The self-fertilizing mangrove killifish *Kryptolebias marmoratus* as a model fish for breeding

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The Self-fertilizing Mangrove Killifish
*Kryptolebias marmoratus* as a Model Fish
for Aquacultural Study

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Mangrove killifish (*Kryptolebias marmoratus* Poey)

- distribute mangrove swamps from Florida to Brazil
- about 5 years lifespan, reaching 3-5 cm
- about 3 months to mature (short generation time)
- Euryhaline (0-60 ppt)
- 18-35(>) °C
- Tolerant to ammonia

[Image of fish]

www.youtube.com
“Fish in a log!”

http://www.biol.vt.edu/faculty/turner/rivmar/
Kryptolebias marmoratus

- Functional Hermaphrodite
- Self-fertilization
  (Harrington 1961 Science)

Test animal for various aspects in the marine teleosts

Lee et al. (2008) J Fish Biol
Advantage

- Easy to keep
- Easy to handle
- Track individual record

1. Test for new live feed
2. Model for breeding
1. Testing new live feed
Feeding behavior of newly hatched larva (day 0) to various food items (1 hr)

<table>
<thead>
<tr>
<th>Food item</th>
<th>Feeding success (%)</th>
<th>Ingestion (/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Artemia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. franciscana</em></td>
<td>100</td>
<td>2.6±0.5</td>
</tr>
<tr>
<td><em>Protozoa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fabrea salina</em></td>
<td>8.6±3.7</td>
<td>2.4±1.4</td>
</tr>
<tr>
<td><em>Rotifer</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-type</td>
<td>100</td>
<td>37.6±11.5</td>
</tr>
<tr>
<td>S-type</td>
<td>100</td>
<td>21.8±4.8</td>
</tr>
<tr>
<td>SS-type</td>
<td>100</td>
<td>42.8±22.5</td>
</tr>
<tr>
<td><em>Copepod</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acartia tsuensis</em></td>
<td>35.6±4.9</td>
<td>14.8±5.8</td>
</tr>
<tr>
<td><em>Tigriopus japonicus</em></td>
<td>2.2±5.0</td>
<td>0.2±0.4</td>
</tr>
<tr>
<td><em>Cladoceran</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Diaphanosoma celebensis</em></td>
<td>35.3±6.8</td>
<td>4.4±0.5</td>
</tr>
<tr>
<td><em>Moina mongolica</em></td>
<td>3.5±4.8</td>
<td>0.8±1.1</td>
</tr>
<tr>
<td><em>Pellet</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 and 700 µm</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Growth (10 days)

Nutrition (HUFA: mg/100 g·DW)

<table>
<thead>
<tr>
<th>Diet</th>
<th>EPA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acartia tsuensis</td>
<td>14.2</td>
<td>26.1</td>
</tr>
<tr>
<td>Artemia franciscana</td>
<td>17.3</td>
<td>0</td>
</tr>
<tr>
<td>DHA-enriched Artemia</td>
<td>71.4</td>
<td>57.9</td>
</tr>
</tbody>
</table>

Grageda et al. (2008) Aquaculture
### 2. Test animal for breeding study

<table>
<thead>
<tr>
<th>Clonal lineage</th>
<th>PAN-RS</th>
<th>DAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>country</td>
<td>Panama</td>
<td>Belize</td>
</tr>
<tr>
<td>growth</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>aggression</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>GH mRNA</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>fecundity</td>
<td>high</td>
<td>low</td>
</tr>
</tbody>
</table>

Production of hybrid

PAN-RS strain
hermaphrodite (36.7 mm)

DAN strain
primary male (29.4 mm)

F1

selfing

Artificial insemination

Fish from the same parent

PDHy

F2

selfing

selfing

PAN-RS
Control (N=14)

PDHy
(N=12)

DAN
Control (N=13)

Growth of $F_2$

- **PAN-RS** (n=14, 40.6±1.9 mm)
- **DAN** (n=12, 32.1±1.1 mm)
- **PDHy** (n=36, 33.6±3.7 mm)

canonical discriminant analysis

Value 2 (morphology)

Value 1 (growth, behavior)

PDHy II (medium)

PDHy I (high growth)

PDHy IV (low growth)

PDHy III (medium)

PAN-RS

DAN

PDHy
canonical discriminant analysis

Value 2 (morphology)

Value 1 (growth, behavior)

PAN-RS
DAN

PDHyI line (high growth)
PDHyII line (medium)
PDHyIII line (medium)
PDHyIV line (low growth)
Use of Mangrove killifish *Kryptolebias marmoratus*

- Trace individual records
  - Growth, behavior, nutritional study
- Establishment of clonal lineage (homozygote)
  - Different traits
    - Growth
    - Agonistic interactions
- Hybrid lineage (recombinant inbred strain)
  - Useful traits for aquaculture
  - Genetic markers
Bluefin tuna
2.7 m, 367 kg

Thank you!