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MORTALITY ASSESSMENT OF ATLANTIC SEA SCALLOPS (*PLACOPECTEN MAGELLANICUS*) FROM GRAY-MEAT DISEASE

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ABSTRACT The sea scallop *Placopecten magellanicus* (Gmelin, 1791) fishery is the most valuable wild scallop fishery in the world. Landings are primarily adductor muscles, which are ideally creamy white and firm. Recently, an increasing number of dark brown to gray, flaccid, and stringy meats have been caught in areas of Georges Bank and the mid-Atlantic, causing concern in industry and management. The mortality of gray-meat scallops was investigated, and an anesthetization procedure was developed allowing assessment of meat color in live scallops for laboratory experiments. The mortality rate of gray-meat scallops compared with white-meat scallops was tested. Gray-meat infection was swift and fatal; 26 of the original 28 gray-meat scallops died, whereas only 1 of 28 white-meat scallops died. The gray-meat condition was clearly associated with apicomplexan parasite infection intensity. Gray-meat scallops were identified visually, with image analysis computer software, and cell counts of apicomplexan nuclei. Secondary infections and stressors, including the effect of senescence, boring worm (*Polydora* sp.), and boring sponge (*Cliona vastifica*), were examined. Shells exhibiting high levels of boring sponge and worm damage had significantly higher incidence of gray-meat scallops. The prevalence of gray meats in a scallop population changes the shell height–meat weight relationship and the estimates of natural mortality and fishing effort used in stock assessments. Understanding the impacts of this disease and how to manage the fishery in its presence is important for the future of the fishery.

KEY WORDS: apicomplexan, giant scallop, Georges Bank, mid-Atlantic, fisheries, mass mortality, *Placopecten magellanicus*

INTRODUCTION

Normal sea scallop adductor muscles are creamy white and firm. Recently, ''gray meats'' that are stringy, dark, and distasteful have been observed in scallops on Georges Bank and in the mid-Atlantic (Fig. 1). Severity of discoloration progresses from the normal white to light brown, dark brown, and ultimately gray. Gray-meat Icelandic scallop adductor muscles have a meat weight lower than expected for a given shell height (Kristmundsson et al. 2011). The adductor muscle of gray-meat scallops is weak and often tears from the shell or stretches away with the mantle from the attachment site when being harvested (Fig. 2).

Scallops with gray and brown muscles have been reported from Nova Scotia to Narragansett Bay and were associated with intracellular prokaryotes (rickettsia-like bacterium) in the gills, extensive invasion of the shell by boring sponge (*Cliona vastifica*) and polychaete worms (*Polydora* sp.), and age, that is, large, old scallops (Stevenson 1936, Medcof 1949, Gulka et al. 1983, Ballou 1984). The presence of discolored scallop meats was commonly associated with mortalities. Fishermen claimed that regular fishing helps reduce gray-meat scallops by removing them from the population.

Gray-meat scallops were observed in the Nantucket Lightship Closed Area (NLCA) in a 2004 to 2005 mass mortality event. The mortality may have been caused by synergistic effects of senescence, parasitism by shell borers, and prokaryotic infection (Stokesbury et al. 2007). Part of the NLCA is a rotational access area, which contained a high abundance of large scallops (Stokesbury 2002, Harris & Stokesbury 2006, NEFMC 2015). When permitted access to this area, fishermen found old scallops with deteriorating meats, and dead ''clappers'' (dead scallops with shells still articulated) (Stokesbury et al. 2007). Clappers indicate natural mortality, as the shells are separated during harvest in the fishery (Feder & Christensen 1966). Gray-meat scallops were falling out of their shells and hanging from the dredge during commercial fishing operations. The biomass lost during this mass mortality was equivalent to ~6,484 mt of harvestable meats worth ~US$100 million ex-vessel (Stokesbury et al. 2007).

The extended southern portion access area of Closed Area I (CAI) was closed to fishing for 8 of 10 years (2001 to 2010) with the exception of one trip per full-time vessel in 2005 and 2007 (NEFMC 2015). When the area was opened to fishing in 2011, fishermen found gray-meat scallops and numerous clappers (Cuddy 2011). Only 31.9% of CAI total allowable catch was collected in 2013 due to the poor quality of scallop meats, leading to an early closure of the access fishery (NEFSC 2014). Fishermen that were allocated pounds from this area were not able to collect their share, and pounds have yet to be reallocated to another area. The biomass of scallops in the area was lost and the industry has not yet recovered those earnings [Federal Register (USA) 2014].

In 2000, wild populations of the Icelandic scallop *Chlamys islandica* experienced small, dark meats and abnormally high mortality in adult scallops (Kristmundsson et al. 2011). The most extensive mortalities were observed in scallop stocks with limited fishing (Kristmundsson et al. 2015). Pathological analysis revealed infections of two previously unknown apicomplexan parasites, one of which affects muscle tissue and targets the adductor muscle (Kristmundsson et al. 2011). The unexpected collapse of this valuable resource resulted in a commercial fishing ban in Icelandic waters that has been in effect since 2003 (Kristmundsson et al. 2015). The apicomplexan

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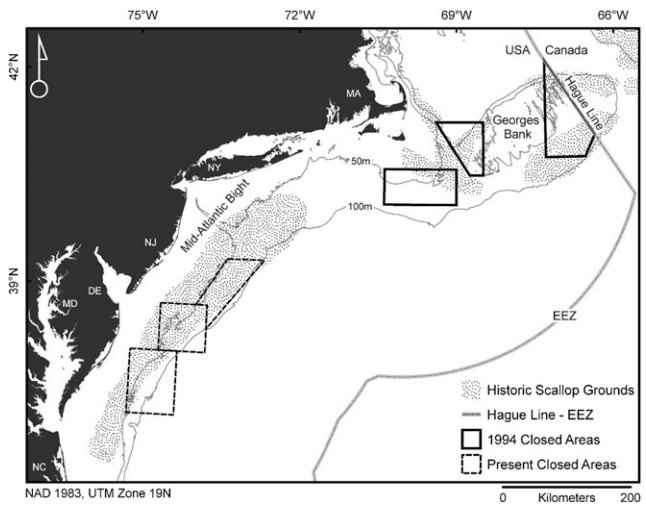

Figure 1. U.S. scallop range from Cape Hatteras, NC, to Georges Bank and the Gulf of Maine. Stippled regions represent the major commercial scallop aggregations; solid black lines depict the groundfish closure areas established in 1994 on Georges Bank and the mid-Atlantic. The solid gray lines represent the 50- and 100-m depth contours and the U.S. EEZ (adapted from Stokesbury et al. 2010).

parasite was found in all sampled Iceland scallops (*C. islandica*) from Icelandic and Faroese waters, and in queen scallops (*Aequipecten opercularis*) from Faroese waters, and infections were commonly intense. Genetically identical parasites were found in lighter intensity levels in both king (*Pecten maximus*; detection frequency 90%) and queen scallops (40%) from Scottish waters, which showed no signs of disease or abnormal mortality (Kristmundsson et al. 2011).

This apicomplexan is a newly proposed genus and species infecting *Chlamys islandica*, *Aequipecten opercularis*, and *Pecten maximus* and was genetically confirmed as the same parasite infecting muscle tissue of *Placopecten magellanicus* (A. Kristmundsson & M. Freeman, University of Iceland, Reykjavik & University of Malaya, Kuala Lumpur, personal communication, 2011). In these three scallop species, developmental forms of the parasite, including both sexual and asexual stages, were found in a single host (Kristmundsson et al. 2011). The parasite targets the adductor muscle and causes progressive myodegeneration. Zoites, the mobile, infective stage of the apicomplexan, were abundant in all muscle tissue, including the adductor muscle, of moderately and heavily infected individuals (Kristmundsson et al. 2011). The most heavily infected tissue was the adductor muscle, containing large clusters of zoites in necrotic areas of individually infected muscle cells and inside hemocytes (Kristmundsson et al. 2011). Hemocytes are the primary immune defense mechanism for invertebrates, but lack immunological memories. Two routes of exposure seem logical, one being excretion through the kidney and exposure to environment from feces, and the other from a dead decaying scallop (Kristmundsson et al. 2015).

Total exploitable biomass is estimated from a stock-wide video and a dredge survey (NEFSC 2014). When total exploitable biomass is estimated from video surveys, counts and measurements of scallops from images of the sea floor are used to estimate the size-specific mean density for the entire population (Stokesbury et al. 2004, Rothschild et al. 2009, Stokesbury 2012). The size-specific mean density is then multiplied by the area of the survey to get size-specific total number of scallops in the population. When total exploitable biomass is estimated from dredge surveys, all scallop catch is put into baskets, then depending on the total volume of the catch, a fraction of the baskets is measured for shell height frequency and expanded to the number of baskets caught. The size-specific total number is then multiplied by the meat weight of scallops for each size group based on a shell height–meat weight relationship (Rothschild et al. 2009). Scallop shell height–meat weight relationships change with location, seasonally, and

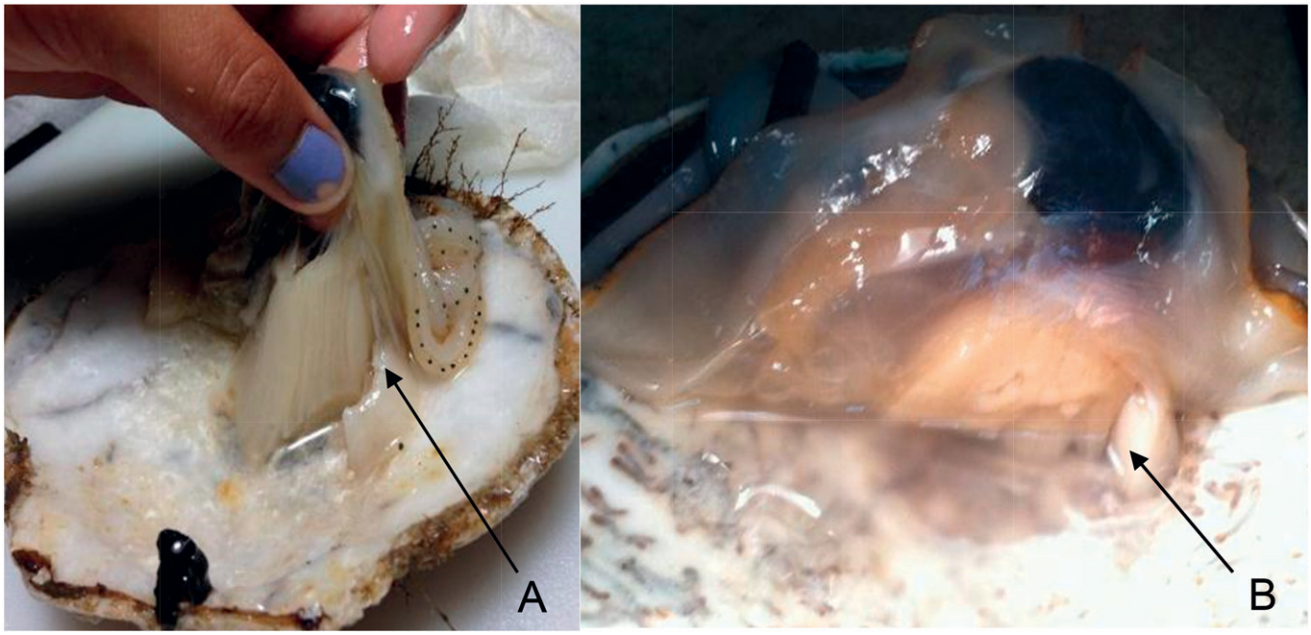

Figure 2. Examples of gray-meat adductor muscles stretching with (A) viscera and (B) tearing from the shell.

annually, but variability in health is not considered due to lack of information (Caddy 1989, MacDonald et al. 2006, Sarro & Stokesbury 2009, Hennen & Hart 2012, Truesdell et al. 2016). The catch at size analysis model is used in the stock assessment for sea scallops to estimate spawning stock biomass, recruitment information, and fishing mortality.

The objective of this study was to investigate the mortality of gray-meat scallops. The mortality rate of gray-meat scallops compared with white-meat scallops was tested under optimal laboratory conditions. Darkening of adductor muscle color was quantified and correlated to intensity of parasitic infection. Secondary infections and stressors, including the effect of senescence, boring worm (*Polydora* sp.), and boring sponge (*Cliona vastifica*), were explored as associated stressors in scallops.

MATERIALS AND METHODS

Scallops were collected in cooperation with commercial fishing vessels. Adult scallops (≥90 mm) were collected from CAI, CAII, the NLCA, and adjacent to those areas by CFF in March, April, May, and June 2014 and CAII in April 2014 and CAI in June 2014 (Fig. 1). Scallops were collected in August, September, and November 2015 from CAII and along the northern edge of the bank (Fig. 1). In December 2015, white-meat scallops from Hudson Canyon were collected (Fig. 1). Scallops collected in 2014 were kept on deck in water baths with flowing surface water until entering New Bedford Harbor (MA). Water was removed from the containers and scallops were covered with cool, wet towels and transported to the seawater laboratory at the School for Marine Science and Technology in New Bedford, MA. Scallops were acclimated to test tanks for a minimum of 48 h to eliminate sick or injured individuals. Scallops collected in 2015 were used for the preliminary anesthetization experiment as well as the 4-month laboratory experiment. Scallops from the 2015 collections were dissected and used immediately for GIMP images, parasite intensity counts, determination of age (size), and intensity of boring studies.

To assess the recovery of gray-meat scallops compared with white-meat scallops in a controlled laboratory experiment, it was necessary to determine if the scallops were white or gray, while they were still alive. Anesthetics have frequently been used to avoid stress and to relax bivalve molluscs. Dead Sea Works $MgCl_2$ was chosen as the anesthetic for the present study based on cost and efficiency (Suquet et al. 2009).

The appropriate concentration of Dead Sea Works $MgCl_2$ and time needed to anesthetize *Placopecten magellanicus* were determined using observations from five trials. Each anesthetic trial was performed on four scallops in a fish tote suspended in seawater tanks. Scallops were observed for 3–16 h in different concentrations ranging from 35 to 50 g Dead Sea Works $MgCl_2$ to 1 l of water. With 50 g/l Dead Sea Works $MgCl_2$, 2 l seawater (salinity 33–36) and 3 l of freshwater were required to maintain salinity levels at 35–38 (Suquet et al. 2009). Four scallops per tote with 35 g/l of Dead Sea Works $MgCl_2$ for 5–5.5 h was established as the anesthetic protocol for adult *P. magellanicus*. The 20-l water bath included a mixture of fresh water (13 l) and incoming sea water (7 l) to reach the desired salinity.

A color chart for defining white-, brown-, and gray-meat scallops was created to assist in color identification (Fig. 3). The adductor muscle, composed of two parts, allows the scallop prolonged shell closure (smooth muscle) and supplies the force for jet propulsion (striated muscle) to avoid predators (Chantler 2006). The smooth muscle of the scallop generally did not show signs of infection, allowing for the comparison in color of the phasic muscle, which progressively darkens with higher infection. Once anesthetized, the scallops opened, allowing a clear observation of the meat. There was also a large space between the two muscles in gray-meat scallops; in a white-meat scallop, the muscles lie closely together (Fig. 4). The smooth muscle of *Placopecten magellanicus* was not viewed microscopically for parasitic intensity; however, the parasite was found in the smooth muscle as well as the phasic muscle in Icelandic scallops (Kristmundsson et al. 2015).

Using the anesthetic protocol and color guide, 246 scallops were anesthetized and observed by two observers [investigator (Observer 1) and colleagues (Observer 2)] to determine if meat quality could be assessed accurately under anesthesia. Meat color was divided into 1 of 5 categories: white, light brown, brown, gray, and salmon. Salmon scallops occur due to pigmentation from the female gonad (zeaxanthin), a side effect of maturation and development (Bourne & Bligh 1965). Observations were recorded and then the lower shell of the scallop was removed providing a full view of the adductor muscle to obtain muscle color, confirming or rejecting the initial assessments.

Overall, observations under anesthesia for Observer 1 were correct 88.6% of the time, and Observer 2 was correct 80.9% of the time when compared with the actual muscle color. Chi-square test results indicated a significant difference in initial and actual observations for Observer 2, but not for Observer 1. Removing the perceived light brown scallops greatly reduced the difference for Observer 2.

Once a procedure was established to determine adductor muscle color with anesthesia, the effect of an optimal and restricted diet on white- and gray-meat scallops was tested in a laboratory experiment. Maintained conditions included flow (~9 cm/s), substrate (sand), and temperature (10–15°C) to provide the most suitable conditions for growth along with an optimal or restricted diet.

Each scallop was anesthetized and measured for shell height (maximum distance from umbo to shell edge, in mm), assessed for levels of worm and boring parasitism (0, low, medium, and high) (Figs. 5 and 6), and examined for muscle color. If both researchers categorized a scallop as white, brown, or gray, it was tagged with a Peterson tag identification number and put into a designated tank (Harris & Stokesbury 2006).

To avoid uncertainty, white and stringy scallops, as well as light salmon- or salmon-colored scallops, were eliminated from this study. Because of their ambiguity, and for a larger sample size, brown and gray scallops were grouped together for this experiment. Light brown scallops appeared to represent the beginning stages of gray-meat infection and were removed due to uncertainty when assessing the muscle quality under anesthesia. In addition, if the observing researchers disagreed on the muscle color (excluding differentiation of gray or brown), the scallop was not used for the experiment.

Twenty-eight tagged scallops were separated equally into two, 300-gallon tanks for both gray- and white-meat scallops resulting in a density of 14 scallops in each tank. An extra tank with reserve white, tagged scallops was maintained under optimal conditions to replace any scallops (in gray or white

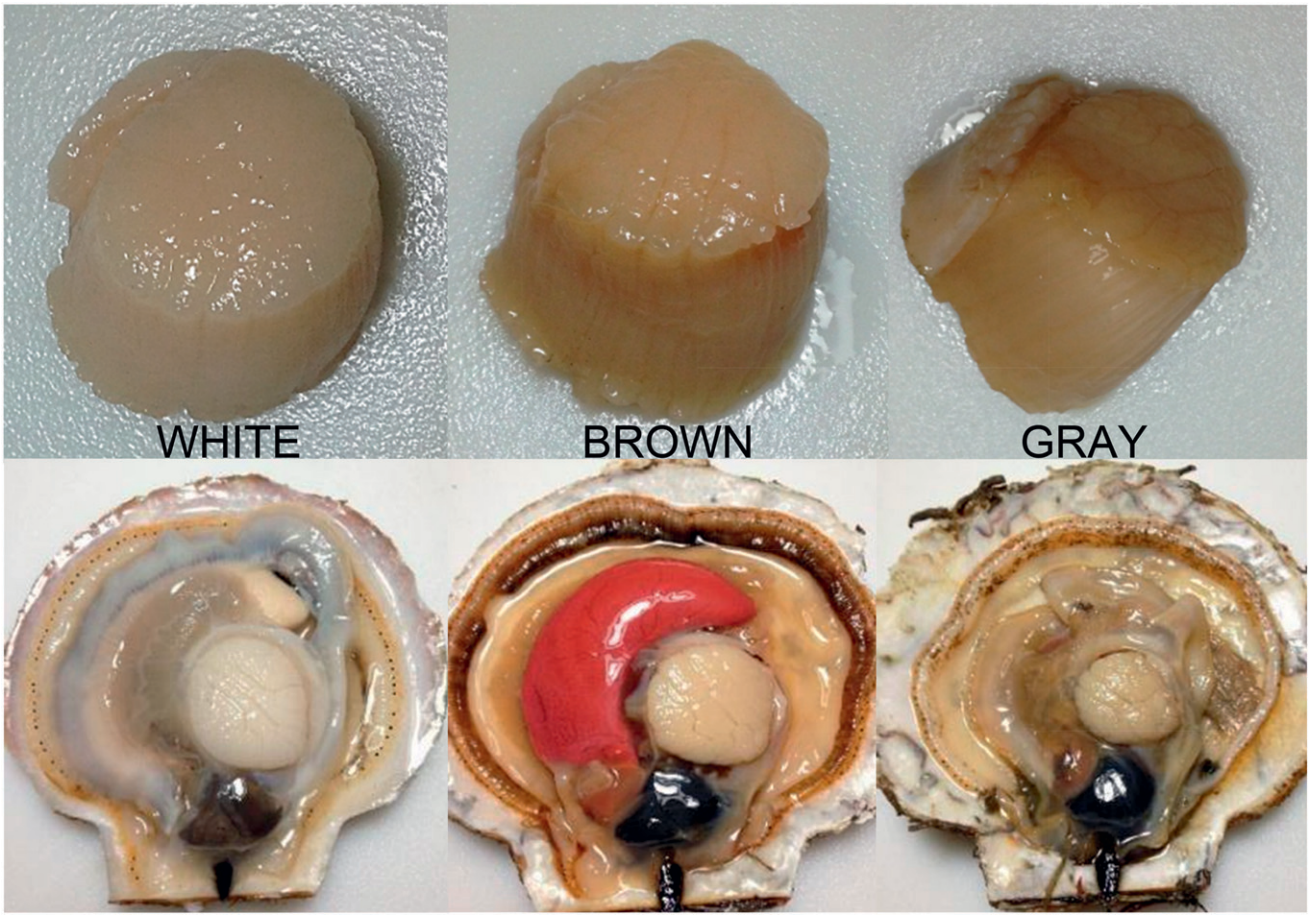

Figure 3. Adductor muscle color identification chart. Each opened shell view corresponds to the picture of the meat removed above it.

tanks) that died during the experiment. This eliminated changes in density dependence for food.

The scallops were fed a mixed diet twice daily to avoid high concentrations of phytoplankton. The diatoms *Chaetoceros neogracilis* and *Thalassiosira weisfloggi* as well as the flagellate Tahitian *Isochrysis* aff. *galbana* are indigenous to the waters of Georges Bank and the mid-Atlantic and are suitable food sources for the growth of shellfish, and are recommended for sea scallop aquaculture (Thompson et al. 1993, Pilditch & Grant 1999). Optimally fed scallop tanks received 2.5 l of cultured phytoplankton per day, equivalent to an initial density of ~15,000 cells/ml in the tank and $\sim 1 \times 10^9$ cells/scallop/day (Pilditch & Grant 1999). The restricted-diet tanks were fed half of the optimal diet, 1.25 l of cultured phytoplankton per day, equivalent to an initial density of ~7,500 cells/ml and $\sim 5 \times 10^8$ cells/scallop/day. The flow of the tank was set to 1 gallon per minute so that most phytoplankton cells were retained in the tank until the scallops filtered them out. Any accumulated pseudofeces were siphoned from the tanks weekly, and filter socks were washed daily and bleached weekly.

At the end of the experiment ($n = 124$), all scallops were measured for shell height and wet meat weight (blotted dry adductor muscle, in g). A linear regression analysis of covariance (ANCOVA) was performed on the shell height–meat weight data for scallops from the laboratory and compared with scallops collected directly from the field. Scallop meats were preserved in 10% neutral buffered formalin for analysis of infection intensity.

Identification of gray-meat scallops can be difficult, because it is a subjective process and darkening is progressive (Fig. 3). A measure of darkness seen in gray-meat scallops was quantified and compared with that of white-meat scallops using GIMP software (2.8.14).

Pictures of scallop adductor muscles were all taken under the same light conditions (3,264 × 2,448 pixels). Meats were placed individually on a white cutting board with a 14-W CFL (60-W equivalent) light bulb 38.1 cm above the table. An initial experiment tested the capabilities of GIMP color determination and the color curve tool. The peak of the color curve corresponds to a quantitative interpretation of darkness on the X axis. The axis ranges from 0 to 255 with 0 equal to black, and 255 equal to pure white. Four white-meat, three brown-meat, and three gray-meat scallops were used to establish protocol for color determination in GIMP. The best procedure required desaturation of the image and using the ellipse selection tool to select an area without glare. About 17 gray-meat and 20 white-meat scallops from the field were examined in GIMP for color determination. To assess the precision of GIMP color determination, six light-brown-meat and six brown-meat scallops were also evaluated.

As parasitic infection intensity increases, the adductor muscle of the scallop seems to darken in color. To link darkening

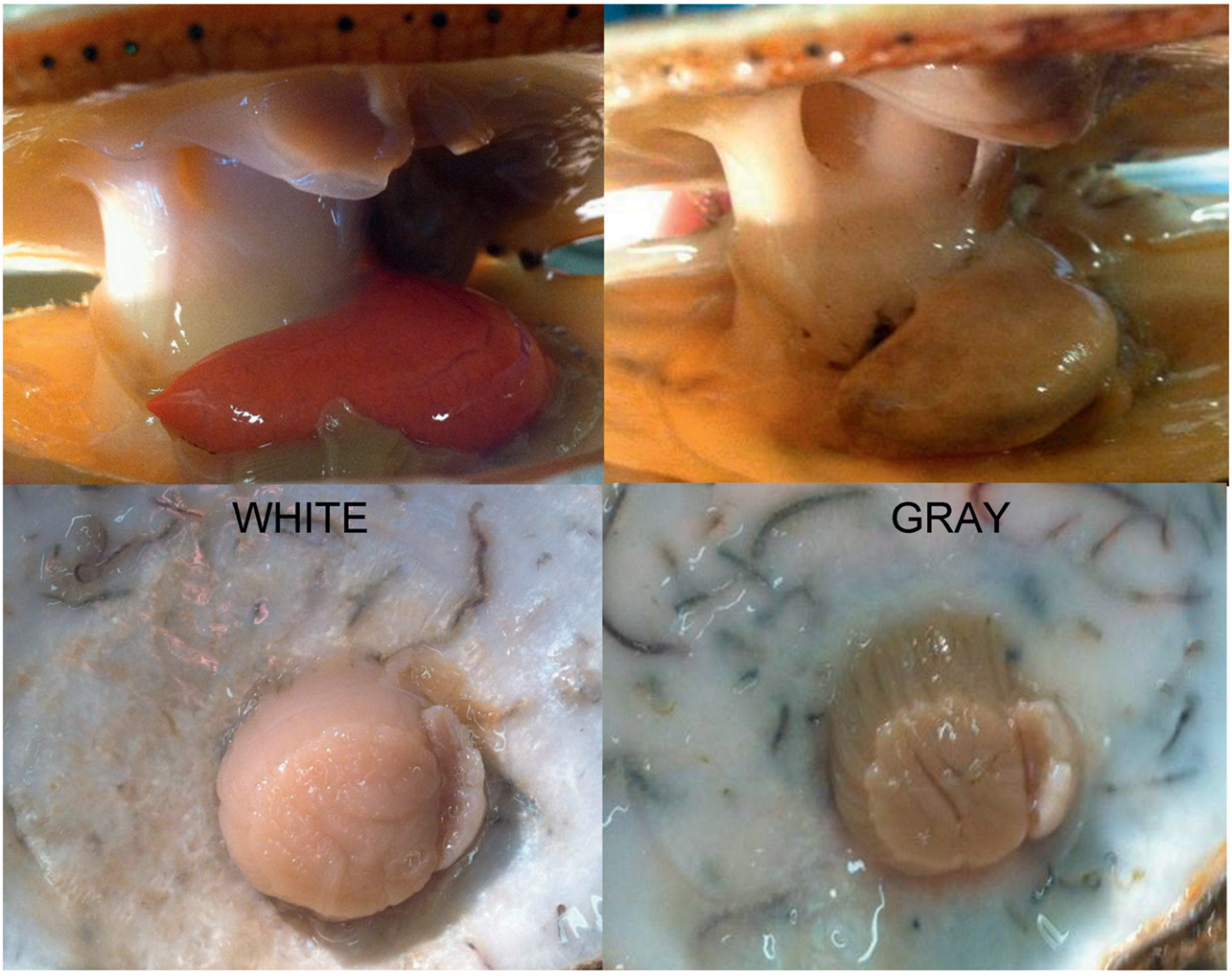

Figure 4. Difference in spacing and color between the catch muscle "C" and phasic muscle "P" is demonstrated for white- and gray-meat scallops under anesthesia; each live scallops shell view corresponds to the picture of the meat removed below it.

to parasitic intensity, infection intensity was explored microscopically for white and gray scallops ($n = 28$). Half of the scallops were from the optimal/restricted diet laboratory experiment, and the other half were collected from the field. In each group, half were white and the other half gray. These data allowed comparisons between laboratory- and field-collected scallops of the same meat quality, as well as within laboratory and field collections of white- and gray-meat scallops.

The adductor muscles of the scallops were cut creating three longitudinal sections. The sections were approximately 3–4 mm thick and were cut down in length to fit into the embedding cassettes. The samples were stored in 80% ethanol for 12+ h before being transferred into the Autotechnicon Mono Tissue Processor. The Autotechnicon dehydrated, cleared, and infiltrated the tissue with paraffin wax over a 12-h period (Procedure modified from Luna 1968). The samples were then oriented longitudinally and embedded in a wax block and stored at 2°C until cutting.

A microtome was used to produce 7-µm sections that were attached to slides using a Histobond solution in a 6°C water bath (10 ml Histobond/l water). Two or three tissue sections from each meat section were attached to each slide. There were six slides (two nonconsecutive slices of each of the three longitudinal sections) for each scallop with two or three tissue sections on each slide. One slide from each scallop section was sent to Mass Histology Services (7 Lenora Street, Worcester, MA) to be stained using the May-Grünwald Giemsa staining method.

Stained slides were returned to the laboratory and observed microscopically under 400× magnification (10× ocular), with a diameter field of view of 0.45 mm. Three random field of view images were captured by taking a picture through the microscope. These pictures were examined using a custom application developed to digitize video footage of the ocean floor, primarily for scallop counts (Carey & Stokesbury 2011). The pictures were examined and the number of nuclei including parasite stages, hemocytes, and muscle cell nuclei were counted for each of the nine fields of view for each of the 28 scallops. Analysis of variance (ANOVA) was used to determine significance of GIMP readings between meat quality categories and to examine differences between parasite intensity counts of white- and gray-meat scallops within and between laboratory- and field-collected scallops.

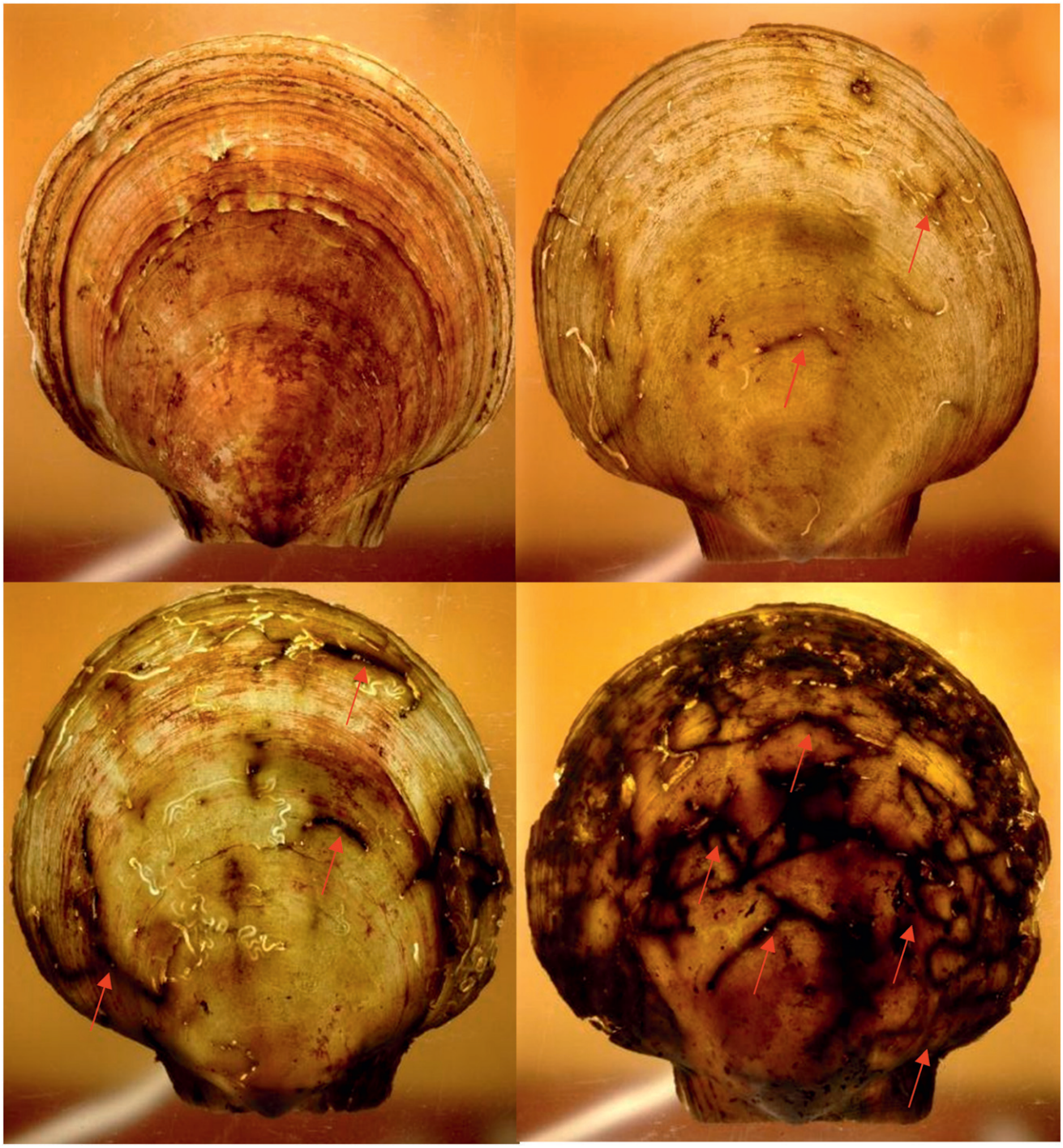

Figure 5. Gradation of intensity of boring worm (*Polydora* sp.) invasion (red arrows) in the upper shells classified as 0 (top left), low (top right), medium (bottom left), and high (bottom right).

To complete the study, historically associated stressors of gray-meat scallops were examined including boring infestation levels and age. Scallop meat color and infestation by boring organisms were examined to analyze if boring intensity or size are related to darkened meat color. Scallops were examined for boring worm (Fig. 5) and boring sponge (Fig. 6) infestation (classified as 0, low, medium, and high), meat color, and the shell height and meat weight (g). Shell height was used as a proxy for age and measured in mm. This proxy is supported in management since growth functions are used to estimate shell growth and predict shell height at age of sea scallops; however, there is variation in growth by factors such as the area and depth the scallop was collected from (Harris & Stokesbury 2006). This information allows determination of correlations among age, parasitism, and color. An ANOVA was carried out to determine a significance or correlation between adult scallop size and meat quality.

The contingency table created with white-meat scallop data produced the expected values of infection intensity for

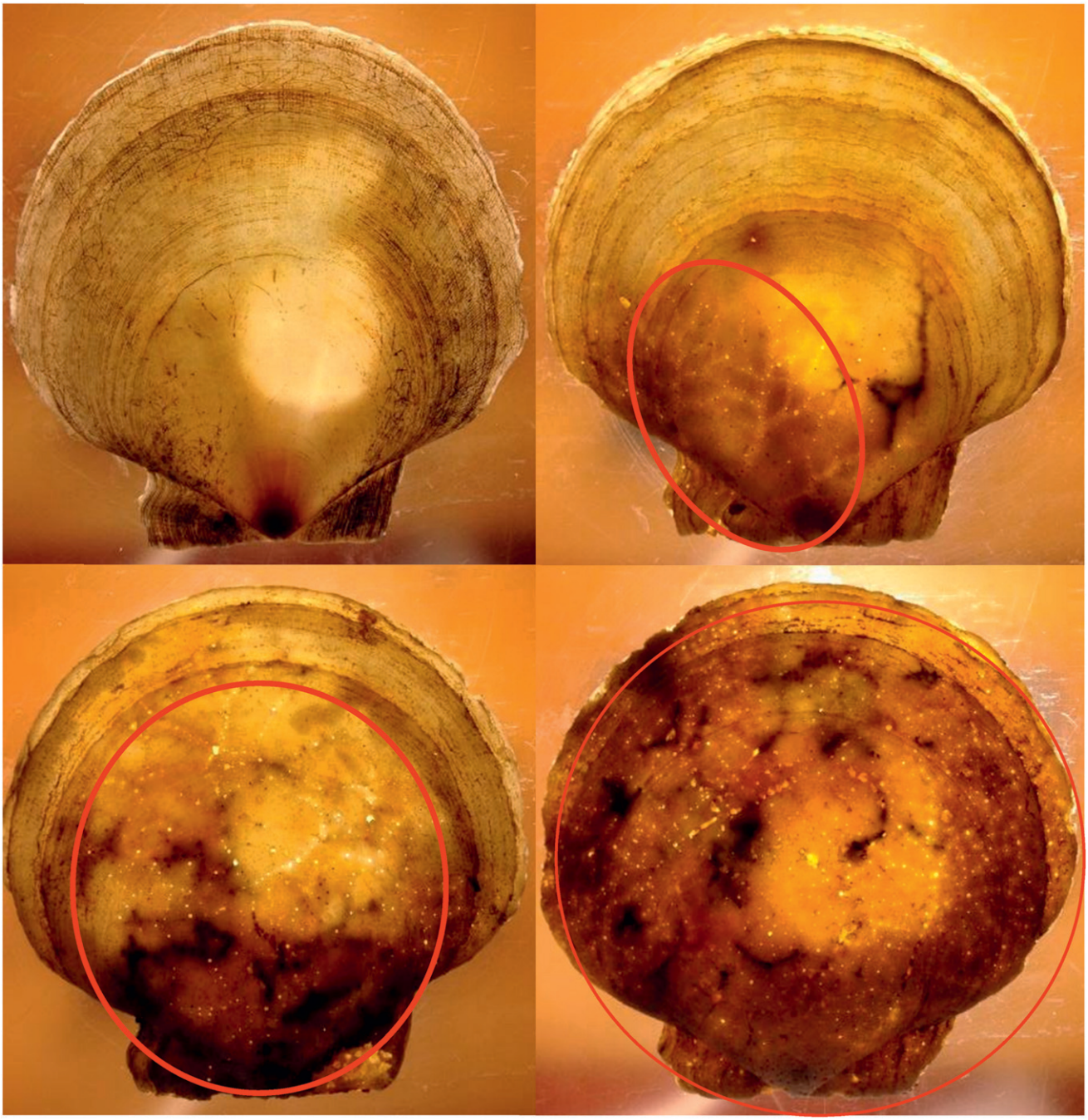

Figure 6. Gradation of intensity of boring sponge (*Cliona vastifica*) invasion (red circles) in the lower shells classified as 0 (top left), low (top right), medium (bottom left), and high (bottom right).

light-brown-, brown-, and gray-meat scallops, separately and combined as infected individuals. Chi-square statistic will use these observed and expected values to determine if boring is significantly different between white and gray scallops.

RESULTS

Over the course of the feeding experiment, 13 of the 14 original gray-meat scallops in both tank 1 (restricted diet, gray) and tank 2 (optimal diet, gray) died (Fig. 7). Since each mortality was replaced with a white-meat scallop, 16 new white-meat scallops were added into tank 1, 3 of which died, whereas 5 of 18 replacements died in tank 2. The three white replacements in tank 1 lived for 17, 62, and 93 days. The shortest lived replacement was still white at mortality, the 62-day replacement mortality was light brown, and the scallop that lived in the gray-meat tank for 93 days was gray when it died. In tank 2, the five replacements lived in the gray-meat tank for 7, 8, 25, 27, and 90 days. The two shortest lived replacements were still white at mortality and the scallop that lived for 25 days in the tank was light brown, the mortality at 27 days was gray, and the scallop that lived for 90 days was brown.

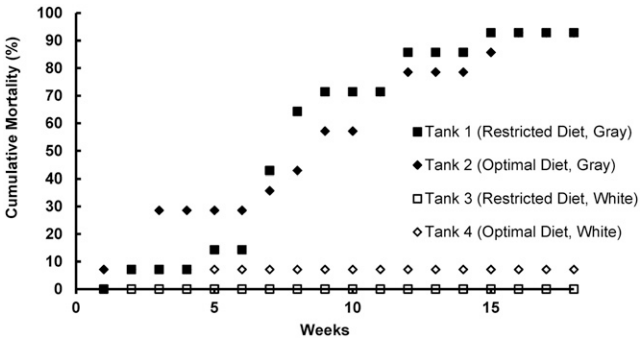

Figure 7. Cumulative mortality of original 14 scallops per tank over the 18-wk optimal/restricted diet laboratory experiment.

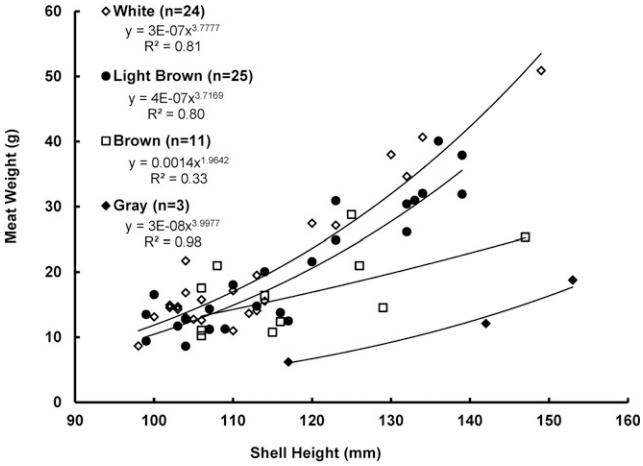

Figure 8. Shell height–meat weight data with scallops from the end of the optimal/restricted diet laboratory experiment fit with power trend lines.

The original gray-meat scallops in tank 1 (restricted, gray) lived for an average of 52 days (SD = 22.85; $n = 13$). The white-meat replacements in the gray tank 1 lived for an average of 58 days (SD = 31.20; $n = 3$). On average, the original gray-meat scallops in tank 2 (optimal, gray) lived for 54 days (SD = 32.84; $n = 13$). The white-meat replacements in the gray tank 2 lived for an average of 31 days (SD = 30.45; $n = 5$). Tank 3 (restricted, white) experienced no mortalities, whereas tank 4 (optimal, white) only experienced a single mortality on day 8 of the experiment.

At the end of the experiment, scallops from all tanks were dissected. Tanks 1 and 2 only had one original scallop for the duration of the experiment, whereas tanks 3 and 4 had all original scallops except for the one replacement in tank 4. In tank 1 (restricted, gray), only one scallop was still white with six light brown, five brown, and one gray scallop. The last original gray-meat scallop in this tank seemed moribund, having no response to being removed from water, and a very small and stringy gray meat. In tank 2 (optimal, gray), five scallops were still white, four light brown, three brown, and one gray. The last original gray-meat scallop in this tank was equally moribund. Tank 3 had only two white scallops remaining, nine light brown, two brown, and one gray. Tank 4 had eight white scallops remaining, five light brown, and one brown scallop. At the end of the experiment, there were seven remaining stock tank scallops, of which six were white and one light brown.

As darkening progressed (white to light brown to brown to gray), meat weight per unit length decreased in the laboratory samples (Fig. 8). A linear regression ANCOVA using shell height and meat weight with color as the interaction factor showed no significant differences between slopes (ANCOVA; $F_{(3,55)} = 1.95$; $P = 0.133$). There was a significant difference between white and gray scallop adductor muscle weights ($P < 0.001$) as well as between white and brown ($P < 0.05$), but not between white and light brown ($P = 0.07$).

When compared with shell height–meat weight data from field-collected scallops (larger sample size), the same trends appeared (ANCOVA; $F_{(31,55)} = 2.23$; $P = 0.087$). All infected categories (light brown, brown, and gray) were highly significant compared with white-meat scallops ($P < 0.001$). This included a significant difference between white and light brown shell height–meat weight ratios (Fig. 9).

The peak of each desaturated ellipse color curve was recorded for 49 scallops. White-meat scallops had an average peak of 130 (SD = 7.03; $n = 20$), 123 for light brown scallops (SD = 5.76, $n = 6$), 109 for brown scallops (SD = 6.45; $n = 6$), and 105 for gray scallops (SD = 6.61; $n = 17$). There was a highly significant difference in darkness of muscle between groups (ANOVA; $F_{(3,45)} = 42.19$; $P < 0.001$; Tukey $P < 0.001$). There was, however, no significant difference between white and light brown scallops, or between brown and gray scallops ($P > 0.05$).

May-Grünwald Giemsa stains the muscle tissue pink and nuclei blue. Distinguishing muscle nuclei from hemocyte and various parasite stage nuclei was difficult. The number of nuclei, including parasite stages, hemocyte and muscle cell nuclei, was counted (collectively) and averaged for each study group. The field-collected gray-meat scallops had an average of 164 dots per image (SD = 85.87; $n = 7$), whereas the laboratory gray-meat scallops had an average of 217 dots per image (SD = 114.06; $n = 7$). The field-collected white-meat scallops had an average of 24 dots per image (SD = 17.90; $n = 7$), whereas laboratory white-meat scallops averaged 120 dots per image (SD = 36.69; $n = 7$).

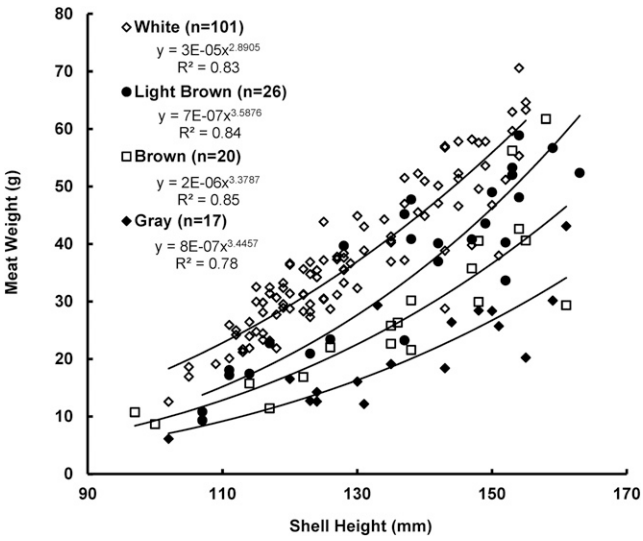

Figure 9. Shell height–meat weight data with scallops collected from the field fit with power trend lines.

There was a significant difference in parasite intensity between gray-meat scallops in the laboratory and the field (ANOVA; $F_{(1,62)} = 0.57$; $P < 0.05$; Tukey $P < 0.001$) and a highly significant difference between white-meat scallops in the laboratory and the field (ANOVA; $F_{(1,62)} = 0.24$; $P < 0.001$; Tukey $P < 0.001$).

There were 242 white-meat scallops, and 112 light brown-meat, 33 brown-meat, and 20 gray-meat scallops were examined for senescence and intensity of boring in relation to meat color. White-meat scallops had an average shell height of 134 mm (SD = 15.63), 143 mm for light brown scallops (SD = 14.41), 135 mm for brown scallops (SD = 16.47), and 139 mm for gray scallops (SD = 15.52). There was only a significant difference in shell height between light brown and both white and brown meats. There was no significant difference between size and scallop muscle color in adult scallops (ANOVA; $F_{(3,403)} = 8.02$; $P < 0.001$).

Intensity of boring data was grouped in the contingency tables for each of the four color categories. White-meat scallops ($n = 242$) had 62% with 0 to low infestation levels of boring sponge and worm, 10% with low worm and high sponge, 10% with high worm and low sponge, and 17% with high sponge and high worm infestation. This ratio was used to create chi-square expected results for each infected color category (light brown, brown, and gray) as if boring intensity was not linked to meat darkening (Table 1). Infestation levels for each color category were all significantly different than expected ($P < 0.05$). The expected and actual intensity of boring levels were combined for all discolored scallops ($n = 165$), and differences were highly significant (ANOVA; $F_{(3,162)} = 5.35$; $P = 0.10$; Tukey $P < 0.001$). Infected scallops ($n = 165$) had 25% 0 to low infestation level of boring sponge and worm, 20% with low worms and high sponge, 15% with high worms and low sponge, and 40% with high sponge and high worm infestation.

DISCUSSION

Gray-meat infection was swift and fatal; 26 of the original 28 gray-meat scallops died. Of the original 28 white-meat scallops in the optimal- and restricted-diet tanks, only one scallop died. There seemed to be variability in progression of the disease; for the five replacement mortalities in tank 2, the amount of time in the gray-meat tank did not fully correspond to the meat quality and color observed after death. One white scallop spent 27 days in a gray-meat tank and turned gray, another spent 90 days in a gray-meat tank but was only brown.

Scallops with gray meat had a smaller meat weight per shell height compared with white-meat scallops. In Icelandic scallops, as infection intensity increased, moisture levels increased, and protein levels diminished (Kristmundsson et al. 2011). This appeared true for sea scallops collected from the field and held in the laboratory, although scallops in the laboratory were in the poorest condition. It is likely "optimal conditions" in a laboratory were below natural healthy conditions.

Whether a scallop adductor muscle is white or gray was determined by GIMP. The range for gray-meat scallops was $x = 82$–113, and white-meat scallops $x = 120$–147, so there was a threshold for gray-meat at $\sim x = 115$. It was difficult to determine between less distinct shades, that is, white to light brown or brown to gray, with the image analysis system used.

Microscopically, all field-collected white-meat scallops were infected by the apicomplexan, but in low intensities and with no physiological or visual evidence. Infection is not synonymous with disease. There may be an infectious agent but the host is not technically diseased until the host cells, tissues, or organs are damaged or destroyed, resulting in an abnormal state of function as well as showing signs and symptoms of disease (Snieszko 1973). The emergence, prevalence, and severity of the disease state of an animal is based on the symbiotic interaction of the pathogen (density, pathogenicity), host (shell quality, nutritional factors), and environment (ocean temperatures, currents, microbial communities) (Snieszko 1973).

Darkened scallop muscle color was associated with apicomplexan parasite infection intensity. Comparing total counts of nuclei was the best proxy to represent infection levels without being able to differentiate nuclei cell types. The necrosis of muscle tissue associated with gray meat could also correlate with a loss of nuclei.

In this study, scallops with shells exhibiting high levels of boring sponge and worm damage had significantly higher rates of gray meats than less-impacted animals, although the correlation does not equal causation. High levels of boring organisms also existed in some scallops that showed no signs of infection.

Senescence did not appear to correlate with increased darkening of the meat, although sample sizes were low for brown- and gray-meat scallops. Using shell height as a proxy for age is risky, and does not take into account differences in growth rates (Merrill et al. 1965, Barber et al. 1988, Rothschild et al. 2009). Further, the inclusion of scallops less than 90 mm could change the outlook for the effect of senescence, and a difference in infection intensity could be observed between scallops of fishery size compared with those that are too small.

In sea scallop management, shell height–meat weight relationships allow for the conversion from numbers of scallops at a given size to equivalent meat weights. There are three different relationship models: one for the open and closed areas of

TABLE 1.

Intensity of boring worm and boring sponge from white scallops and all infected scallops combined.

	Boring sponge					
	White		Infected		Expected	
Boring worm	0/low	Medium/high	0/low	Medium/high	0/low	Medium/high
0/low	150	25	41	33	102	17
Medium/high	25	42	25	66	17	29

Expected intensity of infected scallops if boring organisms did not influence meat darkening. Measure of intensity of boring worms is presented vertically and intensity of boring sponge is presented horizontally.

Georges Bank, and a separate relationship for the mid-Atlantic (NEFSC 2014).

The shell height–meat weight equation differed significantly for each of the four categories of scallop meat color (infection) examined. In areas with gray-meat scallops using a single shell height–meat weight ratio derived from healthy "white-meat" scallops will likely produce an overestimate of biomass. Precise, area-specific data on the impact and intensity of gray-meat infection would be necessary to accurately estimate marketable, harvestable biomass. As an example, different proportions of gray-meat scallops were inserted into scallop counts, and shell height measurements were made from the School for Marine Science and Technology scallop video survey in 2014 for access areas on Georges Bank, CAII, and NLCA, to determine the effect on the immediate biomass estimate.

In 2014, the NLCA contained 3,891 mt of exploitable scallop biomass with an average meat weight of 17.33 g (SE = 3.77). Counts and measurements of scallops less than 90 mm were transformed to biomass using the traditional shell height–meat weight equation used in stock assessments (NEFSC 2010). Representing an extreme case, if the population of adults (>90 mm) in the area was entirely gray meat, only 1,835 mt of biomass would be expected and the average meat weight fell to 8.18 g (SE = 1.78). If half of the scallops greater than 90 mm exhibited gray meat, only 2,863 mt of biomass would be expected with an average meat weight of 12.75 g (SE = 2.77).

Closed Area II in 2014 contained 2,963 mt of exploitable scallop biomass with an average meat weight of 18.27 g (SE = 5.73). Again, the 50th SAW (2010) shell height–meat weight relationship was used on scallops less than 90 mm. If all scallops greater than 90 mm had gray meat, the expected biomass would be 1,564 mt, with an average meat weight of 9.64 g (SE = 3.02). In the area, if half of the scallops greater than 90 mm were gray, average biomass would be 2,263 mt, with a meat weight of 13.95 g (SE = 4.37). These examples indicate the importance of an accurate shell height–meat weight equation in forming applied harvestable biomass estimates. An overestimation of biomass in an area will result in increased allocations of harvest, causing economic lose and overfishing. Further, scallops exhibiting signs of gray-meat infection will perish, so an estimate of natural mortality of scallops should include infection rates in stock assessment.

The presence of gray-meat scallops in areas changes how fishing vessels operate, forcing them to high grade (discard diseased meats) or move from an area. In 1994, Fisheries Management Plan Amendment 4 changed the management strategy from meat count regulation to effort control within the U.S. EEZ. Landings per unit effort changes with strong recruitment events, as well as low catch rates. The majority of gray-meat scallops that are shucked tear with the viscera, or are rejected due to poor quality and infected meats, fall back to the sea floor. Estimates of landings per unit effort would be assumed to be lower than expected with the introduction of diseased scallops; however, this estimate does not take into account diseased individuals.

Future work should include transmission trials to understand if the parasite can live freely in the water column, and for how long. Tracking the occurrence and prevalence of gray-meat scallops would be very advantageous in understanding transmission and other factors leading to infection as the impact of the disease is likely region specific. Additional research on how gray-meat disease affects fecundity is needed to determine how disease may affect reproductive abilities and therefore recruitment models for the stock. Gray-meat disease is a threat to a very productive industry, and more research needs to be conducted to understand how to manage it.

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