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research paper

Development of rapid diagnostic techniques for idiopathic blindness in the American lobster, *Homarus americanus*, from eastern Long Island Sound

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ABSTRACT.—Idiopathic blindness is a condition that afflicts approximately 50% of the lobsters, *Homarus americanus* H. Milne-Edwards, 1837, in Long Island Sound (LIS). The condition occurs in lobsters from LIS and Narragansett Bay, but has lower prevalence levels in the Gulf of Maine. Grossly, the condition presents as patches of cloudy, gray-colored regions in the eyes of afflicted animals. Histologically, the ommatidia show signs of altered pigment distribution, necrosis of the optic nerves and rhabdoms, and hemocyte infiltration through the protective basement membrane separating the ommatidia from the optic nerves. Severe lesions show areas with necrotic ommatidia and nearly complete loss of the underlying associated optic nerves. We assessed a rapid, nondestructive, diagnostic technique for determining blindness in lobsters. We compared the use of an otolaryngoscope (o-scope) with stereomicroscopy on live, frozen, and histologically-fixed eyes. Live lobsters from Narragansett Bay, Rhode Island, and off southern Massachusetts were assessed with the o-scope. Right eyes were analyzed via standard histological procedures. Left eyes were frozen and stored at −80 °C, and later thawed and reassessed for blindness. The o-scope had good sensitivity and specificity in diagnosing blindness in the laboratory with good inter-observer comparisons among trained staff. Initial results indicate that the etiological agent of idiopathic blindness is present throughout a large portion of the sound, and that lobsters are probably continually exposed to it. The use of the o-scope as a diagnostic tool will help us better understand the distribution of idiopathic blindness in lobsters from the New England region.

The American lobster, *Homarus americanus* H. Milne-Edwards, 1837, is fished extensively from Long Island Sound (LIS), USA, north to Newfoundland and the Gulf of St. Lawrence, Canada. Combined annual landings are valued at US$750 million to US$1.7 billion (NMFS 2017, Fisheries and Oceans Canada 2017). Prior to 1999, the fishery for lobsters in southern New England, mostly within LIS and the smaller sounds to the east, was a small but significant component of the US fishery, generating annual revenues of $40 million. Due to poor recruitment, in part due to epizootic shell disease, landings in LIS are now at an all-time low, and there is significant concern over the sustainability of the stock off southern New England (Wahle et al. 2009).

During investigations into the 1999 mortality event in LIS, a new condition, known as idiopathic blindness, was described in lobsters from western and central parts of the sound (Maniscalco and Shields 2006, Magel et al. 2009). Idiopathic blindness is a condition with an unknown etiology, but it arises from degenerative changes to the ommatidia (photoreceptor cells) and their optic nerves. Both eyes are typically affected in an individual (Maniscalco and Shields 2006), and any degree of degeneration results in nearly complete blindness (Magel et al. 2009). Initially, the condition was reported in >50% of animals, but only in small sample sizes (51 and 19) from western and central LIS, respectively. More recently, the prevalence was reported as >54% in a larger sample (90) of lobsters from Narragansett Bay, with lower prevalence and severity in animals (n = 19) from the Gulf of Maine (Shields et al. 2012). Physiologically, lobsters with only moderate blindness (approximately 20% affected area) have nearly complete loss of vision in the affected eye (Magel et al. 2009). Given the high prevalence levels and small affected area required to severely impair vision, we estimate that roughly half of the mature lobster population within LIS have significant loss of vision due to this condition.

Lobster eyes are vital to homeostasis and survival. They are important in the endocrine system, because the eyestalk sinus gland and associated nervous tissues produce neuro-hormones that regulate vital processes: molting, reproduction, osmoregulation, and blood sugar levels. Lobsters from southern New England (SNE) show signs of endocrine disruption resulting in changes to molting, loss of reproductive potential, and possible metabolic aberrations (Tarrant et al. 2012). These alterations may be due to epizootic shell disease (Laufer et al. 2012), but the role of blindness cannot be ruled out. Although *H. americanus* does not rely heavily on vision for feeding, blindness may affect mating, molting, and the loss of photoreception is likely to impair diel foraging behaviors. We know little about blindness in lobsters, but we do know that vision and the hormone-regulating glands in the eyestalks are affected by it.

Our first objective was to develop a rapid, nondestructive diagnostic tool for determining blindness in lobsters. At present, the only diagnostic tool for blindness is histological assessment, which requires removing the eye and eyestalk of each lobster and undertaking histological evaluation. Gaten (1988) developed an infra-red, light-based method for assessing blindness in Norway lobsters, *Nephrops norvegicus* (Linnaeus, 1758), affected by sunlight-induced blindness. We adapted Gaten's method using an otolaryngoscope (o-scope), a nondestructive technique, and compared it with histological assessment to assess its sensitivity and specificity for use in field applications with American lobsters. Our second objective was to demonstrate the utility of the o-scope in determining the spatial extent of blindness in lobsters from Narragansett Bay and Rhode Island Sound. As part of this investigation, we trained several laboratory personnel in the use of the o-scope and then compared their findings to determine inter-observer variation for this method.

Materials and Methods

Collection of Animals.—American lobsters were obtained from fishers from December 2016 to April 2017. Lobsters were collected with commercial lobster traps from four locations within Narragansett Bay, Rhode Island, and Rhode Island Sound. Lobsters were assessed dockside as described below, they were then shipped via FedEx overnight from the University of Rhode Island to the Virginia Institute of Marine Science (VIMS). They were processed immediately upon arrival, with the exception of a batch of 22 lobsters that were housed together in a large aquarium and processed over the course of 10 d.

Otolaryngoscopy (O-scope) and Stereomicroscopy.—Lobsters were sexed, measured (carapace length in mm), and examined for injury, shell disease, or other conditions. Multiple observers were trained in the use of the o-scope and asked to score the presence of blindness and estimate the relative area affected (%) for each eye when present. Scoring was blinded between observers until after assessments, corrections were allowed during the training period (eight lobsters), but not thereafter. Both eyes were then examined for blindness with an otolaryngoscope (Dr. Mom LED pocket otoscope). Areas in the eyes with observable lesions were subjectively scored by percentage of area affected. Several photographs were taken with the macro setting on an Olympus Tough TG-4 digital camera. After the assessment with the o-scope, the lobsters were killed by dislocation of the carapace, and their eyes removed and assessed with a stereomicroscope with a dual fiber optic lamp. Left eyes were evaluated as above and then stored at −80 °C for assessment with the stereomicroscope at a later date. Right eyes were evaluated as above, fixed in Bouin's solution for 48 hrs, and then assessed again with the stereomicroscope prior to their being processed for histology. Later in the study, when freezing was shown to be ineffective in determining blindness, the left eyes were no longer frozen, rather they were assessed with the o-scope, fixed for histology, and then processed as below.

The methodology for evaluating lobster eyes in the field differed somewhat from that in the laboratory. Lobsters were collected and held in a holding car, or dockside receiver, until they could be evaluated. Lobsters were then evaluated in a semi-open pilothouse of a lobster boat where the lighting was not controlled. Lobsters fresh out of the water had some condensation on the eyes, which created a glossy coating that looked similar to that of a lesion. To avoid confusion, the eyes of lobsters were patted dry and then examined.

Histological Sampling of the Eyes.—Eyes fixed in Bouin's solution were decalcified following Luna (1968), bisected using a clean, single-edge razor, and then processed for histology using standard histological methods, with the exception that the initial 70% ethanol storage was no longer than 6 hrs prior to initiating the tissue process. Sections were cut at 6–7 μm and stained with Mayer's hematoxylin and eosin. Eyes were initially sectioned on a coronal plane. After reading the histological sections and comparing them with the o-scope results, the left eyes of a subset of 22 animals were sectioned on a transverse plane for additional comparisons.

Because the coronal plane crosses down the vertical midline of the eye, some lesions were poorly detected by histological sampling. In these cases, tissue recuts were done to go deeper into the mediolateral areas of the affected eyes. Recuts often showed signs of blindness that were missed on the first evaluation. Because of this,

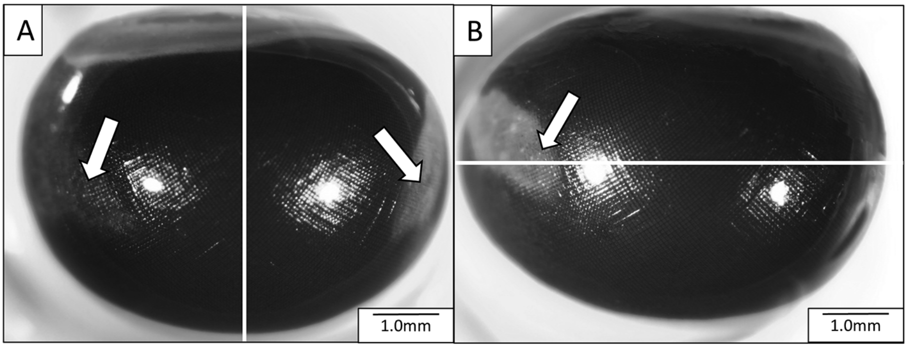

Figure 1. Two section planes (indicated by bars) superimposed over the left and right eyes of the same animal. (A) Right eye showing the absence of an intersection between the traditional coronal plane and the mediolateral lesions (arrows) in the right eye. (B) Left eye with a line indicating the transverse plane intersecting a mediolateral lesion (arrow). Note the two light spots are reflections from the fiber optic light source. Stereomicroscopy.

we compared the left eyes of some animals (*see* below) using the transverse plane with the right eyes using the coronal plane (Fig. 1). The transverse plane gave a more comprehensive view of the area of the lesion, particularly those located in mediolateral and ventral areas of the eye.

Statistical Analyses.—For most statistical analyses, blindness was scored as present or absent and analyzed using 2 × 2 contingency tables for specificity, sensitivity, and goodness of fit. Specificity refers to the proportion of healthy eyes correctly identified as healthy. Sensitivity refers to the proportion of eyes with blindness correctly identified as blind. The relative area of blindness for each eye was compared between o-scope evaluation and histological evaluation using correlation and model 2 regression (RStudio v3.2.4 with the package lmodel2; *see* https://CRAN.R-project.org/package=lmodel2). The Fleiss kappa analysis, Cohen's kappa, pairwise agreement scores between observers, and associated scores between observers were calculated using Recal 3 for multiple observers (http://dfreelon.org/utils/recalfront/recal3/).

Results

We evaluated 129 eyes from 65 different animals using the o-scope and comparisons on different diagnostic assessments, including stereomicroscopic analysis of fresh, frozen, and Bouin's fixed eyes, as well as the "gold standard" of microscopic analysis of histological samples (Table 1). One animal was missing an eye with the distal eyestalk capped by an obvious scar. In the first two sets of lobsters obtained for

Table 1. Disposition of lobster eyes in this study by diagnostic method used to assess idiopathic blindness.

Diagnostic method	Left eye	Right eye	Total
O-scope	64	65	129
Fresh (stereomicroscope)	48	47	95
Frozen (stereomicroscope)	44	0	44
Bouin's solution (stereomicroscope)	3	27	30
Histology	20	65	85

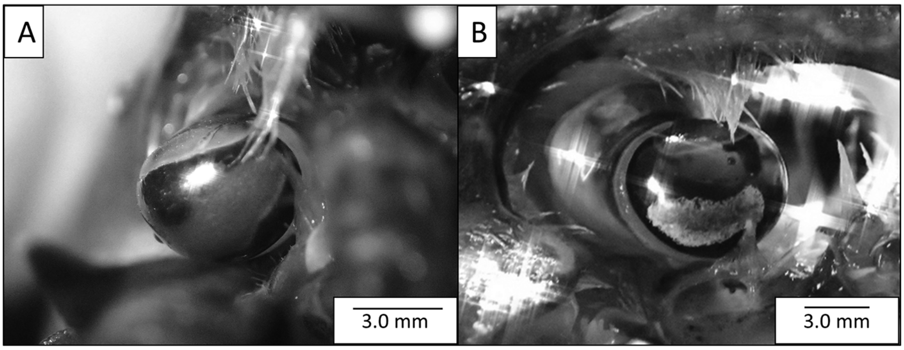

Figure 2. Macrophotographs of live lobsters with two manifestations of idiopathic blindness. (A) A severe case of the more common "cloudy" appearance of the affected ommatidia. This would be evaluated as approximately 60% affected. (B) An example of the less common "white eye" syndrome exhibiting a very distinct white area of diseased ommatida within the affected area. Photos taken with an Olympus Tough 4 camera.

the study, there was initial bias towards collecting animals with blindness so as to refine observer training and techniques. The other sets of animals were collected for other purposes and did not reflect this sampling bias.

Macroscopic Evaluation with the O-scope.—Macroscopically, lobsters with idiopathic blindness had single or multiple lesions indicated by cloudy or gray regions of irregular ommatidia in one or both eyes (Fig. 2A). Evaluation of the eyes was improved in the laboratory by wetting each eye immediately prior to evaluation and by thorough, up-close evaluation of each eye from several angles. Severity ranged from light to heavy with the o-scope, and in most cases, severity matched well with histological assessment (Fig. 3). In cases with light severity, small lesions (approximately 10%) were difficult to observe with the o-scope, but they were sometimes noted against the clear reflective background of the healthy ommatidia and tapetum.

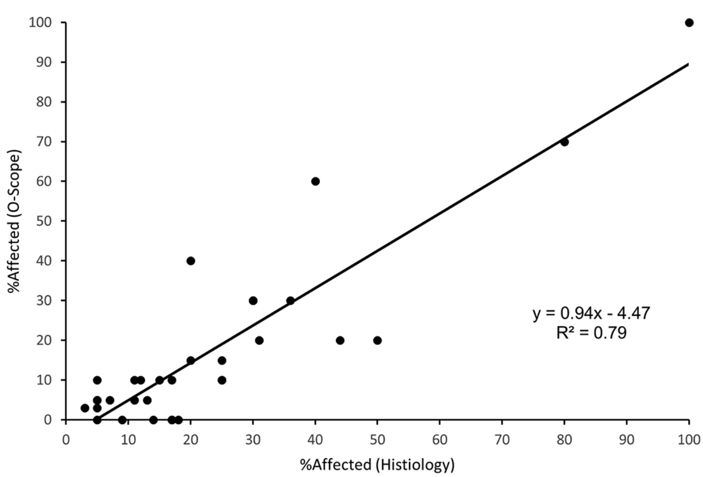

Figure 3. Relationship between the subjectively scored percentages of area affected by the otolaryngoscope with the percentages of the basement membrane area affected calculated from a representative histological section.

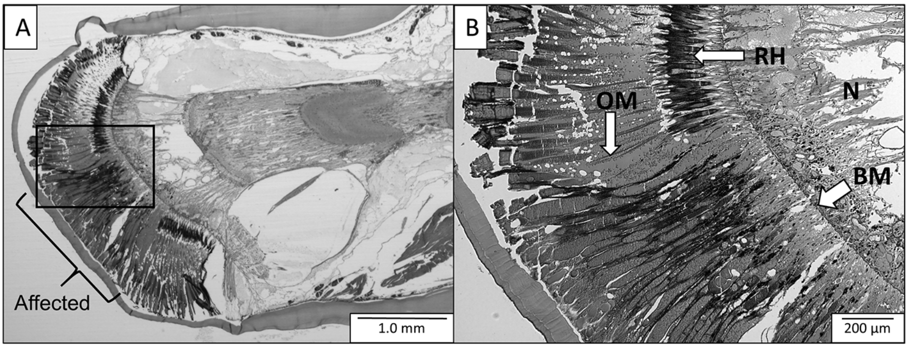

Figure 4. (A) Coronal section of the lobster eye depicting the affected region (bracket) as a centroid, subventral lesion at a low magnification (stereomicroscopy). (B) Inset showing a higher magnification (40×) of the affected area. The altered pigment distribution, obliteration of ommatidia (OM) and rhabdoms (RH) in the center of the lesion area, affected basement membrane (BM), as well as the loss of the underlying optic nerves (N) in the affected region of the eye.

In moderate and severe cases, focal and larger, coalescent lesions were observed in the affected area. These sometimes presented as obvious gray areas that were macroscopically evident. In severely affected individuals, lesions often encompassed the left, central, and right portions of the compound eye, and they were frequently noted in the ventral portion of the affected eye. The dorsal portion of the eye was mostly unaffected.

Several animals also had a condition known as "white eye" (Fig. 2B). The eyes of animals with this condition had bright white splotches limited to the area of affected ommatidia. The relative area affected ranged from 20% to 60% of the affected eye. These animals were included in the assessment of the o-scope and other methods for diagnosis of blindness. The histopathology of this condition was identical to those presenting with idiopathic blindness (*see* below).

Histopathology.—The histopathology of idiopathic blindness was identical to that observed in Maniscalco and Shields (2006) and Magel et al. (2009). Briefly, severity was evaluated as light, moderate, and heavy. In eyes with light severity, single or a few ommatidia were affected. In some cases, the screening pigments surrounding the rhabdom at the base of an individual ommatidium had shifted distally. No infiltration of hemocytes was apparent and there was no apparent degeneration or necrosis of the underlying optic nerve. In moderate and severe cases, large areas of the ommatidia were affected, from 20% to 30% (moderate) or more (severe) of the ommatidia in a histological section (Fig. 4). The screening pigments were displaced distally along the affected ommatidia or were clumped in gross aggregations proximal to the basement membrane around necrotic or degenerating optic nerve fibers. Hemocyte infiltration was often observed in the ommatidial region and the ommatidia frequently exhibited degeneration. In oblique sections, the affected basement membrane was not observed, but affected ommatidia and displaced screening pigments were often apparent and indicative of idiopathic blindness. In moderate and severe cases, hemocyte infiltrates were frequently observed in edematous arterioles located proximal to the basement membrane in the glial zone surrounding the optic nerve tracts.

Table 2. Laboratory assessment of right eyes on live lobsters evaluated by otolaryngoscopy (o-scope) and histology (gold standard). The specificity of the o-scope in the laboratory setting was 92%, sensitivity was 79%, positive predictive value was 88.4% and negative predictive value was 84.6%.

	O-scope		
Histology	Blind	Not blind	Total
Blind	23	6	29
Not blind	3	33	36
Total	26	39	65

The area affected by blindness was determined in histological preparations following Maniscalco and Shields (2006). Briefly, the lengths of the affected area of basement membrane and the observed basement membrane were measured with an ocular micrometer in optical units, and the affected areas was then expressed as a percentage.

Diagnostics Assessment of Blindness.—Of the 65 lobsters examined, 46.2% exhibited some form of blindness via histology, 38.5% exhibited blindness in the right eye using the o-scope, 52.3% blindness in at least one eye using the o-scope, and 24.6% blindness in both eyes with the o-scope (Table 2). Two of the eyes were positive for blindness by histological assessment, but the affected area was <1% of the length of the basement membrane; therefore, these eyes were considered below the detection limit (i.e., negative) for o-scope assessment. In the laboratory, all animals were assessed in a darkened room by one of us (ATO). All histological preparations were read by two of us (ATO and JDS) with 99% matching. In the laboratory setting, the specificity, or true negatives diagnosed correctly by the o-scope, was 92% (33/36). The sensitivity, or true positives diagnosed correctly by the o-scope, was 79% (23/29). The lesion area (%) in histology was positively correlated ($r^2 = 0.79$, $P < 0.001$) with the lesion area subjectively estimated with the o-scope (Fig. 3). Although somewhat variable, the subjective rating of affected area using the o-scope was consistent with the histological assessment.

The comparison between right and left eyes from the subset of 22 animals gave information on the prevalence in both eyes (35% via histology), as well as an initial assessment of the section plane. Of the 22 left eyes assessed with histology along the transverse plane, two eyes were discarded due to damage during shipment. Of the remaining 20, one was positive with histology, but had an affected area of <1% of the length of the basement membrane, well below the detection limit for the o-scope, and was therefore counted as negative for the o-scope comparison. The prevalence of blindness in the remaining lobsters was 40% and 55% in the left and right eyes, respectively, via histology, and 38% and 46% in the left and right eyes, respectively, via the o-scope. Blindness occurred in at least one eye in 60% of these lobsters via histology and 50% via the o-scope. The smaller number of eyes cut along the transverse plane (left eyes) did not allow for an optimal comparison between the two section planes. The transverse plane may be preferred over the traditional coronal plane due to its more accurate representation of the mediolateral regions of the eye.

In the field, all animals were assessed under variable conditions of glare, lighting, and dockside constraints by one of us (MH). In this setting, the specificity (69.7%) and sensitivity (75.0%) were lower than those determined under more ideal conditions in

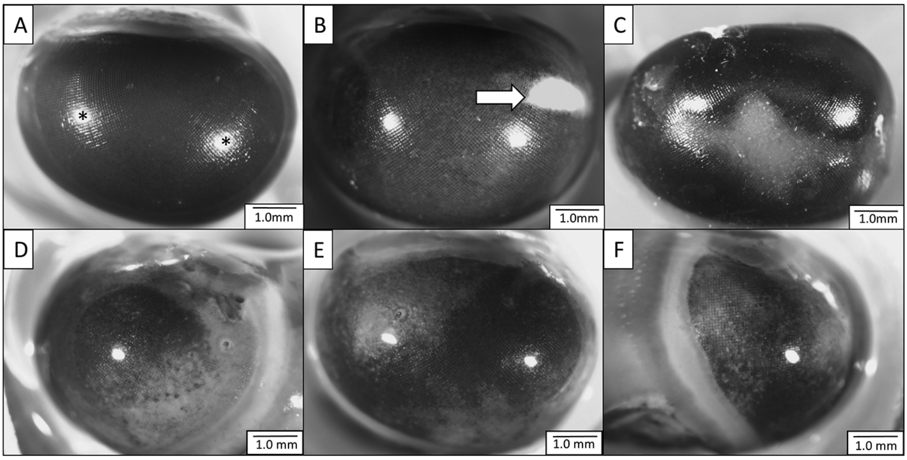

Figure 5. Lobster eyes showing different severity states of idiopathic blindness in Bouin's-fixed specimens. (A) Healthy eye with well-defined ommatidia and no signs of lesions. The two over-exposed regions indicated by the asterisks are reflections from the fiber optic lamp. (B) Mild case of blindness with a medial lateral lesion (arrow) affecting approximately 5% of the eye. (C) Moderate blindness with asymmetric lesions affecting approximately 30% of the eye. (D-F) Same eye from a lobster showing a complex coalescing lesion from (D) the left medial side, (E) the center, and (F) the right medial side. These lesions collectively affected approximately 50% of the eye. Stereomicroscopy.

the laboratory (Table 2). The field assessments resulted in a more variable outcome in o-scope evaluations than in the more controlled environment of the laboratory.

We examined inter-observer comparisons between histology and the two best trained observers, one in the laboratory setting and the other in the field. The comparison indicated a moderate agreement (Kappa = 0.520), with good mean pairwise agreement (76.6%) between observers, but with very good agreement between the histological and laboratory assessments (89.5%). In addition, the o-scope evaluation of lobsters was undertaken by five observers in the laboratory setting. Training consisted of an initial assessment of eight lobsters by the group, followed by discussion and then reassessment of cases with large disagreements in relative area of blindness. Other sets of lobsters were sampled in a blinded fashion without concurrent discussion. Because the evaluations were based on observer availability, the largest data set (n = 55 lobsters) consisted of three observers. The inter-observer comparison with these three observers (ATO, BNB, JP) indicated a moderate agreement (Kappa = 0.497), with good mean pairwise agreements (76.1%) between observers, with the best agreement only modestly better (78.2%). The o-scope evaluation of lobsters in the field was conducted by MSH who underwent the same training to identify blindness lesions as the aforementioned observers, but with a different set of lobsters for the training period only. For the inter-observer comparisons, the agreement between histological and dockside assessments (71.9%) was below that of the laboratory assessment (89.5%).

Stereomicroscope Diagnosis of Fixed Eyes.—All of the lobster eyes were fixed and processed for histology, but a subset of 27 right eyes were examined for blindness prior to histological processing. The picric acid in Bouin's solution was an effective counterstain providing excellent contrast of affected tissues in the eyes (Fig. 5). Of the eyes assessed after fixation, 66.7% (18) exhibited some form of blindness

Table 3. Laboratory assessment of right eyes fixed in Bouin's solution and then evaluated by stereomicroscopy and histology (gold standard). The specificity of the stereomicroscopy using Bouin's fixed samples was 100%, sensitivity was 94.4%, positive predictive value was 100% and negative predictive value was 90%.

	Stereomicroscopy		
Histology	Blind	Not blind	Total
Blind	17	1	18
Not blind	0	9	9
Total	17	10	27

via histology and 63% (17) were evaluated as having idiopathic blindness by stereoscopic microscopy (Table 3). The specificity, or true negatives diagnosed correctly by fixation and stereomicroscopy, was 100%. The sensitivity, or true positives diagnosed correctly by the o-scope, was 94.4% (17/18). The comparison between the fixed eyes and the histology produced a near perfect Cohen's Kappa of 0.919 and average percentage of agreement of 96.3%. That is, stereoscopic assessment of the eyes fixed in Bouin's solution provided an excellent alternative to histological assessment.

Frozen Eyes for Diagnosis.—The eyes frozen and later evaluated for blindness (n = 44) matched poorly with data from the o-scope (data not shown). Given the poor initial results of this method, we began evaluating blindness in the left eyes of newly received lobsters using histological methods, with the exception that these eyes were sectioned on the transverse plane (*see* histological assessment below).

Field Assessment of Lobsters with the Otolaryngoscope.—Idiopathic blindness was relatively common in the waters of Narragansett Bay and Rhode Island Sound. On five sampling periods, 145 lobsters were evaluated with the o-scope for blindness (Table 4). Prevalence levels with this technique ranged from 18.2% to 60.0%. Using the specificity and sensitivity calculated from histological assessment of dockside evaluations, the prevalence was corrected using a simple formula ($\text{prevalence}_{\text{o-scope}}$ × specificity divided by sensitivity). Because the specificity was higher than the sensitivity, but both were close to 1.0, the corrected prevalence was only marginally lower than the estimated prevalence using the o-scope. This accounted for the misclassifications compared to the gold standard of histology. The corrected prevalence ranged from 16.9% to 55.7%. In addition, the presence of the white eye condition was noted. Although the data are sparse, there may be a temporal or spatial component to the distribution of blindness in the area around Rhode Island.

Table 4. Assessment with the otolaryngoscope for the prevalence of idiopathic blindness in lobsters from Narragansett Bay (Castle Hill, Kettle Bottom, and Newport Bridge) and Rhode Island Sound in 2017. Frequencies are given for animals exhibiting blindness (blind) and the white eye syndrome. Both eyes were used in the assessment. Prevalence values includes the white eye condition which is an acute manifestation of the syndrome. O-scope prevalence refers to the raw prevalence uncorrected for specificity and sensitivity. The corrected prevalence is adjusted by specificity and sensitivity using the formula ($\text{prevalence}_{\text{o-scope}}$ × specificity)/sensitivity. n = sample size.

Date	Location	n	Blindness	White eye	Not blind	O-scope prevalence	Corrected prevalence
30 January	Castle Hill	33	6	0	27	18.2	16.9
2 April	Kettle Bottom	25	8	0	17	32.0	29.7
20 April	Castle Hill	36	13	3	20	44.4	41.3
8 May	Rhode Island Sound	11	0	3	8	27.3	25.3
31 May	Newport Bridge	40	24	0	16	60.0	55.7

Discussion

We evaluated the use of the otolaryngoscope as a rapid noninvasive tool for diagnosing idiopathic blindness in the American lobster, *H. americanus*. In the laboratory setting with highly trained personnel, the high sensitivity (79%) and specificity (92%) of the o-scope indicated that it was very effective in diagnosing moderate and severe cases of the syndrome. In the field setting, the lower specificity (69.7%) indicates that it should be used with some caution, even in the hands of highly trained personnel, because of issues controlling lighting and contrast. Nonetheless, specificity and sensitivity can be used to give adjusted estimates of prevalence from field samples. In other words, misclassifications can be corrected using specificity and sensitivity when compared to the "gold standard," in this case histological assessment.

The o-scope provides several advantages over histology for assessing lobsters for blindness. It provides a noninvasive assessment tool that is relatively easy to learn, is much faster than traditional methods, and can be applied to real-time surveys and disease progression studies. With proper training in the laboratory setting, it can have a high specificity and sensitivity as a diagnostic tool when compared to histological assessment (i.e., 92% of o-scope assignments were correct in the present study).

Although the o-scope presents a noninjurious, modestly error-prone method to evaluate lobsters, if the animals are to be killed and necropsied for other analyses, then fixation with Bouin's solution and subsequent stereomicroscopic examination of the eyes can provide a nearly perfect match with histological assessment. Fixing the eyes in Bouin's solution enhances the contrast of the affected region of the eye without altering the dimensions of the lesions. Although the method is invasive and time consuming, it allows for the eyes to be shipped and stored in ethanol and does not exclude the option of additional histological assessments. Assessments of the freshly-dissected eyes with the stereomicroscope were comparable to that of the o-scope, but were also invasive and time consuming. Freezing the eyes at −80 °C rendered them undiagnosable, but preserved the tissues for other studies (e.g., presence of contaminants); therefore, if the eyes are to be frozen, they should be diagnosed prior to freezing. Histology remains the "gold standard" for diagnosing idiopathic blindness in lobsters. It elucidates underlying pathology and can provides estimates of lesion area based on the involvement of the actual ommatidia.

Nephropid lobsters, such as *Homarus* spp., have reflecting superposition compound eyes (Gaten et al. 2013). Light-induced blindness is a condition known to occur with *N. norvegicus* (Loew 1976), and the pathology is similar between *N. norvegicus* and *H. americanus* (Maniscalco and Shields 2006). Nonetheless, light sensitivity can be excluded as the primary causative factor concerning idiopathic blindness in *H. americanus*. Light-induced eye damage has yet to be reported in *H. americanus* (Shelton et al. 1985) and is not expected to occur, as the species commonly inhabits shallow waters with diel exposure to sunlight, unlike *N. norvegicus*. Furthermore, Magel et al. (2009) held *H. americanus* under ambient light intensities without inducing damage to the eye and we have held this species for longer periods (6–7 mo) under controlled light conditions without observing increased prevalence levels of blindness (Shields unpubl data).

Blindness is widespread in the southern New England fishery (Tables 4, 5) and its spatial distribution is indicative of a widespread phenomenon. Although

Table 5. Prevalence of idiopathic blindness from histological samples from different studies. LIS = Long Island Sound, Lobsters from Maine were sampled from Down East and South Coast were pooled for the analysis. *n* = sample size.

Location	Blind via histology (%)	*n*	Study
Western LIS	56.9	51	Maniscalco and Shields 2006
Central LIS	52.6	19	Magel et al. 2009
Narragansett Bay, RI	54.4	90	Shields et al. 2012
Mount Desert Rock, ME	15.8	19	Shields et al. 2012
Down East and South Coast, ME	2.9	35	Shields and Gillevet 2014
Vineyard Sound, MA	7.7	70	Barris et al. 2018

contaminants are a possibility, exposure to hypoxic reactive metals from seasonally occurring benthic hypoxia events may be more likely. Under hypoxic conditions (<16% oxygen saturation), Mn and other metals become bioavailable as metal oxides are reduced (i.e., to the ionic state, Mn^{2+}), which is then released from sediments into burrows and epibenthic water layers (Aller 1994). Both LIS and Rhode Island Sound experience benthic hypoxia and anoxia for prolonged periods of time within the inshore environments. However, lobsters that remain in their burrows below the benthic boundary layer may be subjected to more pronounced anoxic and hypoxic conditions due to their proximity to the anoxic zone and highly reduced benthic sediments.

Mn^{2+} is an established neurotoxin in crustaceans and has been shown to accumulate in nerves, hemolymph, and other tissues of *Homarus gammarus* (Linnaeus, 1758) and *N. norvegicus* (Bryan and Ward 1965, Baden and Neil 1998). Baden et al. (1999) showed that accumulation of Mn in *N. norvegicus* reaches maximum concentration in most tissues, including the brain, after only 5 d of exposure, and elimination from the tissues occurs rapidly when removed from the source. Draxler et al. (2005) used Mn concentrations in *H. americanus* as an index of exposure to reducing conditions. Assuming that *H. americanus* demonstrates a similar mode of Mn uptake and elimination to that of *H. gammarus* and *N. norvegicus*, most bioavailable Mn will be expelled from the lobsters shortly after benthic oxygen levels have returned to normal; however, they may sequester it in the exoskeleton of the antennules, which could then be used as a biomarker for exposure (Baden and Neil 2003). If the optic nerves are affected by exposure to Mn, then it is unlikely that the damage to the ommatidia is reversible. As lobsters molt, new rows of ommatidia are added along the margins of the eyes not the in the middle (Shelton et al. 1981). Therefore, centroid lesions would likely not be healed. In the Norway lobster fishery off Scotland, light-induced blindness did not affect mortality rates (Chapman et al. 2000), but its effect on the American lobster remains to be determined.

In summary, the otolaryngoscope provides a rapid assessment tool for evaluating blindness in lobsters. In the laboratory, it provided very good comparisons between histological and stereomicroscopic evaluations. Although its specificity and sensitivity are modest in field applications with bright lighting and glare, it can reliably resolve moderate and severe cases of idiopathic blindness, and therefore, may have suitable application for studies examining spatial and temporal aspects of the disease syndrome.

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