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Identification of a *Mycobacterium* sp. as the causative agent of orange nodular lesions in the Atlantic sea scallop *Placopecten magellanicus*

Catherine Grimm[1], Carl Huntsberger[2], Kathryn Markey[1], Susan Inglis[3], Roxanna Smolowitz[1,*]

[1]Aquatic Diagnostic Laboratory, Roger Williams University, One Old Ferry Road, Bristol, RI 02809, USA
[2]Coonamessett Farm Foundation, 277 Hatchville Road, East Falmouth, MA 02536, USA
[3]University of Massachusetts-Dartmouth, SMAST, 200 Mill Road, Fairhaven, MA 02719, USA

ABSTRACT: The Atlantic sea scallop *Placopecten magellanicus* is an economically important species in the offshore fisheries on the east coast of the USA. Recently, animals collected from waters ranging from Massachusetts to Maryland have shown variably sized (up to 1 cm in diameter) orange nodular foci, predominantly in the adductor muscle tissue, but also in other organs. Histological evaluation of the nodular lesions showed rod-shaped bacteria that stain acid-fast positive and Gram-positive. PCR methodology was employed to identify the causative organism of the nodules as a *Mycobacterium* sp. using analysis of the partial 16S gene and the 16S-23S internal transcribed spacer region. Based upon genotypic findings, the causative bacterium fits well into the genus *Mycobacterium*.

KEY WORDS: Atlantic sea scallop · PCR · *Placopecten magellanicus* · *Mycobacterium* sp. · Orange nodules

INTRODUCTION

Atlantic sea scallop *Placopecten magellanicus* populations found in the northwest Atlantic have supported a valuable wild fishery in recent years. Between 2003 and 2011 landings exceeded 50 million pounds (22.7×10^6 kg) annually. Total fleet revenues in 2011 were $585 million (NOAA 2013). The range of this species extends from Cape Hatteras to Newfoundland, with the bulk of the fishing effort occurring on localized sea scallop beds in the mid-Atlantic Bight and on Georges Bank (Black et al. 1993, NEFMC 2014, Posgay 1957).

Annual sea scallop fishery harvest allotments are defined by management and based on predetermined recruitment patterns of the stock (Naidu & Robert 2006). Unfortunately, sea scallop populations suffer from irregular recruitment creating a discrepancy between yearly allotments; for example, the projected catch for 2013 and 2014 was 45.8 million pounds (20.8×10^6 kg) annually, a significant decrease from the 2010 harvest numbers of 59.1 million pounds (26.9×10^6 kg) (NEFMC 2014, NOAA 2013). The decrease in projected catch from 2010 to 2014 is hypothesized to be a result of poor recruitment (NEFMC 2014).

Maintaining a fishable stock requires an accurate estimate for natural mortality, which includes mortality rates for aging and predation, as well as disease. Disease is often overlooked or not calculable as a cause of decreased or decreasing populations in marine animals. Additionally, the identification of disease as a cause of decreased populations, or poor recovery of populations, depends on alert observations by fisherman and researchers. In 2011, orange nodules were observed in the adductor muscle of sea scallop fished from the north-west Atlantic coast.

*Corresponding author: rsmolowitz@rwu.edu

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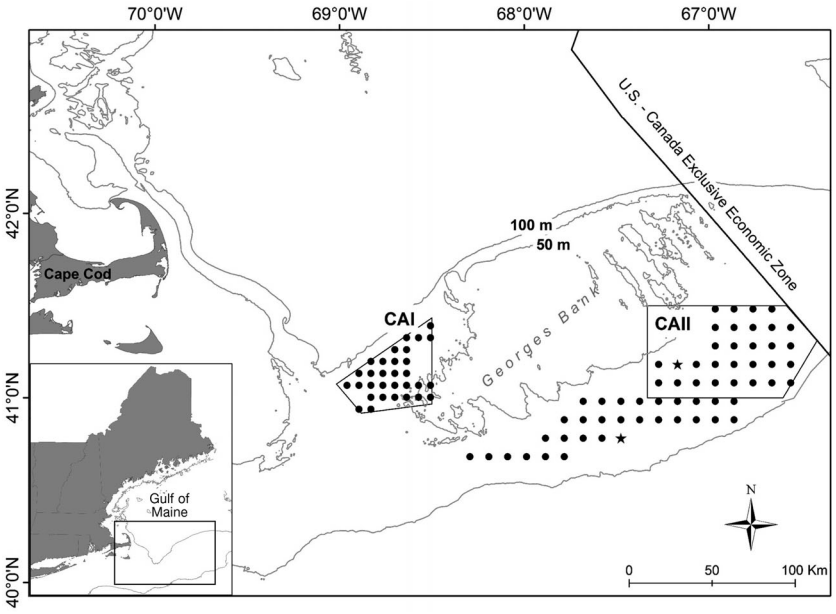

Fig. 1. Location of fixed survey stations for the CFF Seasonal Bycatch survey in Closed Area 1 (CAI; 31 stations), Closed Area II (CAII; 30 stations), and open area stations (30 stations). Scallops *Placopecten magellanicus* with orange nodules were rarely collected outside of CAI; stars represent those few sites (Stn 310 in the open area and Stn 226 in CAII)

From November 2012 to March 2014, Coonamessett Farm Foundation (CFF) researchers, as part of the seasonal bycatch survey, monitored and collected tissues from fished sea scallops. Potential impacts upon the health of the natural populations, the quality of marketable meat, and the cause of these lesions were of concern. This report describes and identifies the cause of orange nodules in Atlantic sea scallops from areas on, and adjacent to, Georges Bank, in the western Atlantic Ocean.

MATERIALS AND METHODS

Sources of samples

Atlantic sea scallops with visibly identifiable orange nodules were collected during the seasonal bycatch survey that routinely sampled fixed fishing stations within the scallop access areas on Georges Bank (Fig. 1). The stations were concentrated in the Closed Area One access area (CAI), the Closed Area Two access area (CAII), and open fishing grounds between the 2 closed areas. At each station, 2 commercially rigged scallop dredges were simultaneously towed. The catch was evaluated for abundance of sea scallops and groundfish. A 1-bushel (~35.2 l) subsample of the sea scallop catch was measured for shell height and meat weight at each station. At randomly selected stations (35 of the 91 stations), twelve sea scallops were indiscriminately selected from the subsample for dissection and processing as described in the next subsection. During dissection the sea scallops were also examined for any abnormalities. The number of sea scallops with orange nodules was recorded and used to calculate the percentage of affected scallops for that tow (see Table 3). When time allowed, additional animals were examined for orange nodules which increased the number examined per tow in some sites/times (see Table 3). All sea scallops positive for orange nodules from December 2012 to July 2013 were macroscopically examined for tissue location of nodules (see Table 1, n = 18) but animals collected after July 2013 were also examined for the number of nodules in each tissue (see Table 2, n = 26). Orange nodules were excised and fixed for histological examination and molecular evaluation.

Histopathology

Tissues containing nodules from each animal identified as positive (n = 27) were fixed in 10% formalin in seawater (Howard et al. 2004). Samples of fixed tissue were embedded in paraffin, sectioned, and stained with haematoxylin and eosin. Sections from all animals were stained with Ziehl-Neelsen acid-fast stain and selected cases were stained with Brown and Brenn Gram stain (Mass Histology Service) (Howard et al. 2004). Stained sections were evaluated for location, appearance and staining characteristics on an Olympus BX40 compound microscope with an attached Olympus DP 25 camera.

DNA extraction and PCR amplification

DNA was extracted from the orange nodules using Qiagen DNEasy Blood and Tissue Kit (Qiagen #69504), according to the manufacturer's protocol. Quantification was performed using a Thermo Scientific NanoDrop #2000c spectrophotometer. Samples were also analyzed for quality with a desirable 260nm/280nm ratio between 1.80–2.00.

PCR amplification of a 540 bp fragment of the 16S-23S internal transcribed spacer region and partial 23S region was performed using genus-specific mycobacterial primers ITS-F (5′-TGG ATC CGA CGA AGT CGT AAC AAG G-3′) and ITS-R (5′-TGG ATC CTG CCA AGG CAT CCA CCA T-3′) (Integrated DNA Technologies) (Park et al. 2000, 2005). The PCR protocol began with an initial denaturation step of 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min; with a final elongation step of 72°C for 10 min (Park et al. 2000).

Amplification of the 924 bp fragment of the 16S gene was performed with genus-specific mycobacterial primers T_{39} (5′-GCG AAC GGG TGA GTA ACA CG-3′) and T_{13} (5′-TGC ACA CAG GCC ACA AGG GA-3′) (Integrated DNA Technologies) (Talaat et al. 1997, Yadav et al. 2003). The PCR protocol was carried out with an initial denaturation step of 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min; with a final elongation step of 72°C for 10 min (Yadav et al. 2003).

All initial reactions were carried out in 12.5 µl containing 400 nM of each primer, 10.5 µl of Platinum® PCR Supermix (22 U ml^{-1} *Taq* DNA, 22 mM Tris-HCl [pH 8.4], 55 mM KCl, 1.65 mM $MgCl_2$, and 220 µM dNTP; Invitrogen # 11306-016) each with 1 µl of template DNA. These 12.5 µl reactions were used as an initial screening to determine the best samples to perform large-scale PCR reactions for genetic analysis. *Mycobacterium marinum* DNA (ATCC BAA-535D-5) was used as the positive control for all reactions. The products were visualized through gel electrophoresis (1.5% agarose), stained with GelRed (Biotium #41003) in 1× TAE buffer, run at 80 V for 1 h and visualized under UV light.

Sequence and phylogenetic analysis

Large-scale PCR reactions for sequencing were carried out in a total volume of 50 µl with the same concentrations as previously mentioned. Five discrete samples were chosen based on the occurrence of macroscopically identifiable nodules in the adductor muscles and amplification in the small-scale PCR reactions for both targeted gene sequences. Volumes of each reagent were extrapolated accordingly, using 1 µl of template DNA and both primer pairs. Gel electrophoresis, as described in the previous section, was used to isolate the desired product. The DNA extraction from the agarose gel protocol followed the manufacturer's instructions for the Wizard SV Gel and PCR Clean-Up System protocol (Promega #A9281). Single-strand Sanger sequencing was prepared using 0.42 µM of one primer (forward or reverse) and 2.5 ng of DNA per 100 bp, with a final volume of 24 µl. Purified PCR product was processed through Sanger sequencing at the Rhode Island Genomics and Sequencing Center, University of Rhode Island.

Initial genetic similarities were determined using BLAST (Madden 2013). Neighbor-Joining Phylogenetic trees were constructed using the Maximum Composite Likelihood model in MEGA v6 software with the addition of related sequences from GenBank (Tamura et al. 2013). Statistical confidences in tree branches were generated by performing 500 bootstrap replicas.

Culture

Culture of nodules occurred at sea as infected tissues were identified. Foci were sampled by sterilely cutting into the muscle lesions and sampled using a sterile loop. The loop was streaked on Middlebrook 7H10 Agar (Sigma-Aldrich #M0303-500G) in slant tubes (n = 10). The tubes were incubated in ambient air temperature for 2 d (on board the boat) before returning to the laboratory where they were maintained at room temperature for 14 d.

RESULTS

Gross results

In affected sea scallops, orange nodules were present macroscopically, most commonly in the adductor muscle (Fig. 2, Tables 1 & 2). For the samples collected after September 2013, abundance of macroscopic nodules by tissue type was recorded (Table 2). In those individuals exhibiting severe infections in the adductor muscle, orange nodules in the mantle and gonads were usually grossly identified.

Histology

All orange nodules showed similar microscopic characteristics. Orange nodules collected from the adductor muscles were composed of a haemocytic response and consisted of a central caseous core of necrotic debris intermingled with some intact

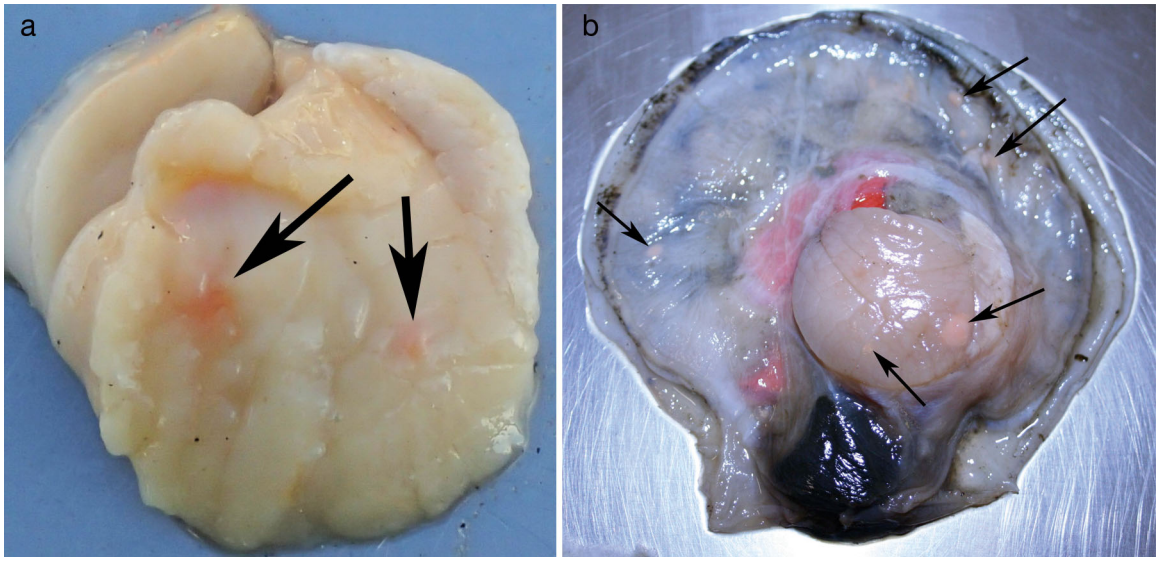

Fig. 2. Atlantic sea scallop *Placopecten magellanicus* showing orange nodules (arrows) in (a) adductor muscle only and (b) adductor muscle and mantle

Table 1. Specific host tissue in which nodules were identified and collected during the seasonal survey. Samples were collected on survey trips conducted between December 2012 and July 2013. Only presence (X) or absence (0) of nodules was recorded for each tissue. Station numbers match the seasonal bycatch survey grid stations (see Fig. 7)

Year	Month	Station/ animal no.	Location of macroscopically visible nodules/animal		
			Muscle	Gonad	Mantle
2012	December	101-1	0	X	0
		135-1	X	0	0
		119-1	X	0	0
		119-1	X	0	0
		118-1	X	0	0
2013	January	129-1	X	0	0
	March	128-1	X	0	0
		129-1	X	0	0
		136-1	X	0	0
		119-1	X	0	0
	April	310-1	X	0	0
	June	226-1	X	0	0
		126-1	X	0	0
		128-1	X	0	0
		128-2	X	0	0
	July	119-1	X	0	0
		136-1	X	0	0
		136-2	X	0	0

haemocytes. This core was circumferentially surrounded by several layers of round to oval haemocytes then delineated externally by a layer of squamous (flattened) appearing haemocytes (Fig. 3). Such inflammation is termed 'encapsulation' in older invertebrate descriptions but is also termed granu-

Table 2. Total number of macroscopically identified nodules by tissue for each sea scallop sample collected during the seasonal bycatch survey from September 2013 until March 2014. More detailed observations were conducted during these trips than in previous trips. Station numbers match the seasonal bycatch survey grid stations (see Fig. 7)

Year	Month	Station/ animal no.	No. of visible macroscopic lesions/tissue/animal		
			Muscle	Gonad	Mantle
2013	September	136-1	2	0	0
		136-2	4	0	1
		136-3	4	0	0
		136-4	2	0	0
		135-1	1	0	0
		112-1	3	0	0
		119-1	2	0	0
		119-2	5	0	0
	October	132-1	4	1	0
		132-2	3	0	0
		132-3	3	0	0
		131-1	3	0	0
		119-1	5	0	0
	December	125-1	1	0	0
		124-1	4	0	0
		112-1	4	0	0
		119-1	3	0	0
2014	January	126-1	1	0	0
		136-1	11	5	1
		136-2	8	0	0
		136-3	4	0	0
	March	135-1	1	2	0
		136-1	2	0	0
		136-2	1	0	0
		136-3	10	0	0
		127-1	10	0	0

loma formation in newer literature and as used in vertebrate pathological descriptions. Numerous rod-shaped, acid-fast positive and Gram-positive bacteria were present within the inner layers of intact haemocytes surrounding the caseous centers and were both free and within remaining intact haemocytes in the caseous centers (Fig. 4). In most cases the nodules appeared to effectively sequester the bacteria, but in some instances the lesions were not well contained by the response; these cases showed haemocytic inflammation, myonecrosis, and mild oedema.

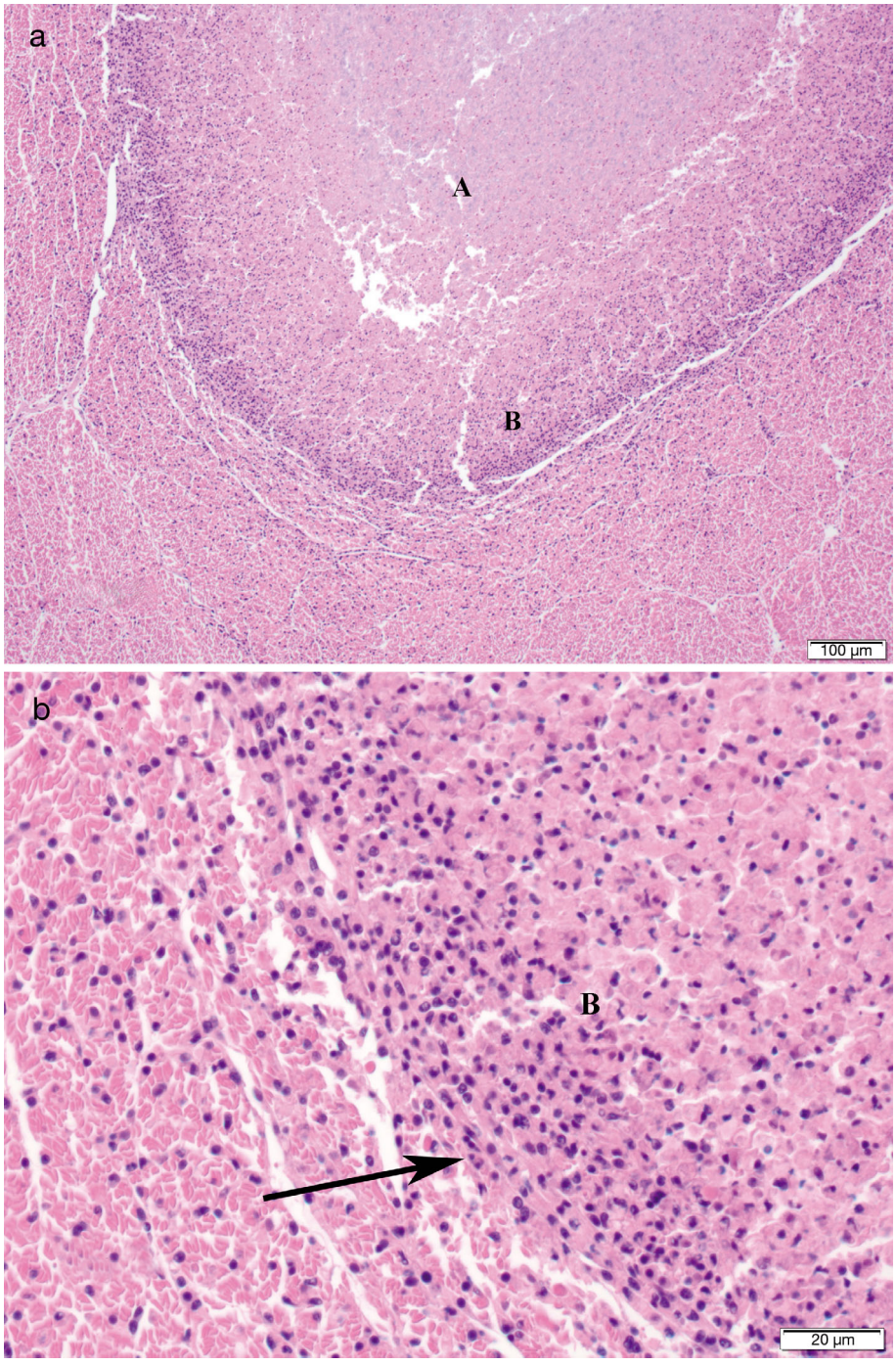

Fig. 3. Histological sections of a sea scallop *Placopecten magellanicus* showing a granuloma (encapsulation) in the adductor muscle at (a) low and (b) high magnification (haematoxylin and eosin stain of paraffin-embedded tissue). A: caseous central debris; B: haemocytes surrounding debris; arrow: peripherally flattened haemocyte layer

In several animals, areas of haemocytic inflammation were noted in other tissues. Such foci were especially common in the sinusoids and connective tissues adjacent to the intestinal loops, style sac, and gastric epithelium, but were also noted in the digestive gland, kidney, gills and mantle. These other foci consisted of smaller granulomas ranging in diameter from 300 to 50 µm or less and usually did not contain a core of necrotic tissue as in the large granulomas. Sections from animals containing granulomas in other tissues were examined using acid-fast stain and showed acid-fast positive short rod-shaped bacteria and acid-fast debris in the haemocytes (Fig. 5). No other organisms were identified in the affected tissues in any animals examined.

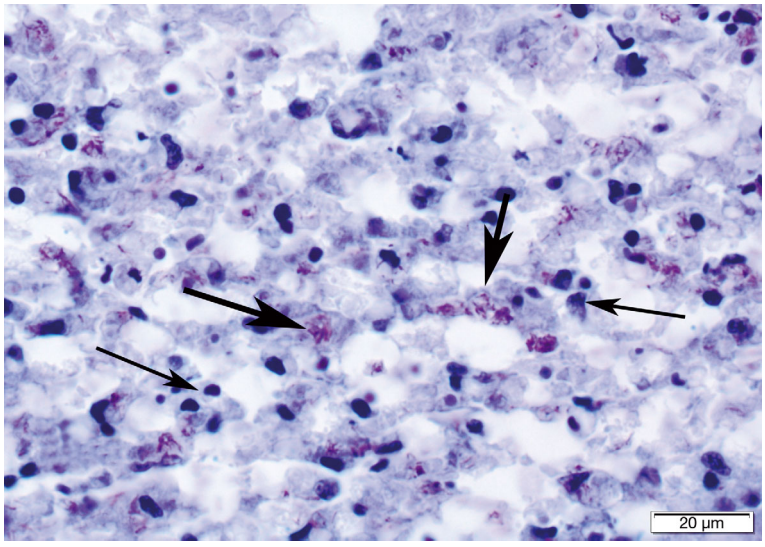

Fig. 4. Scallop *Placopecten magellanicus* adductor muscle showing acid-fast positive bacteria in intact haemocytes (large arrows) and free in the debris in the center of a granuloma. Haemocytes without visible bacteria are also visible in the granuloma (small arrows) (Ziehl-Neelsen acid-fast stain of paraffin-embedded tissue)

Sequence and phylogenetic analysis

The 12.5 µl and 50 µl PCR reactions were both successful in amplifying the 16S and 16S-23S internal transcribed spacer region. The 16S-23S internal transcribed spacer region showed more consistent electrophoresis results between separate assays. A representative electrophoresis gel can be seen in Fig. 6.

Samples from 5 animals (also examined histologically) were sent for sequencing for each of the 2 genetic segments sequenced. The genetic variation between the different samples within each distinct sequence was <15 nt (3%).

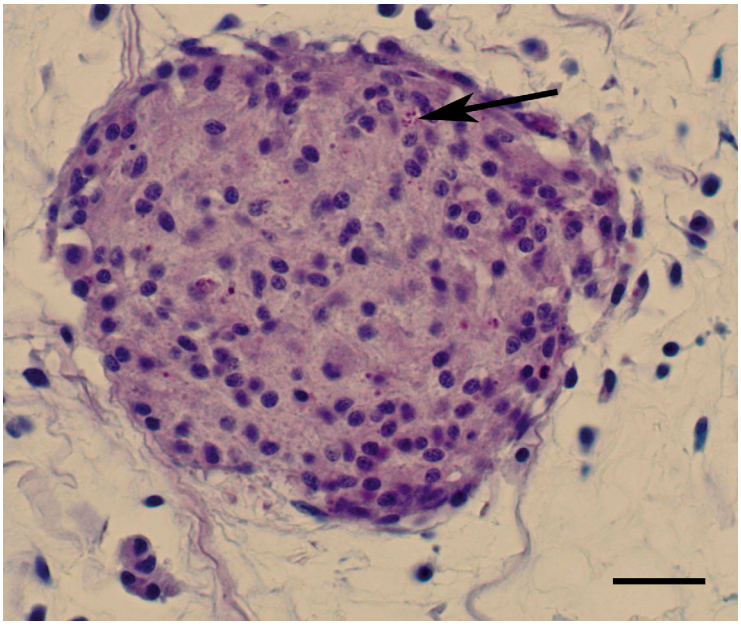

Fig. 5. Photomicrograph showing acid-fast positive bacteria (arrow) in haemocytes forming a granuloma in perigastric connective tissue (Ziehl-Neelsen acid-fast stain of paraffin embedded tissue). Scale bar = 20 µm

Culture results

No bacterial colonies grew on Middlebrook 7H10 Agar. Lack of growth on the media is consistent with past attempts to culture *Mycobacterium* sp. from natural sources. The lack of mycobacterial growth does not negate its presence in the orange nodular lesions. Culturing fastidious organisms on fishing boats, at sea for extended times, is problematic. Future work by CFF will use new methods and equipment to address this problem.

DISCUSSION

Mycobacterium is an acid-fast staining bacterium that proliferates within phagosomes in the host cell (McMurray 1996). Several species of *Mycobacterium* are pathogenic in humans and are considered terrestrial disease threats, such as *M. tu-*

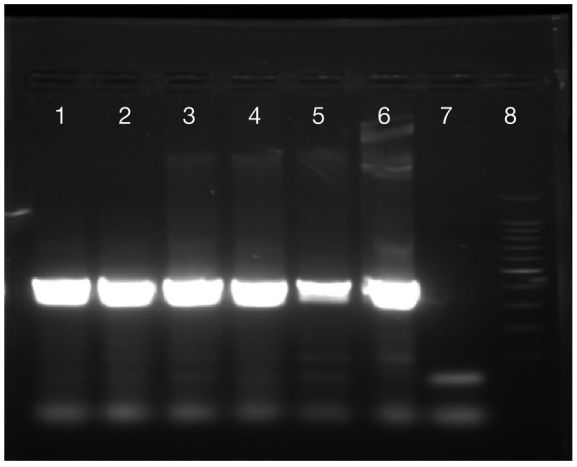

Fig. 6. Gel electrophoresis of a 12.5 µl volume PCR representing the Internal Transcribed Spacer (ITS) sequence, using primers ITS-F and ITS-R. Lanes 1–5: wild-caught scallop *Placopecten magellanicus* specimens with orange nodules. Lanes 6 and 7: positive and negative control, respectively. Lane 8: DNA ladder used for reference

berculosis (tuberculosis) and *M. leprae* (leprosy) (Bruijnesteijn van Coppenraet et al. 2004). *Mycobacterium* spp. can also cause significant problems in wild marine environments and in cultured fish. Mycobacteriosis in fish can be caused by various *Mycobacterium* spp.: *M. marinum, M. fortuitum, M. chelonae, M. pseudoshottsii,* and *M. shottsii* (Talaat et al. 1997, Selvaraju et al. 2005, Gauthier et al. 2010). Commonly aquacultured animals, such as the zebrafish *Danio rerio*, experience *Mycobacterium* sp. infections resulting in morbidity and mortality (Peterson et al. 2013). Mycobacterial infections, identified in Chesapeake Bay striped bass *Morone saxatilis* in 1984, are primarily caused by *M. pseudoshottsii* and *M. shottsii* (Jacobs et al. 2009, Gauthier et al. 2011).

In 1989, orange nodular lesions were identified in the adductor muscle and other tissues in cultured yesso scallop *Patinopecten yessoensis* (Bower & Meyer 1992, Bower et al. 1992) in British Columbia, Canada. Histologic and electron microscopic evaluation of the nodules showed lesions described as pustular reactions with necrotic cores. Low numbers of intracellular bacteria were noted in the pustules and were consistent with either *Mycobacteria* sp. or *Mycoplasma* sp. (Bower et al. 1994, p. 104–105), however the causative organism was never determined. The disease was suggested to be caused by stress resulting from poor culture practices, but no lesions occurred in the following year when the same culture practices were employed. Laboratory infection in which homogenates from the pustules were injected into yesso scallops resulted in mortality in 2 to 4 wk, but infection by immersion or cohabitation was not successful (Bower & Meyer 1992, Bower et al. 1992).

Orange nodular lesions were noted in fished wild sea scallops from the Damarascotta area of Maine in October 2008 and May 2009. The bacterium isolated from the lesions was found to have 99.5% homology with *Williamsia maris*, a non-acid fast staining bacterium (D. Bouchard, University of Maine, pers. comm. to R. Smolowitz).

Review of the catch data from the bycatch survey showed that prevalence of the infection may be location-specific; the eastern portion of Georges Bank showed the lowest prevalence with no infected samples observed during processing of the 12 random sea scallops per bushel/tow when outside of CAI (Fig. 1). Only when >12 animals were examined per tow in these other areas were infected sea scallops identified (2 scallops were observed with orange nodules when a larger sample size was evaluated but the number of sea scallops examined was not recorded). CAI showed the highest concentration of infected sea scallops (Fig. 7, Table 3). The central part of CAI showed higher rates of infection at the beginning of the study, but later surveys showed decreased infection rates coupled with a higher percentage of natural mortality indicators. There were

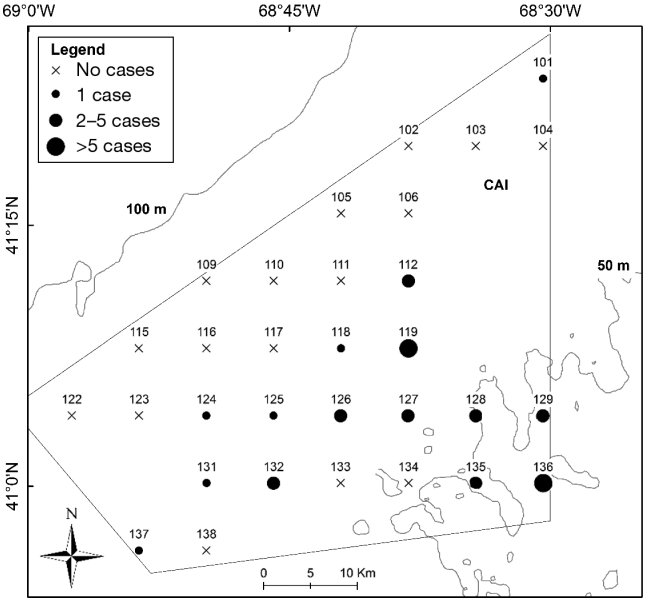

Fig. 7. Location and prevalence of collected scallops *Placopecten magellanicus* with observed orange nodules from November 2012 to March 2013 (12 trips). Each station is labeled with the station number which was consistent during the period of collection. As noted in the 'Materials and methods' the number of scallops sampled for orange nodules at each station for each trip was not consistent

Table 3. Prevalence (%) of orange nodules in scallops *Platopecten magellanicus* (n = 12 per station) identified during tows. Only stations which were positive for orange nodules inside CAI (15/31) are shown. Additional scallops at each station were evaluated when time allowed; this data is shown in parentheses: (number positive/total number examined, percentage of total)

Year	Month	Station														
		101	112	118	119	124	125	126	127	128	129	131	132	135	136	137
2012	Nov		0		0					0		0	0	0	0	
	Dec	0	0	0	16.7					0		0	0	8.3	0	
2013	Jan	0	0		0					0		0	0	0	0	0
	Mar		0		8.3					8.3		0	0	8.3	8.3	8.3
	Apr	0	0		0		0			8.3		0	0	0	0	0
	Jun		0		0	0				16.7		0	0	16.7	0	0
	Jul	0	0		0					0			0		16.7	
	Sep	0	0 (1/88, 1.1)		8.3	0					0	0		8.3 (1/31, 3.2)	16.7 (5/30, 16.7)	0
	Oct		0.00		0 (1/88, 1.1)	0						8.3	8.3 (3/22, 14)	0	0	0
	Dec		0 (1/30, 3.3)		0 (1/18, 5.5)		8.3					0	0	0	0	0
2014	Jan		0	0				8.3		0		0	0		16.7 (3/20, 15)	0
	Mar		0		0		0	8.3	16.7			0		0	0	

also many other factors influencing the sea scallop population during the time of this survey, including heavy commercial fishing, potential movement of stocks, spawning and metamorphic success, as well as other diseases. However, this data suggests there may be an association between mycobacterial infection and mortality.

(a)

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GAGTGCGGCT GGATCACCTC CTTTCTAAGG AGCACCACGA AAAGTATCTC
AATTCCTGAG ATACAGGCCG TGTGGAGTTC GCGTCTGTAG TGGACGGGAA
CTGGGTGCGC AACAACAAAC AAAACTACTG GACACACTAT TGGGCCCTGA
GGCAACAGCC GGTCAATTTG GGGCTGTTGT CCCCTCCATC TTGGTGGTGG
GGTGTGGTGT TTGAGAATTG GATAGTGGTT GCGAGCATCA AATGGATGCG
TTGCCCGCGT TGGTAGCGAA TTCATTTTGT GTAATTTTGT TCTTTGGTTT
TATTGTGTTT GTAAGTGTCT AAGGGCGCAT GGTGGATGCC TTGGCAGGAT
CCAA
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(b)

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TGGGTCTAAT ACCGGATAGG ACCACGGGAT GCATGTCCTG TGGTGGAAAG
CGATTAGCGG TGTGGGATGG GCCCGCGGCC TATCAGCTTG TTGGTGGGGT
GATGGCCTAC CAAGGCGACG ACGGGTAGCC GGCCTGAGAG GGTGTCCGGC
CACACTGGGA CTGAGATACG GCCCAGACTC CTACGGGAGG CAGCAGTGGG
GAATATTGCA CAATGGGCGC AAGCCTGATG CAGCGACGCC GCGTGGGGGA
TGACGGCCTT CGGGTTGTAA ACCTCTTTCA CCATCGACGA AGGTTCGGGT
TTTCTCGGAT TGACGGTAGG TGGAGAAGAA GCACCGGCCA ACTACGTGCC
AGCAGCCGCG GTAATACGTA GGGTGCGAGC GTTGTCCGGA ATTACTGGGC
GTAAAGAGCT CGTAGGTGGT TTGTCGCGTT GTTCGTGAAA ACTCACGGCT
TAACTGTGAG CGTGCGGGCG ATACGGGCAG ACTAGAGTAC TGCAGGGGAG
ACTGGAATTC CTGGTGTAGC GGTGGAATGC GCAGATATCA GGAGGAACAC
CGGTGGCGAA GGCGGGTCTC TGGGCAGTAA CTGACGCTGA GGAGCGAAAG
CGTGGGGAGC GAACAGGATT AGATACCCTG GTAGTCCACG CCGTAAACGG
TGGGTACTAG GTGTGGGTTT CCTTCCTTGG GATCCGTGCC GTAGCTAACG
CATTAAGTAC CCCGCCTGGG GAGTACGGCC GCAAGGCTAA AACTCAAAGG
AATTGACGGG GGCCCGCACA AGCGGCGGAG CATGTGGATT AATTCGATGC
AACGCGAAGA ACCTTACCTG GGTTTGACAT GCACAGGACG CGTCTAGAGA
TAGGCGTTCC
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Fig. 8. Sequence data for the unknown *Mycobacterium* species isolated from scallops *Platopecten magellanicus* collected from the North Atlantic in 2012–2013. (a) Homogenous partial 16S–23S ITS sequence; (b) 16S gene sequence generated from T_{13} and T_{39} primers

Distinct sequences in both the 16S and 16-23S internal transcribed spacer region (Fig. 8) distinguish the samples collected in this study from other published sequences (Figs. 9 & 10). This work shows the causative mycobacterial organisms for each of the infected samples examined in this study is the same species, and that the lesions in scallops from George's Bank may be caused by a genetically distinct mycobacterial species that resembles *M. marinum*. However, further studies must be conducted to better define the potential uniqueness of the *Mycobacterium* sp. identified in these animals.

Orange/pink nodular lesions previously identified in the Atlantic sea scallop fished from the Damarascotta area of Maine were caused by a non-acid fast bacteria, *Williamsia maris*. Identification of cause was inconclusive in other work conducted on the yesso scallop (Bower et al. 1994, p. 104–105). These findings in addition to our work suggest that the macroscopically visible orange color of the nodules are a result of the inflammatory response to injury in the scallop, and not directly related to the specific bacteria or other potentially infectious agents causing the infection.

Attempts to 'wall off' an infected site to halt the spread into other tissue is termed encapsulation in older (Tripp 1960), and some newer, invertebrate

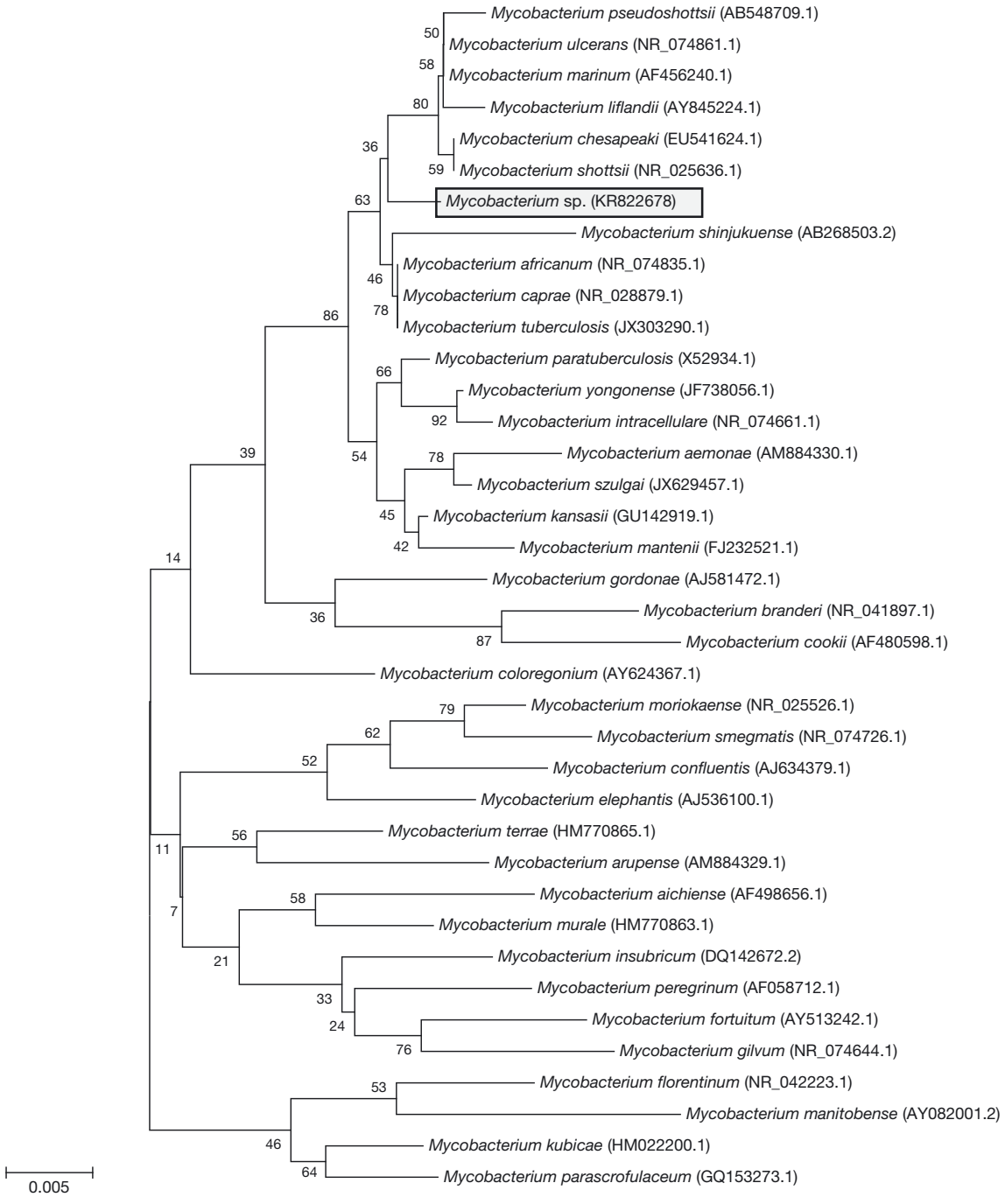

Fig. 9. Neighbor-joining phylogenetic tree of the mycobacterial 16S region, with additional sequences from GenBank (accession nos. in parentheses). *Mycobacterium* sp. (GenBank no. KR822678) represents the homologous sequence isolated from the collected samples of the present study

literature. Granulomas are a vertebrate pathological description of a similar immune response and even though the cell types are different in vertebrates, the function of granuloma formation is the same as encapsulation. Because the term, encapsulation, is not a generally recognizable inflammatory term, the term granuloma is used here. In this disease, granulomas were most commonly macroscopically identified in the adductor muscles of the scallops, but micro- and macroscopic nodules were present in other tissues of at least some infected animals. The common finding of grossly identifiable nodules in the adductor muscle

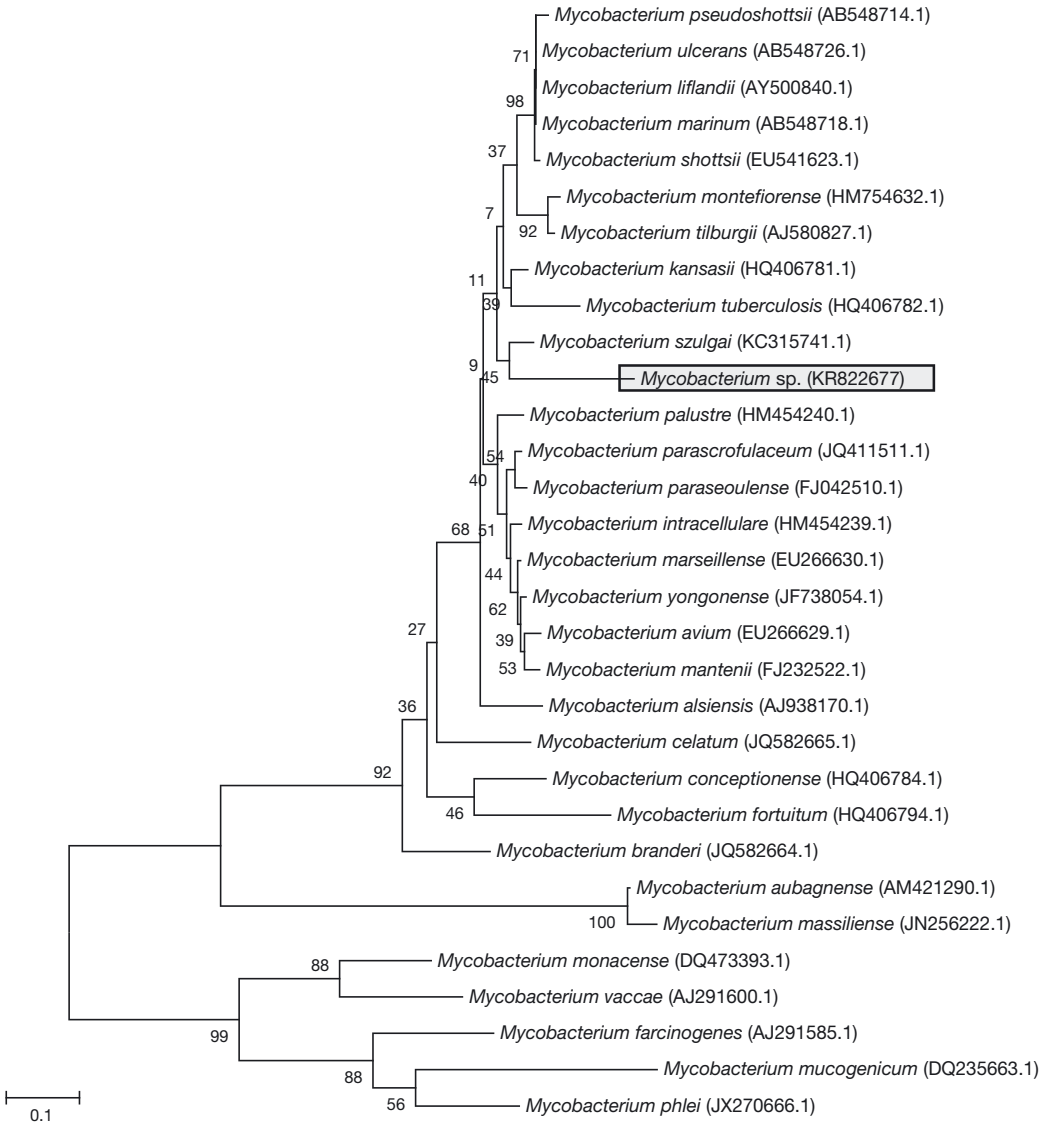

Fig. 10. Neighbor-joining phylogenetic tree of the mycobacterial 16S-23S ITS region, with additional sequences from GenBank (accession nos. in parentheses). *Mycobacterium* sp. (KR822677) represents the homologous sequence isolated from the collected samples of the present study

as compared to other tissues, without associated shell lesions, suggests that the muscle may provide a more permissive focus for pathogen proliferation.

There is speculation as to the method of infection/transmission of *Mycobacterium* sp. Identical species of *Mycobacterium* sp. have been isolated in menhaden *Brevoortia tyrannus* and striped bass (Stine et al. 2010). Menhaden is a common prey item for the striped bass, suggesting infection through the gastrointestinal tract. Salmon were shown to be infected through ingestion of contaminated prey items (Ross & Johnson 1962). Similarly, the identification of small granulomas associated with the gastrointestinal tract in sea scallops may implicate the gastrointestinal tract as the route of infection.

Mycobacteriosis is a cause not only of disease in humans, but also a potential food safety problem. *Mycobacterium marinum*, a surface-loving bacterium, can infect wounds in human extremities (Lewis et al. 2003). Other species of marine *Mycobacterium* have been shown to infect humans after contact with infected waters (Jõe & Hall 1995). Autoimmune diseases and drug therapies reduce human immune functions, increasing systemic health risks to humans coming in contact with the infected tissues (Panek & Bobo 2006).

Possible human impacts resulting from exposure to infected sea scallops are likely low. The highest risk is to fishermen who are shucking the scallops at sea to remove the adductor muscle. Fishermen usually do not retain abnormal meats for sale and other tissues of the shucked scallops are discarded at sea, so it is unlikely that obviously infected meats would be included in the marketable product brought to port. Additionally, the visible orange foci in the sea scallop muscle would result in a commercially unacceptable food product when processed at a restaurant or sold in a fresh seafood store.

George's Bank represents one of the world's largest producers of sea scallops (Naidu & Robert 2006). This study identified a new disease in the Atlantic sea scallop caused by *Mycobacterium* sp. Comparisons of the generated sequences to published sequences do not show specific fidelity to currently identified *Mycobacterium* species through 2 investigated gene sequences. This may indicate a new species of bacterium is responsible for disease in areas inhabited by the northeastern Atlantic sea scallop. Future work will determine the relatedness of this organism to other marine *Mycobacteria* species.

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