The combined effects of elevated CO2 and temperature on the physiological conditions of olive flounder larvae, *Paralichthys olivaceus*

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Climate change: GW and OA
Post-larval Ezo abalone *Haliotis discus hannai* were reared under different pCO2 concentrations for 30 days from just after metamorphosis. (Takami et al., 2010)

Dorsal view of sagittal otoliths of 7-day-old white sea bass grown. (Checkley et al., 2009)
Olive flounder

- Important commercial species in Korea (main aquaculture species)

- their growth and survival during the early life history will respond to a new environment: seawater warming and ocean acidification
Aquaculture production of commercial species in 2010

- **Flounder**: 40,923 M/T
- **Rockfish**: 20,000 M/T
- **Red Sea Bream**: 15,000 M/T
- **Mullet**: 10,000 M/T
- **Black Sea Bream**: 5,000 M/T
- **Sea Bass**: 0 M/T
To evaluate the combined effects of ocean acidification and global warming on early development stage of olive flounder.
Experimental setting

- Artificial condition
Experimental setting

- 3 different CO₂ concentrations
  - 400 ppm-current day
  - 850 ppm-mild emission (2100)
  - 1550 ppm-strong emission (2100)

- 2 different temperatures
  - 18°C-mean temp. of southern coast area in Korea
  - 22°C-forecast temp. of southern coast area in Korea at 2100 (KORDI, 2004)
Rearing and feeding

- Rearing: from fertilized eggs to metamorphosis (approximately 28 days)

- Feeding: from hatching to 14 days: fed rotifer & chlorella
  - 14 ~ 21 days: rotifer and artemia
  - 21 ~ 28 days: artemia only
Sampling and measurement

- Sampling: 10 ind/tank every 3~5 days (measuring length)
- At 28th day: All live fishes were preserved in alcohol after measuring length and weight
- Skeleton malformation check
- Whole body section-histomorphology
all sample data – test of normality.

- One way ANOVA and Two way ANOVA.
  (Tool : minitab 16)
At normal CO2 condition- High > Low (p<0.05)
However, High CO2 condition- High < Low (p<0.05)
CO2, temp. Interaction- p<0.01
Results(2)- weight

- At normal CO2 condition- High > Low (p<0.05)
- However, High CO2 condition- High < Low (p<0.05)
- CO2, temp. Interaction- p<0.05
Daily Growth rate
CO2 Only

- Early larval stage (hatching~14 days)
  - 18°C / Control: $y = 0.1593x + 2.3802$, $R^2 = 0.6632$
  - 18°C / Medium: $y = 0.0819x + 2.6569$, $R^2 = 0.663$
  - 18°C / High: $y = 0.1255x + 2.5273$, $R^2 = 0.5472$

- Late larval stage (14 days~settlement)
  - 18°C / Control: $y = 0.6669x - 7.2298$, $R^2 = 0.8912$
  - 18°C / Medium: $y = 0.7514x - 8.9291$, $R^2 = 0.893$
  - 18°C / High: $y = 0.8798x - 9.8654$, $R^2 = 0.9129$

Higher
Daily Growth rate

CO₂ + Temp.

- **22°C / Control**
  - $y = 0.0855x + 2.6901$
  - $R^2 = 0.6583$

- **22°C / Medium**
  - $y = 0.0801x + 2.6641$
  - $R^2 = 0.512$

- **22°C / High**
  - $y = 0.1466x + 2.4411$
  - $R^2 = 0.7593$

**Early larval stage** (hatching~14 days)

**Late larval stage** (14 days~settlement)

- **22°C / Control**
  - $y = 0.9026x - 11.95$
  - $R^2 = 0.9239$

- **22°C / Medium**
  - $y = 0.9866x - 13.048$
  - $R^2 = 0.9037$

- **22°C / High**
  - $y = 0.8587x - 10.451$
  - $R^2 = 0.9495$

**Higher**
Results(4)- skeleton malformation

- Temp. 22°C
- 1st experiment

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Total sample size</th>
<th>Frequency of malformation</th>
<th>Malformation ind.</th>
</tr>
</thead>
<tbody>
<tr>
<td>400ppm</td>
<td>4 ind.</td>
<td>0~1</td>
<td>2</td>
</tr>
<tr>
<td>850ppm</td>
<td>4 ind.</td>
<td>2~3</td>
<td>3</td>
</tr>
<tr>
<td>1550ppm</td>
<td>4 ind.</td>
<td>2~5</td>
<td>3</td>
</tr>
</tbody>
</table>
Results(5) - skeleton SEM photography

- Normal CO2 condition
- High CO2 condition
Results(6)- histomorphology

A: liver
B: skeleton
C: intestine
Results(7)- rearing condition

- \( p\text{CO}_2 \) of each seawater were different.

<table>
<thead>
<tr>
<th>CO(_2) (ppm)</th>
<th>pH</th>
<th>Temp.</th>
<th>TA</th>
<th>Sal.</th>
<th>( p\text{CO}_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>8.05</td>
<td>22.0</td>
<td>2278.9</td>
<td>32.4</td>
<td>573.6</td>
</tr>
<tr>
<td>850</td>
<td>7.84</td>
<td>22.0</td>
<td>2275.4</td>
<td>32.4</td>
<td>987.9</td>
</tr>
<tr>
<td>1550</td>
<td>7.73</td>
<td>22.0</td>
<td>2267.4</td>
<td>32.4</td>
<td>1296.7</td>
</tr>
</tbody>
</table>
Discussion - temperature

- Usually, temperature impact on fish physiology (Brett, 1969).

- Marine organism growth was increase with increasing temperature within range of optimum temperature (Pörtner and Farrell, 2008).
As our results, some study suggest fishes were affected positively by increased CO₂ concentration in rearing water (Munday et al., 2009).

But, other study suggest fishes were affected negatively by increased CO₂ (Baumann et al., 2011).

Most invertebrate were affected negatively by increased CO₂ concentration in rearing water (Wittmann et al., 2013).
Discussion

- Depending on species, CO₂ and temperature can affect (positively or negatively) their growth, survival and several physiological factors.

- In this study, olive flounder was affected by both factors of temperature and CO₂ (positive- growth, negative- bone density)
Dissolved CO₂ positively affect olive flounder larvae growth (length, weight) at normal temperature (18°C).

But, skeleton formation and density were affected negatively by decreased pH (both temperature).

High temperature (22°C) positively affect growth of larvae at the normal CO₂ condition (400 ppm).
Thank you for your attention.