DIURNAL PATTERNS OF TRYPDIC ENZYME ACTIVITY IN SEA BREAM LARVAE UNDER DIFFERENT FEEDING REGIMES

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Digestive processes in larval stages
Daily rhythm of Tryptic enzyme activity of sardine larvae in nature

Mean length
Mean Tryptic activity

Trypsin (hydrolysed MCA, nmol min\(^{-1}\), larva\(^{-1}\))

Sampling Time (hours)

Start 12h 24h 36h 48h

Length (mm)
Phase I: yolk-sac stage and beginning of exogenous feeding

Phase II: “critical” larval stage with decreasing trypsic activities, poor growth and high mortality rates

Phase III: sufficient production of trypsin (at optimal food supply), high growth rates

Phase IV: beginning of metamorphosis, during which trypsic activity is being partly replaced by the activity of pepsin in the developing stomach
Trypsin is the most significant protease in the early larval stages and is considered to be a key enzyme in the digestive process.

This short-term experiment was conducted to evaluate the impact of different dietary treatments on the diurnal digestive trypsic enzyme activity in gilthead sea bream (*Sparus aurata*) larvae.
Larval Rearing Facility

- Stocking density 75 larvae L$^{-1}$ in 65 L rearing tanks
- 20 hour short-term experiment (06:30 – 01:30)
- 4 different feeding regimes (live feed and MD)
- 24 hours illumination

- 4 different age groups (21 & 26 & 34 & 44 dph)
5.99
6.74
8.22
13.26

26 & 44 dph
Every 15 min
30 mg/feeding event
4 Artemia ml⁻¹

21 & 26 & 34 dph
26 dph

26 dph

44 dph

6 R.ml⁻¹+1.3 A.ml⁻¹

4 & 6 & 15 Rotifers ml⁻¹
- n=6 sea bream larvae sampled from each group in each hour
- The tryptic enzymatic activity of 1680 individual larva sample was analyzed

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatments</th>
<th>Age</th>
<th>Length</th>
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<tbody>
<tr>
<td>21 dph</td>
<td>Rotifer</td>
<td></td>
<td></td>
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<tr>
<td>26 dph</td>
<td>Rotifer + Artemia</td>
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<tr>
<td>34 dph</td>
<td>Rotifer + MicroDiet</td>
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<tr>
<td>44 dph</td>
<td>X</td>
<td>Artemia</td>
<td>MicroDiet</td>
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</tbody>
</table>

- Tryptic enzyme activity
Results

All data points are the means of n=6 sea bream larvae

- **R21** Control
  - Ø 7.05 mm

- **R26** Control
  - Ø 7.24 mm

- **R34** Control
  - Ø 8.11 mm

Tryptic enzyme activity (hydrolysed MCA, nmol min⁻¹, larva⁻¹)
Results

All data points are the means of n=6 sea bream larvae.
Results

All data points are the means of n=6 sea bream larvae

Sampling time

Tryptic enzyme activity (hydrolised MICA, nmol min⁻¹, larva⁻¹)

Fed with Rotifer + Artemia
Fed with Microdiet
Fed with Rotifers
Unfed larvae

RA 26
MD 26
R 26
Control

Ø 6.76 mm
Ø 6.40 mm
Ø 7.05 mm
Results

All data points are the means of n=6 sea bream larvae
Results

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Results

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Sampling time

Tryptic enzyme activity (hydrolised MCA, nmol min^-1, larva^-1)
Conclusions

The results indicate that no matter what kind of diet was applied, there is a limited digestive capacity in sea bream larvae at some point in time during the day.

There is an existence of endogenous rhythm as internal biological clock in daily rhythm.

The results should be considered as species-specific development stage.

These findings should be considered in feeding schedules for younger larval stages.
Outlook

In order to investigate the digestive enzyme capacity, a comparison study should be done between continously and not continously fed larvae.

Other enzymes (Pepsin, Amylase, Lipaz) should be investigated in order to evaluate the digestive processes development.
Thanks for your attention!
Vielen Dank!

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