Ontogeny and characterization of some intestinal enzymes in cobia

*Rachycentron canadum* larvae

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About cobia

- Cobia is a pelagic, migratory marine fish
- Widely distributed
  - Tropical waters
  - Subtropical waters
  - Warm temperate waters apart from the Eastern Pacific Ocean
Cobia is a good candidate for marine farming

- Fast growth rate: 4-6 kg within one year
- Good meat quality: high n-3 HUFA content

Widely farmed:
- Asia
- Americas and Caribbean regions
• Juvenile production of cobia is the main bottleneck
• Stomach of cobia larvae become functional during 12-20 dph, and pancreatic enzymes increased during this period (Faulk et al. 2007)
• Attempts to early weaning of cobia larvae (from 16 dph) with limited success
• No study on intestinal enzymes of cobia sofar
• Better understanding digestive capacity is needed
Objectives

• Ontogeny of the three intestinal enzymes alkaline phosphatase (AP), leucine aminopeptidase (LAP) and leucine-alanine peptidase (leu-ala) in cobia larvae fed live feed

• Whether early weaning of larvae to a microdiet from 17 dph affects the gut maturation process?
Materials and Methods
Larvae rearing and diets

• Exp took place at RIA1 facility, at Cua Lo town, Vietnam
• Larvae were reared in two 500 L-cylindrical composite tanks
• Initial stocking density was 45 newly hatch larvae/L
# Water quality

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
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<tbody>
<tr>
<td>Ambient water temperatures (°C)</td>
<td>26.3 ± 0.2 (23.5-30.0)</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>31.0 ± 0.3 (28-33)</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>5.4 ± 0.3</td>
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<tr>
<td>pH values</td>
<td>7.6- 8.0</td>
</tr>
<tr>
<td>Total ammonia-nitrogen (mg/ L)</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>14h light: 10h dark</td>
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</table>
Larval rearing and diets

- Microdiet Otohime (17-33 dph)
- Enriched EG Artemia (9-27 dph)
- Enriched EG Artemia (9-33 dph)
- AF Art.
- Rotifers (2-12 dph)
- Micro algae (1-12 dph)

Days post hatch:

0 5 10 15 20 25 30 35
Sampling and dissection for enzyme analysis

- **LF treatment:**
  - on 2, 4, 7, 10, 13, 17, 23, 26, 30, 33 dph

- **L-MD treatment:**
  - From 23 dph

- Frozen in liquid nitrogen then stored at -80°C.
- Thawed on ice, dissected for digestive tract
Sampling and dissection for enzyme analysis

- **2-4 dph larvae**
  - 20 larvae/sample
  - Digestive tract was removed by cutting away the head and tail

- **Larvae > 4 dph**
  - 5-15 larvae/sample
  - Only digestive tract was dissected out

- **3 samples of larvae/sampling day**
Results: Growth

![Graph showing growth over days post hatch with Co-feeding indicated]

- **LF**
- **L-MD**

Days post hatch vs. Standard length (mm)
## Survival

<table>
<thead>
<tr>
<th>Age (dph)</th>
<th>Survival rate (%)</th>
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<tbody>
<tr>
<td></td>
<td>LF</td>
</tr>
<tr>
<td>10&lt;br&gt;(^1)</td>
<td>67.7 ± 6.7</td>
</tr>
<tr>
<td>20&lt;br&gt;(^1)</td>
<td>36.1 ± 10.6</td>
</tr>
<tr>
<td>30&lt;br&gt;(^2)</td>
<td>19.8</td>
</tr>
</tbody>
</table>

\(^1\) The survival rates were measured by volumetric method (n =5)

\(^2\) Counting remaining larvae at 30 dph.
Activity of intestinal Alkaline Phosphatase

Specific activity

Individual activity
Specific activity of bbm Alkaline Phosphatase

Days after haching

Specific activity

bbm AP (mU/ mg protein)
Individual activity of bbm Alkaline Phosphatase
Activity of intestinal Leucine Aminopeptidase

Specific activity

Individual activity
Activity of bbm Leucine Aminopeptidase

Specific activity

Individual activity
Activity of Leucine-alanine

Specific activity

Individual activity
Gut maturation index

Comparison of Gut maturation index for LF and L-MD at different developmental stages (23, 26, 30, and 33 dph).

- **LF** (dark green) shows a general increase in Gut maturation index from 23 dph to 33 dph.
- **L-MD** (light green) consistently exhibits a higher Gut maturation index compared to LF across all stages.

Significant differences are indicated by lowercase letters (a, b), with a indicating a significant difference at 23 dph, and b indicating a significant difference at 30 and 33 dph.

**bbm AP/leu-ala**

- **23 dph**: LF (b) and L-MD (a)
- **26 dph**: LF (b) and L-MD (a)
- **30 dph**: LF (b) and L-MD (a)
- **33 dph**: LF and L-MD (b)

Notable increase in Gut maturation index for LF and L-MD between 23 and 30 dph.
Gut maturation index

![Graph showing gut maturation index with bars for different days post hatching (dph) and two conditions: LF and L-MD. The graph indicates a decrease in LAP/leu-ala ratio over time with a significant difference at 30 dph for L-MD compared to LF.](image-url)
Conclusions

• All the studied intestinal enzymes in cobia larvae were detected before onset of first feeding and their individual activity increased as larval growth

• LF larvae attained intestinal maturation on 26 dph (SL 17.0 ± 0.6 mm, 699 degree days), L-MD larvae delayed until 30 dph (SL of 20.0 ± 0.7 mm, 814 degree days), associated with lower survival rate

• Prolonged feeding of cobia post-larvae with Artemia after 28 dph reduced digestive capacity compared to those fed the microdiet
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