What Causes Gray Meat in the Atlantic Sea Scallop *Placopecten magellanicus* in Georges Bank Closed Areas?

Scallop RSA Project: NA12NMF4540036

Kevin D.E. Stokesbury, Susan D. Inglis

Collaborators: Arni Kristmundsson, Mark A. Freeman
What are “Gray Meat” Scallops?

Clinical Signs:

Discolored: Brown ➔ Gray, small, loosely bound adductor muscle vs. White, compact adductor muscle
History of Occurrence

Since 1936: Periodic reports of scallops with reduced, darkened (gray) adductor muscles:

- Stevenson (1936) described gray, stingy meat in scallops from the Bay of Fundy
  - related the condition to senescence.
- Medcof (1949) described darkened meat color in scallops off Digby, Nova Scotia
  - attributed to chronic infestation of older scallops in high densities by boring sponges (Clinoa sp.)
- Gulka et al. (1983) associated with a mass mortality in 1979-80 in Narragansett Bay, Rhode Island
  - Unidentified prokaryotic infestation
- Stokesbury et al. (2007) reported gray meat in scallops during a mass mortality in NLCA in 2004-2005.
  - Attributed to synergistic effect of senescence and parasitism by shell borers, and prokaryotic infection.
- Inglis and Stokesbury (2012) reported gray meat as incidental finding in laboratory experiment looking at effect of ration on shell carbonate $^{13}$C signature.
Impact on Fishery

- Associated with reduced harvestable biomass and mass mortality events.
- Commonly observed in rotational management areas on Georges Bank following periods of fishing closures.
**Objective:**
Examine possible causative agent(s) for gray meat quality.

1. **AGE** - Gray scallop meat is a result of old age and senescence in sea scallops.
   - Gray meat samples collected Bycatch Seasonal Survey 2012, 2013, 2014 (Coonamessett Farm Foundation).

2. **NUTRITION** - Scallops with gray meat exhibit poor nutritional status.
   - Frozen gray, brown, white adductor muscle submitted for proximate analysis.

3. **DISEASE** - Gray scallop meat results from parasitism by shell borers and/or prokaryotic infection.
   - Adductor muscle, mantle, and gill samples from gray, brown and white meat scallops collected for histology and DNA
   - Shells assessed for boring sponge and polychaete infestation.

4. **HABITAT** - Identify habitat characteristics associated with scallops with gray meat
   - SMAST video survey library
   - Video analysis of “nearest neighbors stations” to gray meat locations in CAI and CA II (YT Survey stations)
Methods:

Scallops with white, brown and gray meat color were collected from the rotational management areas on Georges Bank and analyzed for:

1. Shell height and meat color (n=613)
   - Age
2. Reproductive stage (n=395)
   - Senescence
3. Shell height : meat weight (n=395)
   - Condition/Nutrition
4. Adductor muscle composition (n=88)
   - Condition/Nutrition
5. Histopathology and molecular analysis (n=80)
   - Disease

Laboratories:
Kennebec River Biosciences
Dept. of Fish Diseases at the Institute of Pathology, University of Iceland
Seasonal Bycatch Survey Stations (Coonamessett Farm Foundation Scallop RSA NA13NMF4540011) in Georges Bank where randomly selected scallop samples were collected for shell height (mm): meat weight (g) and meat quality and histopathology (circled stations).
The percent of the shell height meat weight samples that contained discolored meat per station for CAI, CAII and open areas from Sept 2013-March 2014. The number of scallops observed at each station were red > 50%, yellow 25-49%, blue 1-24%, green 0%.
RESULTS: Age

Analysis 1:

The shell height for targeted gray meat scallops collected in 2012, 2013, 2014 (n=218).
RESULTS: Age

Analysis 2:

Pearson's Chi-squared test

<table>
<thead>
<tr>
<th>Sample</th>
<th>X²</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1</td>
<td>209.7</td>
<td>195</td>
<td>0.22</td>
</tr>
<tr>
<td>CA2</td>
<td>152.5</td>
<td>130</td>
<td>0.09</td>
</tr>
<tr>
<td>Open</td>
<td>85.0</td>
<td>70</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Pearson's Chi-squared test with simulated p-value did not change significance
RESULTS: Suggest some correlation but not causation

Analysis 3: GAMS Model: shell height - meat quality - location

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>AIC</th>
<th>Delta AIC</th>
<th>Deviance Explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>quality ~ s(ShellHt) + Location</td>
<td>7.27</td>
<td>1605.60</td>
<td>0.00</td>
<td>13.70%</td>
</tr>
<tr>
<td>quality ~ s(ShellHt, by = Location)</td>
<td>5.67</td>
<td>1694.76</td>
<td>89.16</td>
<td>8.63%</td>
</tr>
<tr>
<td>quality ~ s(ShellHt)</td>
<td>5.4</td>
<td>1697.48</td>
<td>91.88</td>
<td>8.49%</td>
</tr>
<tr>
<td>quality ~ Location</td>
<td>3</td>
<td>1764.14</td>
<td>158.54</td>
<td>4.51%</td>
</tr>
<tr>
<td>quality ~ 1 (Intercept only model)</td>
<td>1</td>
<td>1843.20</td>
<td>237.61</td>
<td>0%</td>
</tr>
</tbody>
</table>
RESULTS: Reproductive Stage

STAGE:
• White meat scallops (n=1753)
  - 6.37% PS, 4.28% S, 40.10% L, 23.56% D and 25% R.
• Gray meat scallop (n=48)
  - 10.42% PS, 8.33% S, 46.17% L, 22.92% D and 12.16% R.
• Brown meat scallops (n=287)
  - 8.71% PS, 13.59% S, 48.78% L, 12.89% D and 16.03% R.

➤ suggests scallops with gray and brown meat follow white meat or “normal” scallop reproductive cycles.

BUT GSI:

Significant difference between gray and white meat (n=71; p≤ 0.05)
White: 16.18 ± 1.72SD
Gray: 11.43 ± 2.16SD
No significant difference between brown and white.

Needs further assessment
RESULTS: Meat weight

The log-transformed shell height meat weight relationship between gray and white meat scallops in CAI, n=663 (left) from September 2013-March 2014.
The log-transformed shell height meat weight relationship between gray and white meat scallops in CAII, n= 867 from September 2013-March 2014
RESULTS: Meat weight

- There was a significant reduction in meat yield (ANOVA p<0.001) in both areas.
- Scallops reported as “brown” in the survey were included in the gray meat category.

Regression summary statistics for CAI and CAII

|          | Estimate | Std. Error | t value | Pr(>|t|)  |
|----------|----------|------------|---------|-----------|
| **CAI**  |          |            |         |           |
| Gray     |          |            |         |           |
| (Intercept) | -4.3815 | 0.8419     | -5.205  | 6.56E-06  |
| SH       | 2.674    | 0.3978     | 6.722   | 5.17E-08  |
| White    |          |            |         |           |
| (Intercept) | -3.90039| 0.1339     | -29.13  | <2e-16    |
| SH       | 2.55677  | 0.06353    | 39.79   | <2e-16    |
| **CAII** |          |            |         |           |
| Gray     |          |            |         |           |
| (Intercept) | -5.8278 | 0.8484     | -6.869  | 1.17E-08  |
| SH       | 3.3891   | 0.3913     | 8.66    | 2.25E-11  |
| White    |          |            |         |           |
| (Intercept) | -4.54568| 0.15217    | -29.87  | <2e-16    |
| SH       | 2.8523   | 0.07168    | 39.79   | <2e-16    |

Significance codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’
RESULTS: Muscle Composition

Map of sample collection locations for proximate analyses. The red shading represents samples from the general area.
The moisture:protein ratio (mean percent wet weight ± SD) found in white, brown and gray meat scallops. In Atlantic sea scallops a ratio of 4.0-4.9 is considered “normal” by the FDA and USDA.
RESULTS: Muscle Composition

Proximate composition (mean percent wet weight ± SD) of adductor muscle in Atlantic sea scallops from Georges Bank. There was a significant reduction in % protein and carbohydrate and inverse increase in moisture content in brown and gray meat compared to white meat scallops n=88; (ANOVA, p<0.05).

<table>
<thead>
<tr>
<th>Analysis</th>
<th>White (n=33)</th>
<th>Brown (n=26)</th>
<th>Gray (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>77.86 ± 2.56</td>
<td>80.82 ± 2.33</td>
<td>90.33 ± 3.05</td>
</tr>
<tr>
<td>Protein</td>
<td>17.68 ± 1.68</td>
<td>14.81 ± 2.57</td>
<td>6.97 ± 1.01</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>2.56 ± 0.87</td>
<td>0.62 ± 0.92</td>
<td>0.08 ± 0.76</td>
</tr>
<tr>
<td>Ash</td>
<td>2.90 ± 0.24</td>
<td>3.67 ± 0.14</td>
<td>2.77 ± 0.14</td>
</tr>
<tr>
<td>Lipid</td>
<td>0.08 ± 0.02</td>
<td>0.08 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>M:P</td>
<td>4.40 ± 0.89</td>
<td>6.46 ± 1.36</td>
<td>12.96 ± 4.2</td>
</tr>
</tbody>
</table>
RESULTS: Disease

Infection by newly identified apicomplexan parasite causing severe histopathology in muscle tissue

IDENTICAL rDNA sequence to a new genus and species of apicomplexan parasite observed in 3 other scallop species:
1. Iceland scallop, *Chlamys islandica* in Icelandic waters (associated with stock collapse)

![Graph showing stock index over time](image)

Reproduced from Eiriksson et al 2000

2. Queen scallop *Aequipecten opercularis*, the Faroe Islands (high rates of natural mortality)
3. King scallop *Pecten maximus*, West Coast of Scotland (parasite observed in low levels)
# Comparison of apicomplexan species described from molluscs

<table>
<thead>
<tr>
<th>Name of apicomplexan</th>
<th>Host Class/species</th>
<th>Infected organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bivalve clam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudoklossia pelseneeri</td>
<td>Donax sp.</td>
<td>Kidney</td>
</tr>
<tr>
<td>P. glomerata</td>
<td>Tellina sp.</td>
<td></td>
</tr>
<tr>
<td>P. (Merocystis)</td>
<td>Tapes floridas</td>
<td>Kidney</td>
</tr>
<tr>
<td>Unnamed</td>
<td>Tapes virginius</td>
<td></td>
</tr>
<tr>
<td>Margolisiella kabatai</td>
<td>Tellina tenuis</td>
<td>Ovary</td>
</tr>
<tr>
<td>Unnamed</td>
<td>Protothaca staminae</td>
<td>Kidney</td>
</tr>
<tr>
<td>Unnamed</td>
<td>Protothaca staminae</td>
<td>Kidney</td>
</tr>
<tr>
<td>Bivalve cockle</td>
<td>Tridacna crocea</td>
<td>Kidney</td>
</tr>
<tr>
<td>Pseudoklossia sp. 8</td>
<td>Cerastoderma edule</td>
<td>Kidney</td>
</tr>
<tr>
<td>Bivalve Scallop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. pectinis</td>
<td>Pecten maximus</td>
<td>Kidney</td>
</tr>
<tr>
<td>Many species10</td>
<td>Argopecten irradians</td>
<td>Kidney and other organs</td>
</tr>
<tr>
<td>Pseudoklossia sp.11</td>
<td>Argopecten irradians</td>
<td>Kidney and other organs</td>
</tr>
<tr>
<td>P. pectinis-like12</td>
<td>Argopecten irradians</td>
<td>Kidney</td>
</tr>
<tr>
<td>Unnamed13</td>
<td>Argopecten irradians</td>
<td>Kidney and other organs</td>
</tr>
<tr>
<td>Margolisiella islandica14</td>
<td>Chlamys islandica</td>
<td>Heart</td>
</tr>
<tr>
<td>Bivalve mussel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. semiluna15</td>
<td>Mytilus spp.</td>
<td>Kidney</td>
</tr>
<tr>
<td>Gastropoda</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. haliotis*16</td>
<td>Haliotis spp.</td>
<td>Kidney</td>
</tr>
<tr>
<td>Polyplacophora</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. patellae*17</td>
<td>Acanthochites fasciularis</td>
<td>Intestine</td>
</tr>
<tr>
<td>P. chitonis*17</td>
<td></td>
<td>hepatopancreas</td>
</tr>
</tbody>
</table>

From Kristmundsson et al 2011
### Sea scallops vs. Iceland scallops

**Similarities**

<table>
<thead>
<tr>
<th>Macropscopic signs</th>
<th>Developing oocysts</th>
<th>Sporozoites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea scallop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceland scallop</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DNA confirmation: same parasitic infection**
All apicomplexan stages (both sexual and asexual stages): free sporozoites, trophozoites, meronts, merozoites were found in *Placopesten* adductor muscle samples
Histopathology

- Apicomplexan found in all muscular tissues
  - Intracellular in muscular tissues
  - Free in the extracellular space
    - Inside hemocytes
- Adductor muscle most heavily infected tissue
- Observe hemocyte neoplasia
  - “Gray scallops” most heavily infected
  - Some white scallops lightly infected

Effect of Parasite on Muscle Tissue

- Causes severe histopathological changes in adductor muscle as well as other muscular tissues
- Focal or disseminated necrosis
- Observe hyalinization and myoliquefaction of muscle tissues
General sequence of changes observed in muscle tissues:

- The pathology results suggest the following progression of the disease but controlled laboratory experiments are required.

- Hemocyte infiltration with no detectable changes in the muscle fiber structure, hemocytes morphologically normal but at 2-3X normal density.

- Reduction in the diameter of muscle fibers (thinning), appearance of apicomplexan zoite stages in pockets among fibers and structures.

- Further thinning of muscle fibers, increased numbers of neoplastic hemocytes, concentrated in pockets and in conjunction with zoites.

- Extensive thinning of muscle fibers, gaps among fibers.

- Fused muscle fibers, initially observed nearer to shell attachment sites, then deeper into muscle tissue.
SUMMARY

- Increase in myodegeneration with muscle discoloration

<table>
<thead>
<tr>
<th>Adductor Muscle Color</th>
<th>Muscle Degeneration</th>
<th>Hemocyte Neoplasia</th>
<th>Apicomplexon Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>none</td>
<td>rare</td>
<td>rare</td>
</tr>
<tr>
<td>Brown</td>
<td>slight-thinning of fibers (stringy)</td>
<td>elevated, low-moderate</td>
<td>moderate</td>
</tr>
<tr>
<td>Gray</td>
<td>moderate to severe with deterioration of muscle attachment sites</td>
<td>high</td>
<td>extensive -secondary infections in some samples</td>
</tr>
</tbody>
</table>
Adductor muscle
Massive, disseminated apicomplexan infection
Adductor muscle
Cysts in adductor muscle – presumably an early oocyst development
Adductor muscle

Early trophozoites inside muscle fibres

Free merozoites
Adductor muscle

A cluster of sporozoites associated with muscular degeneration
Muscular necrosis associated with a cluster of sporozoites in adductor muscle
Adductor muscle
Liquefactive necrosis
Adductor muscle
Hyalinization
Muscle fibers become hyalinized into a glossy homogeneous mass
Testis

Normal and necrotised supporting tissue (stroma)
Transmission of Infection

- All known apicomplexan stages observed in scallop host

**Suggests** no intermediate host - monoxenous life cycle → **DIRECT TRANSMISSION**

- Pseudofeces?
- Decomposition of infected tissue?

Site of entrance via the oral feeding route or gills?
Possible autoinfection

**Need** verification through controlled infection transmission studies.

IMPORTANT AS SCALLOP FISHERMEN SCHUCK AND DISCARD TISSUE AT SEA
Polychaete (*polydora*) and *cliona* infections:

- Qualitative observations suggest a correlation of shell health with gray meat.
- Quantification of this observation is addressed in Megan Levesque’s MS research.
RESULTS: Habitat

- Gray meat stations were characterized by:
  - a predominantly sand substrate with shell debris.
  - very few clappers observed.
  - Sea stars were the most abundant predator in gray meat areas.

This analysis is being expanded and continued in the 2014 RSA project entitled “Tracking the Occurrence of Gray Meat in Atlantic Sea Scallops, *Placopecten magellanicus*” and the Saltonstall Kennedy Grant “Combining Fishermen's Knowledge with Oceanographic and Economic Models to Locate and Predict Gray Meat Outbreaks in Atlantic Sea Scallops”

Percent occurrence of substrate in locations where gray meat were found in Closed Area 1 (CAI) and Closed Area II (CAII) in 2011 and 2012 (n=13 stations and 52 quadrats).
Percent occurrence of different habitat characteristics in locations where gray meat were found in Closed Area 1 (CAI) and Closed Area II (CAII) in 2011 and 2012 (n= 8 Stations and 32 quadrats).
Current Research

University of Iceland: Publication submitted to name new apicomplexan
• Develop in situ hybridization – a very specific staining based on DNA staining using probes specific for this apicomplexan species.
• Design a quantitative real time PCR for this pathogen DONE √

University of Massachusetts Dartmouth:
I. Laboratory studies.
Virulence Studies: (Megan Levesque MS; Spring 2016 completion)
• cumulative mortality experiment DONE √
• colorimetry to quantifying the meat color designations
• determine the parasite intensity associated with terminal pathological changes.
• analyze occurrence of shell parasites with gray meat
Tissue Discard Studies:
• determine the effect of discarding gray meat tissue at sea on the spread of the infection to new hosts
• test if fresh water soak can reduce the virulence of the parasitic infection in tissues
University of Massachusetts Dartmouth:

II. Develop temporal and spatial map of gray meat outbreaks in Atlantic sea scallops
   • Interviews with scallop fishermen

III. Overlay map with environmental variables to understand affect of abiotic / biotic stressors on parasite:

   e.g. Scallop density, water temperature, depth stratification, substrate, bathymetry, sediment stability.
IV. Determine range and prevalence of the parasitic infection in Atlantic sea scallops:

Canadian waters - Georges Bank
✓ Parasite confirmed

Gulf of Maine - Inshore
✓ Parasite confirmed

Note:
- Samples from weathervane scallop *Patiniopecten caurinus* to be tested for apicomplexan

Ryan Burt. Alaska Dept. Fish and Game
Summary

- Gray meat caused by newly identified apicomplexan parasite that targets all muscle tissue, but concentrates in adductor muscle.

- Same parasite found in Iceland, Queen and King scallops with differing stock consequences.

- Likely direct transmission of parasite between hosts possible high impact on fishery. Infection transmission trials required.

- Age, nutritional stress as well as secondary infections and shell parasites (*cliona* and *polydora*) may be covariates reducing overall fitness.

- Combination of physiological and site specific environmental conditions that supports the proliferation and transmission of this parasite likely responsible for “gray meat” outbreaks.
Acknowledgements:

• New England Commercial Scallop Fishermen.
• SMAST-Kevin Stokesbury and Steve Cadrin Labs, Connamesset Farm Foundation and VIMS, Dave Rudders and Bill DePaul for assistance in collecting samples.
• Dr. Stephen Smith (DFO) - Canadian samples
• Caitlin Cleaver (Hurricane Island Foundation) - Gulf of Maine samples

Funding Source: Scallop Research Set Aside (RSA)- NOAA/NA12NMF4540036
1. **Tracking the Occurrence of Gray Meat in Atlantic Sea Scallops, Placopecten magellanicus**
   Scallop RSA : NA14NMF4540080
   End Date: April 2016
   Kevin D.E. Stokesbury, Susan D. Inglis, Daniel Georgianna

   **Support** for MS Student- Megan Levesque

   **Collaborators**- Arni Kristmundsson, Mark A. Freeman: University of Iceland
   Kevin St. Martin: Rutgers University

2. **Combining Fishermen's Knowledge with Oceanographic and Economic Models to Locate, Evaluate, and Predict Gray Meat Outbreaks in Atlantic Sea Scallops**
   Saltonstall Kennedy: NOAA-NMFS-FHQ-2015-2004246
   End Date: September 2017

   Daniel Georgianna, Susan Inglis, Gavin Fay, Kevin Stokesbury: SMAST
   Kevin St. Martin: Rutgers University
   Min-Yang Lee: Northeast Fisheries Science Center, NOAA

   **Collaborators**- Arni Kristmundsson, Mark A. Freeman: University of Iceland
Can Infected Scallops Recover?
Megan Levesque

**Pilot Study:** Nonlethal method to categorize meat color and quality
- $\text{MgCl}_2$ Dead Sea Works

Photo: Megan Levesque, 2015
Summary Laboratory Experiment:

Tanks:  White meat - Full Ration
       - Half Ration
       Gray/Brown meat - Full Ration
                   - Half Ration

Diet:
Full Ration = 2.5 L cultured phytoplankton/day
Half Ration = 1.25 L cultured phytoplankton/day
Tracking Gray Meat Using Fisherman’s Knowledge

- Conducted Captain’s Workshop August 2014 to develop interview questions and protocols
- 28 interviews completed to date (goal 40 interviews)

Fishermen were asked questions about gray meat attributes:
- Temporality of gray meat occurrence
- Causes/environmental conditions
- Spatiality of gray meat areas (i.e. small or large areas)
- Severity of problem
- Changes in fishing practices due to gray meat
- Management practices—causes and solutions
- Direction for scientists

Attribute Information provided about each location

<table>
<thead>
<tr>
<th>ID (e.g. B1)</th>
<th>Year</th>
<th>Season</th>
<th>Place from map</th>
<th>Lat/Long, from map</th>
<th>Shell Debris yes or no</th>
<th>Scallop size category</th>
<th>Density low, avg, high</th>
<th>Gray: few, too many</th>
<th>Shell holes: yes or no</th>
<th>Condition: encrusted yes or no</th>
<th>Other Comments:</th>
</tr>
</thead>
</table>
Preliminary Results:

- 116 individual gray meat locations assessed so far

Preliminary number of results for gray meat locations reported per period
QUESTIONS