

The impact of heat stress and pesticide exposure on honeybee immune response

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The increase in honeybee colony losses is a complex problem caused by a variety of stressors, including pesticides and the growing impacts of climate change. Consequences of climate change are elevating global temperature and more frequent occurrence of heat waves, which makes heat stress an increasingly relevant concern. Additionally, bees are exposed to pesticides through contaminated nectar, pollen, and water during foraging, as well as residues in wax and bee bread. There is limited research on the effect of heat stress, and the combined impact of heat stress and pesticide exposure on honeybee immune response.

To address this gap, we simulated realistic field conditions in the laboratory. Cages were divided into 3 main groups: the control, the acute heat stress group, and the group subjected to acute heat stress while being fed with sublethal doses of acetamiprid. Heat stress was applied on three consecutive days by exposing the bees to 42°C for two hours. Before sampling, half of the cages from each group underwent an immune challenge by mechanical wounding. To evaluate the immune response, we measured total and differential hemocyte counts, quantified antimicrobial peptides (apidaecin, abaecin, hymenoptaecin, and defensin 1) in the hemolymph, and analyzed their relative gene expression in bee abdomens. Additionally, we measured total protein content in the hemolymph to evaluate overall physiological condition.

We found that wounding the cuticle of the abdomen strongly induces the gene expression of antimicrobial peptides and elevates their quantity in hemolymph across all groups. Heat stress alone did not significantly affect antimicrobial peptide expression or their quantity in hemolymph. However, the combination of heat stress and acetamiprid exposure resulted in a significant increase in antimicrobial peptide levels. Furthermore, total protein content in the hemolymph was significantly higher in both stressed groups compared to the control, which suggests an altered physiological state under stress.

Our findings suggest that heat stress alone did not significantly affect immunity, however, when bees face both heat stress and pesticide exposure, their immune response amplifies. This could lead to immune exhaustion over time and cause bees to be less capable of facing pathogens or other stressors.

THE IMPACT OF HEAT STRESS AND PESTICIDE EXPOSURE ON HONEYBEE IMMUNE RESPONSE

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Heat stress: A Growing Threat to Honeybees

Climate change is happening faster than previously predicted (Xu et al., 2018):

- Rise in global temperatures
- More frequent, prolonged, and severe heatwaves



- Hive margins overheating
- Foragers most vulnerable

Negative impact on:

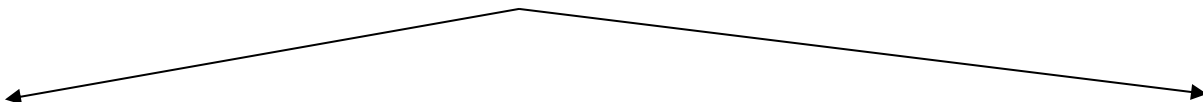
- Growth & development
- Reproduction & longevity
- Foraging & pollination
- Immune system

Cumulative effect of heat stress and insecticides

- **Acetamipride:** The only neonicotinoid insecticide still approved for outdoor use in the EU
- Systemic effects (binds to nicotine-acetylcholine receptors)
- **Sublethal exposure in bees has been linked to:**
 - altered physiology (Shi et al., 2020)
 - changes in behavior, memory, and learning (Abuagla et al., 2024)
 - modulation of immune-related gene expression (Christen et al., 2016)

Honeybee immune system

- Social immunity
- Individual immunity: Innate immune system



Cellular – **hemocytes**

- Immune cells circulating in the hemolymph
- **Marker of cellular immunity**

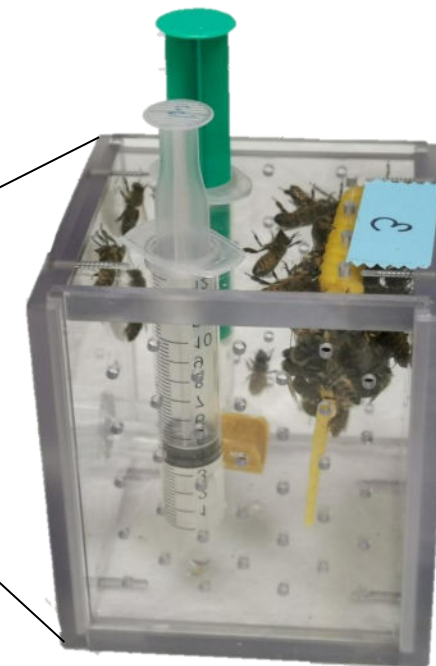
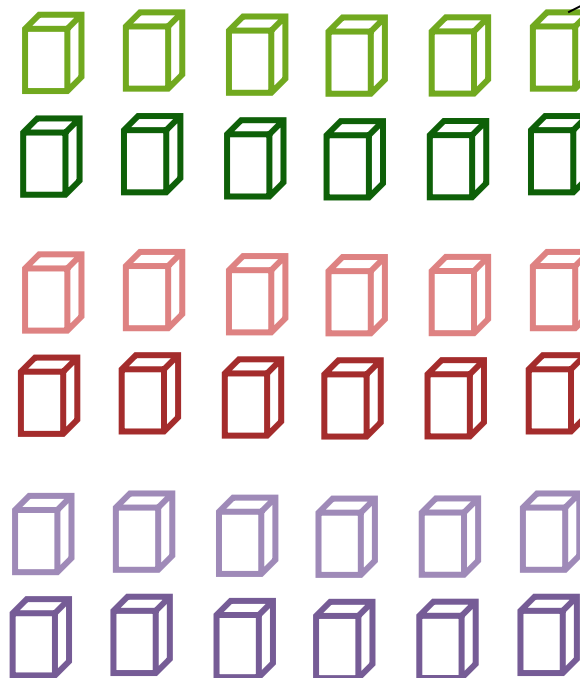
Humoral - **antimicrobial peptides**

- Released in the hemolymph after stress or infection
- Bind pathogens and inactivate them
- **Fast broad response → markers of humoral immunity**

The effect of **heat stress**, and **heat stress + acetamiprid** on the honeybee immune system?

MATERIALS AND METHODS

CTRL	Control
CTRL – IMN	Control + immunization
HS	Heat stress
HS – IMN	Heat stress + immunization
HSA	Heat stress + acetamiprid
HSA – IMN	Heat stress + acetamiprid + immunization

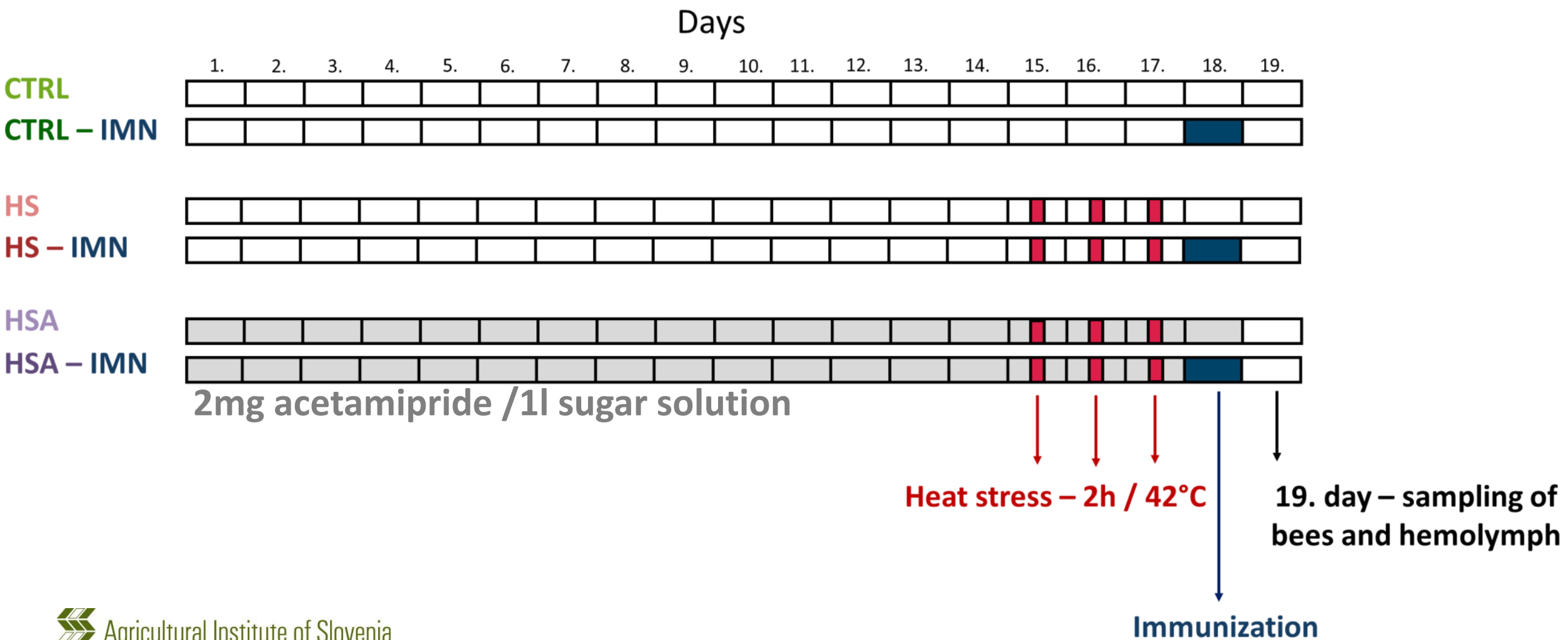


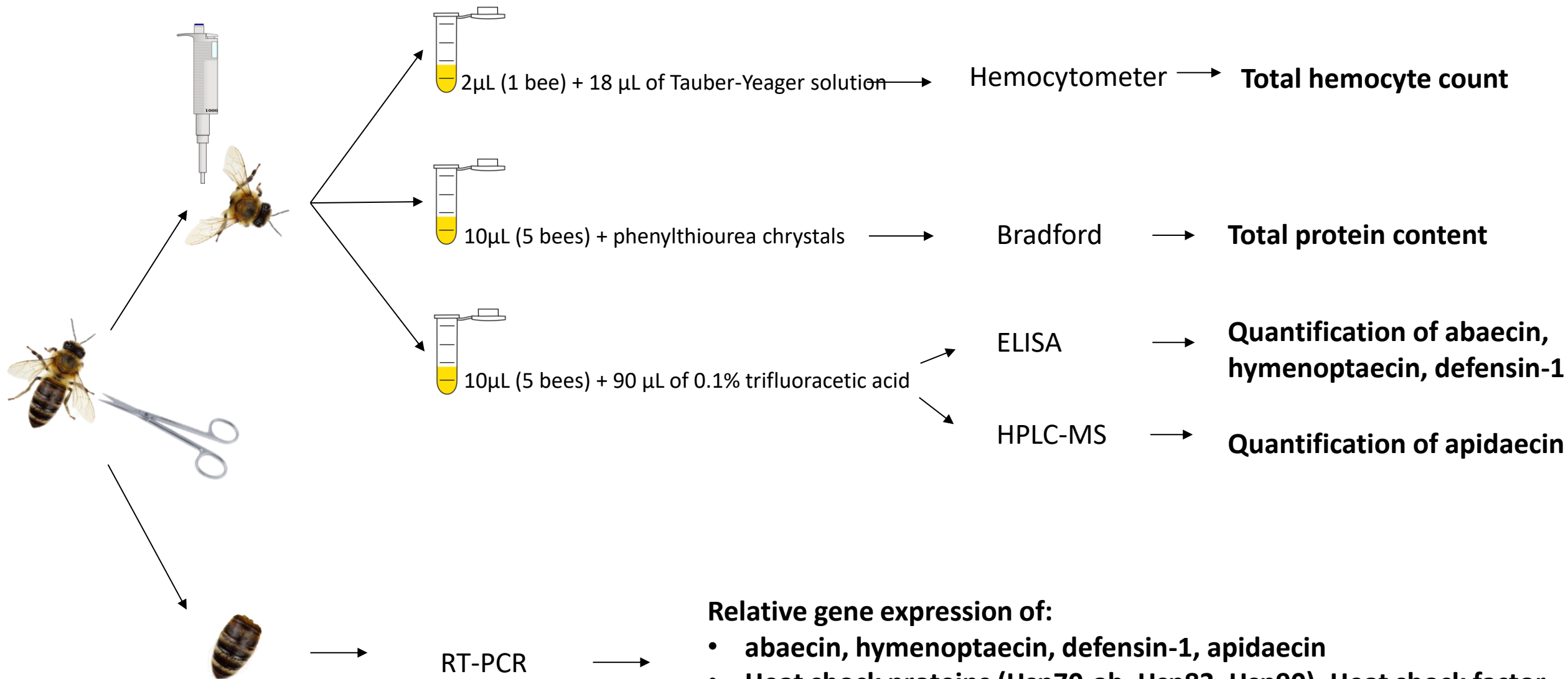
Immunization = immune challenge

- Thin needle injury between 3. and 4. abdominal tergite
- To assess both baseline physiological responses and the capacity to mount an immune response under stressors



Experimental design

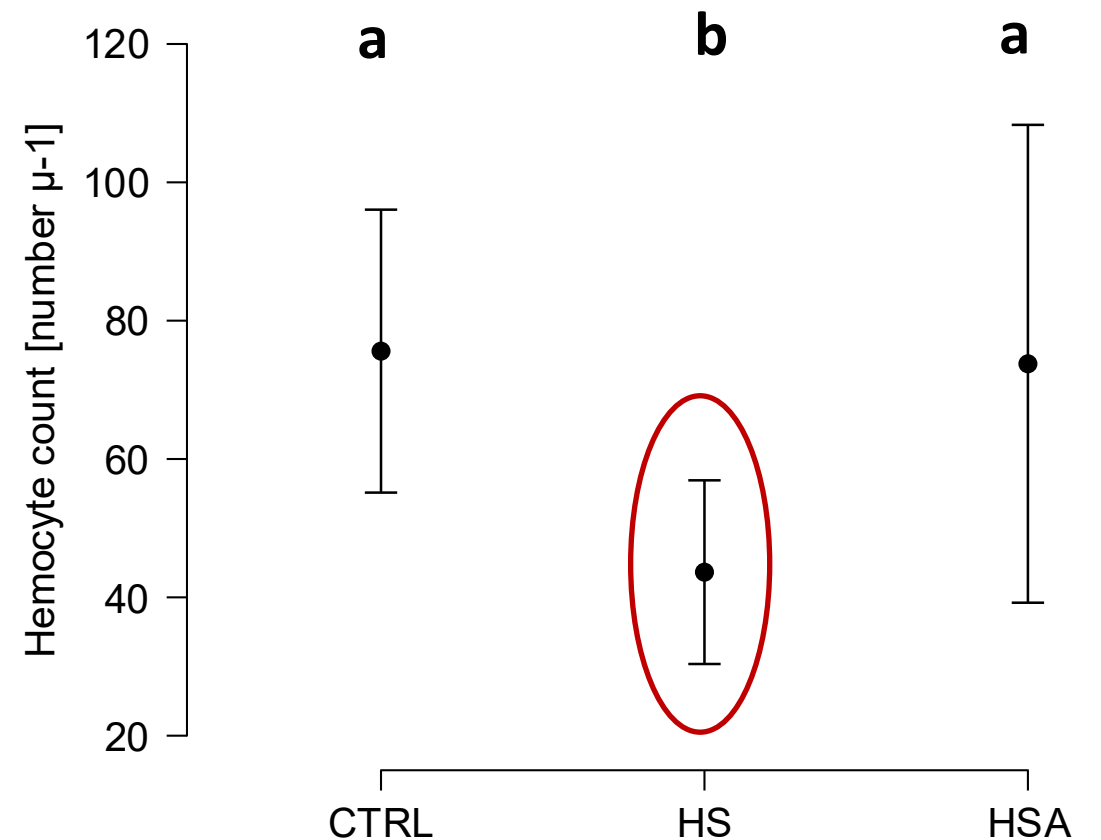
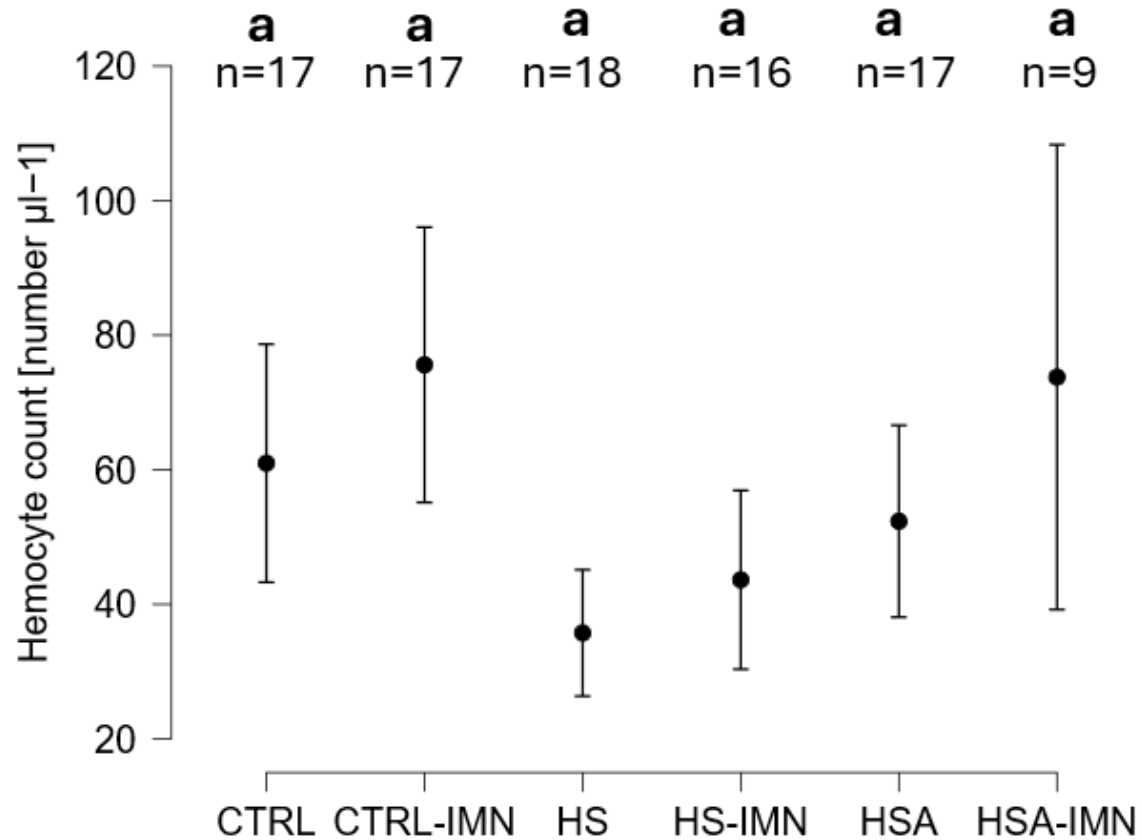




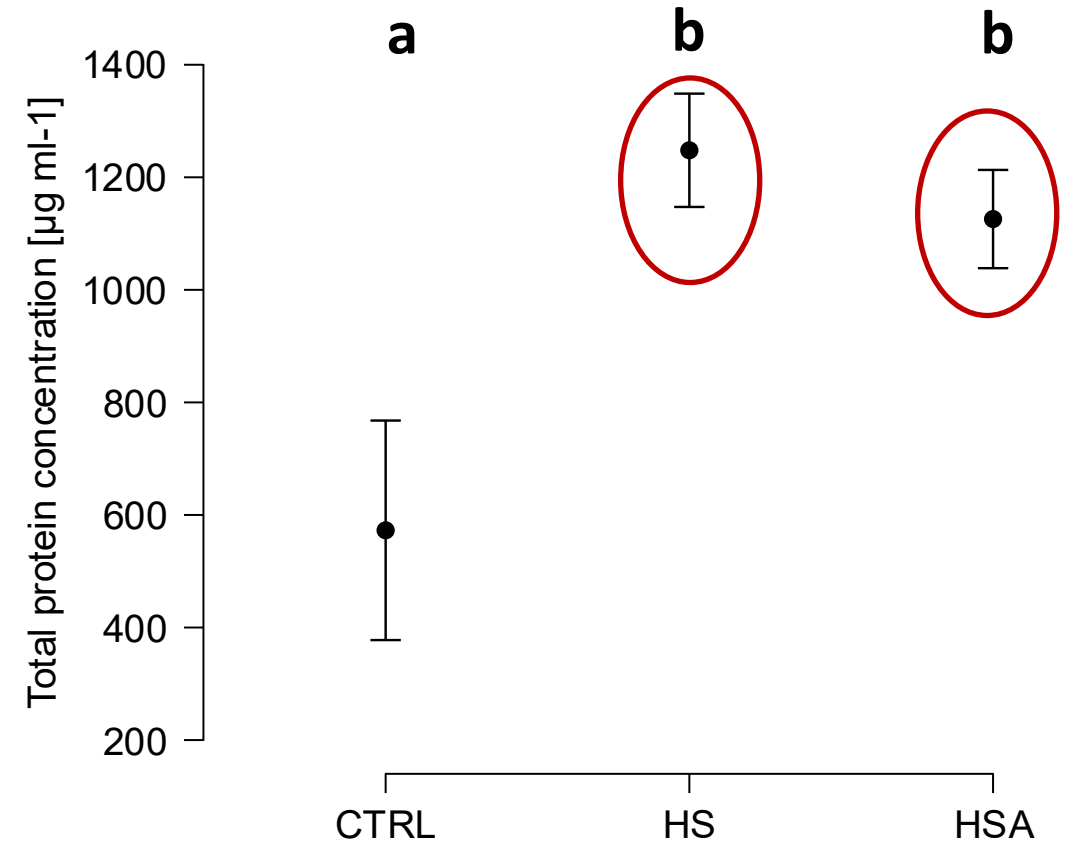
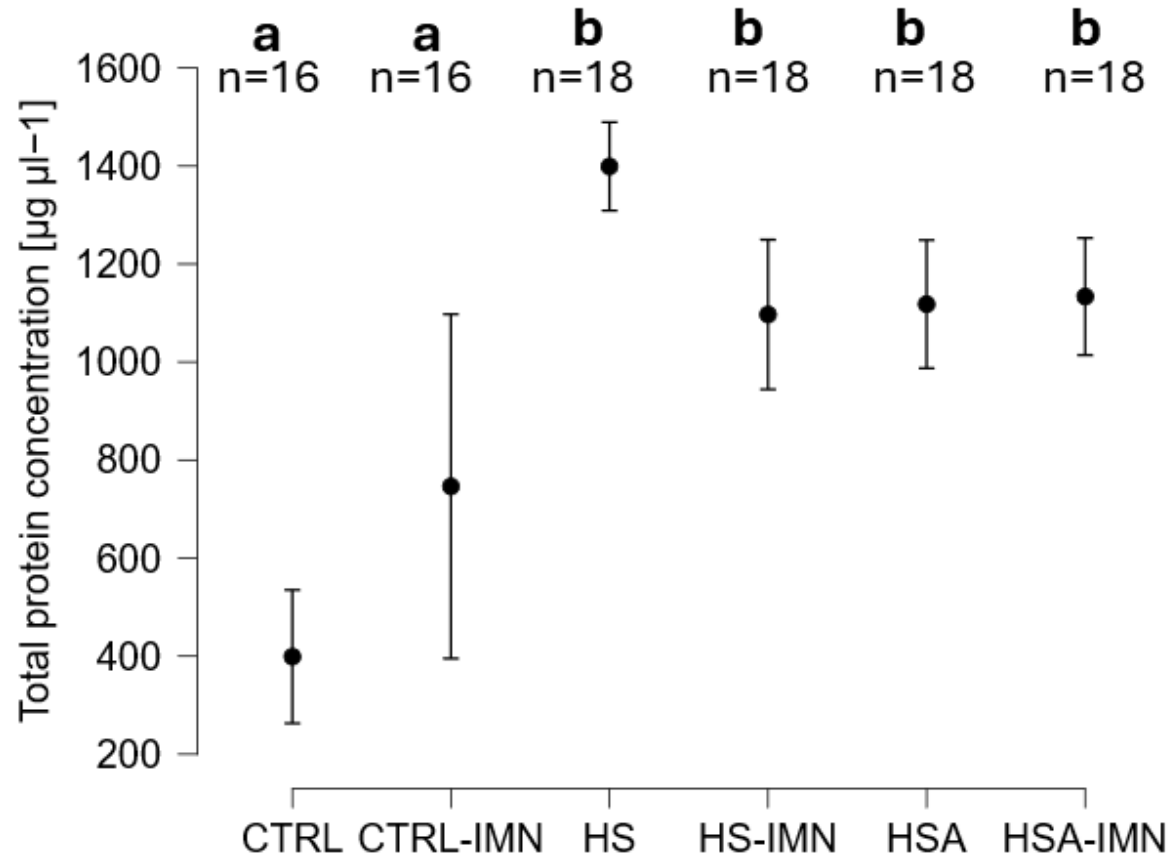
Relative gene expression of:

- abaecin, hymenoptaecin, defensin-1, apidaecin
- Heat shock proteins (Hsp70-ab, Hsp83, Hsp90), Heat shock factor
- Vitellogenin
- Catalase
- Superoxidase dismutase 2

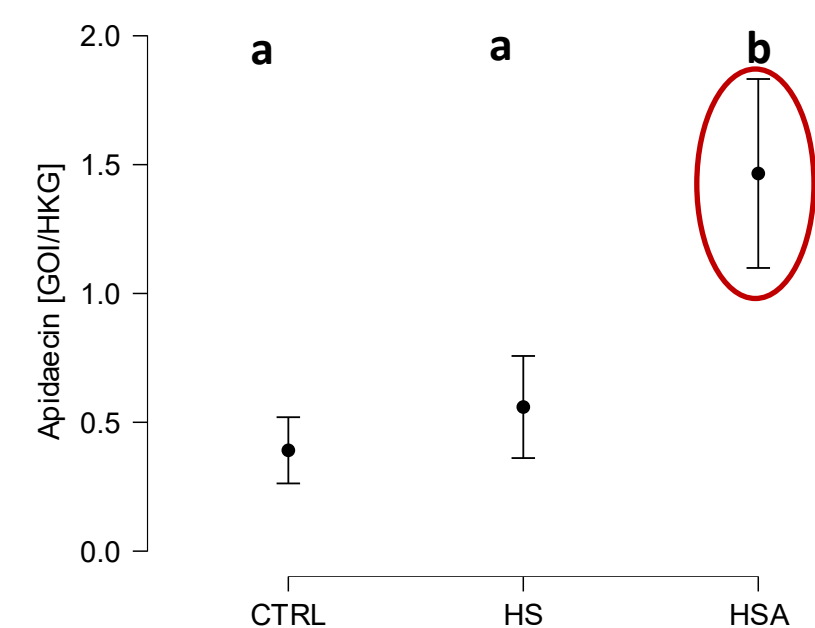
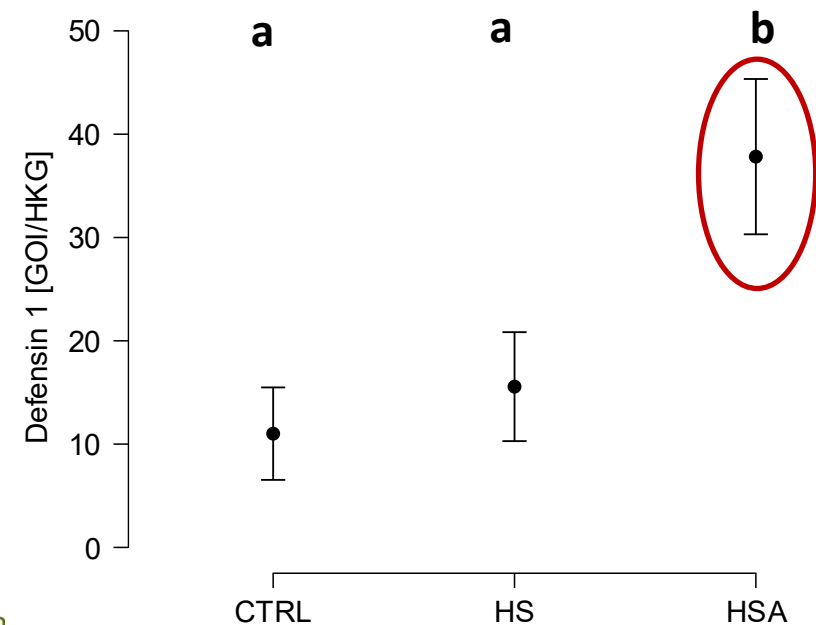
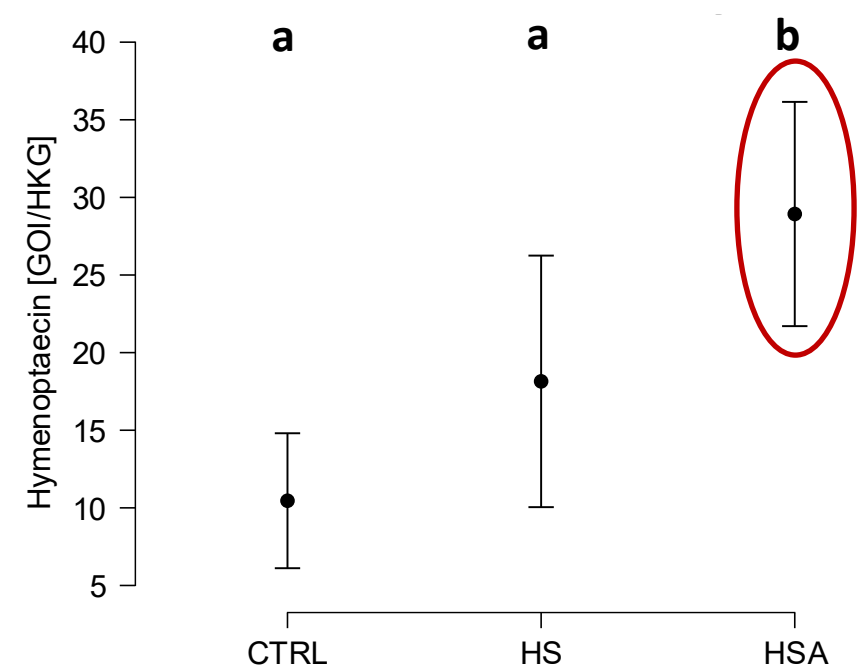
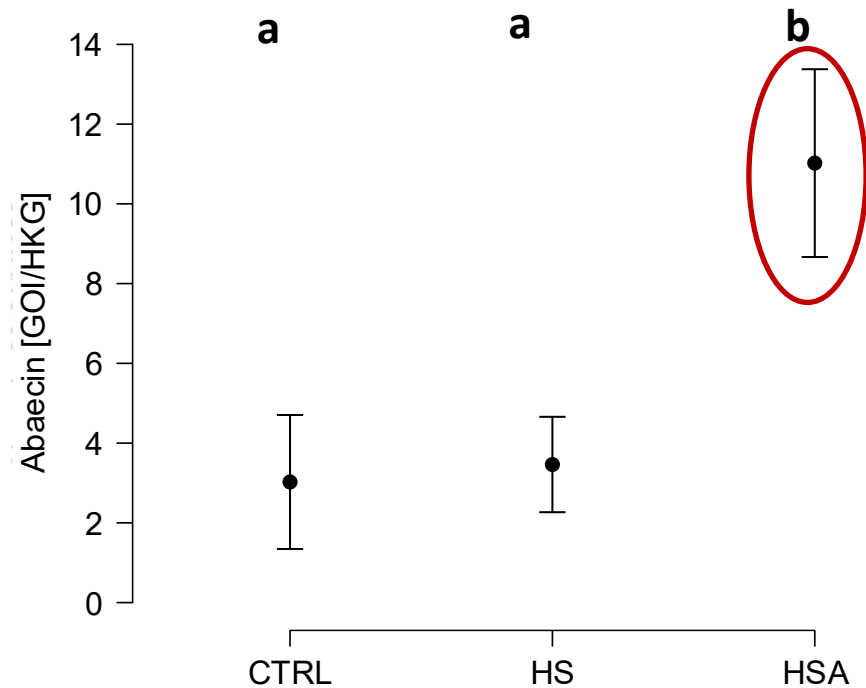
Total hemocyte count



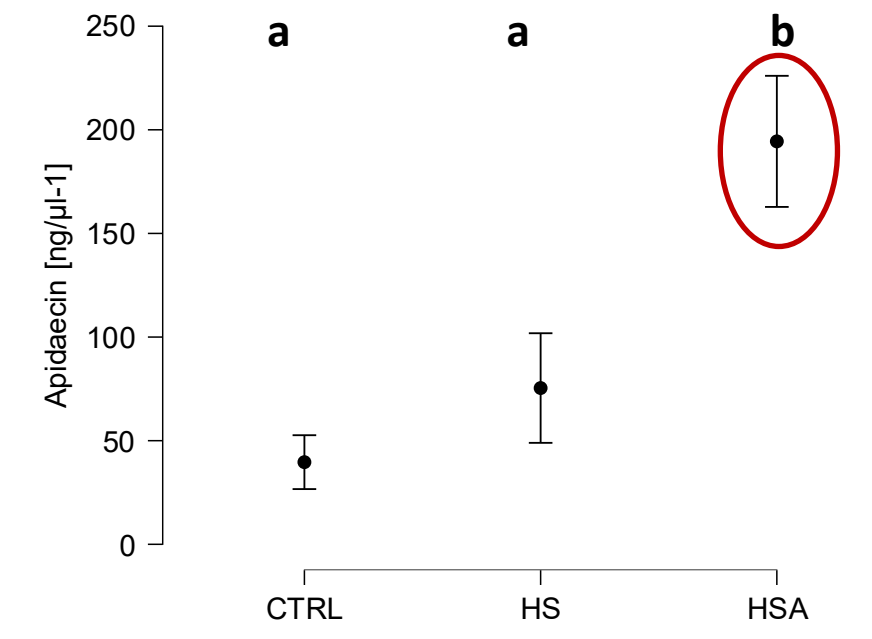
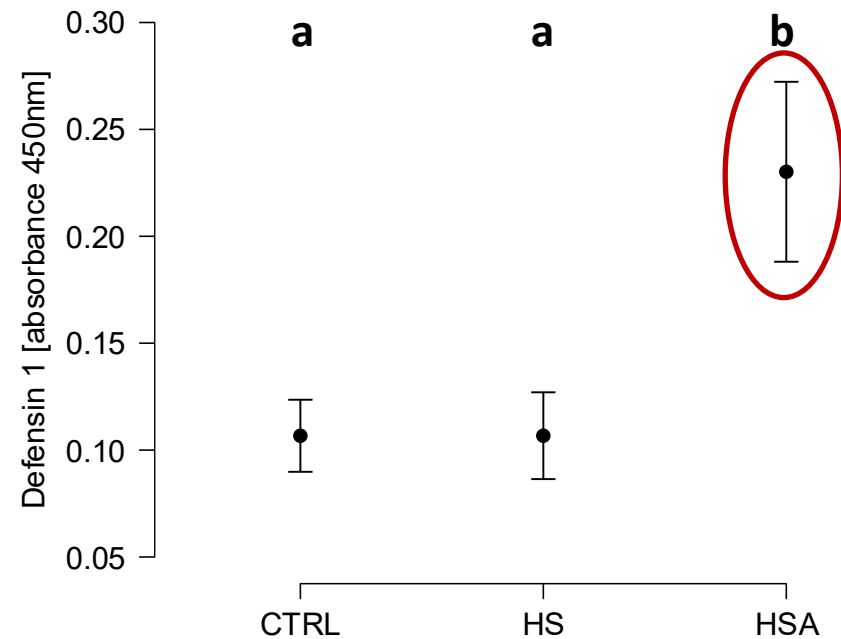
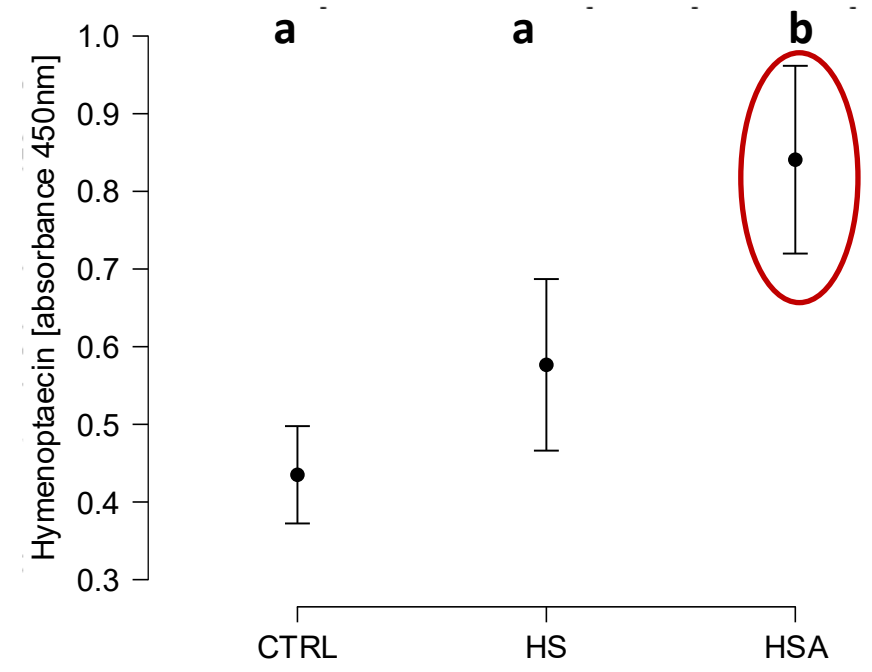
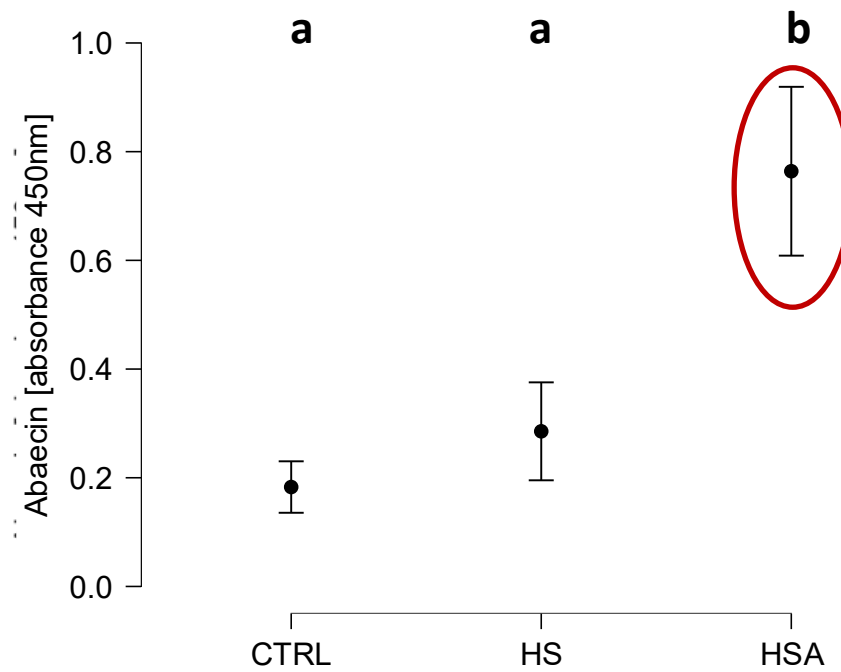
Total protein concentration



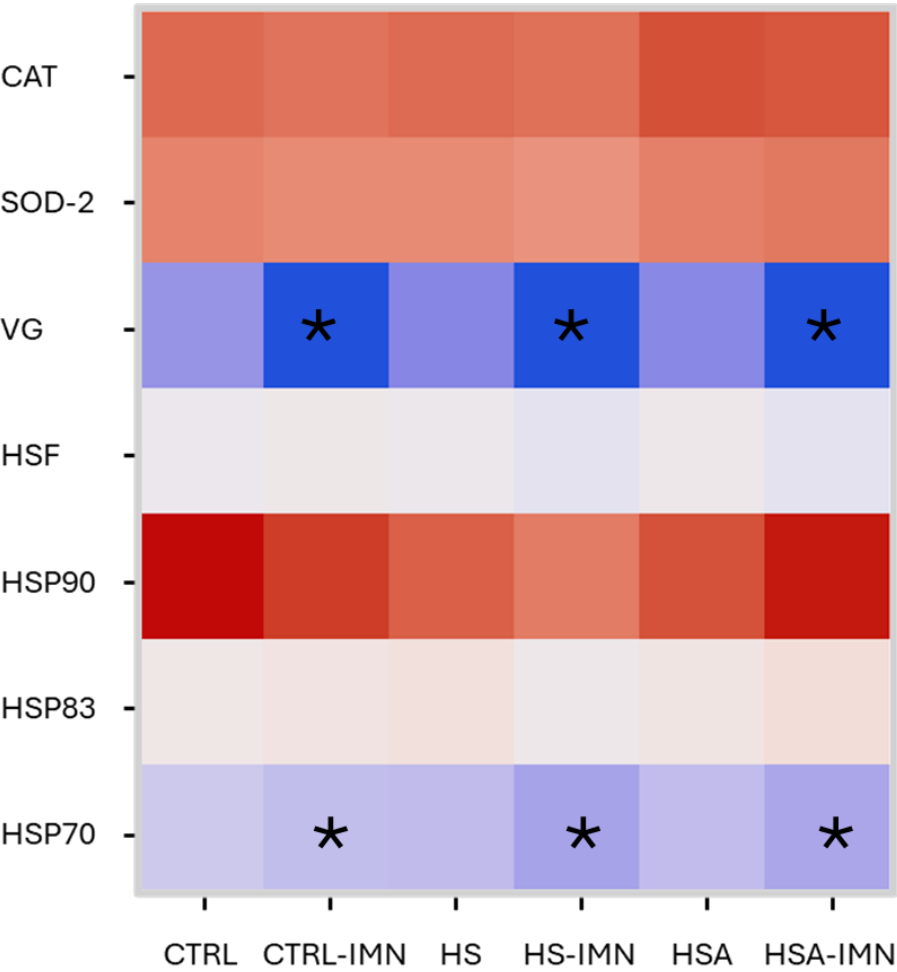
Antimicrobial peptides: gene expression



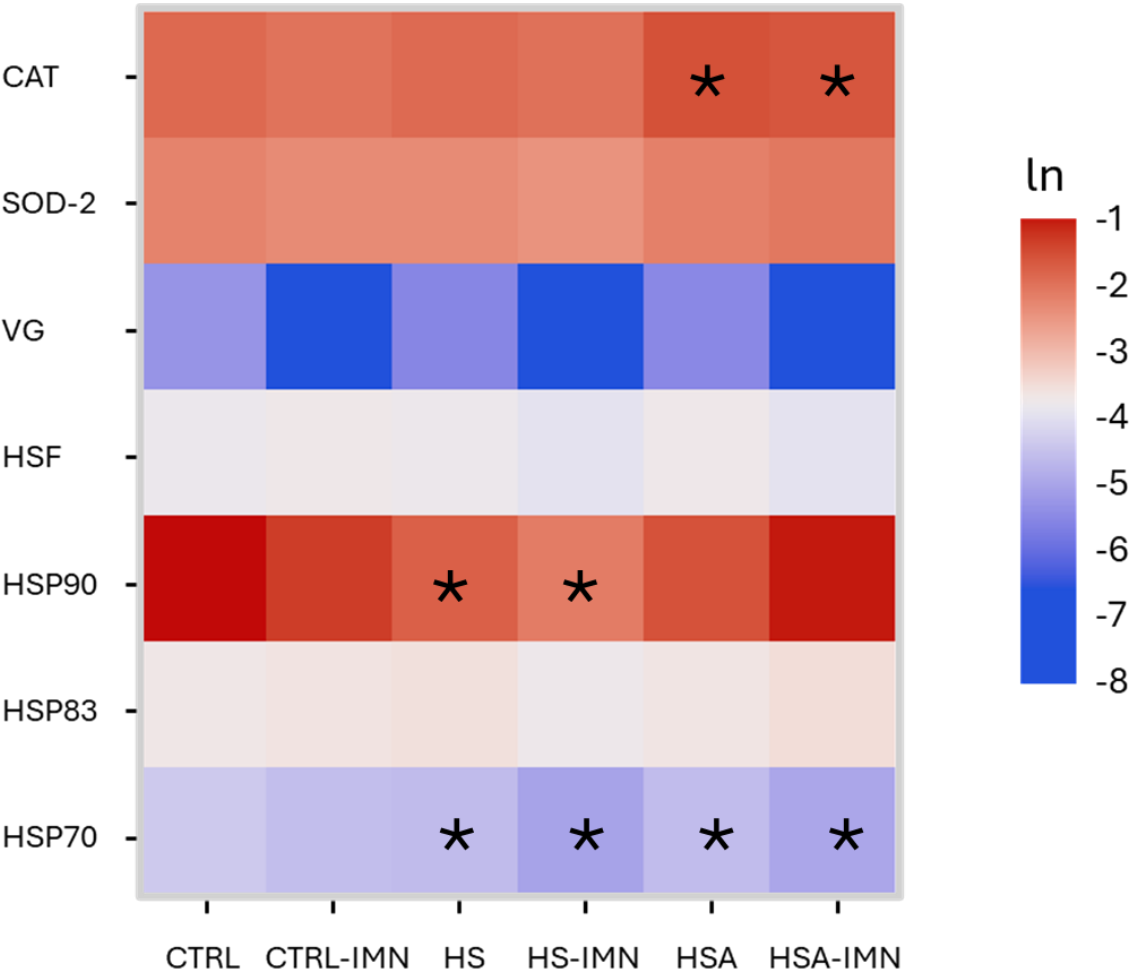
Antimicrobial peptides: concentration in the hemolymph



Catalase, Superoxide dismutase 2, Vitellogenin, Heat shock factor, Heat shock proteins (HSP90, HSP83, HSP70): gene expression



* Significant effect of immunization



* Significant effect of treatment

Conclusions

- **Heat stress:** ↓ hemocytes - reduced immunocompetence
- **Heat stress + acetamiprid:** ↑ AMPs, ↑ catalase (oxidative stress) - cumulative effect
- **Mechanical injury:** ↑ AMPs, ↓ Vitellogenin: trade-off between longevity and immune activation?
- **Stressors alter physiology:** ↑ proteins, Hsp70, and Hsp90 changes

Thank you for your attention!