Control of Metamorphosis in Flatfish

K. Pittman*, J. Solbakken¹ & K. Hamre²

*Dept of Fisheries and Marine Biology, University of Bergen, Norway
¹. Austevoll Mariculture Station, Inst of Marine Research, Bergen
². Division of Nutrition, Directorate of Fisheries, Bergen. Austevoll
Farmed Flatfish juveniles exhibit:

- Pigment abnormalities
- Lack of eye migration
- Skeletal abnormalities
- Weaning mortality

Fundamental common factor?
Control of metamorphosis

= control of juvenile quality
Experiments

1. Natural zooplankton vs standard enriched Artemia
   1 egg batch  ➔  4 groups

2. Exogenous T4
   continuously and at Pre-, Pro, Climax & PC Metam

3. Photoperiod 24 hrs light or 12L:12D
   at 22 days and at 12, 21, 30 and 42 DPF
Feeding with Artemia or Zooplankton

Malpigmentation: Artemia 93%   Zooplankton 32%
Lack of Eye migration:         90  12
Dorsal fin “comb”:            82  33
From: Hamre, Opstad, Espe, Solbakken, Hemre & Pittman, submitted
Feeding with Artemia or Zooplankton
Feeding with Artemia or Zooplankton

From Solbakken et al. submitted
### FATTY ACIDS IN PREY

<table>
<thead>
<tr>
<th></th>
<th>Artemia</th>
<th>Zooplankton</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARA (20:4 n-6)</td>
<td>2.1±0.1(^a)</td>
<td>0.7±0.2(^b)</td>
</tr>
<tr>
<td>EPA (20:5 n-3)</td>
<td>4.9±0.3(^a)</td>
<td>18.6±3.2(^b)</td>
</tr>
<tr>
<td>DHA (22:6 n-3)</td>
<td>6.3±0.9(^a)</td>
<td>28.5±4.8(^b)</td>
</tr>
<tr>
<td>(\Sigma) Saturated</td>
<td>21.3±1.2</td>
<td>22.5±1.0</td>
</tr>
<tr>
<td>(\Sigma) Monoenes</td>
<td>27.3±0.2(^a)</td>
<td>10.4±4.0(^b)</td>
</tr>
<tr>
<td>(\Sigma) n-3</td>
<td>34.5±0.4(^a)</td>
<td>55.9±4.8(^b)</td>
</tr>
<tr>
<td>(\Sigma) n-6</td>
<td>8.1±0.4</td>
<td>4.3±3.3</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>1.3±0.1</td>
<td>1.6±0.4</td>
</tr>
<tr>
<td>EPA/ARA</td>
<td>2.3±0.0(^a)</td>
<td>29±10 (^b)</td>
</tr>
</tbody>
</table>
Feeding with Artemia or Zooplankton

(In prey)

<table>
<thead>
<tr>
<th></th>
<th>Wild zooplankton</th>
<th>Artemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iodine (ug g dw⁻¹)</strong></td>
<td>350 +/- 19 (n=5)</td>
<td>0.5 +/- 0 (n=4)***</td>
</tr>
<tr>
<td><strong>Selenium (ug g dw⁻¹)</strong></td>
<td>2 +/- 0.2 (5)</td>
<td>2.2 +/- 0.1 (4)</td>
</tr>
<tr>
<td><strong>Phenylalanine (mg g protein⁻¹)</strong></td>
<td>32 +/- 2 (5)</td>
<td>39 +/- 1 (4)</td>
</tr>
<tr>
<td><strong>Tyrosine (mg g protein⁻¹)</strong></td>
<td>37 +/- 3 (5)</td>
<td>37 +/- 1 (4)</td>
</tr>
</tbody>
</table>

from: Solbakken, Berntssen, Norberg, Pittman & Hamre (submitted)
Feeding with Artemia or Zooplankton (In larvae)

From Solbakken et al. and Hamre et al. (2001) submitted
Feeding with Artemia or Zooplankton
(In larvae)

From Solbakken et al. and Hamre et al. (2001) submitted
Feeding with Artemia or Zooplankton

(Iodine (ug g dw⁻¹) in larvae)

From: Solbakken, Berntssen, Norberg, Pittman & Hamre submitted
Feeding with Artemia or Zooplankton

Graphs showing the changes in T4 and T3 (ng larve⁻¹) over days post first feeding.
Window of metamorphosis

critical size about 18 mm for halibut?

17.99 mm SL

Foto: Ø. Sæle
Exogenous Thyroxine - continuous

Solbakken et al. (subm.)
## Exogenous Thyroxine - stages

<table>
<thead>
<tr>
<th>Age (DPF)</th>
<th>SL (mm) ±</th>
<th>Stage</th>
<th>MH (mm) ±</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>15.3 ± 0.6</td>
<td>Pre-M - Pro-M</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>37</td>
<td>18.2 ± 1.9</td>
<td>Pro-M - MC</td>
<td>4.4 ± 1.1</td>
</tr>
<tr>
<td>62</td>
<td>29.3 ± 4.2</td>
<td>Late MC - JUV</td>
<td>7.2 ± 1.1</td>
</tr>
</tbody>
</table>
Exogenous Thyroxine - stages

<table>
<thead>
<tr>
<th>DPF</th>
<th>SL (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exogenous</td>
</tr>
<tr>
<td>T4</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>15.3 ± 0.1 (40)</td>
</tr>
<tr>
<td>22</td>
<td>16.3 ± 0.1 (60)</td>
</tr>
<tr>
<td>29</td>
<td>17.3 ± 0.2 (49)</td>
</tr>
<tr>
<td>37</td>
<td>18.2 ± 0.3 (30)</td>
</tr>
<tr>
<td>44</td>
<td>22.2 ± 0.2 (56)</td>
</tr>
<tr>
<td>51</td>
<td>24.8 ± 0.4 (56)</td>
</tr>
<tr>
<td>62</td>
<td>29.3 ± 0.8 (28)</td>
</tr>
<tr>
<td>69</td>
<td>32.4 ± 0.5 (56)</td>
</tr>
<tr>
<td>76</td>
<td>38.0 ± 0.9 (56)</td>
</tr>
</tbody>
</table>
Photoperiod

Continuous 22DPF
Photoperiod - continuous

![Graph showing frequency of larvae (%) under different photoperiod conditions: Normal, Ambi, Pseudo. The graph indicates a higher frequency of larvae under pseudo-continuous photoperiod compared to normal and ambient conditions.](image-url)
Photoperiod - ages

Solbakken et al. (in prep.)
Photoperiod - ages

Days after first feeding

EMI/SL

LL
LD 12
LD 21
LD 30
LD 41

Solbakken et al. (in prep.)
Photoperiod

ages

size vs age
### Age vs Size: example from diet expt

<table>
<thead>
<tr>
<th>Age</th>
<th>Dag 5</th>
<th>Dag 14</th>
<th>Dag 21</th>
<th>Dag 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64-13.91</td>
<td></td>
<td>144-14.01</td>
<td></td>
<td>183-13.69</td>
</tr>
<tr>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>74-13.74</td>
<td></td>
<td>143-15.62</td>
<td>18217.39</td>
<td>203-16.8</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65-14.74</td>
<td></td>
<td>135-16.26</td>
<td>173-19.16</td>
<td>221-18.8</td>
</tr>
</tbody>
</table>

Ø. Sæle
Hypothalamus-pituitary-thyroid axis

photoperiod
melatonin
thyroid

TRH
TSH

T3 T4
Thyroid follicle

T3 Bound T4
Plasma

T3 Deiodination T4
Receptors

Up or down regulation DNA

Development Growth Reproduction

Diet (+iodine?)
Diet (+selenium)

Fig: Ø. Sæle
Proposed pathway to pigmentation and settlement

phenylalanine (essential a.a.)

diet

phenylalanine 4-monoxygenase

dopa

dopaquinone

Monophenol monooxygenase

Monophenol monooxygenase

melanin

Dopaquinone

tyrosine

tyrosine 3-monoxygenase

iodine

diet (\& seawater)

thyroxin (T₄)

Deiodinases with selenocystein (types 1\& 3 ID)
(type 2 ID)

diet

triiodothyronine (T₃)

Deiodinases (type 1 \& 3 ID)

pigmentation

neural + skeletal change
Aseasonal Reproduction

Artemia

Jan
June
Dec
Conclusions

1. Dietary micronutrients important for endocrine control of metamorphosis (iodine & selenium)

2. Peak deiodinase activity in 24-26 mm size, after metamorphosis has started - T4 more impt initially?

3. Window of opportunity 18-20 mm size (pre-pro metamorphosis)

4. Photoperiod induces heterochrony of eye migration, hemoglobin and pigmentation effects

5. Exogenous thyroid has positive effect on skeletal growth at pre-pro metamorphosis, among other effects
Conclusions

6. Order of metamorphic events is i) neural ii) growth and skeletal change iii) circulation changes iv) pigmentation finally established

7. Heterochrony is more related to age than size

8. Stage dependent response to photoperiod-melatonin-thyroid stimulation?

Future: effects on immunology (IgM), TRs, TRIPs