How do Atlantic herring early life stages cope with ocean warming and bacterial infection?

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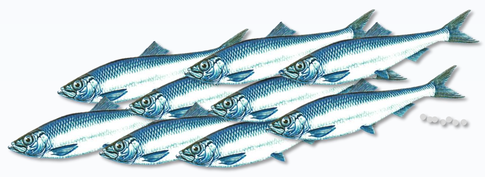
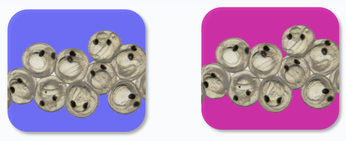
what did we do?



Western Baltic Spring-Spawning Herring

Temperature at start: 8°C



Normal T (+0.1°C/d) **High T (+0.3°C/d)**

hatch

Bacterial challenge

Normal T **High T**

9 replicates / treatment

sampling*

*3 days post challenge = 5 dph = yolk depletion

Full transcriptome gene expression

Microbiota analysis of whole larvae

what did we see?

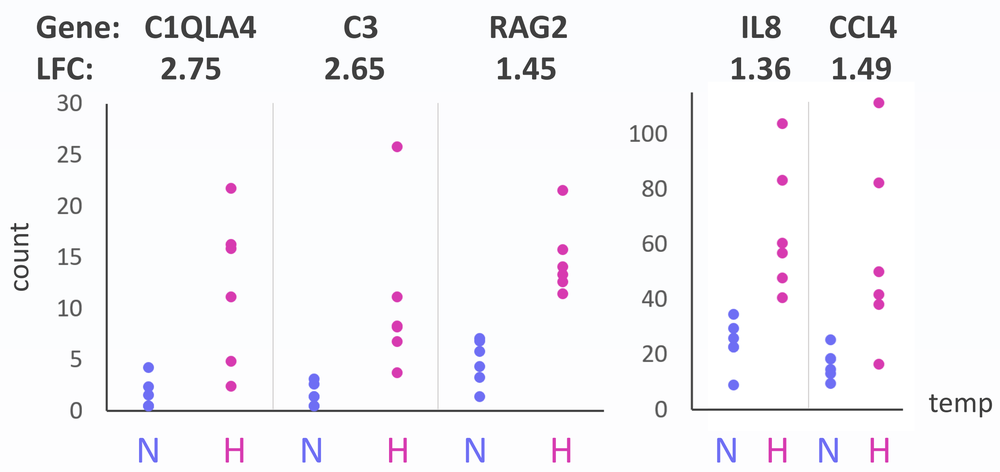


Fig. 1: DEGs Normal vs High temperature (no bacteria, n = 6 larvae/treatment)

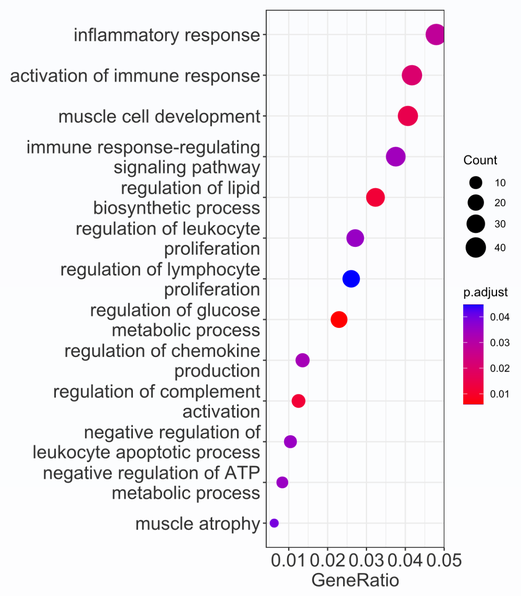


Fig. 2: GO terms (examples for biological processes)

Full transcriptome gene expression analysis

- **Bacterial challenge effect**: only 27 DEGs in the *V. anguillarum* & 42 DEGs in the *V. alginolyticus* treatment compared to the control (no bacteria)
- **Temperature effect**: **2810 DEGs** (1437 up- & 1373 downreg.) identified at the **high temperature** (examples see Fig. 1)

  → **GO analysis** identified over-represented biological processes: DEGs were significantly enriched in **507 GO terms** (incl. immune response, metabolic processes, muscle growth, mitosis; examples see Fig. 2)

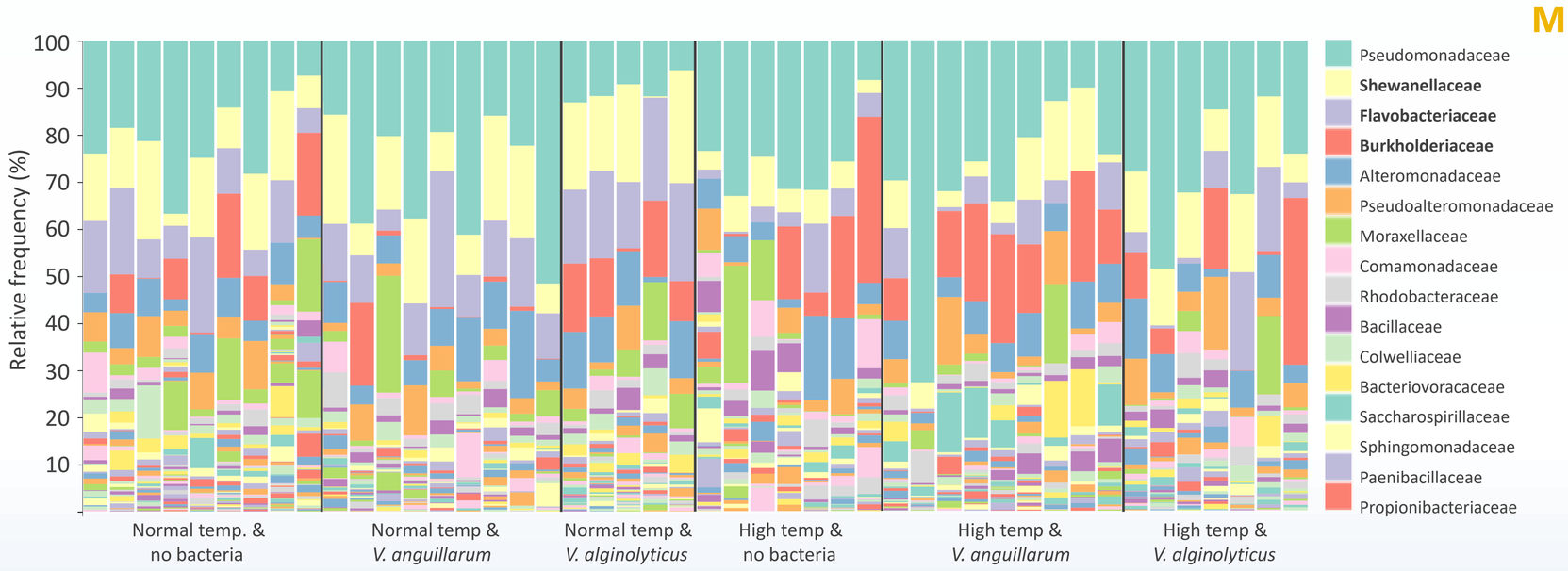


Fig. 3:Taxonomy classification of reads at the family level (n = 5-9 larvae/treatment)

Microbiota analysis (16s rRNA)

- The **temperature** and the **bacterial challenge** had a **significant effect** on the larval microbiota (the added *Vibrio* strains were removed before running a Permanova based on Bray-Curtis distances: temp $F = 3.35$, $P = 0.001$, bacterial challenge $F = 1.36$, $P < 0.05$, temp x bact chall $F = 0.99$, $P > 0.05$)
- Shewanellaceae & Flavobacteriaceae more abundant at normal temperature and Burkholderiaceae more abundant at high temperature (see Fig. 3)

- **Microbiome diversity highest** at **normal temperature control group** (no bacterial challenge) (two-way Anova: Shannon diversity: temperature $F = 5.71$, $P < 0.05$, bacterial challenge $F = 4.45$, $P < 0.05$, temp x bact chall n.s.)
- **Richness** & **evenness** are **higher at the normal temperature** (two-way Anova: richness: temperature $F = 7.88$, $P < 0.01$; evenness: temperature $F = 4.2$, $P < 0.05$, bacterial challenge n.s. and temp x bact chall n.s.)

what does that mean?

- The **temperature** had a **strong effect** on the **gene expression** of Western Baltic Spring-Spawning Herring larvae while the bacterial challenges using two different *Vibrio* species had a minor effect → most likely the Baltic Sea water temperatures during spring (even in years with a spring heat wave) are too low to cause a severe *Vibrio anguillarum* or *Vibrio alginolyticus* infection.
- The **higher temperature** and the **bacterial challenges** both **affected the larval microbial community** (see Bray-Curtis distances) showing that not only an increased temperature but also the presence of *Vibrio anguillarum* and *Vibrio alginolyticus*, respectively, lead to a change in the entire larval microbial community
- The higher temperature had a negative effect on the **microbial diversity, richness** & **evenness** → might be a disadvantage for the development of the herring larvae

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