Preliminary results on repeated non-lethal heat shock on juvenile Asian seabass *Lates calcarifer*

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Aim: To investigate the effectiveness of repeated non-lethal heat shock as a non-antibiotic strategy to improve survival, growth, and immune resilience of juvenile Asian seabass under hatchery microbial challenge conditions.

Problem statement

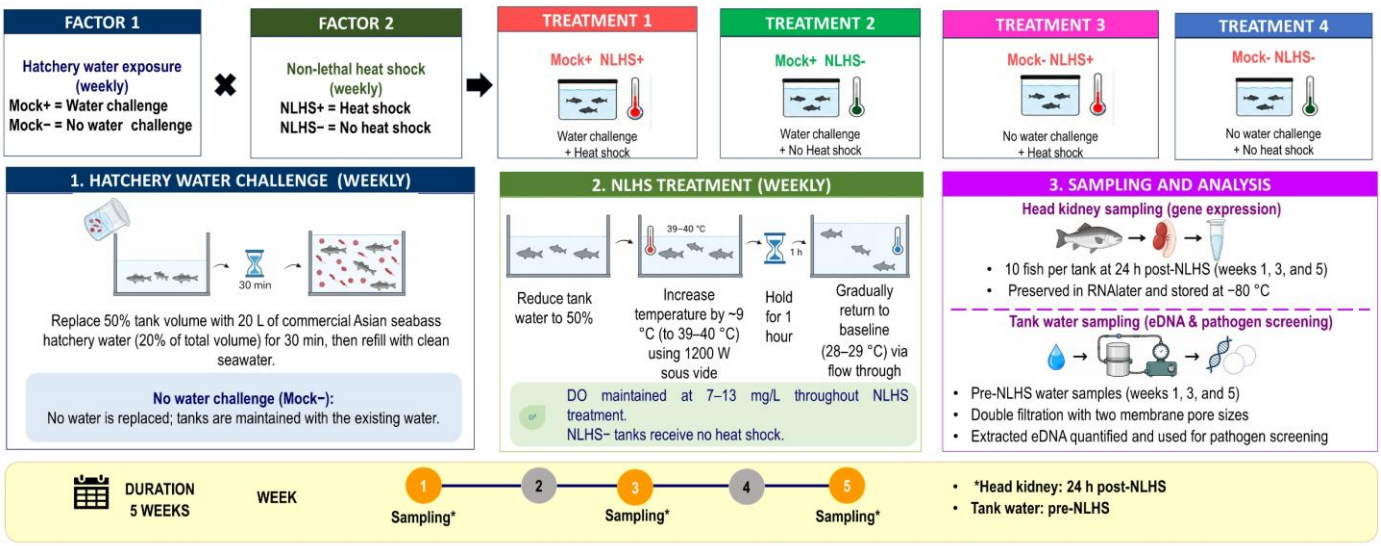
- Juvenile Asian seabass survival in hatcheries remains low, with only approximately 34% of individuals surviving to day 30 post-hatch, making mortality a major challenge during early rearing stages (SEAFDEC, 1998).
- Non-lethal heat shock (NLHS) is a promising non-antibiotic strategy to improve fish robustness by enhancing stress tolerance and stimulating innate immune responses, thereby supporting safer transition into grow-out systems (Limsuwan et.al., 2014; Michel, 2018; Michel, 2022).

Objectives

- To evaluate the effect of repeated weekly NLHS on survival and growth performance of juvenile Asian seabass (*Lates calcarifer*) under hatchery water microbial challenge.
- To characterize the transcriptional response of stress and immune-related genes in head kidney following repeated NLHS and hatchery water exposure.
- To detect and identify key bacterial pathogens in tank water eDNA filtration.

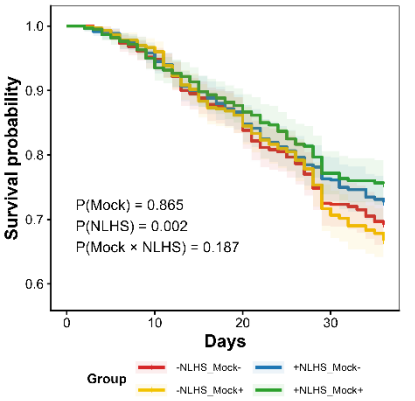
Experimental approach

Five-week 2 × 2 factorial trial (n = 3 tanks/treatment; 200 fish/tank)



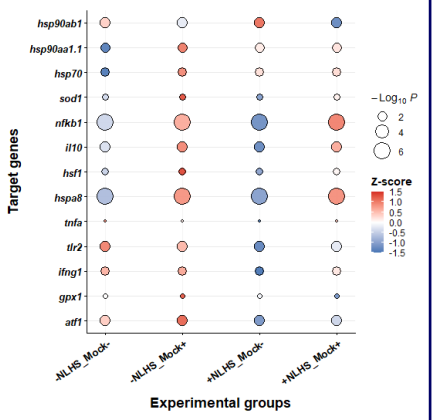
Results & Discussion

Survival and fish performance



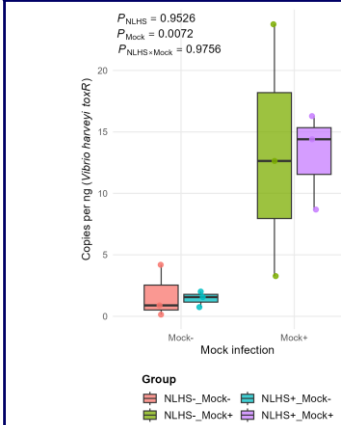
- NLHS improved survival of juvenile Asian seabass (p = 0.002).
- Mock+ exposure lowered survival when fish did not receive NLHS.
- Weekly NLHS may help maintain survival under continuous microbial exposure.

Gene expression analysis



- Mock+ exposure had the strongest effect on gene expression.
- Increased expression was observed in several immune- and stress-related genes, including *nfkb1*, *il10*, *tlr2*, *hsp70*, and *hspa8*.
- Only a few genes significantly affected in response to NLHS, while most showed no change.

eDNA relative abundance



- Mock+ treatments showed higher levels of *Vibrio harveyi toxR*-positive DNA compared to Mock− treatments, regardless of NLHS.
- This suggests that hatchery water remained a source of pathogen-associated DNA throughout the trial (Zhang et. al., 2020).

Conclusion

- Weekly NLHS significantly improved juvenile seabass survival, supporting thermal priming as a promising practical hatchery management strategy.
- NLHS fish showed reduced immune- and stress-gene expression (*tlr2, tnfa, ifng1, hsp70, hsp90aa1*) during water challenge, suggesting HSP70/90-mediated dampening of NFκB-driven inflammation while preserving core defence pathways (*nfkb1, il10, sod1*) (Kumar et. al., 2023; Mackenzie et. al., 2025).
- eDNA confirmed persistent *Vibrio harveyi* in hatchery water, highlighting environmental surveillance as a useful biosecurity tool.
- **NLHS offers a practical, chemical-free approach to improving fish health and hatchery performance.**

References:

SEAFDEC, 1998
Limsuwan et. al, 2014
Michel, A., 2022
Michel, A., 2018
Zhang et. al., 2020
Kumar et. al., 2023
Mackenzie et. al., 2025

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