Effectiveness of AQUI-S20E on Marine Fish: Sedation to Handleable

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Fish Sedatives

- **Agents that, with increasing concentration and duration...**
  - Calm an animal and then cause successive loss of mobility, equilibrium, consciousness, and reflex action

- **Routinely used in fisheries to minimize stress to fish when...**
  - Collecting tissue samples or morphometric data
  - Surgically implanting tags
  - Sorting (field and hatchery); staging; transportation

- **Fish are sedated to...**
  - Minimize mechanical damage
    - Epithelium/internal tissues
  - Reduce physiological consequences of handling
    - Generalized stress response
  - Address animal welfare
    - Pain or nociception
Sedative Options

- **MS-222** - approved by FDA
  - 21-d withdrawal period
- **Clove Oil**
  - Not approved by FDA for use on fish
- **CO2 (gas/alka seltzer/sodium bicarbonate)**
  - Not approved by FDA; low regulatory priority; not all fish respond well
- **AQUI-S 20E (10% eugenol)**
  - Potential immediate-release sedative
Immediate-release Sedatives

- **It’s impractical to hold fish after sedation**
  - Potential to severely constrain field activities
  - Use of clove oil or MS-222 off label violates parts of the FFDCA
- **AQUI-S 20E is the best option**
  - Currently available under the USFWS INAD exemption authorization
    - Immediate release for field use on freshwater finfish; 3-d w/d for all other uses
  - Initial approval
    - Sedation of freshwater salmonids to handleable – 2017
  - Effective
    - Sedate fish within 5 min
    - Preferably within 1 – 3 min
    - Lose equilibrium & ability to swim
  - Safe to fish
    - 1× dose: 3 – 4 min ≥ Time to sedation
    - 1.5× dose: 2 – 3 min ≥ Time to sedation
AQUI-S20 is effective for Freshwater fish.

<table>
<thead>
<tr>
<th>Target Dose</th>
<th>Sedation</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg/L (4 salmonids/14°C)</td>
<td>2.0 min (range, 1.3 – 3.2)</td>
<td>6.2 min (range, 3.6 – 10.8)</td>
</tr>
<tr>
<td>40 mg/L (4 coolwater species/18°C)</td>
<td>2.1 min (range, 1.4 – 3.1)</td>
<td>8.0 min (range, 5.9 – 10.2)</td>
</tr>
<tr>
<td>60 mg/L (4 warmwater species/24°C)</td>
<td>1.4 min (range, 0.9 – 1.7)</td>
<td>8.0 min (range, 5.2 – 12.9)</td>
</tr>
</tbody>
</table>

Effectiveness Technical Section complete (May 16, 2013)
Catfish survival (%)

100 mg/L eugenol – to 4.5 min
(Sedation – 0.25 min)
150 mg/L eugenol – to 4.0 min
(Sedation - 0.25 min)

Yellow perch survival (%)

80 mg/L eugenol – to at least 10.5 min
(Sedation - 1 min)
120 mg/L eugenol – to 6.0 min
(Sedation – 0.75 min)

Rainbow trout survival (%)

40 mg/L eugenol – to 6.5 min
(Sedation – 1 min)
60 mg/L eugenol – to 3.5 min
(Sedation – 0.5 min)
Marine fish sedation

• Demonstrate that AQUI-S20E effectively sedates marine fish to handleable in seawater
  o CVM concurred protocol
  o Virtually identical to the protocol used for freshwater fish

• Generate data to complete effectiveness technical section
  o Test 6-8 different fish species

• Do marine fish respond similarly to freshwater fish?
  o Test fish at:
    ▪ Similar temperatures, doses
  o Compare times to sedation/recovery
    ▪ Freshwater fish data
# Fish species tested

<table>
<thead>
<tr>
<th>Done</th>
<th>To be tested (possibly)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Warmwater</strong></td>
<td></td>
</tr>
<tr>
<td>Black Sea Bass</td>
<td>Red Drum</td>
</tr>
<tr>
<td>Cobia</td>
<td>Atlantic Salmon</td>
</tr>
<tr>
<td>Pompano</td>
<td>White Sea Bass</td>
</tr>
<tr>
<td><strong>Coldwater</strong></td>
<td></td>
</tr>
<tr>
<td>Sablefish</td>
<td>Yellowtail Amberjack</td>
</tr>
<tr>
<td>Steelhead Trout</td>
<td></td>
</tr>
</tbody>
</table>

4 MUMS grant applications approved for funding
## Testing

<table>
<thead>
<tr>
<th>Species</th>
<th>Size (cm)</th>
<th>Dose</th>
<th>Water temp (ºC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Eugenol</td>
<td>MS222</td>
</tr>
<tr>
<td>Pompano</td>
<td>9.9 ± 1.1</td>
<td>30</td>
<td>120</td>
</tr>
<tr>
<td>Pompano</td>
<td>15.7 ± 1.3</td>
<td>30</td>
<td>120</td>
</tr>
<tr>
<td>Cobia</td>
<td>16.0 ± 1.2</td>
<td>30</td>
<td>120</td>
</tr>
<tr>
<td>Black Seabass</td>
<td>19.6 ± 2.1</td>
<td>30</td>
<td>120</td>
</tr>
<tr>
<td>Steelhead</td>
<td>26.7 ± 1.7</td>
<td>25</td>
<td>80</td>
</tr>
<tr>
<td>Sablefish</td>
<td>25.4 ± 3.8</td>
<td>60</td>
<td>180</td>
</tr>
</tbody>
</table>

30 fish tested separately with AQUI-S20E or MS222  
Sedated in static bath, exchanged after every fish  
Recovered in flow through (pompano/cobia) or static (all others)  
Assessed behavior during sedation and recovery; water temp and DO - every fish  
Eugenol dose verification - every sedation bucket
<table>
<thead>
<tr>
<th>Species</th>
<th>Time to sedation</th>
<th>Time to recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eugenol</td>
<td>MS222</td>
</tr>
<tr>
<td>Pompano (S)</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Pompano (L)</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Cobia</td>
<td>2.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Black Seabass</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Sablefish</td>
<td>1.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Steelhead</td>
<td>1.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Marine warmwater fish treated with 30 mg/L eugenol and 120 mg/L MS222 - freshwater with 60 mg/L eugenol and 150 mg/L MS222
Steelhead trout in seawater time to sedation comparable to freshwater salmonid times - all tested at 25 mg/L eugenol
Sablefish (deep water fish) response similar to sturgeon - difficult to assess
All fish recovered
Status and next moves

- **Final Study Reports submitted to CVM**
  - CVM response 5/16/2016
    - Acceptable as substantial evidence of effectiveness
      - Technical section remains incomplete
    - May consider the TAS study on RBT adequate for demonstrating safety to seawater salmonids

- **DRIBS ‘published’**
  - #48 and #49 on AADAP website

- **Submit MUMS grants to fund remaining studies**
  - Yellowtail Amberjack and White Seabass
    - Hubbs SeaWorld and Research Institute
  - Atlantic Salmon
    - e.g., University of Maine
  - Red Drum
    - e.g., FAU Harbor Branch Oceanographic Institute
Acknowledgements

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- Northwest Indian Fish Commission
  - Matt Stinson