Rigor Mortis

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Rigor Mortis in Fish

Rigor Mortis

- Rigor Mortis is characterized by a succession of muscle contractions
- Rigor can cause changes in muscle quality
  - Shortening (shrinking of fish fillet)
  - Gapping (the individual flakes of muscle come apart giving the fillet a broken and ragged appearance)
  - Drip loss (loss of water from fish fillet)
  - Shortening and drip loss lead to tougher texture
- Determining the optimal conditions for muscle to pass through rigor state with minimum quality loss is important

Rigor Mortis

- During harvest aquatic animals experience high activity levels
  - Metabolism speeds up
  - Oxygen demand increases
  - The animal struggles trying to escape and survive
- Harvest stress can greatly affect quality
  - Bruising or other physical injuries
  - Removal of slime coat
  - Handling conditions post mortem
- Rigor Mortis (Latin “the stiffness of death”) is one of the most drastic changes that occur in the muscle soon after death

Studies on Rigor Mortis of Gulf of Mexico Sturgeon
Post-Mortem Changes

- Changes that occur in fish muscle after death and prior to processing

Rigor Mortis
- First notable change in fish is rigor
- Rigor is a physiological reaction to death
- Muscle path:
  limp/elastic ---) stiff/hard ---) limp/elastic
  (pre-rigor)  (rigor)  (post-rigor)

Post-Mortem Changes

- Noticeable changes in muscle quality that may occur in fish due to rigor:
  - Odor changes: Off-odors may develop during short-term or long-term storage of fish muscle depending on the condition fish was left to undergo rigor. This is normally caused by degradation due to enzymes or bacteria
  - Color changes: Pigments may degrade or partially degrade
    - E.g. Fading of orange color is salmon muscle, development of black spots in shrimp
  - Texture changes: If muscle contractions are strong then either muscle gaping (ripping of muscle structure near bones) or muscle toughening (drip-loss, or water loss) can occur

Post-Mortem Changes

- Onset, duration and resolution of rigor in fish muscle depends on many factors...
  - Amount of struggle in net/on deck fish underwent right before death
  - Killing method
  - Handling practices after harvest
  - Elapsed time between harvest and chilling of the catch
  - Temperature muscle undergo rigor mortis (holding temperature prior to processing)
  - Fish species
  - Fish size
  - Starvation prior to harvest
  - Sexual maturity
  - Overall condition of the animal at harvest (health)
  - Processing of fish pre-rigor, in-rigor or post-rigor

Fish Muscle

- Three types:
  - Striated (voluntary)
  - Smooth or unstriated (involuntary) – e.g. stomach walls
  - Cardiac (heart muscle)

- Mostly interested in the striated muscle that makes up the flesh of the fish. Smooth muscle is used in the edible portion of some mollusks.

Top view of fish muscle in bony fish.
Source: Lagler et al., 1977
Fish Muscle
- Two types of striated muscle found in fish - white meat and dark meat
- Dark meat (also called swimming meat) lies just under the skin
- Shape and amount depends on the species
- Fish that live on the bottom of the ocean have very little or no dark meat
- Fish swimming near the surface have higher amounts
- Used for continuous movement
- White meat used for quick bursts of energy, such as fleeing predators

Fish Muscle Structure
- Fiber is the basic unit of a muscle
  - **Definition of fiber:** Cylindrical cell with several nuclei, bounded by an outer membrane (diameter of fibers ranges between 10 to 100 micrometers and length can vary from few to several centimeters long)
- A single muscle is formed by several bundles of fibers held together by connective tissue

Fish Muscle Structure
- Myotomes in fish are one cell deep W-shaped segments showing one forward flexure and one backward flexure

Dark vs. White Muscle
- Distribution and amounts of dark muscle are different among fish species as shown in Figure below
- Sections through the bodies of several fish showing depth of dark muscle.
  - (a) Herring
  - (b) Mackerel
  - (c) Tuna
  - (d) Haddock
  - (e) Cod
  - (f) Whiting (Hake)
- Source: Love, 1988
Fish Muscle Structure
- Myocommata - connective tissue (collagen)
  distributed in sheaths between myotomes
  - Collagen make up 3% of total muscle proteins
  - Content depends on the overall condition of the fish
  - Collagen is never used as energy reservoir, so
    collagen becomes proportionally higher as fish
    starves because other proteins are being used
  - Also found in the skin
  - Important in quality of fish muscle as food because it
    contributes to tensile strength of muscle

Biochemistry of Rigor Mortis
- What happens to the muscle after the animal dies?
  - Muscle works by converting chemical energy
    into mechanical energy
  - Muscle requires a high energy level to operate the contractile apparatus
  - Energy is obtained from ATP (adenosine triphosphate). Atkinson and Walton (1967)
    defined the energy level of a cell as “energy charge”

ATP: What is it?
- ATP (Adenosine Triphosphate) storage
  and use of energy in living things

ATP: What is it?
- The enzyme ATPase is ATP splitter - it take
  energy from ATP by cutting off the last
  phosphate group of the ATP molecule turning it
  into adenosine diphosphate.
  - In the process splitting energy is released and used in
    the cell to do work (move and build things)
  - When our body consumes carbohydrates and
    other foods, it breaks them down to release
    energy. In many cases, the energy is used to
    reattach the phosphate molecule to the ADP,
    restoring it to ATP. Then the cycle begins all
    over again!
Biochemistry of Rigor Mortis

- Low ATP levels in the muscle is what triggers the onset of rigor mortis
- ATP content of muscles is about 3-5 mg/g of fresh muscle at the moment of slaughter
- Whenever ATP concentration reaches about 1 μmol/g, onset of rigor is observed (Gill et al., 1998).

Biochemistry of Rigor Mortis

- Living muscle: ATP obtained mainly from respiring mitochondria (molecular oxygen)
- Animal is slaughtered: Cessation of circulation
- Development of anaerobic conditions (deprivation of oxygen – respiration in no longer occurring). Metabolism wants to maintain high level of ATP. Cells do not want to die!
- Major anaerobic pathway for ATP production: GLYCOLYSIS

Biochemistry of Rigor Mortis

- Using glycogen to produce energy is possible and this is called GLYCOLYSIS
- Glycolytic enzymes (breaks down glycogen) + Glycogen (Glucose - sugar) produces ATP (ENERGY!)
- ATP broken down through hydrolysis → lowering of muscle pH (becomes more acidic)
- Pre-rigor muscle always has higher pH then post-rigor but if rigor is ‘controlled’, then lowering of pH should NOT cause quality loss

Biochemistry of Rigor Mortis

- “Low pH in muscle no ATP”. Why?
  - pH initially drops slowly in muscle after death, generally followed by a small increase but will generally not return to its pre-rigor level
  - Drop in pH (<5.1-5.5) causes inhibition of glycogen breaking enzymes → DISRUPTION OF ATP PRODUCTION (No more ENERGY)
  - Low levels of ATP – rigor mortis will start
Biochemistry of Rigor Mortis

- ATP degradation leads to formation of flavor enhancers as intermediates
- Nucleotides can enhance flavors in muscle foods
  - E.g.: umami flavor (meat-like flavor, in this case "marine meat") - good for fish muscle quality
  - Bitter flavor - detrimental to fish muscle quality

Attach-pull-release cycle

- Bridges swing back and forward in alternate pattern.
- Myosin (thick filament) and actin (thin filament) become linked by crossbridges
- Each cycle requires the expenditure of 1 ATP molecule

Muscle Contraction

- Sliding filament model
  - (a) Muscle relaxed; (b) Muscle contracted.
  - Thin filament = actin; Thick filament = myosin
  - 'Attach-pull-release' mechanism

Muscle Contraction

- ATP acts as a plasticizer of thick and thin filaments preventing permanent linkage
- Plasticizers are additives that increase the plasticity or fluidity of the material to which they are added
  - e.g. In the making of plastic materials a plasticizer may be added to increase the flexibility of the final product
- Drop in ATP levels leads to impediment of 'sliding' of thick and thin filaments (stops the "attach-pull-release" mechanism
- When concentration of ATP is lowered down, muscle loses its natural extensibility and stiffens up = RIGOR MORTIS
Muscle Contraction and Quality
- The permanent formation of actomyosin (after ATP is depleted) combined to the extent of overlapping between thin (ACTIN) and thick filaments (MYOSIN) will greatly influence toughness in post rigor fish.
- Actomyosin is a complex protein formed by Actin (thin filament in the muscle) and Myosin (thick filaments in the muscle).
  - If there is a lot of overlapping of the filaments, muscle will be tougher.
  - If contraction is too vigorous then filaments may rip and water will “ drip of” the muscle causing gaping and shrinkage.

Fish vs. Warm-Blooded Animals
- Collagen is one of the main components of skin, cartilage, ligaments, tendons, etc…
- Collagen is one of the responsible compounds for skin strength and elasticity – its degradation leads to wrinkles (aging).
- Gelatin (food) is made up of partially hydrolyzed collagen.
- In fish muscle, collagen provides only a weak network of connective tissue, because water provides resistance to gravity force.
- In warm-blooded animals collagen provides a strong network of connective tissue for animals to STAND (fight gravity force).
- Amount of collagen in the fish body is relatively LOW compared to mammals and birds.
- Collagen is the main protein type of connective tissue in animals.
- Collagen is a long structural protein, and its function is quite different from other types of proteins. Tough bundles of collagen (collagen fibers) give the cells structure from the outside.

Fish live in a colder natural environment so the type of collagen is not stable at HIGH temps.
- This is why when fish muscle is cooked it ‘flakes’, its is much more TENDER then muscle of warm-blooded animals.
- The physical arrangement of the muscle fibers in fish is very different then in warm-blooded animals:
  - Shorter fibers then for warm-blooded animals.
  - Fish myotome (muscle served by a single nerve) contains muscle cells that are ONE layer deep. Connected through heat-labile tissue (collagen).
  - No chain of interconnecting cells in the longitudinal direction = Low tensile strength.
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**Rigor and Dependent Variables**

- Most important variables:
  - Fish species
  - Fish size
  - Overall health of the animal (condition)
  - Killing method
  - Amount of struggling prior to death
  - Delay in bleeding the fish
  - Sexual maturity
  - Handling technique after harvest
  - Catch storage temperature
  - Habitat water temperature (tropical vs. arctic)

**Rigor and Dependent variables**

- Fish species
  - Some species take longer than others to go into rigor mostly due to differences in the chemical composition of the muscle.
  - E.g. Fat content: Pink salmon is low in fat vs. king salmon is high in fat
  - Whitefish (white) goes into rigor very quickly and may be completely stiff one hour after death. Fish stored under the same conditions may take as long as 22 hours to develop full rigor.
  - Trawled cod, 18-22 inches long, gutted and stored in ice, usually take 2-5 hours to go into rigor.
- Fish size
  - Small fish usually go into rigor faster than large fish of the same species.
- Condition
  - The poorer the physical condition of a fish (less well nourished fish is before capture), the shorter will be the time it takes to go into rigor because there is very little reserve of energy in the muscle to keep it viable.
  - E.g. Post-spawning fish will enter rigor faster then pre-spawning fish of same species and similar size.

**Rigor and Dependent Variables**

- Common types of killing method for fish
  - Submersion in cold water (0°C)
  - Sharp blow in the head
  - Decapitation
  - Stun with carbon dioxide with further placement of a cut on gill arches
  - Cranial spiking
- Salmon killed by a blow to the head enter rigor about eighteen hours after death, but if stunned by carbon dioxide and killed by bleeding, it becomes rigid in five hours.

**Rigor and Dependent Variables**

- Amount of struggle prior to death
  - Struggle depletes energy, thus fish that struggled a lot right before death will enter rigor faster.
- Handling
  - Manipulation of pre-rigor fish does not appear to affect the time of onset of rigor.
  - Manipulation of tagging of the fish while in rigor can shorten the time they remain stiff.
- Temperature
  - This is perhaps the most important factor governing the time a fish takes to go into and pass through rigor because the temperature at which the fish is kept can be controlled.
  - The warmer the fish, the sooner it will go into rigor and pass through rigor.
  - E.g. Gutted and kept at 32-35°C may take about 60 hours to pass through rigor whereas the same fish kept at 87°F may take less than 2 hours.

Studies on Rigor Mortis of Gulf of Mexico Sturgeon
Rigor and Dependent Variables

- Temperature is critical to the onset of rigor
  - Normally rigor occurs more rapidly if fish are not chilled quickly after death but some controversy on the relationship between rigor mortis progress and temperature
  - Fish species such as sardine, mackerel and plaice had their pre-rigorous periods shortened when stored at 0°C compared to warmer temperatures
  - Other species such as cod, mahi mahi and grunt seem to have the onset of rigor retarded at 0°C

Rigor Mortis Onset and Duration

<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature</th>
<th>Time from landing on deck to entering rigor (h)</th>
<th>Time from landing on deck to end of rigor (h)</th>
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</thead>
<tbody>
<tr>
<td>trawled cod</td>
<td>16°C</td>
<td>24</td>
<td>28-65</td>
</tr>
<tr>
<td></td>
<td>27°C</td>
<td>40-60</td>
<td>64-64</td>
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<tr>
<td></td>
<td>40-44°C</td>
<td>5</td>
<td>46-66</td>
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<tr>
<td></td>
<td>62°C</td>
<td>2-5</td>
<td>10-33</td>
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<td></td>
<td>87°C</td>
<td>1½</td>
<td>1½-3</td>
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<tr>
<td>rested cod</td>
<td>27°C</td>
<td>14-15</td>
<td>37-48</td>
</tr>
<tr>
<td>rested redfish</td>
<td>18°C</td>
<td>12</td>
<td>12-13</td>
</tr>
<tr>
<td>rested whiting</td>
<td>18°C</td>
<td>1</td>
<td>20-30</td>
</tr>
<tr>
<td>rested plaice</td>
<td>18°C</td>
<td>3-10</td>
<td>11-15</td>
</tr>
<tr>
<td>rested codfish</td>
<td>18°C</td>
<td>10</td>
<td>10-15</td>
</tr>
<tr>
<td>rested haddock</td>
<td>16°C</td>
<td>2-4</td>
<td>27-35</td>
</tr>
</tbody>
</table>

Rigor and Fillet Quality

- Examples of how rigor influences muscle quality:
  - White sturgeon post rigor fillets from struggled fish were softer than the ones from anesthetized fish
  - White sturgeon cooked fillets had firmer texture pre rigor than post rigor
  - Onset of rigor in White sturgeon (Acipenser transmontanus) ranges from 72 h to more than 96 h. White sturgeon can take as long as seven days to resolve rigor

Rigor and Fillet Quality

- Examples of how rigor influences muscle quality:
  - Fish filleted before resolution of rigor can shrink up to half of its former length when cooked
  - If the fillet remains attached to the skeleton shortening does not occur unless rigor develops at high temperatures
  - In case of cod this is above 17°C. As rigor tension becomes too strong weakening of the connective tissue and rupture of the fillet occurs, a phenomenon known as “GAPING”.
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Gaping in cod fillets

No Gaping

Severe gaping

Moderate gaping

Rigor and Fillet Quality

- Examples of how rigor influences muscle quality:
  - Salt uptake in Atlantic salmon is influenced by rigor state
  - The equilibrium salt concentrations of pre-rigor, in-rigor and post-rigor mortaris salmon were 0.53, 0.66 and 0.75 g/g salt-free solids (using a 20% sodium chloride brine solution). This mean that post-rigor muscle takes up salt faster
  - Salt distribution inside pre-rigor salmon samples is less uniform than in-rigor and post-rigor

How to Measure Rigor?

- Many physical and chemical methods have been proposed with some methods being more widely accepted than others
- Most chemical methods focus on measuring ATP and its degradation products (nucleotides) and enzymatic activity of specific enzymes
- Physical methods are more diverse and can be very complex in conception

Physical Method of Monitoring Rigor Mortis in Fish (An example)

- Rigor Index - Iwamoto et al. (1987)
  - Most accepted physical measurement to monitor rigor mortis progression over time
  - Simple and quick
  - Not suitable for large fish
  - Combination of Rigor Index and other techniques
    - Sensory evaluation (tactile and visual analysis)
    - Texture measurements (using instruments)
    - pH progression
    - Nucleotide degradation products (ATP, etc.)
Rigor Index by Iwamoto et al. (1987)

- Rigor index (%) = (D₀ - D/ D₀) x 100
  
  D₀ = distance of the base of the caudal fin from horizontal line of the table on prerigor state
  
  D = distance of the base of the caudal fin from horizontal line of the table during rigor state