Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente, Ciclo XXXIX

Interactive and cumulative impacts of CO_2-related ocean changes and anthropogenic pollutants in marine organisms: from cellular mechanisms to physiological tolerance

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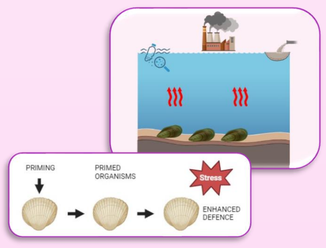
INTRODUCTION

Human activities are the main driver of climate change and marine ecosystem are threatened by multiple combined stressors [1]. These include climate-change-related stressors, as marine heatwaves and ocean acidification, and chemical pollution because of anthropogenic activities [2]. In this context, wild and cultured bivalves as mussels and clams are facing severe challenges that may cause physiological disturbance and stocks decrease: is now of outmost importance to unravel the mechanisms of disturbance of these stressors and organisms tolerance thresholds in order to provide biodiversity conservation and sustainable aquaculture management, understanding cellular and physiological responses involved in organisms' health status and acquisition of stress-tolerance mechanisms [3].
Recently, novel approaches to improve organisms' tolerance are emerging: stress-priming is a promising technique consisting in triggering stress-response mechanisms in organisms exposed at low magnitude of stress, to facilitate the onset of tolerance development towards a forthcoming major stress through molecular and biochemical adaptations [4].

AIMS of PhD

- Understand the mechanisms of tolerance and susceptibility to multiple combined stressors related to pollution and climate change in bivalves
- Explore the applicability and effectiveness of stress-priming techniques in bivalves' populations

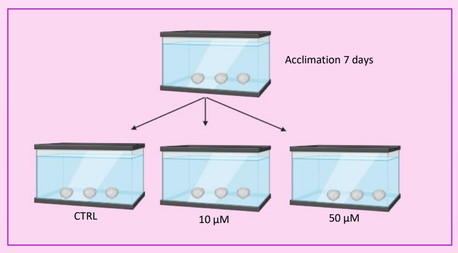


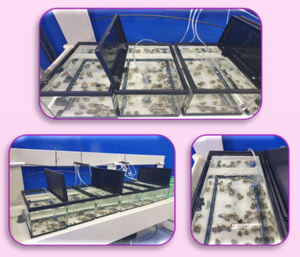
FIELD ACTIVITIES

Sampling of wild mussels along Conero Riviera from sites with different anthropogenic impacts and evaluation of their health status in relation to environmental variability, seasonality and pollution.

LAB ACTIVITIES - STRESS-PRIMING

Selection of best-priming technique: clams *Ruditapes philippinarum,* after a first period of acclimation were assigned to control with clean water and to the two concentration of hydrogen peroxide. Organisms were fed every day with a commercial blend of microzooplankton for filter-feeding organisms. Water was changed four times and the H_2O_2 solution was dosed each times.
After 7 days of exposure, clams were dissected, and tissues collected for biochemical and immunological analyses.

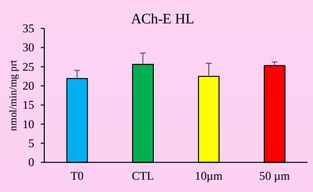


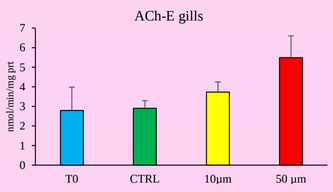


Exposure time: 7 days
Temperature: 18 °C
H_2O_2concentration:10 µM and 50 µM

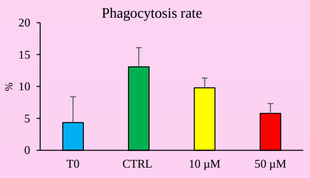
STRESS-PRIMING RESULTS

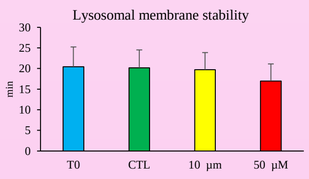
CHOLINERGIC RESPONSE



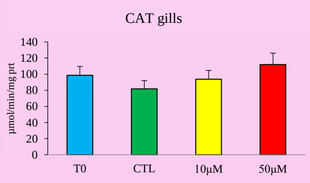


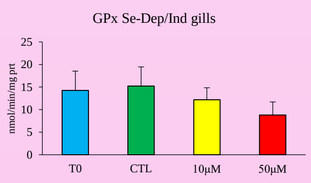
IMMUNITARY PARAMETERS

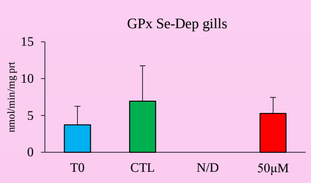


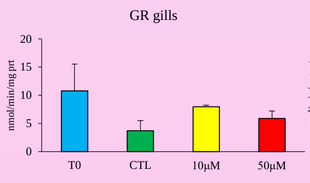


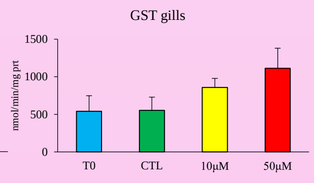
OXIDATIVE STRESS-GILLS



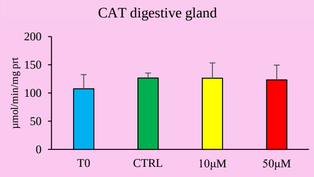


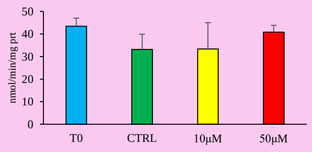


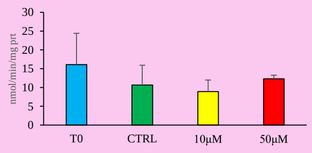


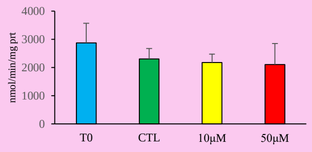


OXIDATIVE STRESS-DIGESTIVE GLAND









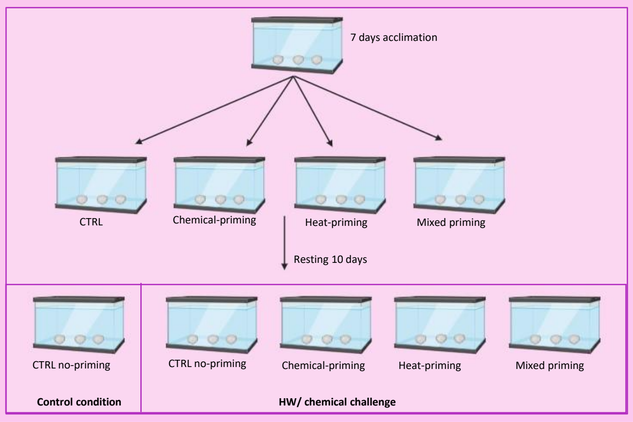
DISCUSSIONS

- Acetylcholinesterase activity shows an increase in gills sample exposed to 50 µM concentration of H_2O_2
- Immunity parameters resulted more sensitive for 50 µM than 10 µM concentration
- Enzymatic activity in gills reported a major response for 50 µM and only for Glutathione Reductase it's shown an increase activity for 10 µM
- Enzymatic activity in digestive gland shows not important difference between the two concentrations tested and control, only for peroxidase 50 µM concentration has an increase

These results demonstrate that 50 µM concentration of H_2O_2 is the best choice for chemical-priming technique; the organisms show a trigger in stress-response mechanisms as wanted. So, this concentration will be used in next experimental activities with stress-priming.
A heat-priming technique is investigated in the next experimental activities alone and combined with chemical-priming.

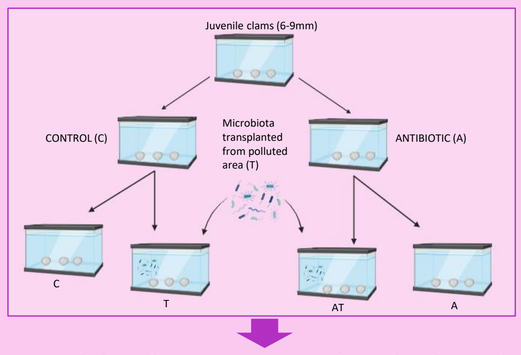
NEXT STEPS

EFFICIENCY OF PRIMING TECHNIQUE



- **Species**: *Ruditapes philippinarum*
- **Chemical-priming** (50 µM H_2O_2), **heat-priming** (30°C) and **mixed priming** for 7 days. Temperature 24°C
- **Resting** at 24 °C for 10 days
- **HW/chemical challenge**: heat waves (32-34 °C) and chemical contaminant (PFOA 2 µg/L) for 7 days
- **Surviving test**: 10 days after the end of the challenge

MICROBIOTA ROLE IN HOST-RESPONSE TO STRESSORS



Combined exposure to chemicals and heat waves

- **Microbiota** of clams (*R. philippinarum*) grown in polluted area will be transplanted in antibiotic-treated clams
- **Exposure to chemical stress** will define the role of microbiota in clams' response to stress

REFERENCES

[1] Nagelkerken, I.; Munday, P.,L., 2016. Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. Global Change Biology, 22: 974-989
[2] IPCC 2021- Climate Change: The Physical Science Basis Contribution of Working Group I to AR6 of the IPCC.
[3] Benedetti, M.; Giuliani, M., E.; Mezzelani, M.; Nardi, A.; Pittura, L.; Gorbi, S.; Regoli, F., 2021. Emerging environmental stressors and oxidative pathways in marine organisms: Current knowledge on regulation mechanisms and functional effects. Biocell, 46(1): 37-49.
[4] Alam, R.; Ehiguese, F.,O.; Vitale, D.; Martin-Diaz, M., L., 2022. Oxidative stress response to hydrogen peroxide exposure of *Mytilus galloprovincialis* and *Ruditapes philippinarum*: Reduced embryogenesis success and altered biochemical response of sentinel marine bivalve species. Environmental Chemistry and Ecotoxicology, 4: 97-105.