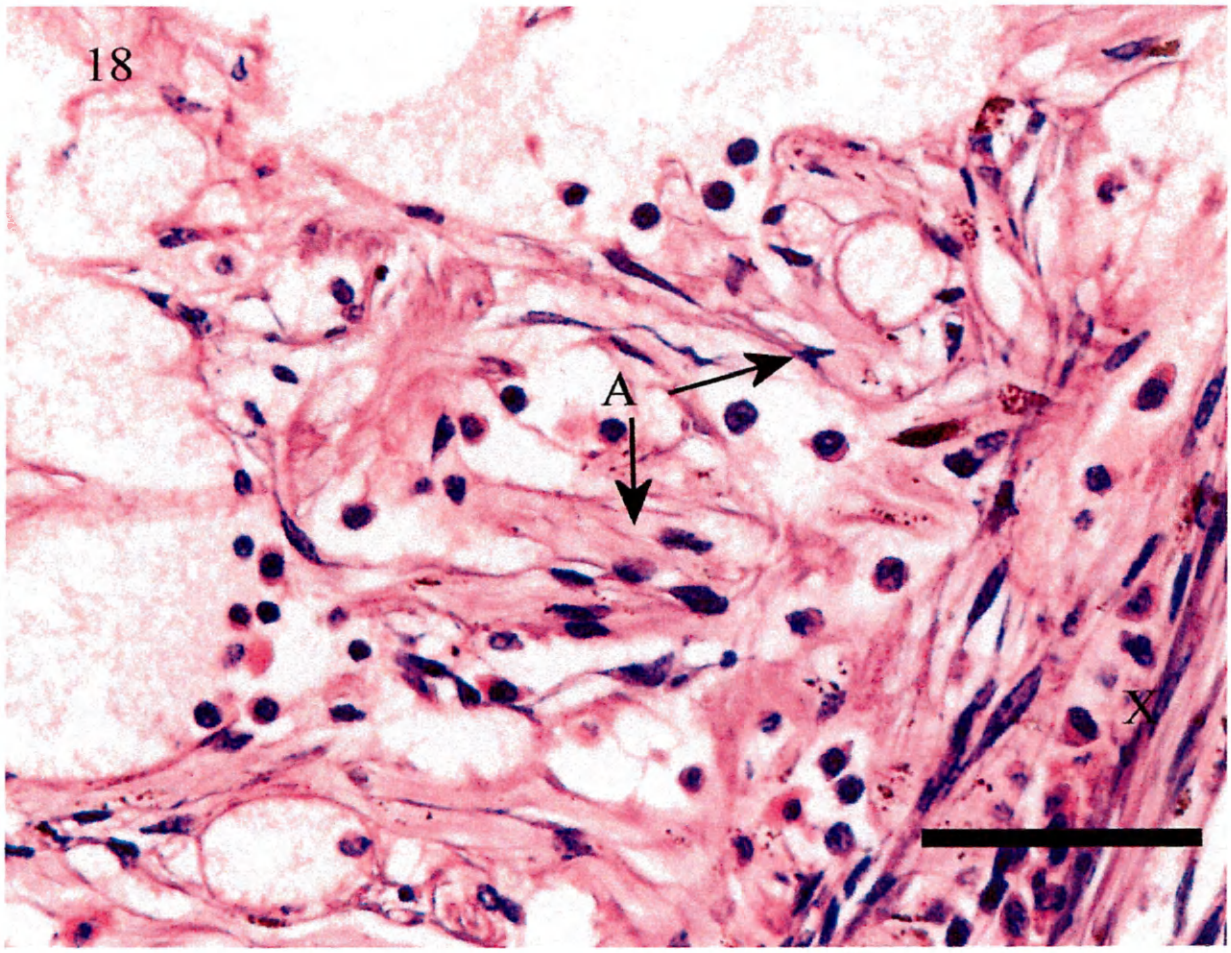

18
A
X

FIGURE 19. Beginnings of new tissue growth proximal to the basement membrane; ON, optic nerve region; BM, basement membrane; OM, ommatidial region; X, new tissue. scale = 100 μm

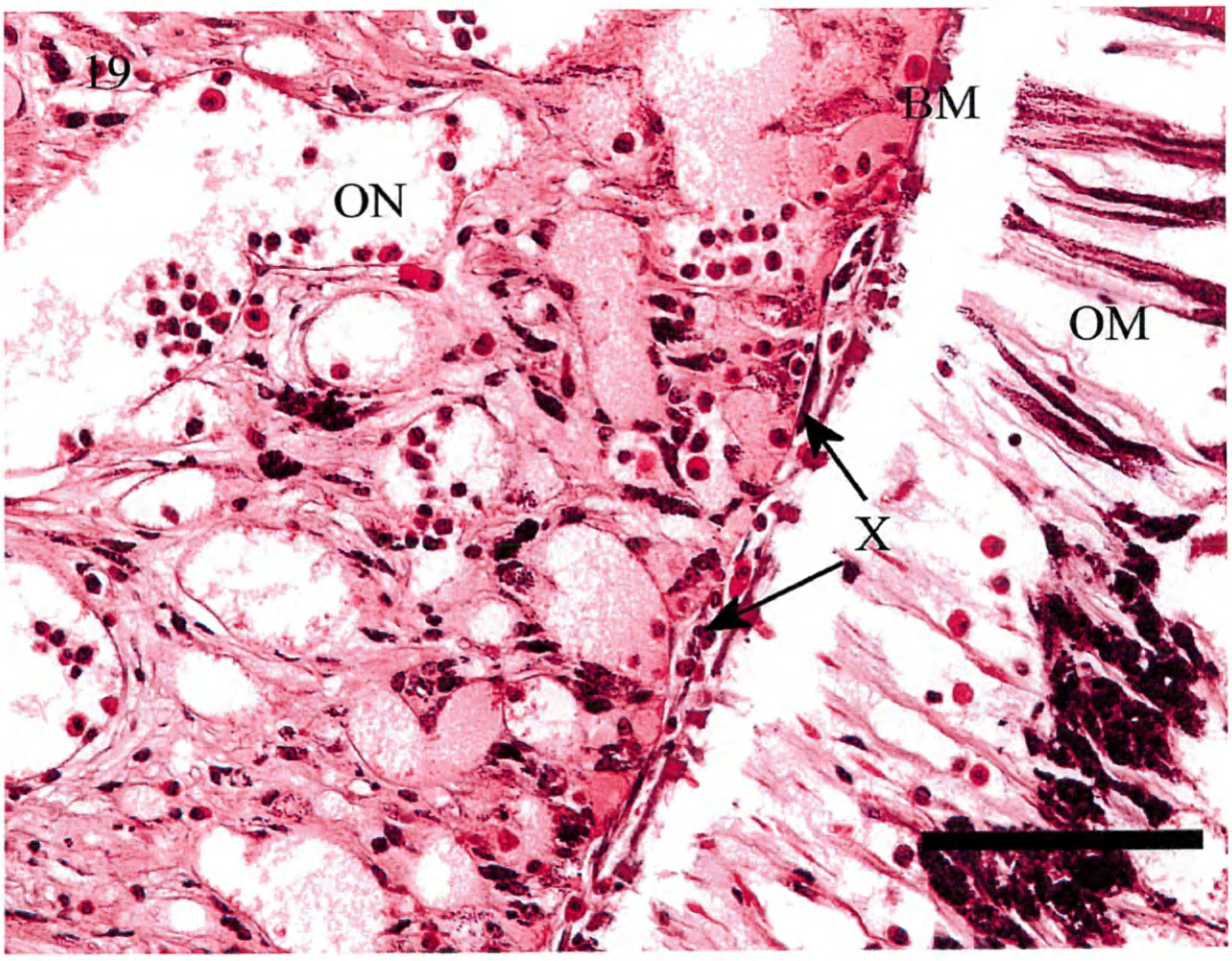
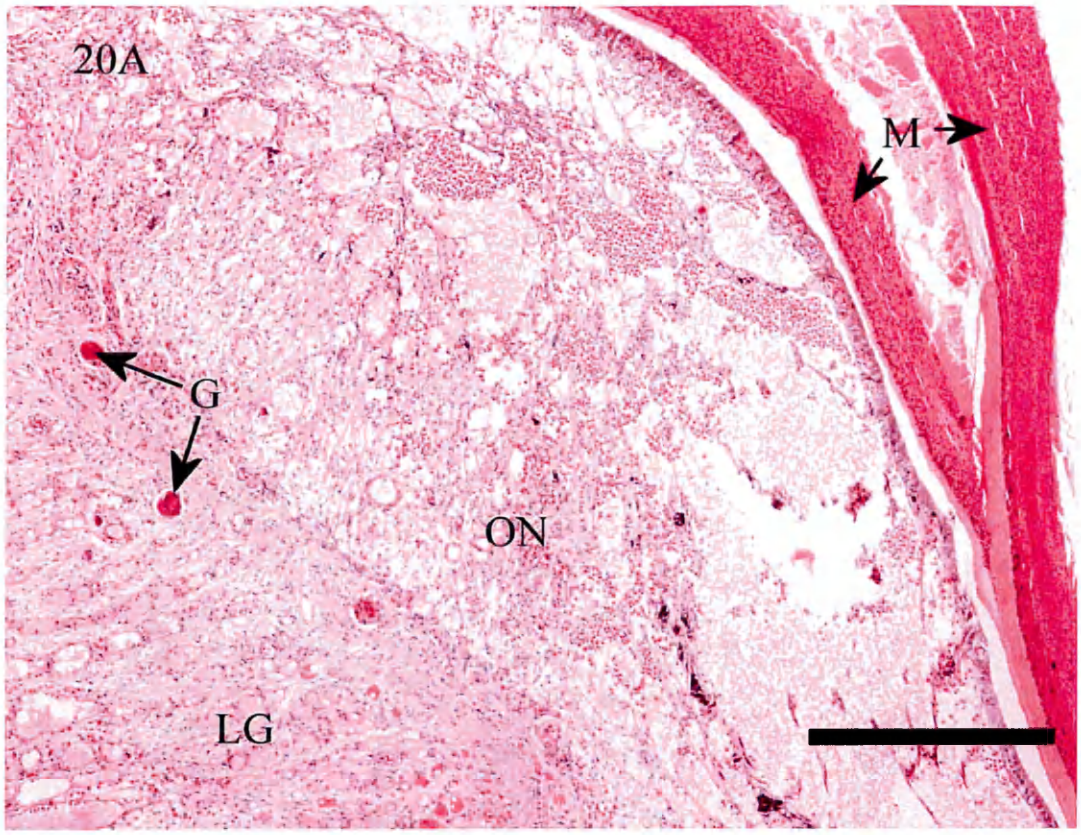

L9
BM
ON
OM
X

FIGURE 20. (A) & (B) Distal portion of a mechanically damaged eye with melanized granulomatous response, M; G, granuloma formation; ON, destroyed optic nerve and ommatidial regions; LG, loss of organization in the lamina ganglionaris; HI, hemocyte infiltration; and B, growth of basophilic tissue. (A.) scale = 500 μm; (B.) scale = 200 μm


20A
M
G
ON
LG

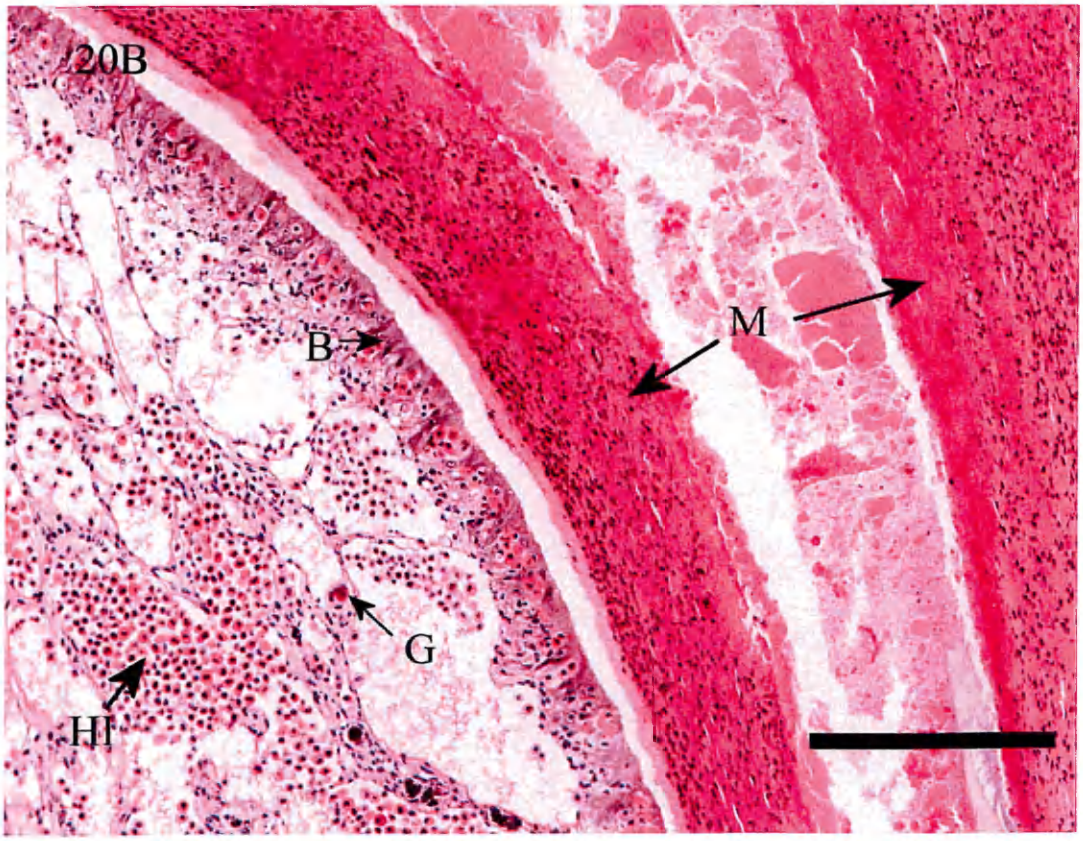

20B
B
M
G
HI

FIGURE 21. Border of optic nerve area and lamina ganglionaris from a mechanically damaged eye; ON, optic nerve region; LG, lamina ganglionaris; G, granuloma; ONLG, border of optic nerve region and lamina ganglionaris. scale = 200 μm

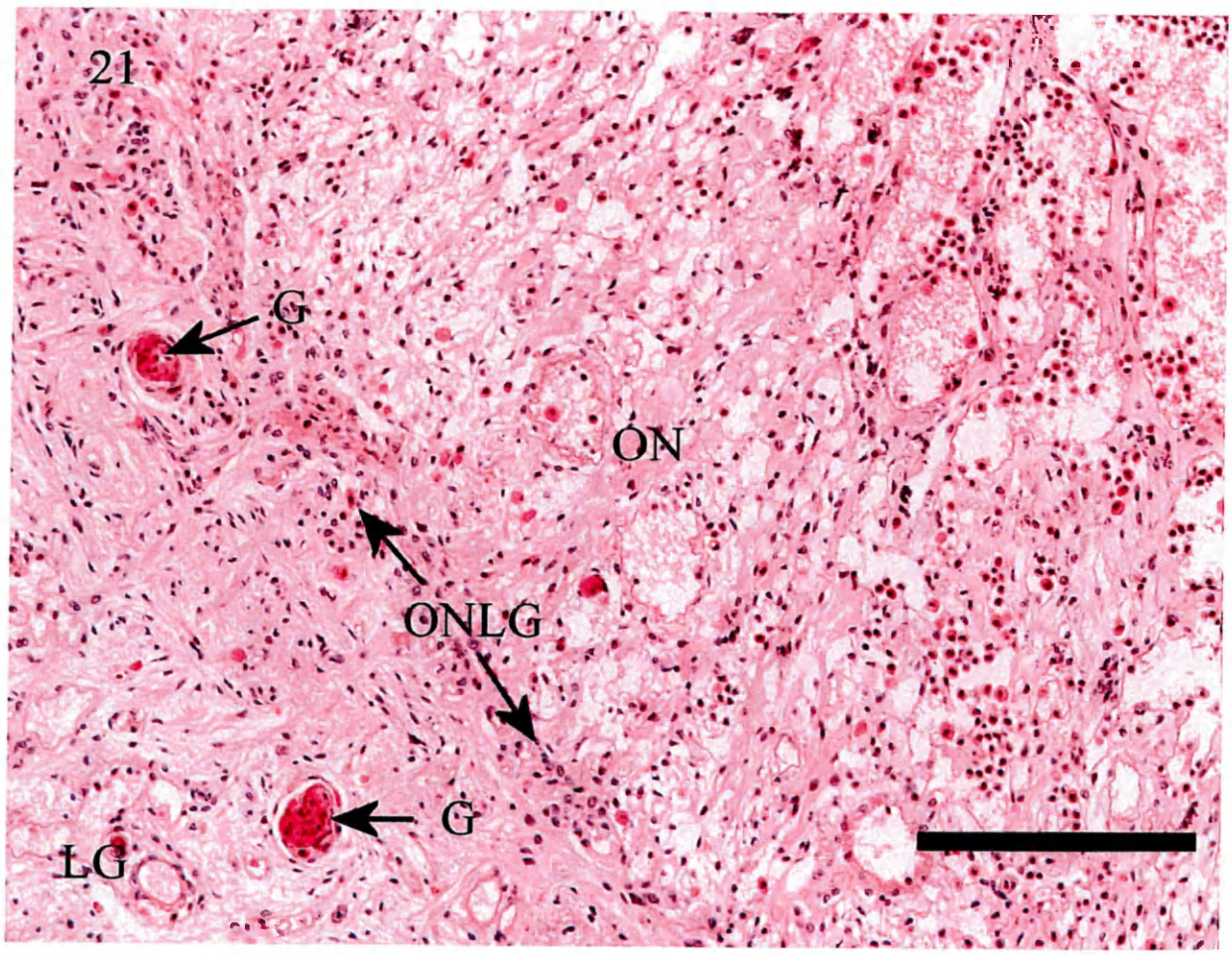
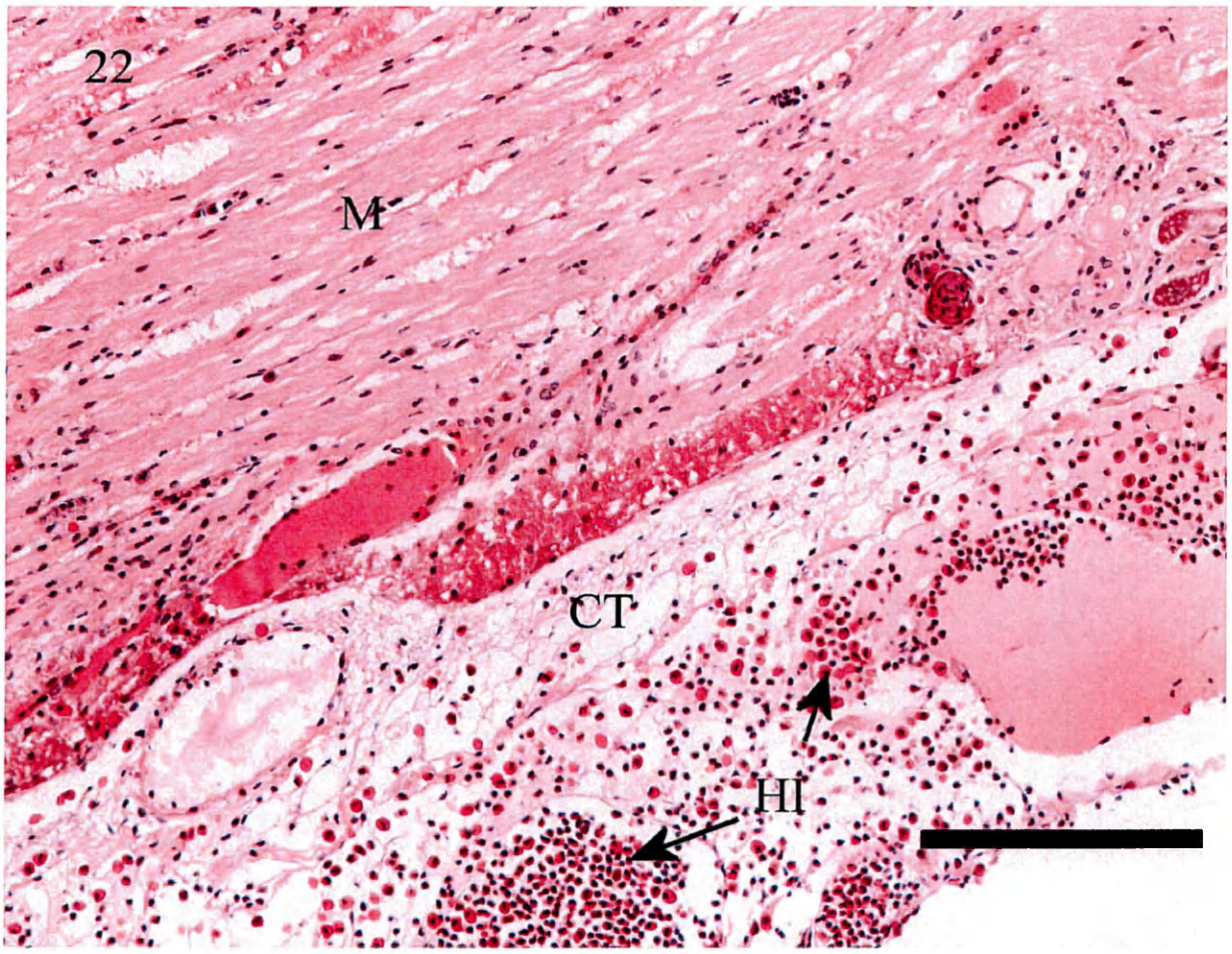

21
G
ON
ONLG
G
LG

FIGURE 22. Medullar area and connective tissue from a mechanically damaged eye; M, medulla; CT, connective tissue; HI, hemocyte infiltration. scale = 200 μm


22
M
CT
HI

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CHAPTER 2: COMPARATIVE PATHOLOGY OF THE EYES AND EYESTALKS OF DECAPOD CRUSTACEANS INFECTED BY SYSTEMIC MICROBIAL AGENTS

ABSTRACT

Although many studies have examined disease-related pathology in crustaceans, the effect of diseases on the eyes and vision of crustaceans is not well understood. However, the eyes of crustaceans are important not only for vision, but also for neurosecretory function. The present study compared eye pathologies associated with various microbial diseases in decapod crustaceans to better understand how diseases affect the eyes and eyestalks and the implications that such pathologies may have on the vision and physiology of the host. The focus of this study was to compare the pathologies of the eyes of two decapods infected with four different pathogens: blue crabs, *Callinectes sapidus,* infected with *Paramoeba perniciosa, Hematodinium* sp., and *Mesanophrys chesapeakensis*, and the Caribbean spiny lobster, *Panulirus argus,* from the Florida Keys with a viral pathogen (PaV1).

In one blue crab examined with a *P. perniciosa* infection, amoebae were present in the connective tissues of the eyestalk and between muscle fibers. They were also present in hemal sinuses, but at a low intensity. However, the presence of amoebae did not alter the architecture of any structures in the eye or eyestalk. There was very little pathology found in the eyes of *C. sapidus* with *Hematodinium* sp. infections. *Hematodinium* cells were present in the connective tissues and hemal spaces of all infected crabs, and could even be seen in small blood vessels within the medullar areas. Granulomas were found in the eyestalks of 38% (11/29) of the *Hematodinium* infected crabs, whereas none were found in the eyes of healthy crabs. The granulomas were small and nonspecific, and individual crabs had only a few granulomas in their eyestalks. Blue crabs infected by *M. chesapeakensis* had ciliates throughout their hemal spaces. The ciliate fed on retinal pigments, and probably the ommatidia; the optic nerve fibers were altered in heavy infections. No granulomas were present in crabs infected by *M. chesapeakensis*. Spiny lobsters, *P. argus*, with PaV1 infections had little pathology in their eyestalk tissues. Infected hemocytes were present in hemal spaces throughout the eyes of infected lobsters. Because pyknosis was observed in the glia, optic nerve fibers, and connective tissues of both healthy and PaV1 infected *P. argus*, it cannot be attributed to PaV1 infection.

Although there was little pathology in the eyes of decapods in this study, microbial agents do cause pathology in the eyes of decapod hosts. The eyes and eyestalks of crustaceans should become a part of routine histological examination in decapods. Although examination of eyes and eyestalks will not necessarily result in diagnosis of disease, their inclusion in research studies is necessary in order to have a complete histopathological view of disease because the eyes of crustaceans are important for both vision and neurosecretory function.

INTRODUCTION

Crustaceans face disease threats from several systemic microbial agents. Viruses, bacteria, rickettsiae, fungi, and protozoans can all result in several systemic diseases (Couch 1983, Johnson 1983). Diseases resulting from pathogenic infections can have varying effects on the host, ranging from fatality—as in Gaffkemia, caused by infections of *Aerococcus viridans* in the lobsters *Homarus americanus* and *H. gammarus* (Johnson 1983)—to changing the physical appearance of the host species, making it unmarketable—as in the hyperparisitism of trematode metacercariae by *Urosporidium crescens* in the blue crab *Callinectes sapidus*, which causes pepper spot disease (Couch 1974, Couch 1983). In several diseases, infection results in economic loss to crustacean fisheries.

Although many studies have examined disease-related pathology in crustaceans (e.g. Johnson 1977, Sparks et al. 1982, Messick and Small 1996, Sheppard et al. 2003, Shields and Behringer 2004), the effects of diseases on the eyes and vision of crustaceans are not well understood. However, the eyes of crustaceans are important not only for vision, but also for neurosecretory function. In fact, the main crustacean endocrine organ, the X-organ sinus gland complex, is located within the ganglionic region of the eye (Carlisle and Knowles 1959, Knowles and Carlisle 1956, Bern and Hagadorn 1965, Lockwood 1967, Charmantier and Charmantier-Daures 1998, Smith 2000). The X-organ sinus gland complex is involved in many aspects of endocrine and neuroendocrine

regulation in crustaceans (Charmantier and Charmantier-Daures 1998) and is believed to control five main areas of crustacean endocrinology: (1) movement and response of retinal pigments, (2) somatic pigmentation, (3) molting, (4) metabolism (including oxygen consumption, blood sugar level, and water metabolism), and (5) reproduction and breeding cycles (Carlisle and Knowles 1959, Knowles and Carlisle 1956, Bern and Hagadorn 1965, Lockwood 1967). Therefore, damage to the eyes and eyestalks could result in severe consequences to the endocrine control of the animal, in addition to causing blindness from direct retinal damage.

The purpose of the present study was to compare eye pathologies associated with various microbial diseases in decapod crustaceans to better understand how diseases affect the eyes and eyestalks and the implications that such pathologies may have on the vision and physiology of the host. Because eyes and eyestalks are involved in many aspects of crustacean growth and development, along with vision, damage to the eyestalks could interfere with molting, reproductive behavior, or foraging ability. The focus of this study was on the comparative pathologies of the eyes of two decapods infected with four different pathogens: *Callinectes sapidus* from Chesapeake Bay infected with *Paramoeba perniciosa, Hematodinium* sp., and *Mesanophrys chesapeakensis*, and *Panulirus argus* from the Florida Keys with a viral pathogen (PaV1).

Disease agents and light are known to cause pathologies in the eyes of crustaceans. Smith (2000) reported on disease-related pathologies in the eyes of the shrimp *Penaeus monodon*. Suppurative inflammation occurred, characterized by edema, hemocyte infiltration, and localized sites of abscesses. The abscesses had necrotic cells

and were found frequently in the dioptric region (Smith 2000). Granulomas were found affecting the ommatidia, ganglia, and other internal structures of the eye. Malacia, characterized by necrosis of nervous tissue, vacuolation, and vascular proliferation in the medulla ganglia were also observed. The most likely causes of these pathologies were determined to be viral infection and vibriosis.

Shelton et al. (1985) reported that light damaged the eyes of *Nephrops norvegicus*, an organism that naturally encounters only dim light. The pathologies associated with light exposure involved damage to the photoreceptor layer, i.e. the ommatidia. Damaged areas were marked by the absence of rhabdoms and retinula cell nuclei, and an abnormal distribution of the proximal shielding pigment (Shelton et al. 1985). When damage was present in the ommatidia, the center of the retinular area was always affected. Light damage in the ommatidia of *Nephrops norvegicus* appears to be irreversible, because one month after a short exposure to light, there was no sign of recovery and the entire retinula cell layer was destroyed (Shelton et al. 1985).

The main objective of this study was to determine which areas, if any, of the eyes and eyestalks of decapod crustaceans exhibit pathologies caused by several systemic microbial diseases. The two overarching null hypotheses for this study were as follows:

H_{01}: The eyes of decapod hosts are not pathologically altered by systemic microbial infections.

H_{02}: Histological examination does not provide evidence indicating that systemic microbial infections alter the capacity for vision in affected decapods.

Paramoebiasis in *Callinectes sapidus*

In the summer of 1965, mortalities of *C. sapidus* were reported in the lower Delmarva Peninsula. Dead and moribund animals were described as having a grayish appearance to the ventral side of the body and appendages; this condition was especially evident in animals that had recently molted (Sprague and Beckett 1966). The disease agent was later identified as a parasitic amoeba, *Paramoeba perniciosa* (Sprague and Beckett 1968; Sprague et al. 1969) and the disease is now known as gray crab disease. *Paramoeba perniciosa* infects blue crabs from as far north as Long Island Sound to the Atlantic coast of Florida (Newman and Ward, Jr. 1973, Johnson 1977). It occurs in waters with a salinity higher than 25‰ (Sawyer et al. 1970).

Paramoeba perniciosa takes two different forms in host tissue. The large form is lobose, often with clear vacuoles in the cytoplasm and is generally 10-20 μm in size. The small form is spherical and about 3-7 μm in size (Johnson 1977, Sawyer 1969). Both forms of the amoeba can be found in a single host, although large forms tend to be found in the connective tissue of the antennal gland, endothelium of blood vessels, and within glia of the nervous system and in hemal spaces of nervous tissues (Johnson 1977). Present in both the large and small forms of *Paramoeba perniciosa* is the secondary nucleus or "Nebenkörper", a defining character for the genus (Sprague and Beckett 1968, Sawyer 1969, Sprague et al. 1969, Couch 1983).

One of the main signs associated with paramoeba infection is the gray discoloration of the ventral exoskeleton, especially in mature males that are infected. However, this trait is only seen in animals that are moribund, and even then it does not

always occur. Animals with heavy infections exhibit lethargy and absent or reduced hemolymph clotting ability. Light and medium infections can only be diagnosed through histology. In addition, the amoebae can only be found in hemolymph smears in heavy to terminal infections (Johnson 1977).

Paramoeba perniciosa can be found in a number of locations in host tissues. Generally, the parasite is found in hemal spaces and connective tissues (Johnson 1977). It also occurs in the antennal gland, Y organ, and connective tissues of the midgut. Amoebae can be found in the hepatopancreas and gill, except in light infections. In heavy infections, amoebae are found in the epidermis, within the glia, hemal spaces, and capillaries of the nervous system, and in muscles. In terminal infections—and rarely in heavy and medium infections—amoebae are located in sinuses of the heart.

Johnson (1977) noted some pathologies common among heavily infected crabs. Hemal spaces were often filled with amoebae, resulting in pressure necrosis to the connective tissues supporting the hemopoietic tissue and Y organ. Necrosis of the heart muscle also occurred, as well as skeletal muscle necrosis. Additionally, there was lysis of epidermal cells and of connective tissue. Terminally infected animals exhibited lysis of the muscle. Karyorrhexis of hemocytes or the secondary nucleus of the parasite was also observed, and was present in light, medium, and terminal infections.

Couch (1983) noted additional pathologies related to paramoebiasis in *C. sapidus*. In heavy infections, most hemocytes were replaced by amoebae in blood sinuses. In the case of advanced infections, the hepatopancreas, gonad, muscle, gills, and hemolymph became filled with amoebae, the amoebae essentially replacing the connective tissue cells

of the animal. In the same animals with advanced infections, amoebae did not invade the peripheral epithelial tissues of the gut, hepatopancreas, hypodermis, and gonad.

Paramoeba perniciosa primarily affects the connective tissue of the host. Therefore, *P. perniciosa* may attack the glial cells, which are support structures in the eyes and eyestalks of *C. sapidus*.

H_{03}: The glial cells of the eyes of *Callinectes sapidus* infected by *Paramoeba perniciosa* do not exhibit any pathologies associated with the infection.

Hematodinium sp. in *Callinectes sapidus*

Members in the genus *Hematodinium* are parasitic dinoflagellates that invade crustacean hosts. The species found in *Callinectes sapidus* is morphologically identical to *Hematodinium perezi* (Shields and Overstreet in press) and has sometimes been referred to as such in the literature. However, because molecular comparisons of the organisms have not been completed, I will refer to the parasite in *C. sapidus* as *Hematodinium* sp.

Hematodinium sp. infects *Callinectes sapidus* along the Atlantic coast of the U.S. from New Jersey to Florida and along the U.S. Gulf coast from Texas to Florida (Newman and Johnson 1975, Couch unpublished as cited in Couch 1983, Messick and Shields 2000). The parasite only affects crabs in salinities over 11‰ (Newman and Johnson 1975, Messick and Shields 2000). Prevalence of infections is highest during

autumn when water is cooler (Messick and Shields 2000), but infections decline below 9°C (Messick et al. 1999.)

The only external sign of *Hematodinium* infection in *C. sapidus* is lethargy or weakness (Couch and Martin 1979, Couch 1983, Messick 1994, Shields and Overstreet in press). Messick (1994) also reported a pink carapace as a clinical sign, but this has not been recorded elsewhere. Internally, opaque muscles (Messick 1994) and milky-white tissues (Newman and Johnson 1975) have been reported in affected crabs. Perhaps the most commonly reported sign of infection is discoloration of hemolymph in infected crabs, which has been described as milky, chalky, opaque, yellow, and brown (Couch and Martin 1979, Couch 1983, Shields and Squyars 2000, Maniscalco personal observation, Shields and Overstreet in press). Advanced infections show a reduced number of hemocytes, with apparent replacement of hemocytes by *Hematodinium* (Newman and Johnson 1975, Messick 1994, Shields and Squyars 2000). In addition, the hemolymph does not clot (Newman and Johnson 1975, Shields and Squyars 2000), possibly due to a decline in hyalinocytes (Shields and Squyars 2000, Shields et al. 2003).

Hematodinium sp. is primarily found in the hemolymph of infected *C. sapidus*, with hemal spaces sometimes being occluded (Couch and Martin 1979, Couch 1983, Messick 1994). The parasite is also found in or between skeletal muscle fibrils, in hemal spaces of the gonads, in the hepatopancreas (Couch and Martin 1979, Couch 1983, Messick 1994, Sheppard et al. 2003), and in the heart (Shields and Squyars 2000, Sheppard et al. 2003). Tissue sections have also revealed signs of host response in the form of nodule formation in hemal spaces (Messick 1994), gill, hepatopancreas, and cardiac muscle (Sheppard et al. 2003). Involvement of the tissues of the eyestalks of

infected crabs had not been examined. *Hematodinium* sp. is known to cause pressure necrosis in tissues of infected crabs, but it is unknown if this, or any other pathology occurs in the eye.

H_{04}: *Hematodinium* sp. infection does not pathologically alter the eyes and eyestalks of *Callinectes sapidus* hosts.

Mesanophrys chesapeakensis in *Callinectes sapidus*

During a survey for diseases and parasites in blue crabs from 1990-1995, a histophagous ciliate, *Mesanophrys chesapeakensis*, was found in 8 of 2500 blue crabs (Messick and Small 1996). The infected crabs were found in Delaware Bay, Chesapeake Bay, and Assawoman Bay, at temperature ranging from 4-12°C and salinities from 2-32‰ (Messick and Small 1996).

Mesanophrys chesapeakensis has a fusiform body, measuring 28-47.6 µm long and 11-18.3 µm wide. The ciliate feeds on the hemolymph, hemocytes, and tissues of its blue crab host (Messick and Small 1996).

Messick and Small (1996) reported that *M. chesapeakensis* was found in the connective tissues and hemal sinuses of the heart, muscle, thoracic ganglion, antennal gland, and hemopoietic tissue of the crab host. The parasite was rare in glial tissues. Ciliates were sometimes seen in the myocardial and interstitial tissues of the heart, cell necrosis was present in some tissues as well. Occasionally, the anterior end of the ciliate was inserted into basement membranes, but did not appear to interrupt their architectural integrity (Messick and Small 1996). Hemocytic infiltration and nodule formation

sometimes occurred. Hemocytopenia, although often found in advanced ciliate infections (Cain and Morado 2001), was not observed, probably because Messick and Small (1996) were only reporting on light infections.

Mesanophrys chesapeakensis is histophagous and feeds on its host tissues, but no reports on pathology exist from the eyestalks of blue crabs.

H_{05}: *Mesanophrys chesapeakensis* will not feed on the tissues in the eyestalk of *Callinectes sapidus*.

PaV1 in *Panulirus argus*

During 1999 and 2000, juvenile spiny lobsters, *Panulirus argus*, were reported as lethargic and moribund, with hemolymph that was thin and milky and did not clot (Shields and Behringer 2004). Shields and Behringer (2004) found infected juvenile lobsters nearshore in western Florida Bay and occasionally along the Atlantic reef tract near the Florida Keys; no infected adults were found, but few adults were included in the survey.

The causative agent was identified as a pathogenic virus, *Panulirus argus* Virus 1 (PaV1), and was described as an icosahedral, presumptive DNA virus with a nucleocapsid of approximately 182 nm and nucleoids approximately 118 nm (Shields and Behringer 2004). PaV1 shares features common to the Herpesviridae and Iridoviridae; however, there are too many morphological differences to place PaV1 in either family (Shields and Behringer 2004).

There are few external clinical signs of a PaV1 infection. Lobsters with heavy infections exhibit lethargy, an inability to right themselves, and infrequent tremors (Shields and Behringer 2004). Internally, heavily infected animals have chalky or milky hemolymph that has a loss of clotting ability (Shields and Behringer 2004).

Lobster hemocytes appear to be the main target of viral infection. In heavily infected lobsters, nearly all of the circulating hyalinocytes and semigranulocytes were infected or destroyed, with infected cells showing an altered appearance, their nuclei exhibiting hypertrophy and Cowdry Type-A inclusions (Shields and Behringer 2004). Circulating granulocytes were not infected (Shields and Behringer 2004). In addition to infected hemocytes, connective tissue cells were noticeably infected by the virus, with pyknotic nuclei common in moderate and heavy infections (Shields and Behringer 2004).

Because PaV1 is known to cause damage to the connective tissues of its host, *Panulirus argus*, the glial cells of the eyes and eyestalks of the infected lobsters could be damaged by the virus.

H_{06}: PaV1 does not pathologically alter the nervous tissue and glial cells in the eyes of infected lobster hosts.

MATERIALS AND METHODS

Collection of Animals

Blue crabs *Callinectes sapidus* were collected in commercial crab pots by local watermen from Wachapreague, Virginia. Additional crabs from the Eastern Shore were collected using crab pots put in place by scientists at the VIMS Wachapreague Laboratory. Collection occurred in June, August, and November 2003, May and June 2004, and January 2005.

Spiny lobsters *Panulirus argus* were collected by hand for a time-course study by divers (Mark Butler and Don Behringer, under permit) from reef tracts in the Florida Keys National Marine Sanctuary.

Dissection and Fixation

Hemolymph from *Callinectes sapidus* was extracted for preliminary disease diagnosis from the base of the 5th pereiopod using a 27 gauge needle with a tuberculin syringe. Approximately 4 drops of hemolymph were placed on a slide and 0.04% neutral red solution (neutral red dye in physiological saline buffer, from Shields and Squyars 2000; NaCl 19.31 g/L; KCl 0.65 g/L; $CaCl_2 \cdot 2H_2O$ 1.38 g/L; $MgSO_4 \cdot 7H_2O$ 1.73 g/L; Na_2SO_4 0.38 g/L; HEPES 0.82 g/L) was added in a 1:1 ratio as a vital stain. The

hemolymph was then examined for parasites--*Hematodinium* sp. is known to take up neutral red in its lysosomes (Shields, personal communication). The preliminary diagnosis was recorded for each animal and carapace length and overall physical condition were noted.

Callinectes sapidus hemolymph was drawn for preliminary diagnosis from over 400 animals. Only 62 of these crabs were processed for histology and further analysis—some were taken as uninfected control specimens, while 29 others were determined to have *Hematodinium* sp. infections. No crabs with apparent *Paramoeba perniciosa* infections were found during preliminary sampling.

Following the initial examination of hemolymph for disease agents, the blue crabs were processed for histology. Hemolymph samples (at least 1.5 ml) were drawn from the animals and placed into microcentrifuge tubes. The fresh hemolymph was allowed to clot for 30 minutes. The clots were then macerated with a tissue grinder and samples were centrifuged at 1500 g for 10 minutes (Fisher Scientific Micro7). Serum was extracted from the microcentrifuge tubes, placed into clean tubes, and frozen at -80°C. The following tissues were removed from blue crabs, placed in tissue cassettes, and fixed in Bouin's solution for approximately 48 hours: hepatopancreas, heart, gonad, gill, skin, antennal gland, ventral nerve, and eyestalk. Tissues were held in 70% EtOH until further processing occurred.

One blue crab with *M. chesapeakensis* was collected during winter sampling in January 2005, and its hemolymph was used to transmit the parasite into two additional crabs. These three crabs were then processed for histology. One blue crab with *P. perniciosa* was collected in a previous study.

Spiny lobsters *P. argus* used in the study were part of a time-course study for PaV1 that occurred from July-September 2003. Ten control and 23 infected animals were examined for the present study. Lobsters were dissected by Shields and Li during the time-course study. Hemolymph was collected and then frozen at -80°C in case it was needed for further examination. Tissues collected from the spiny lobsters include hepatopancreas, gill, heart, hindgut, foregut, and eyestalk. These tissues were fixed in Bouin's solution for approximately 48 hours and then transferred to 70% EtOH.

Histology

Collected eyestalks were decalcified using the formic acid-sodium citrate method described by Luna (1968). Following decalcification, eyestalks were cut in half longitudinally using stainless steel, single-edge razor blades. Eyestalks and other tissues were dehydrated, cleared, and infiltrated in paraffin using the Shandon Hypercenter XP tissue processor. Tissues were embedded in paraffin blocks using a Miles Scientific Tissue Tek embedding console. The tissues were cut into 5 μm sections using ThermoShandon blades and an Olympus Cut 4055 microtome or an American Optical 820 microtome. Slides were stained by hand using a regressive Harris' hematoxylin and eosin procedure based on methods described by Luna (1968). These histology procedures were used for all species examined in this study.

Comparative Histology of the Eye

Hepatopancreas, heart, gill, skin, antennal gland, ventral nerve, and other tissues were used for disease diagnosis, however, the eyestalks were examined in more detail. Areas of interest in the eyestalks were the ommatidia, basement membrane, tegumental glands, optic nerve fibers, lamina ganglionaris, the three medullar areas, sinus gland, neurosecretory cells, connective tissues, and hemal spaces (Figure 1). Tissues were examined using an Olympus BX51 compound microscope and photographs were taken using a Nikon DXM1200 with the aid of the ACT-1 computer program (Nikon).

RESULTS

Paramoebiasis in *Callinectes sapidus*

No crabs were found with *Paramoeba perniciosa* infections, but one sample from a prior study was examined. The parasite was observed within the connective tissues of the eyestalk (Figure 2) and between muscle fibers (Figure 3). Amoebae were present in the hemal sinuses (Figure 4), but the intensity was low. While some amoebae were present between fibers of the optic nerve tract (optic peduncle) (Figure 5), proximal to the medulla terminalis, there did not appear to be any damage to the surrounding host tissue. No amoebae were observed in the glia of the eye or in the ommatidia. Furthermore, the presence of amoebae did not alter the architecture of any structures in the eye or eyestalk.

Hematodinium sp. in *Callinectes sapidus*

Twenty nine blue crabs infected by *Hematodinium* sp. were identified. There was very little pathology found in the eyes of these crabs. *Hematodinium* cells were present in the connective tissues and hemal spaces of all infected crabs, and could even be seen in small blood vessels within the medullar areas (Figures 6 and 7). Hemocytes infiltrated into the ommatidia of 10% (3/29) of crabs infected by *Hematodinium* sp. In healthy crabs, this occurred in 22% (4/18) of crabs. In one (3%) of the infected crabs,

Hematodinium cells had penetrated into the ommatidia. Granulomas were found in the eyestalks of 38% (11/29) of the *Hematodinium* infected crabs, while none were found in the eyes of healthy crabs. The granulomas were small, and individual crabs had only a few granulomas in their eyestalks. In one crab infected with *Hematodinium,* a large aggregate of parasites and host hemocytes (Figure 8) was found in the sinus gland of the blue crab host.

During the study, 11 blue crabs were found to have a presumptive virus that infected their hemocytes (Figure 9). In these crabs, 18% (2/11) had hemocyte infiltration in their ommatidia, and 36% (4/11) had granulomas in their eyestalk tissues. No pathology was observed in the ommatidia

Mesanophrys chespeakensis in *Callinectes sapidus*

In the three blue crabs with *M. chesapeakensis*, there were ciliates throughout the hemal sinuses and blood vessels of the eyestalk. Two crabs had light infections, and one moribund crab had a heavy infection. In both light and heavy infections, *M. chesapeakensis* was observed in the ommatidia. The ciliates had consumed retinal pigments, as observed in their phagosomes (Figure 10). In the heavy infection, the ommatidia were altered throughout the entire region (Figure 11), and in light infections there were smaller areas of altered cells. Altered regions were filled with cellular debris, and the rhabdoms were absent. Crystalline tracts had also been destroyed. In infected crabs, the retinal pigments were not as well dispersed as they were in healthy crabs. Ciliates were present between fibers of the lamina ganglionaris in both light and heavy

infections, but the medullar regions were unaffected with the exception of ciliates in the blood vessels. Finally, in the heavy infection, optic nerve fibers were altered and lost the straight, taut appearance of healthy fibers, becoming more ragged (Figure 12). No granulomas were observed in any crabs infected with the ciliates.

PaV1 in *Panulirus argus*

The 23 spiny lobsters *Panulirus argus* with PaV1 infections had little pathology in their eyestalk tissues. Infected hemocytes were present in hemal spaces throughout the eyestalks of infected lobsters (Figure 13). There was pyknosis in the glia of 30% (7/23) of the infected lobsters, but pyknosis occurred in the glia of 60% (6/10) healthy lobsters (Figure 14). Otherwise, glial cells did not present with any pathology. Pyknosis also occurred in nuclei along the optic nerve fibers that run between the ommatidia and the lamina ganglionaris in 48% (11/23) of PaV1 infected lobsters and 40% (4/10) of healthy lobsters (Figure 15). Pyknosis was also observed in the connective tissues of one infected lobster (4%) and one healthy lobster (10%). One PaV1 infected lobster had a granuloma in its connective tissues; none were found in any healthy lobster eyes.

DISCUSSION

The eyes of the blue crab and the spiny lobster were largely unaffected by systemic microbial diseases. Only one agent, the ciliate *M. chesapeakensis* caused severe pathology in the crab eyes. *Mesanophrys chesapeakensis* is a histophagous parasite and feeds on host tissue (Messick and Small 1996), which was evident here by the parasite feeding on the retinal pigments of the ommatidia. *Hematodinium* sp. and *P. perniciosa* are not histophagous; therefore, they present very different pathologies in the host.

The eyes of blue crabs are largely unaffected by *P. perniciosa, Hematodinium* sp, and a presumptive, unidentified virus. Although *Hematodinium* cells were present throughout the hemal spaces of infected blue crab eyestalks, no edema or pressure necrosis was observed in the surrounding tissues. The retina, ganglia, and other nervous tissues in the eyes of infected crabs appeared healthy except for occasional, small granulomas. The presence of cellular aggregates in the sinus gland could potentially disrupt its function, but this pathology was rare and its consequences are unknown. The eyestalk tissues of virus-infected crabs had no pathology other than the presence of granulomas. *P. perniciosa* is a tissue dweller and it was not found in the ommatidia of the blue crab, although it was present in other eye tissues

Granuloma formation is an important and highly prevalent immune response in the eyes of blue crabs infected by microbial agents. Healthy crabs did not have granulomas in their eyestalk tissues, while granulomas were found in the eyes of 38% of

Hematodinium infected crabs and 36% of virus-infected crabs. However, Morado et al. (1988) reported on the ubiquity of granulomas and similar lesions in crustaceans and note that it is a common immune response. Stentiford et al. (2003) found that granulomas only occurred in *Cancer pagurus* infected by *Hematodinium* sp. when there was a co-infection of a yeast-like organism, and hypothesized that *Hematodinium* weakens the host, allowing secondary infections to occur, to which there was a granulomatous response. Bacterial infections are common in decapods infected with *Hematodinium* (Meyers et al. 1987) and perhaps the granulomas in the present study are associated with opportunistic bacteria. No granulomas were observed in the one crab infected with *Paramoeba perniciosa*, and Johnson (1977) reported that they did not commonly occur in amoebic infections.

Hemocytes are not usually seen in the ommatidia of healthy decapods, because the ommatidia likely do not receive their blood supply from a direct capillary system (Johnson 1980). Their presence in the ommatidia indicates a disruption of the basement membrane, which separates the retina from the remainder of the eyestalk (Johnson 1980). In the present study, hemocytes were present in both healthy and diseased blue crab eyes, but the reason for this is unknown. It is possible that the basement membrane was disrupted during the processing of the eyestalk tissue, or that the crabs that were judged to be healthy were compromised by an unknown pathogen, allowing hemocytes to penetrate the basement membrane.

Lobsters infected with PaV1 show little pathology in their eyes. There were occasional infected cells in the connective tissues of the eye, but these may have been hemocytes; glial cells were unaffected. One infected lobster was observed to have a

granuloma in its connective tissue; the granulomatous response occurred less frequently in spiny lobsters than in blue crabs, indicating differences in immune response to pathogens or a variation in immune response to different infectious agents. Pyknosis was observed in the glia, optic nerve fibers, and connective tissues of both healthy and PaV1 infected *Panulirus argus*; therefore, it is possible that the condition is an artifact of fixation. Because it was present in healthy lobsters, PaV1 can be ruled out as a cause of the pyknosis.

Although there was little pathology in the eyes of decapods in this study, viruses and microbial agents do cause pathology in the eyes of decapod hosts. Smith (2000) described significant pathology in the eyes of *Penaeus monodon,* caused by a virus and by *Vibrio* sp. Callinan et al. (2003) reported on a disease, peripheral neuropathy and retinopathy (PNR), that caused minor to heavy mortalities on an Australian shrimp farm. In affected shrimp, peripheral nerve axons and their sheaths were degenerated and necrotic, with necrosis of associated glial cells; and retinular cells and their axons were degenerated and necrotic (Callinan et al. 2003). In addition, the retinular cells that were not destroyed often fused together. Edema occurred in the fasciculated zone and the lamina ganglionaris, and dilated blood vessels and hemocyte aggregations were present in the fasciculated zone, replacing the necrotic axons (Callinan et al. 2003). In advanced cases, there was fragmentation and liquefaction of the crystalline tracts overlying the necrotic retinular cells. The authors believe that PNR would have been recognized earlier if the peripheral nerves and the eyes had been routinely examined histologically. These types of pathologies were not observed in the systemic microbial infections observed in the present study. However, necrosis of the ommatidia of blue crabs infected by *M.*

chesapeakensis, and granuloma formation in the eyes of crabs infected by *Hematodinium* sp. and a presumptive virus were observed, showing that many different types of pathologies can occur in the eyes of crustaceans infected by systemic microbial agents. The eyes and eyestalks of decapod crustaceans should become a part of routine histological examination in decapods, although this will not necessarily result in diagnosis of disease, examination of eyes and eyestalks is necessary in order to have a complete histopathological view of disease because the eyes of crustaceans are important for both vision and neurosecretory function.

FIGURE 1. Longitudinal section of a healthy eye of *Callinectes sapidus*; OM, ommatidial region; BM, basement membrane; ON, optic nerve region; LG, lamina ganglionaris; ME, medulla externa; MI, medulla interna; MT, medulla terminalis; OP, optoc peduncle; NS, neurosecretory cells; S, sinus gland; T, tegumental glands; G, glia. scale = 1000 μm

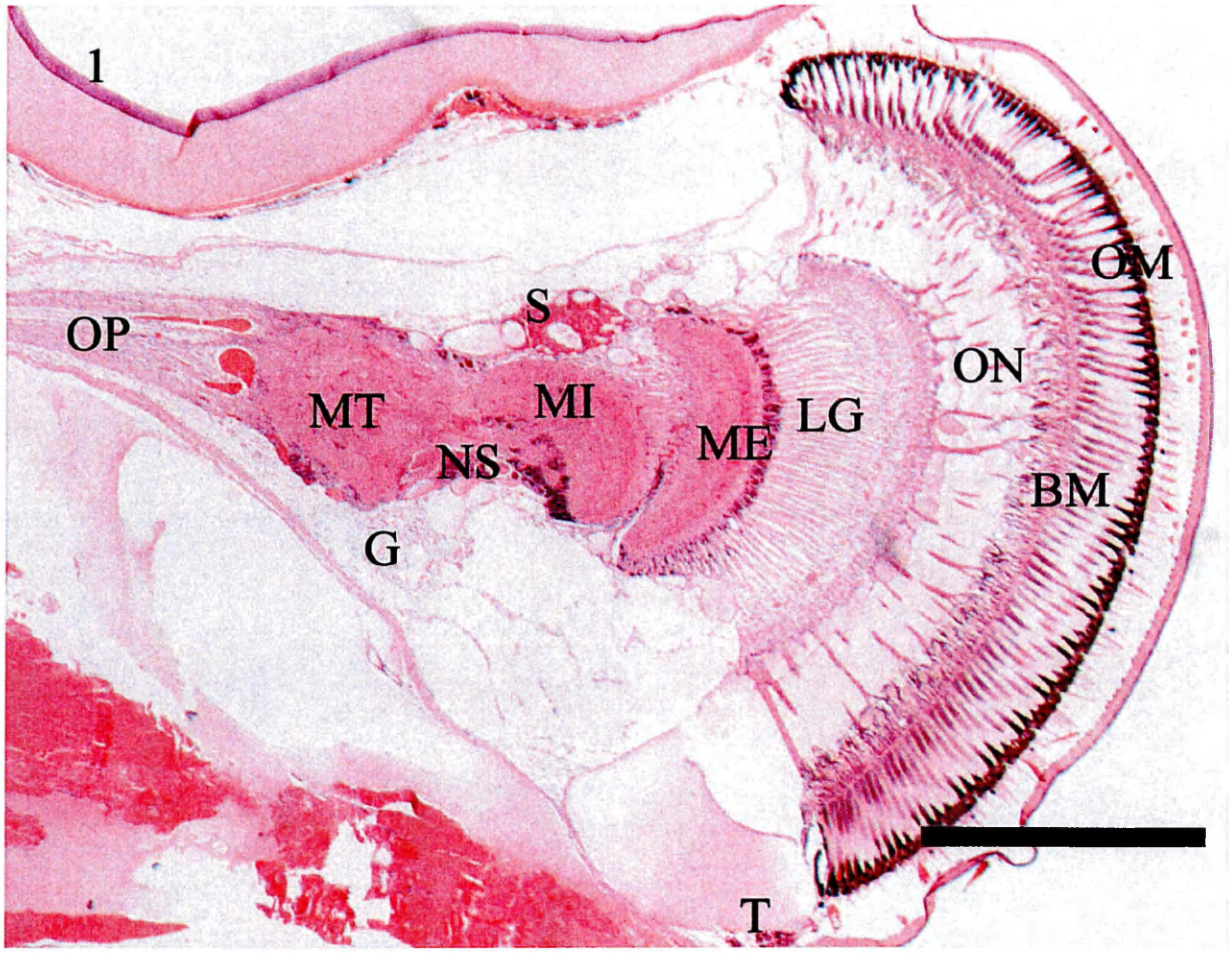
1
OM
S
OP
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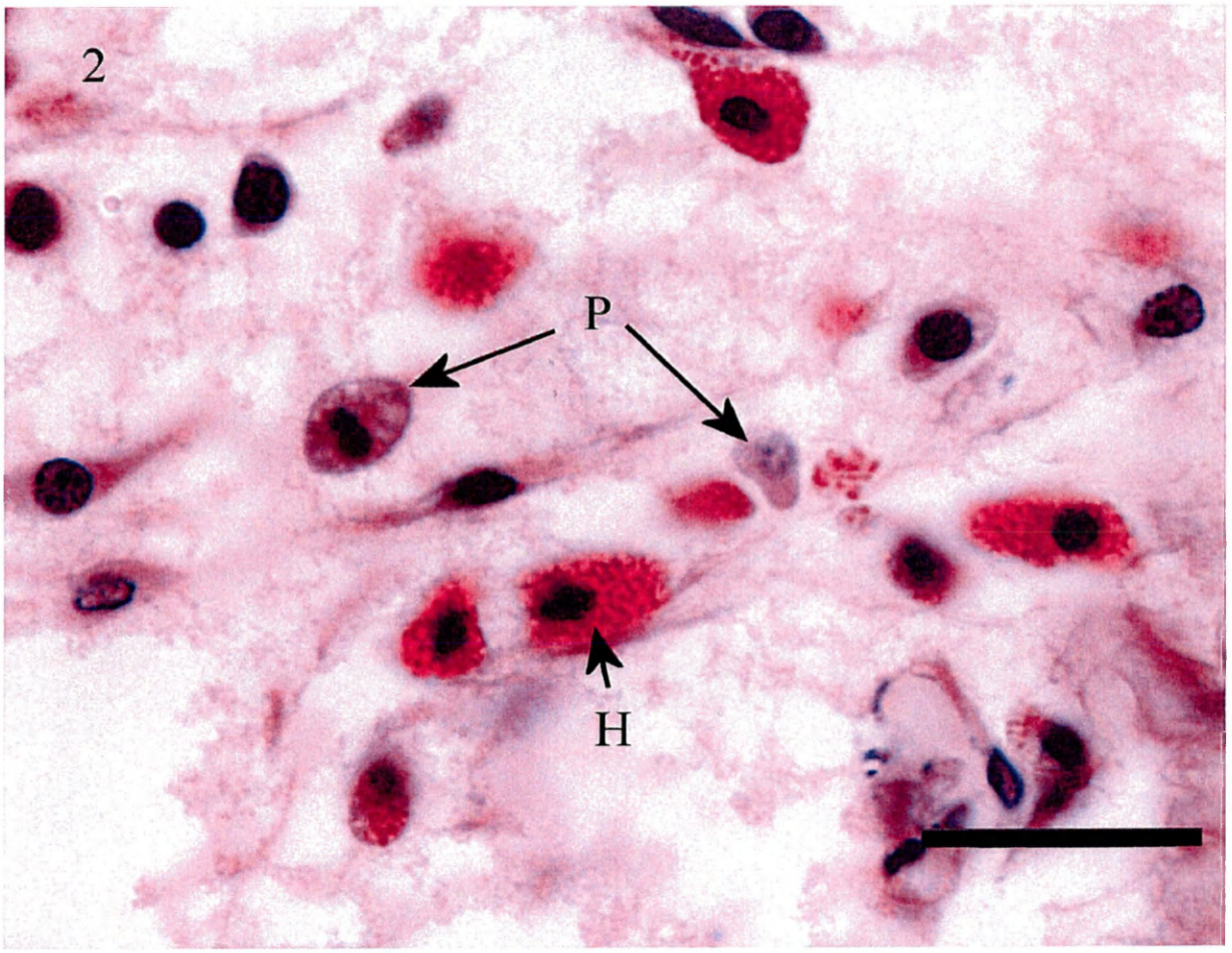
FIGURE 2. *Paramoeba perniciosa* in the connective tissues of the posterior region of the eyestalk in its host, *Callinectes sapidus*; P, *Paramoeba perniciosa*; H, hemocyte. scale = 20 μm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P
H

FIGURE 3. *Paramoeba perniciosa* between muscle fibers of its host, *Callinectes sapidus*; P, *Paramoeba perniciosa*; M, muscle fibers. scale = 20 μm

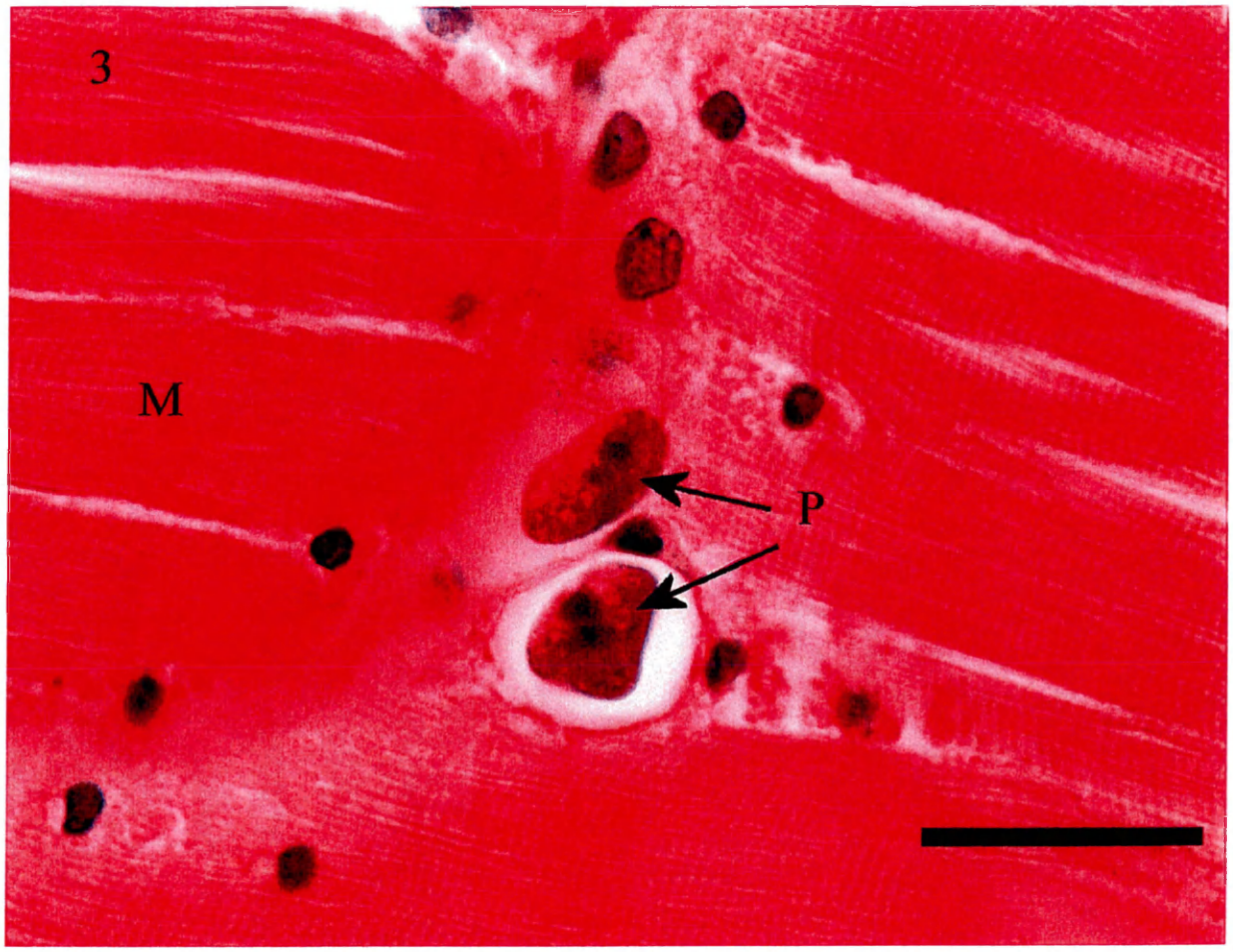
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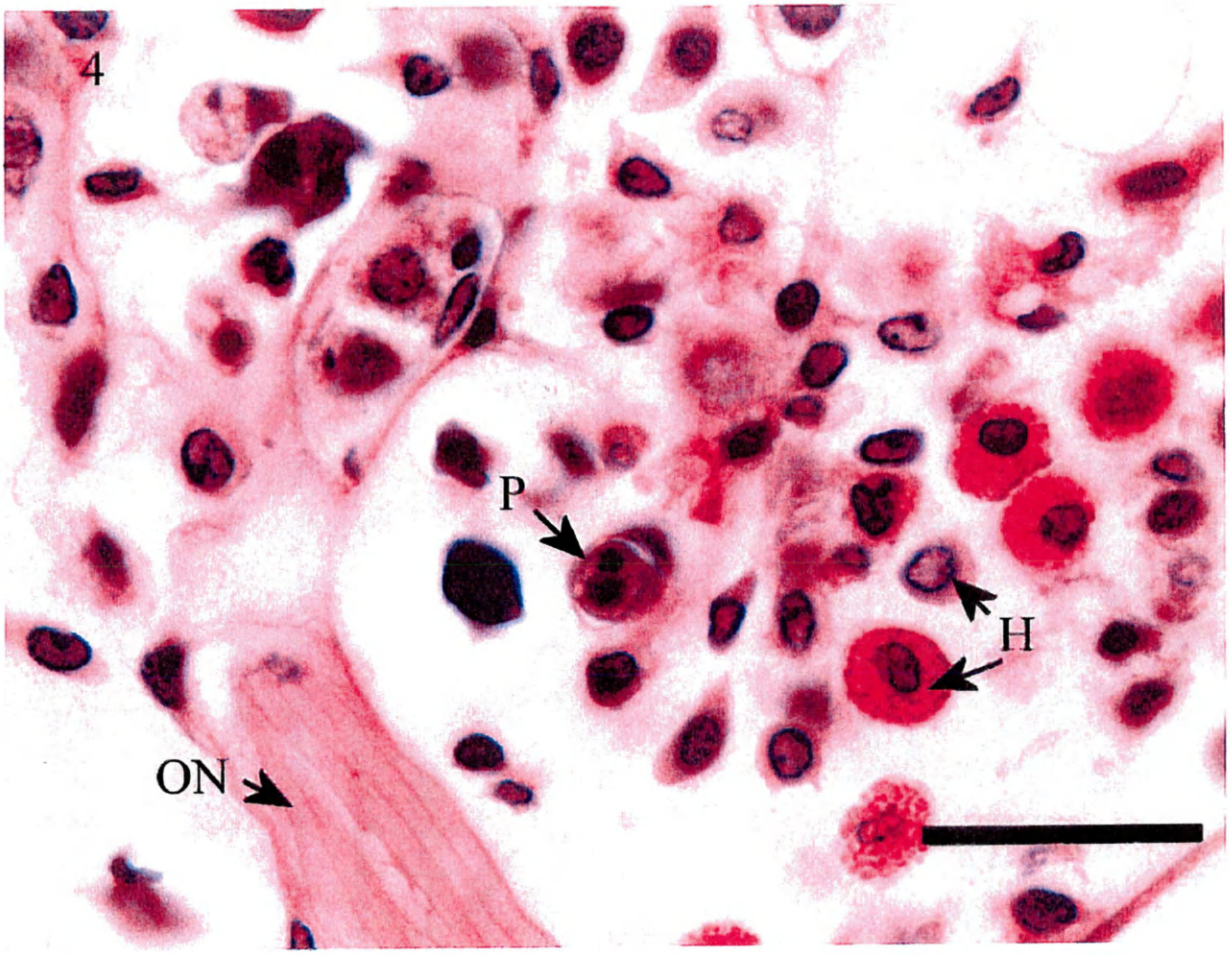
FIGURE 4. *Paramoeba perniciosa* in a hemal sinus of its host, *Callinectes sapidus*; P, *Paramoeba perniciosa*; ON, optic nerve fibers; H, hemocytes. scale = 20 μm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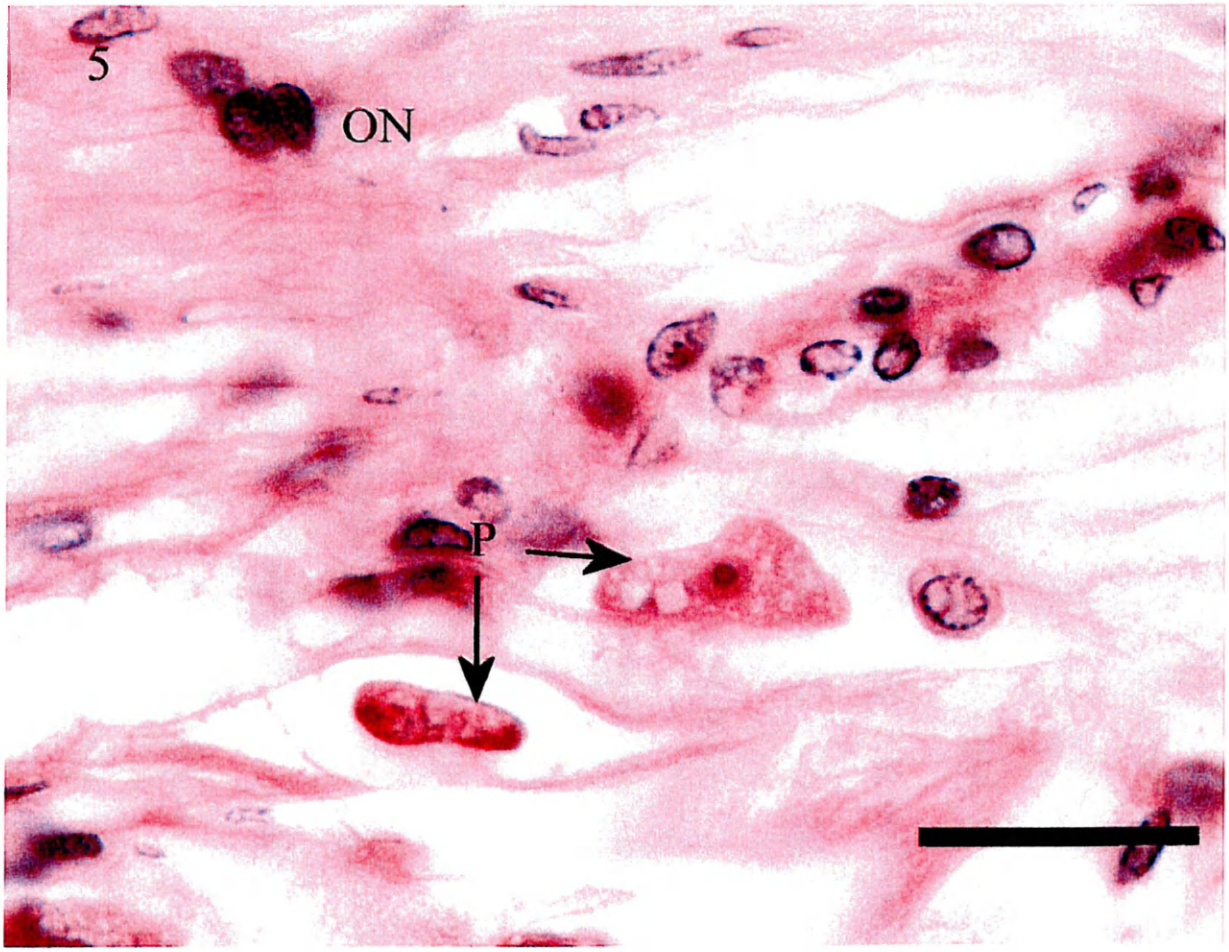
ON

FIGURE 5. *Paramoeba perniciosa* between optic nerve fibers (optic peduncle) in its host, *Callinectes sapidus*; P, *Paramoeba perniciosa*; ON, optic nerve fibers. scale = 20 μm


5
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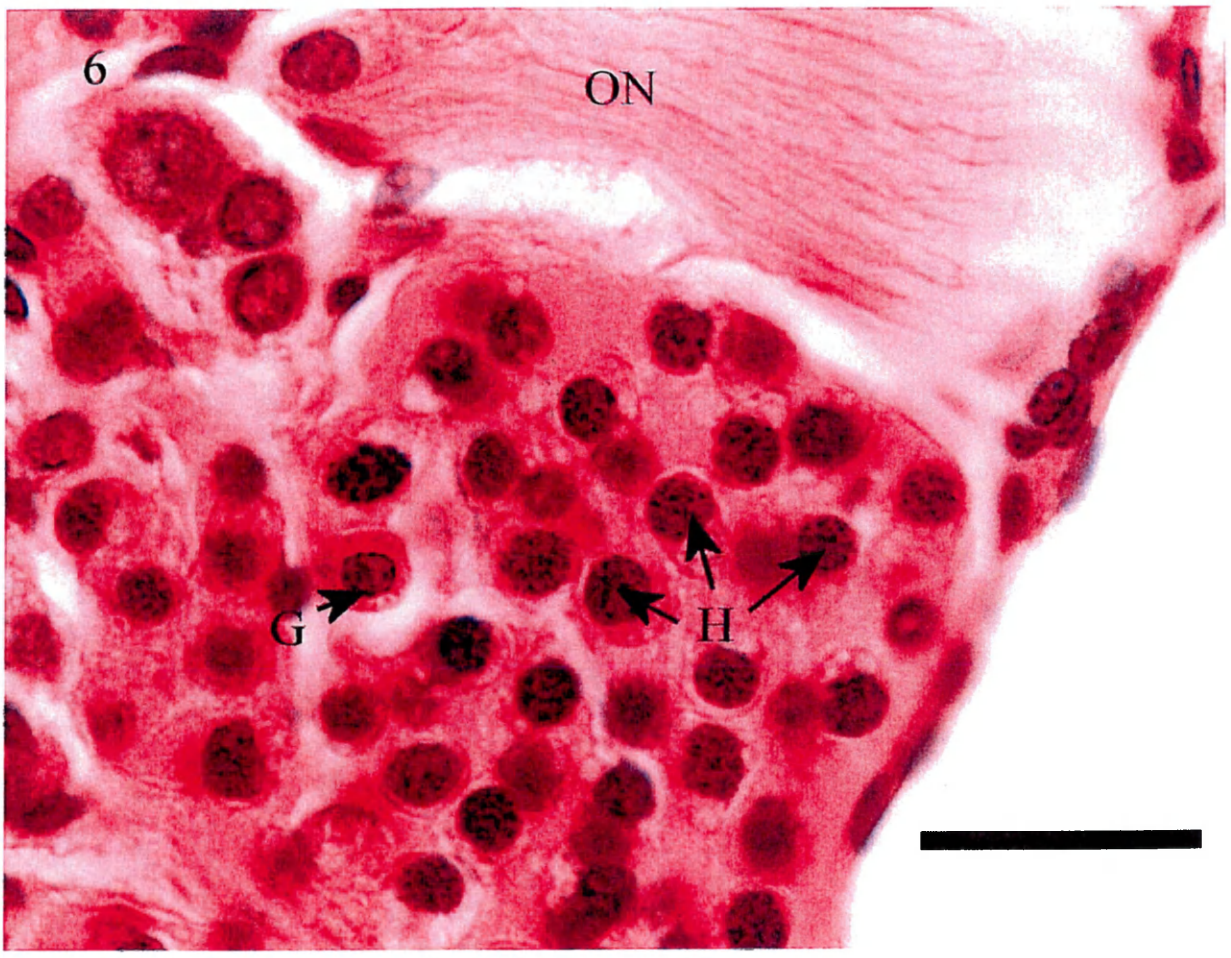
P

FIGURE 6. *Hematodinium* cells in a hemal sinus of its host, *Callinectes sapidus*; ON, optic nerve fibers; H, *Hematodinium* cell; G, granulocyte. scale = 20 μm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G
H

FIGURE 7. *Hematodinium* cells in blood vessels of the medulla interna of *Callinectes sapidus*; H, *Hematodinium* cell. Scale = 20 μm

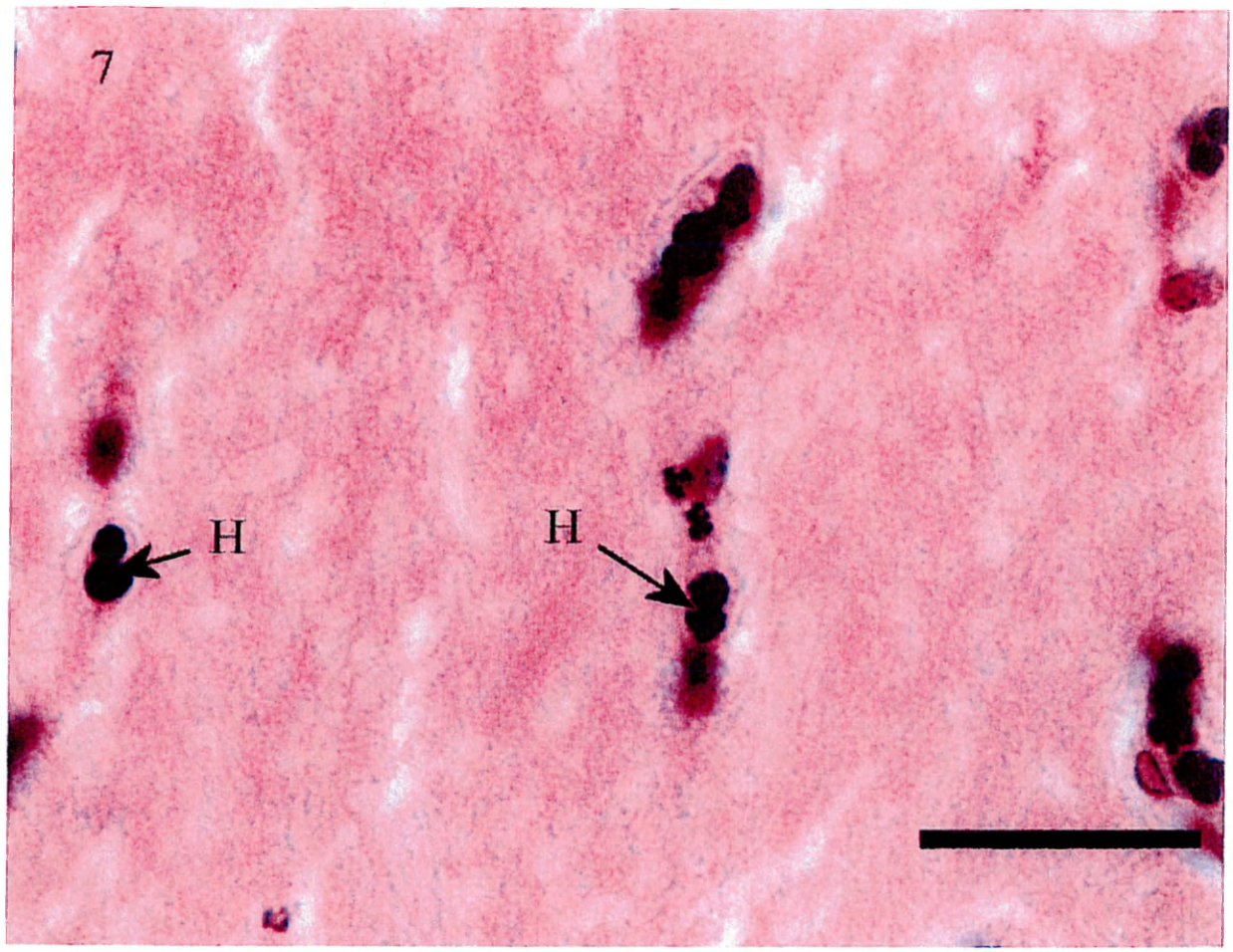
7
H
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FIGURE 8. Aggregate of host hemocytes and *Hematodinium* cells in the sinus gland of *Callinectes sapidus*; A, aggregate; S, sinus gland. scale = 100 μm

8

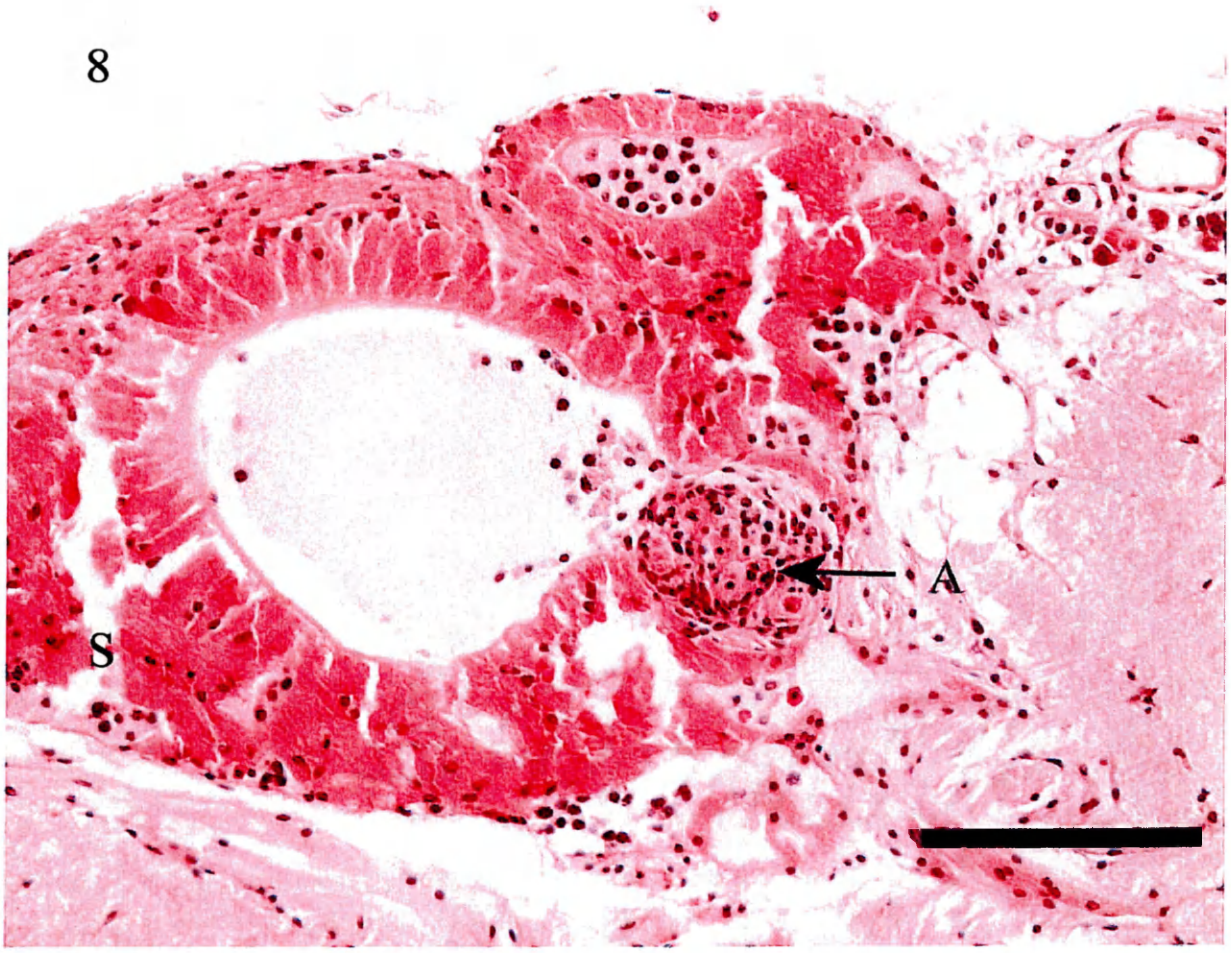

FIGURE 9. *Callinectes sapidus* hemocytes infected by a presumptive virus; P, pyknotic nucleus; I, viral inclusions. scale = 20 μm

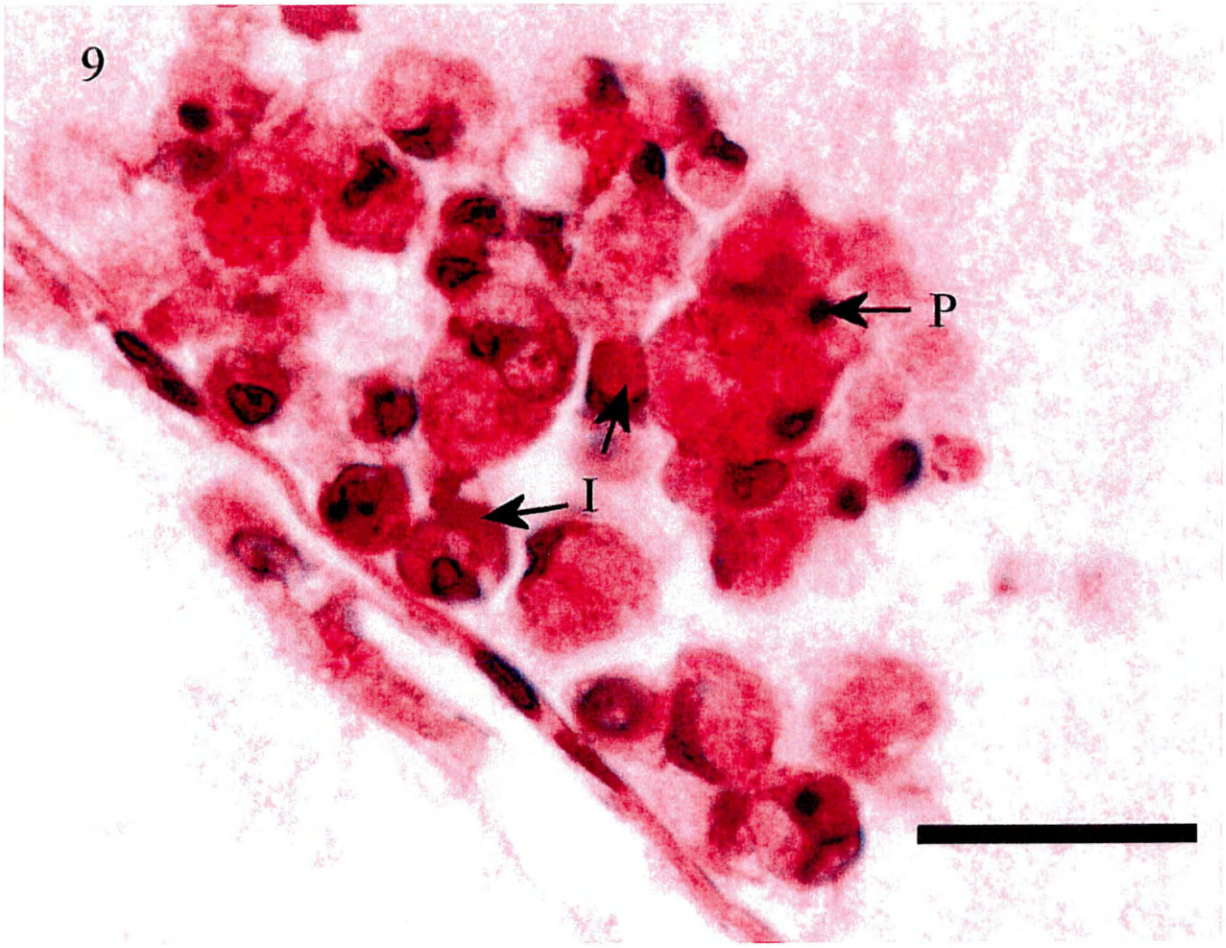
9
P
I

FIGURE 10. *Mesanophrys chesapeakensis* in the ommatidial region of *Callinectes sapidus*. Note black retinal pigment granules in phagosomes inside some ciliates; C, ciliate. scale = 50 μm

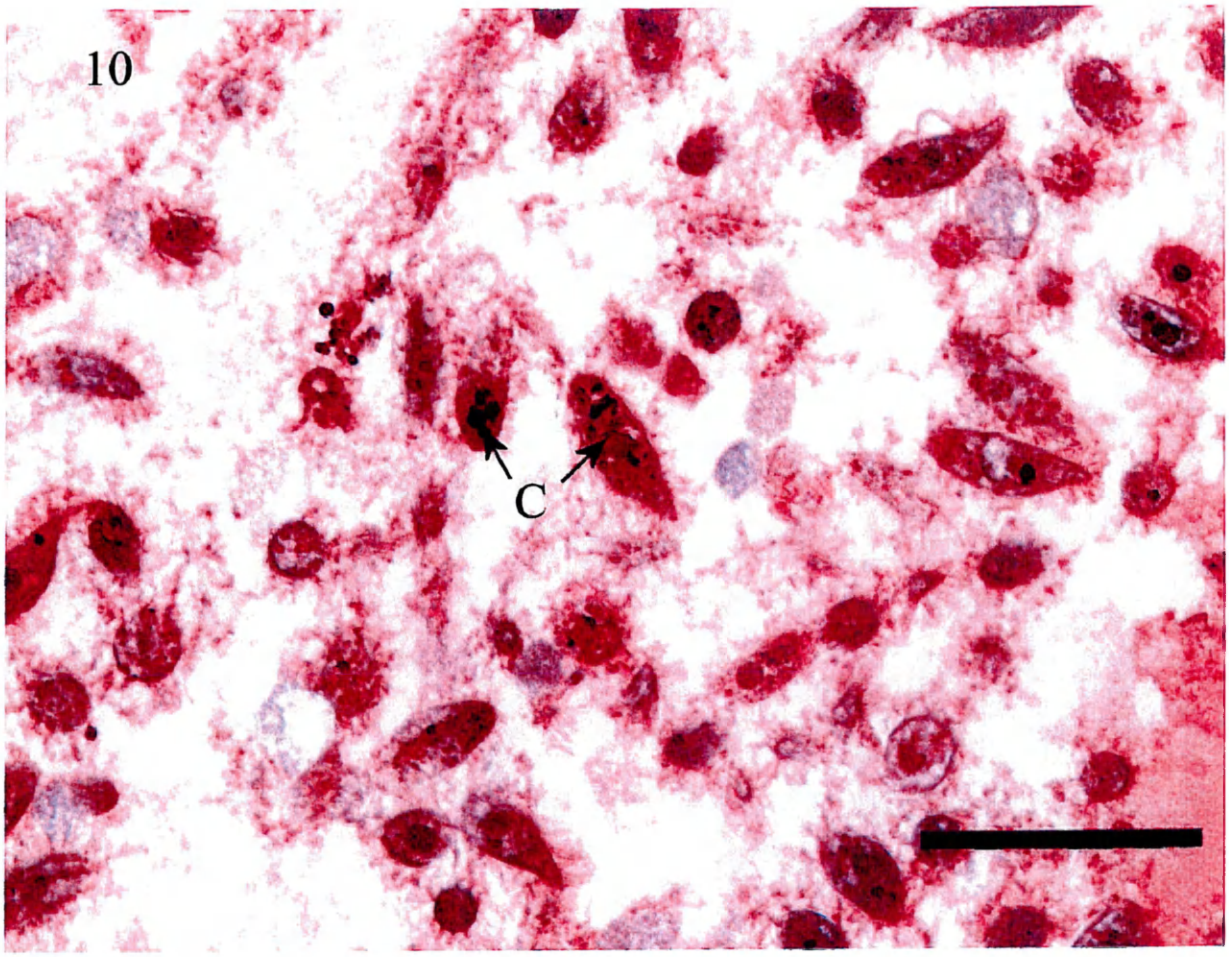
10
C

FIGURE 11. Altered retinular cell layer in the eye of *Callinectes sapidus* infected by a ciliate, *Mesanophrys chesapeakensis*; OM, ommatidial region; ON, optic nerve region; R, retinular cell layer; BM, basement membrane; C, ciliate. scale = 100 μm

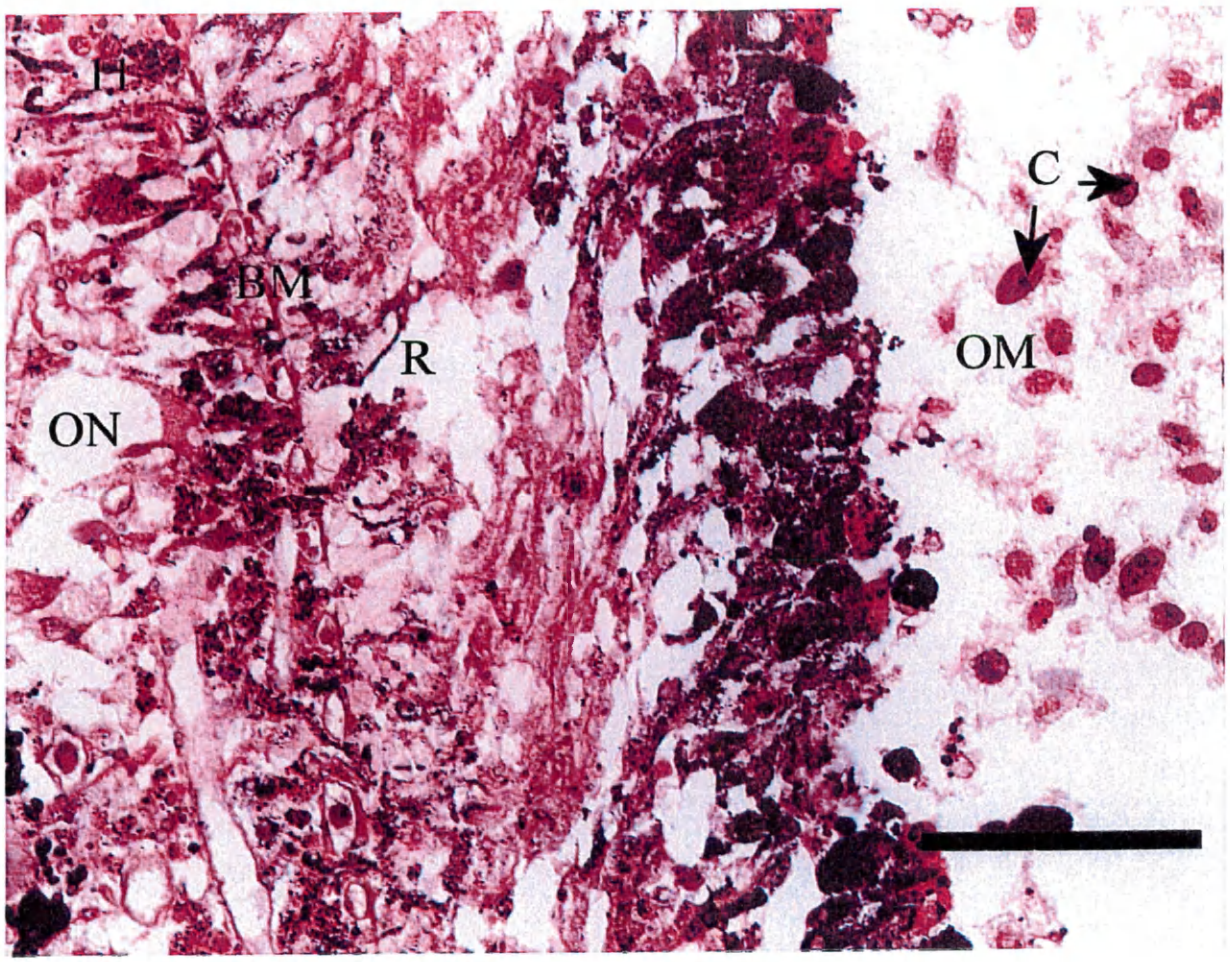
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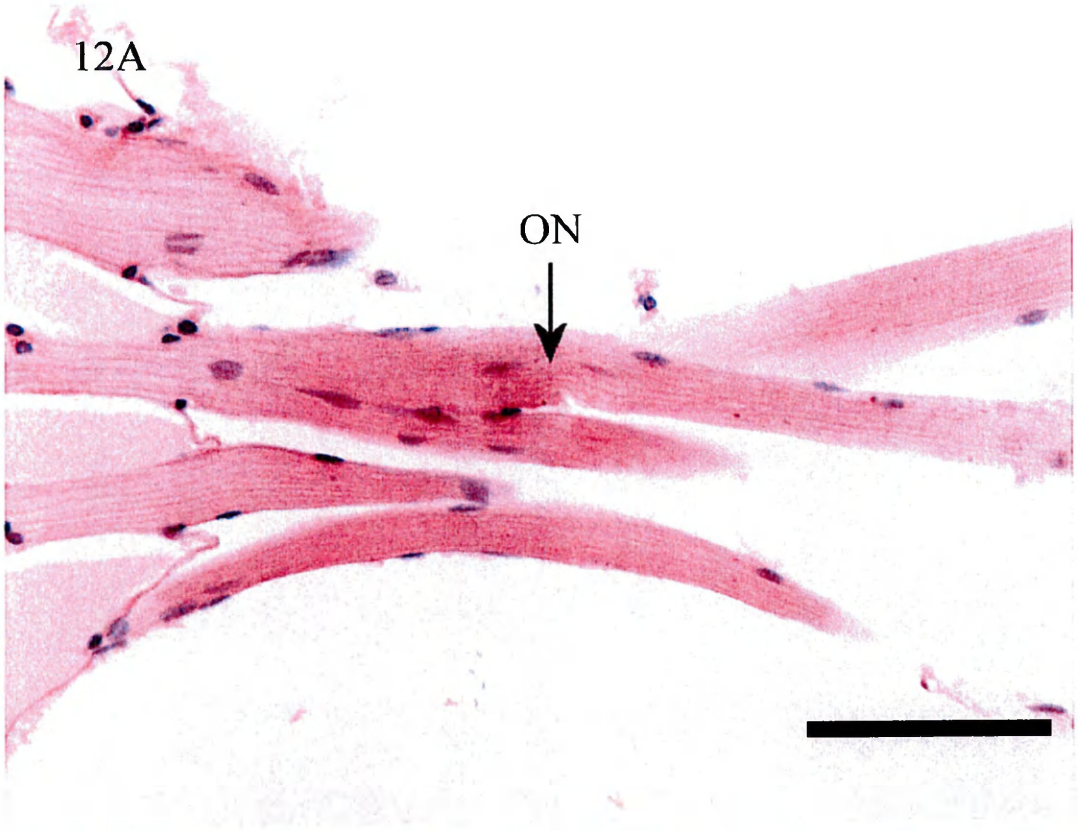
FIGURE 12. Optic nerve fibers from (A) healthy and (B) *Mesanophrys chesapeakensis*-infected *Callinectes sapidus*. ON, optic nerve fibers; C, ciliate; H, hemocyte; P, retinal pigment. scale = 50 μm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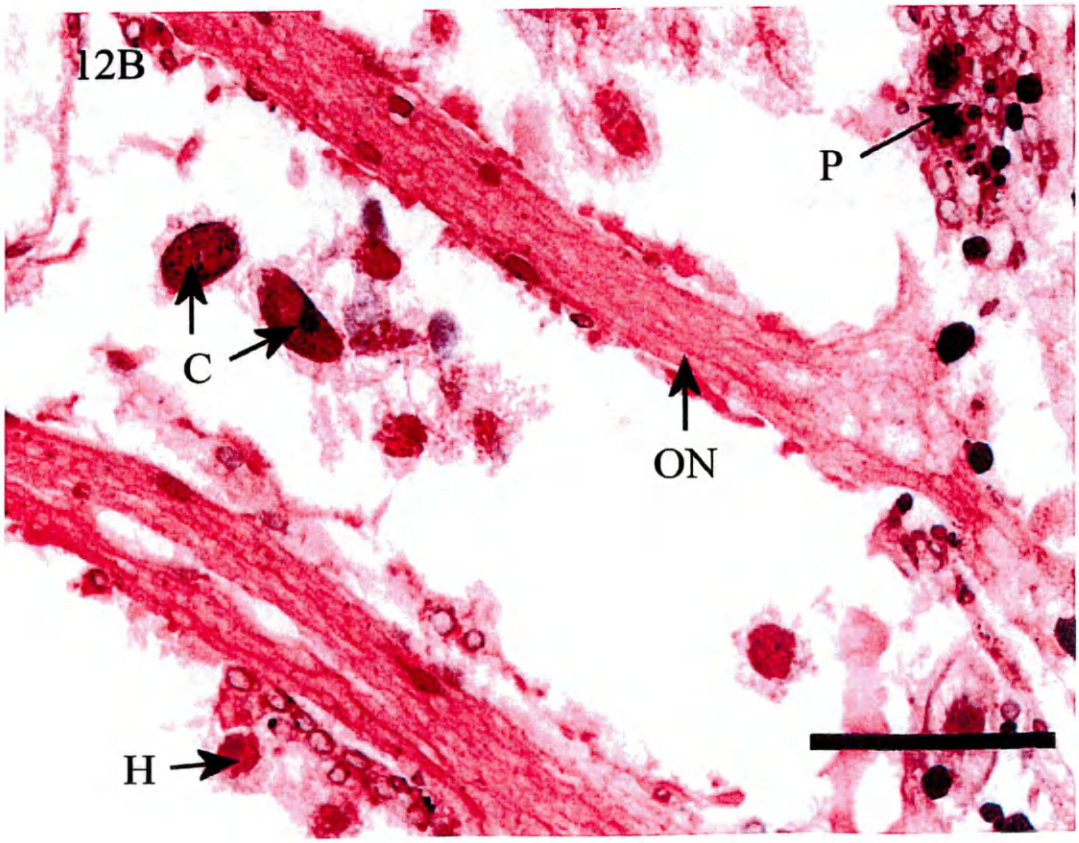
12B
P
C
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FIGURE 13. *Panulirus argus* hemocytes infected with PaV1; I, inclusions. scale = 20 μm

13

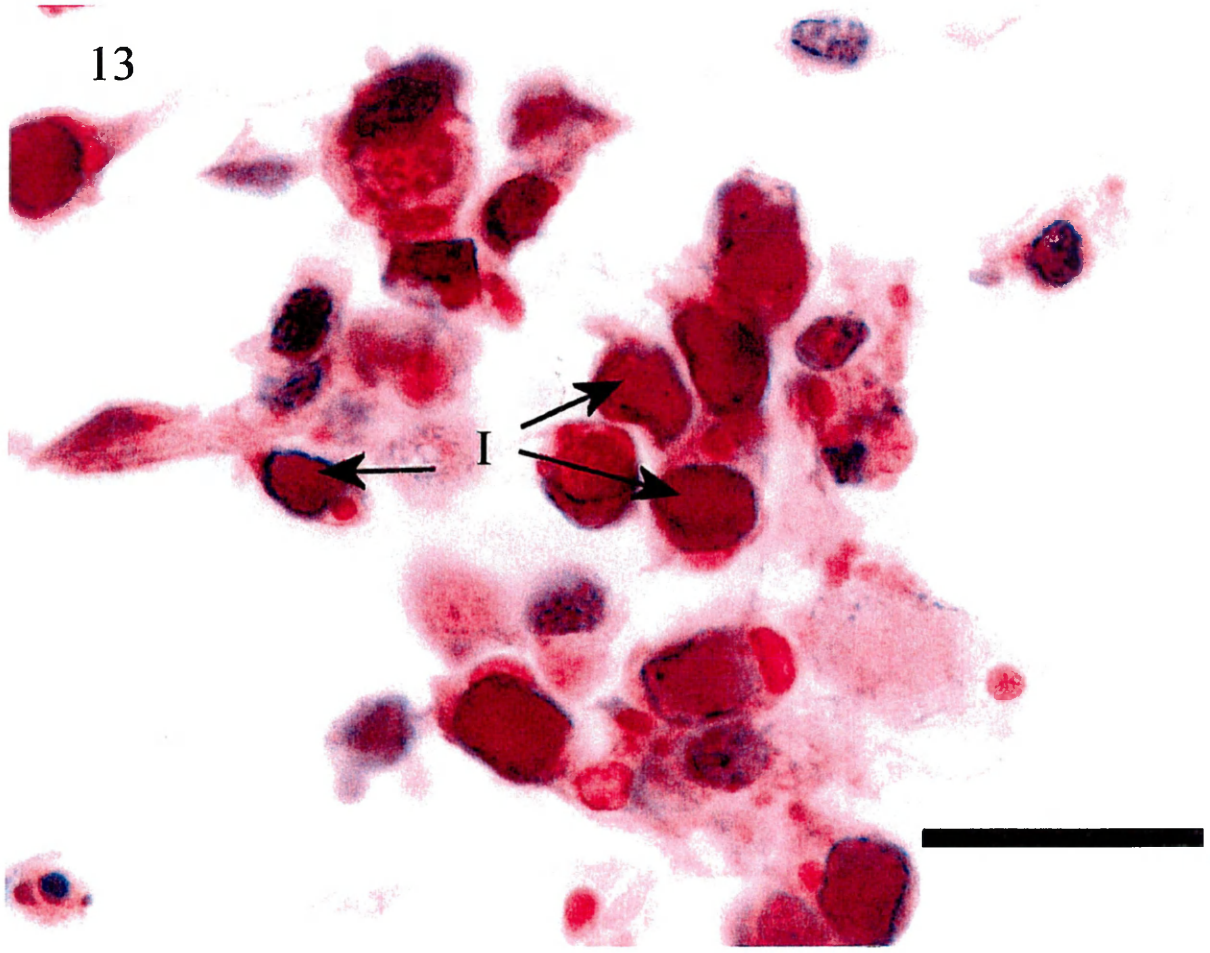
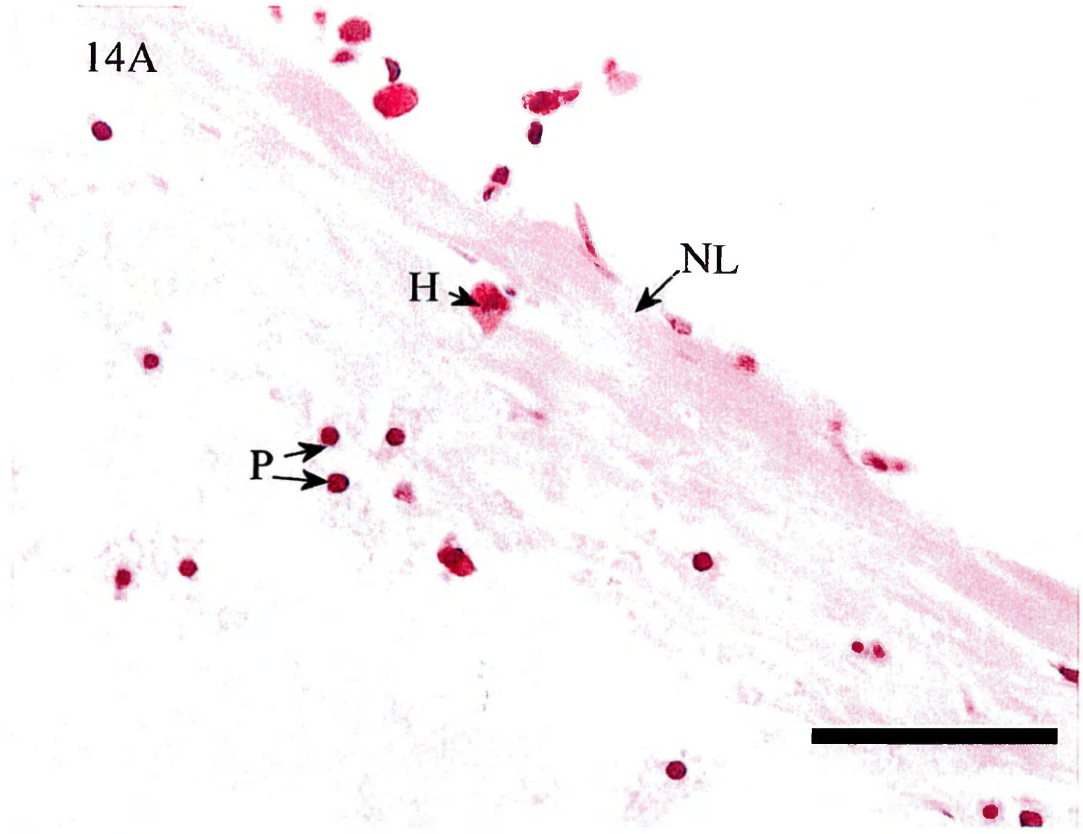


FIGURE 14. Pyknosis in the glia of a healthy spiny lobster *Panulirus argus*. (A) Region of glia inside the neurilemma; (B) Neurosecretory cells in a lobster eyestalk surrounded by glial cells with pyknotic nuclei; P, pyknotic nucleus; NL, neurilemma; H, hemocyte; N, normal nucleus; NS, neurosecretory cell. scale = 50 μm


14A
H
NL
P

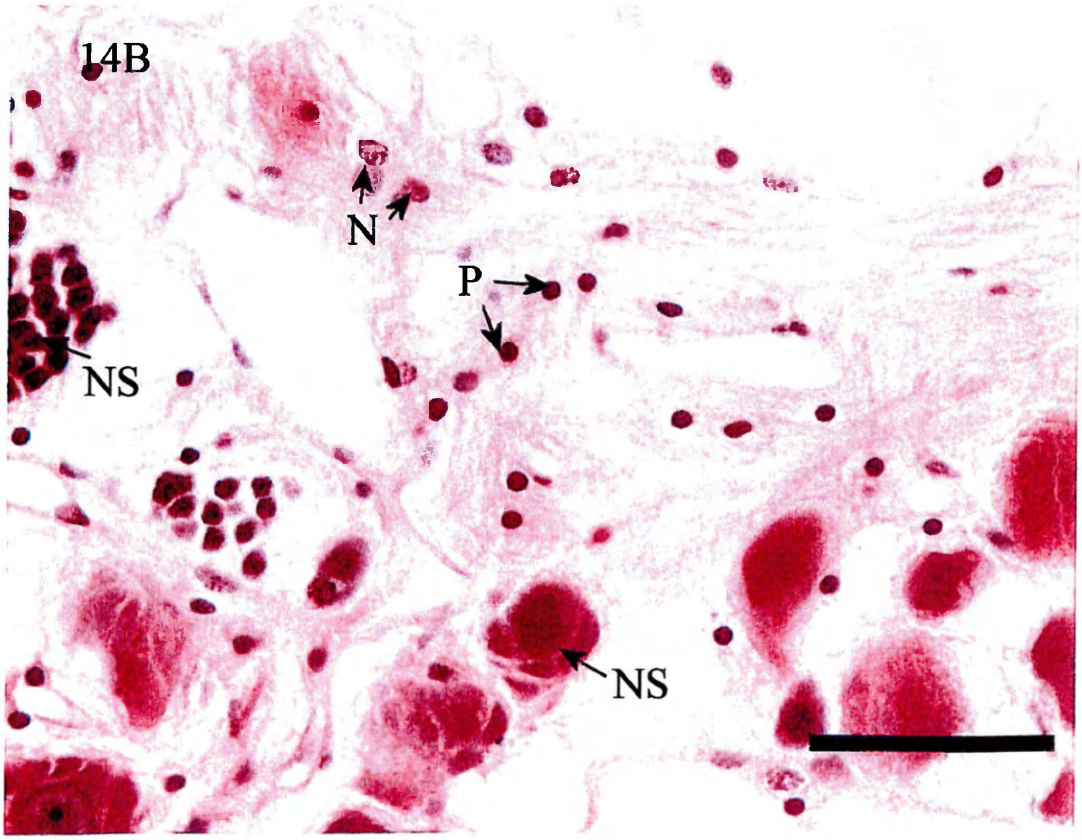
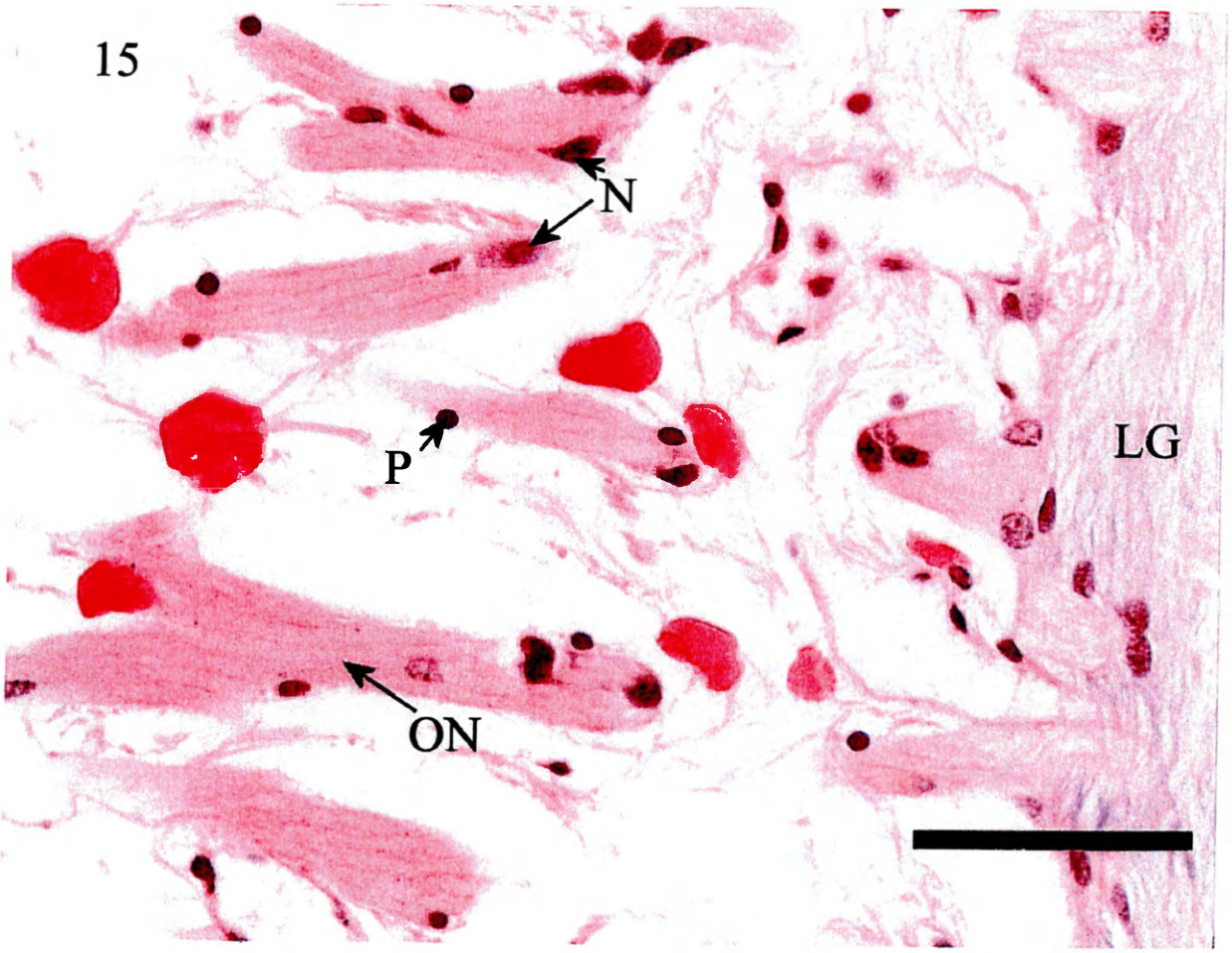

14B
N
P
NS
NS

FIGURE 15. Pyknotic nuclei in the optic nerve region of a healthy spiny lobster *Panulirus argus*; ON, optic nerve fibers; P, pyknotic nucleus; N, normal nucleus; LG, lamina ganglionaris. scale = 50 μm


15
N
P
LG
ON

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