Identification of Biomarkers for Immuno-Ecotoxicity in PAMP-challenged Zebrafish Embryos using Transcriptome Analyses

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Background

Climate change, invasive species and habitat fragmentation pose major challenges for the stability of ecosystems. It is assumed that in stressed ecosystems, pathogens in particular exert strong pressure on populations[1]. In addition, the organisms in the environment are exposed to a large number of directly or diffusively released environmental pollutants such as pesticides or drugs. The ecotoxicological hazard and risk assessment of chemicals, which aim to assess the impact of chemicals in the environment, currently do not include the identification of effects regarding the immunocompetence of organisms[2]. However, studies indicate that chemicals can induce various effects on immune related parameters[3].

Immune challenge development

Currently, there are no reliable methods to identify immuno-ecotoxic modes of action (MoA) in the activated immune system of environmental organisms. The present project aims at the identification of reliable markers for the MoA of immunotoxic chemicals in zebrafish. In a first step, a suitable immune challenge method was established via microinjection of Pathogen Associated Molecular Patterns (PAMPs) into zebrafish embryos to induce an immune system activation:

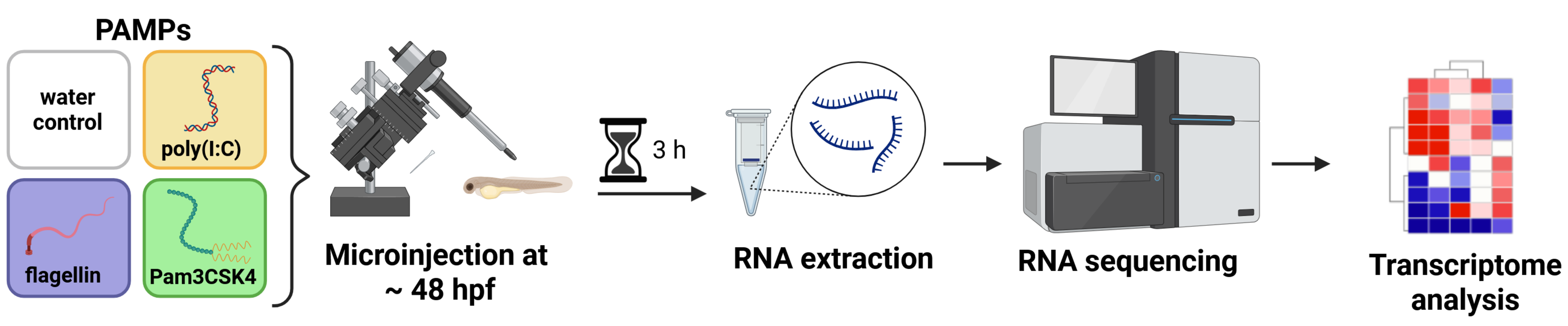
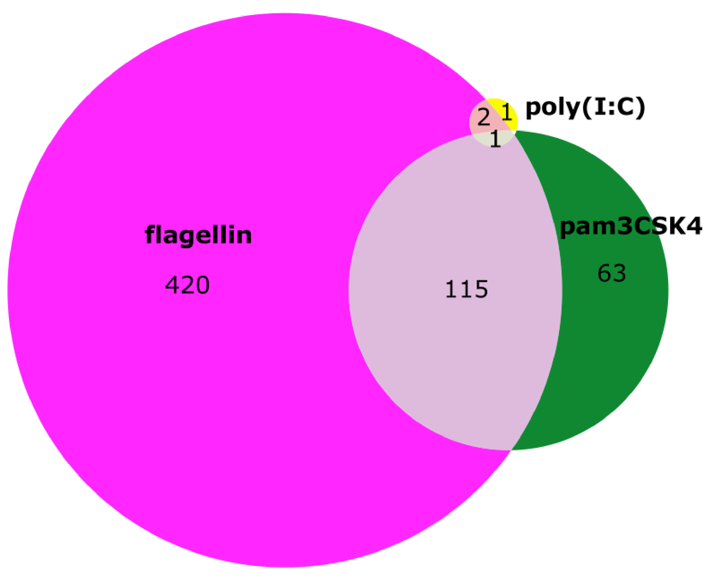


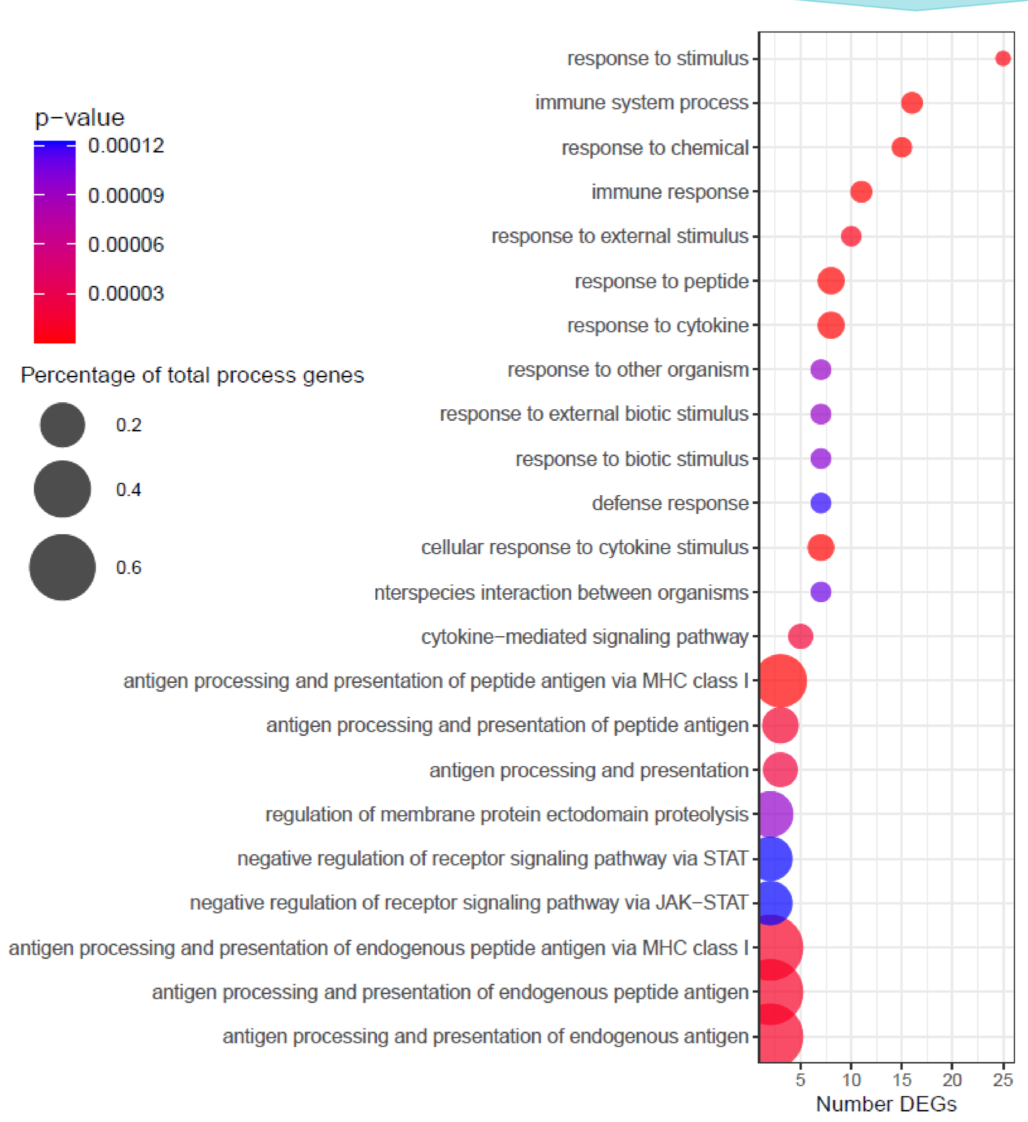
Figure 1: Scheme of the immune challenge experiment performed to identify a PAMP that induces an immune reaction in zebrafish embryos (created in BioRender).



Flagellin injection resulted in a **high number** of Differentially Expressed Genes (DEGs)

→ **Flagellin was chosen as PAMP for main tests**

Figure 2: Venn diagram showing the significantly (padj < 0.05) DEGs of the different PAMP-injection treatments (poly(I:C), flagellin, Pam3CSK4) in comparison to the control.



Flagellin induced **reproducible differential gene expression** in 5 independent experiments; Overlapping DEGs from these experiments show a **specific** influence of flagellin on genes involved in immune processes

→ **The zebrafish immune system is activated by flagellin!**

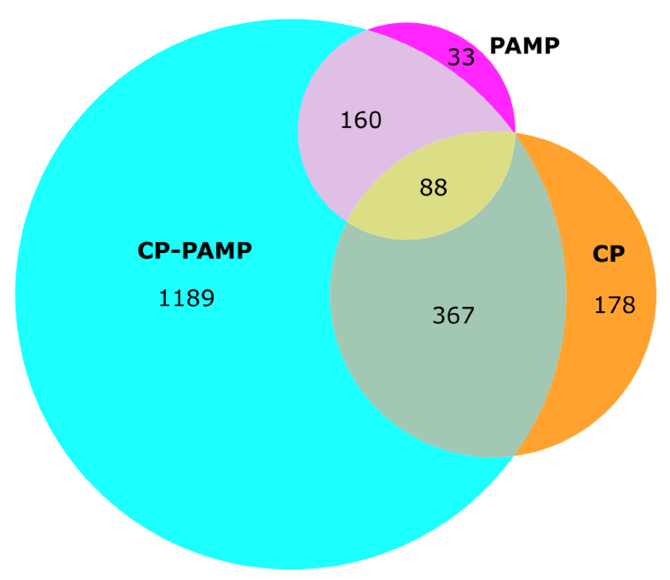
Figure 3: Bubble plot of overrepresented functions of the overlap DEGs of the flagellin injection treatment of 5 independent experiments (analysed with Panther Classification System).

Immune challenge testing

To investigate the influence of chemicals on immune system activation, 48 h exposure of test substances was followed by flagellin challenge and the transcriptome was investigated.

Clobetasol propionate (CP)

Immunosuppressant



__4 treatments per experiment__

- **Control**
- Immune challenge only (**PAMP**)
- Substance exposure only (**CP/DF**)
- Substance exposure followed by immune challenge (**substance (CP/DF)-PAMP**)

Disulfiram (DF)

Negative control (ALDH inhibitor)

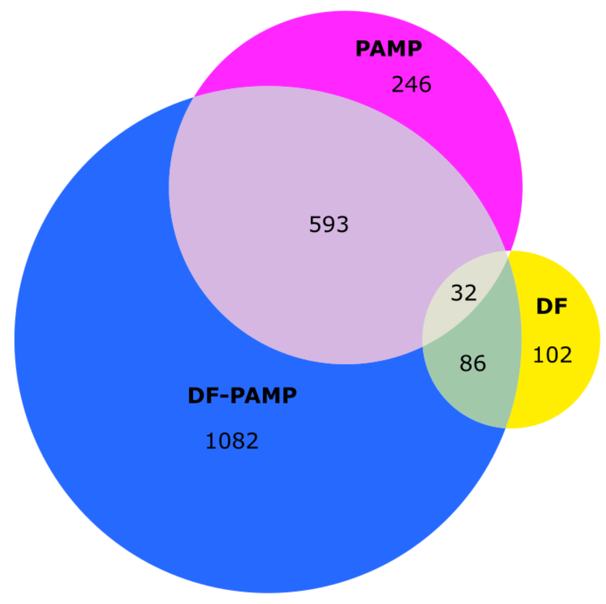
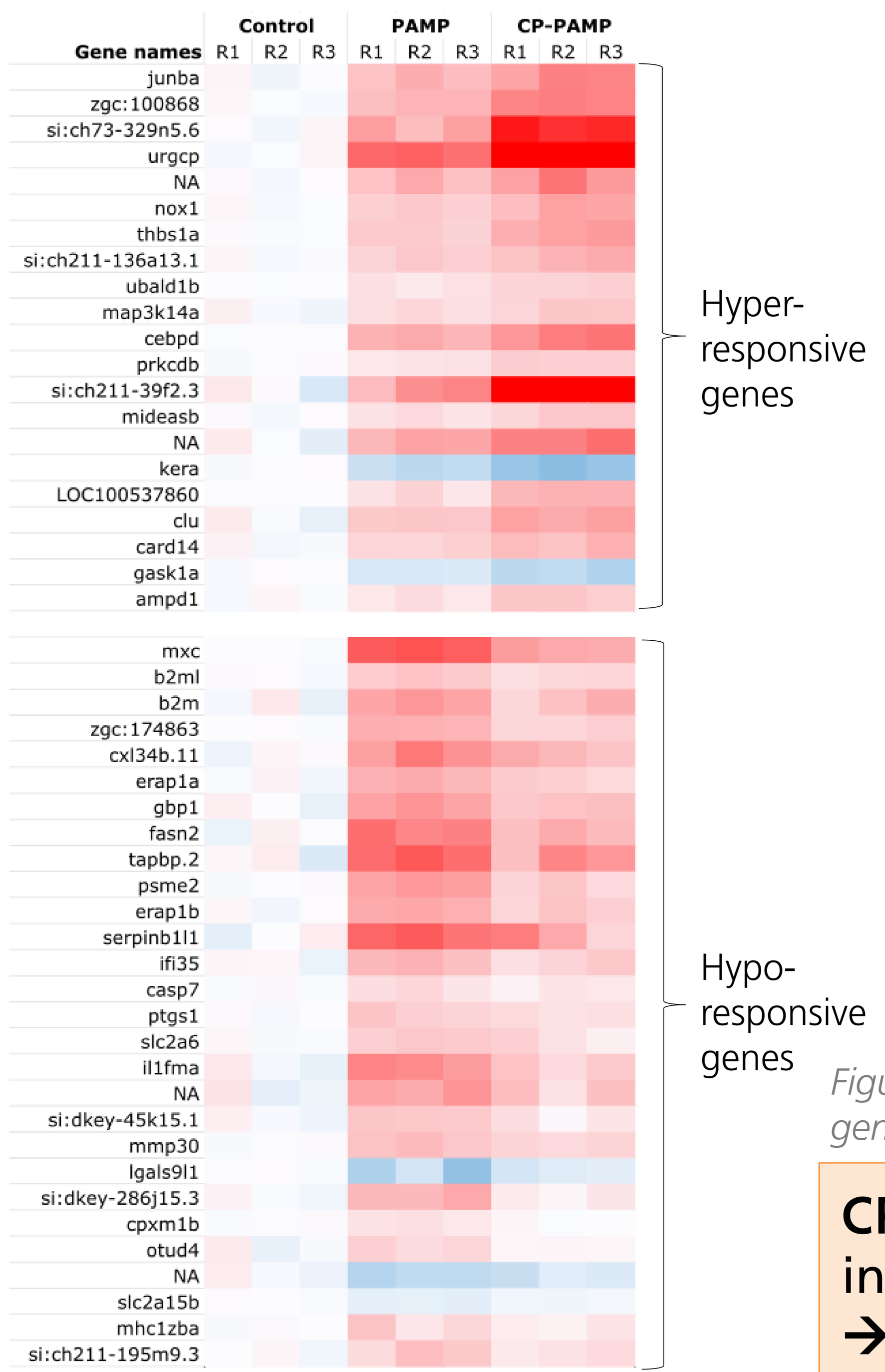


Figure 4, 5: Venn diagrams showing the significantly (padj < 0.05) DEGs of the different main test treatments.

Overlapping DEGs from PAMP exposure from both experiments were extracted. Changes in expression (log_2foldchange (lfc) values) by previous substance exposure were evaluated:
lfc-ratio substance-PAMP to PAMP ≥ 1.5 → **hyper-responsive**
lfc-ratio PAMP to substance-PAMP ≥ 1.5 → **hypo-responsive**



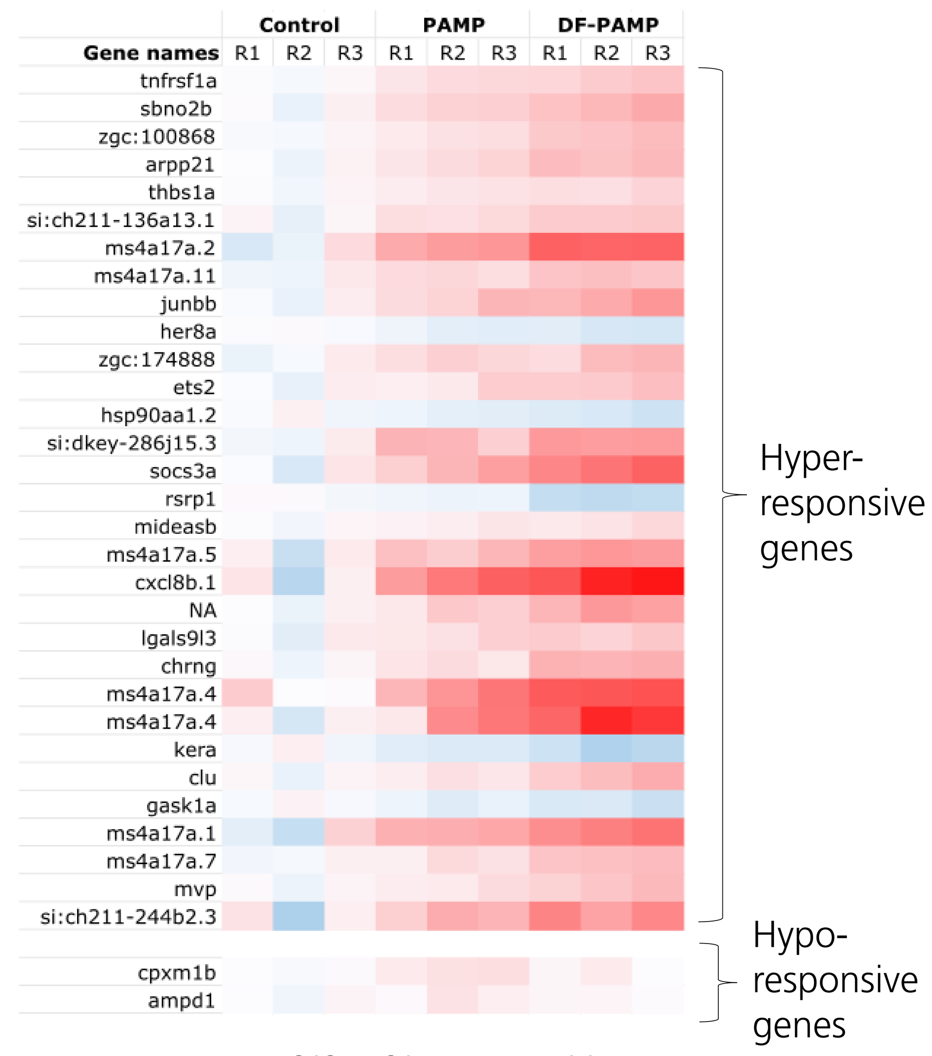


Figure 6: Heatmaps of lfc of hyper- and hypo-responsive genes.

__**Top 10 overrepresented functions of hypo-responsive genes from CP-PAMP**__

- regulation of T cell activation
- regulation of lymphocyte activation
- regulation of leukocyte cell-cell adhesion
- peptide catabolic process
- peptide antigen assembly with MHC class II protein complex
- MHC protein complex assembly
- MHC class II protein complex assembly
- antigen processing and presentation of exogenous peptide antigen via MHC class II
- antigen processing and presentation of exogenous peptide antigen

CP: Hypo-responsive genes are involved in immune processes
→ **immunosuppression!**

DF: No overrepresented GO terms were identified for gene sets and only 2 hypo-responsive genes identified
→ effect on immune activation was unspecific
→ **no immunosuppression!**

→ **The method enables specific detection of immunosuppressive MoA!**

Outlook: Testing of additional substances to validate the method

[1]Vicente-Santos, Amanda; Willink, Beatriz; Nowak, Kacy; Civitello, David J.; Gillespie, Thomas R. (2023): Host-pathogen interactions under pressure: A review and meta-analysis of stress-mediated effects on disease dynamics. In: Ecology letters 26 (11), S. 2003–2020. DOI: 10.1111/ele.14319.
[2]Segner, Helmut; Wenger, Michael; Möller, Anja Maria; Köllner, Bernd; Casanova-Nakayama, Ayako (2011): Immunotoxic effects of environmental toxicants in fish - how to assess them? In: Environmental science and pollution research international 19 (7), S. 2465–2476. DOI: 10.1007/s11356-012-0978-x.
[3]Kataoka, Chisato; Kashiwada, Shosaku (2021): Ecological Risks Due to Immunotoxicological Effects on Aquatic Organisms. In: International journal of molecular sciences 22 (15). DOI: 10.3390/ijms22158305.