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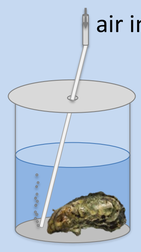
AWI | Biosciences | 1 *Integrative Ecophysiology*, 2 *Shelf Sea System Ecology*, 3 *Coastal Ecology*

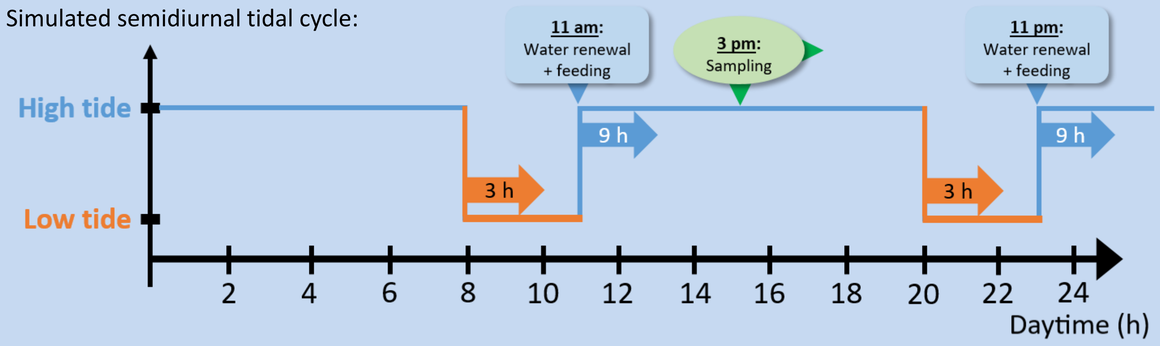
Microplastics and warming: Metabolic disorders in Pacific oysters (*Crassostrea gigas*) from the intertidal zone

Introduction

Microplastics (MP; < 5 mm) have been identified as an emergent environmental threat, especially for filter-feeding organisms such as bivalves[1-6]. Most laboratory studies in the past however used unrealistic MP concentrations[7], experimental setups which simulated just the subtidal habitat, or neglected the potential synergistic risk of MP under climate warming. In contrast to the subtidal habitat, sessile intertidal inhabitants can be subjected to drastic aerial warming during midday low tides[8-9] and may be exposed to relatively high MP concentrations due to sedimentation processes[10]. Considering these habitat specialties, this study focused on potential effects of environmentally realistic MP concentrations on the metabolism of intertidal Pacific oysters (*Crassostrea gigas*, Thunberg 1793).

Experimental Design





- Mix of different-sized polystyrene MP beads (4 µm + 7.5 µm + 10 µm), added during feeding
- Three MP treatments (n = 45 each):
  - 0 µg MP/L → CTR
  - 0.025 µg MP/L → Low MP
  - 25 µg MP/L → High MP
- Sampling of digestive gland and gill tissues (n = 10 – 12 per treatment)
  → after 0, 3, and 12 days of MP exposure (T0, T3, T12) (objective 1)
  → after 16 days of MP exposure, with a single air-exposure warming during the last simulated low tide (16 °C – 26 °C; 3 °C/h) (objective 2)
- Untargeted metabolic profiling based on ^{1}H-nuclear magnetic resonance (NMR) spectroscopy

Objectives

(1) Evaluation of dose- and/or time-dependent effects of polystyrene MP beads on metabolism of intertidal *C. gigas*

(2) Evaluation of MP effects on *C. gigas*' metabolic vulnerability to a single air-exposure warming event during simulated low tide

Preliminary results

Objective 1: Dose- and time-dependent MP effects

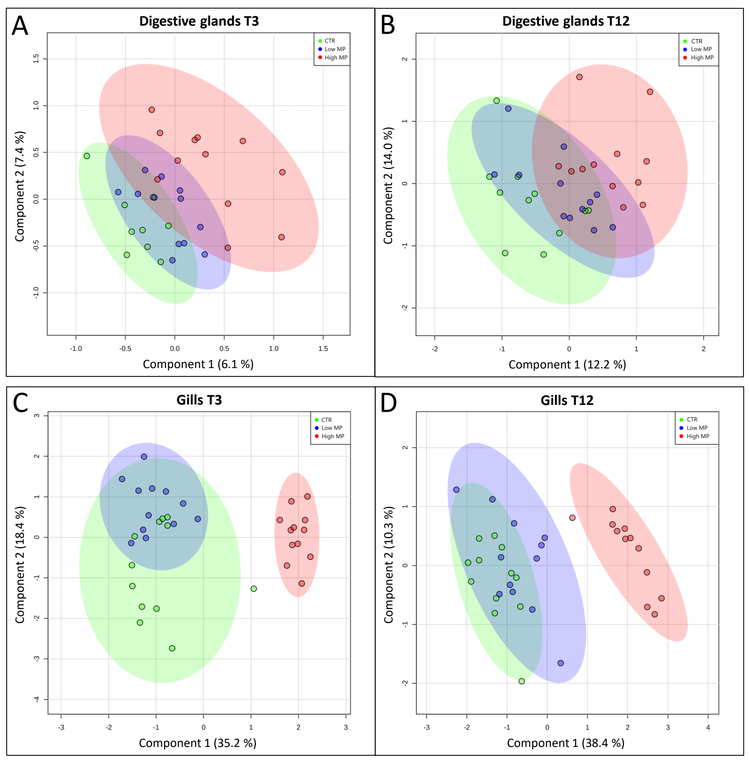


Fig 1 PLS-DA scores plots of ^{1}H-NMR spectra (n = 12 per treatment), showing the dose-dependent impact of MP exposure over time (T3, T12) on the metabolic profile in digestive gland tissue (**A**, **B**) and gill tissue (**C**, **D**) of *C. gigas*. CTR: Control oysters (green); Low MP: 0.025 µg/L (blue); High MP: 25 µg/L (red). Ellipses correspond to a confidence interval of 95 %.

Digestive glands:
- Metabolic profiles of oysters' digestive glands were comparable to each other independent of MP concentration or exposure time

Gills:
- Control oysters and low MP exposed oysters shared a very similar gill metabolome
- Gill metabolome of high MP exposed oysters changed significantly

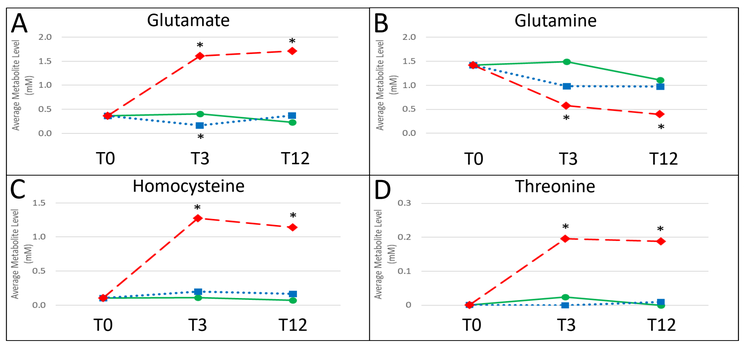


Fig 2 Plot of averaged key amino acid concentrations in gill tissue of *C. gigas* against time (in mM, expressed as means of n = 11 – 12). Control oysters: solid green line (circles); low MP (0.025 µg/L): blue dotted line (squares); high MP (25 µg/L): red dashed line (diamond). Asterisks indicate significant differences in metabolite levels of MP exposed oysters relative to control oysters at the same sampling point (Tukeys; P ≤ 0.05).

Mainly affected pathways in gills:
- Glutamine and glutamate metabolism
- Alanine, aspartate and glutamate metabolism
- Glycine, serine and threonine metabolism
- Cysteine and methionine metabolism

Objective 2: MP effects & warming

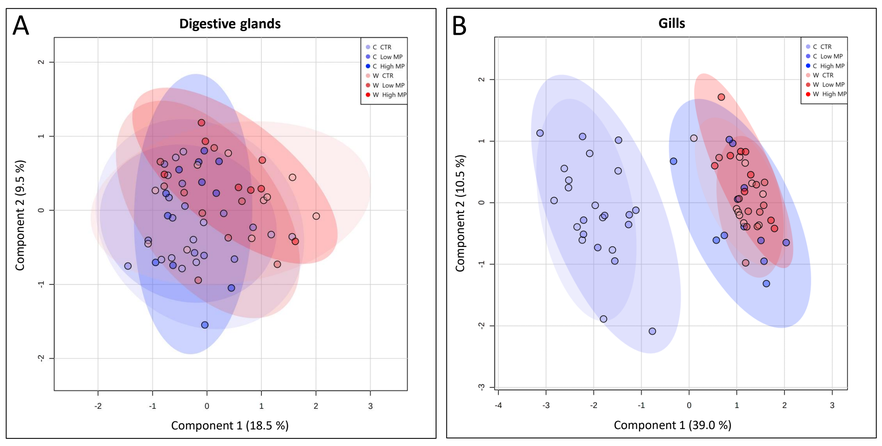


Fig 3 PLS-DA scores plots of ^{1}H-NMR spectra (n = 10 – 12 per treatment) of the metabolic profile in **(A)** digestive gland tissue and **(B)** gill tissue of *C. gigas* from different MP treatments after air-exposure warming during simulated low tide, compared to T12 as reference for no warming conditions. C: No warming (blue shades of colour); W: Warming (red shades of colour); CTR: Control oysters; Low MP: 0.025 µg/L; High MP: 25 µg/L. Ellipses correspond to a confidence interval of 95 %.

- Metabolic profiles of oysters' digestive glands were comparable to each other independent of MP concentration and warming
- Gill metabolome of oysters after air-exposure warming during low tide was clearly separated from oysters sampled without warming event, independent from MP concentration
- High MP concentration induced similar metabolic disorders in gills as warming

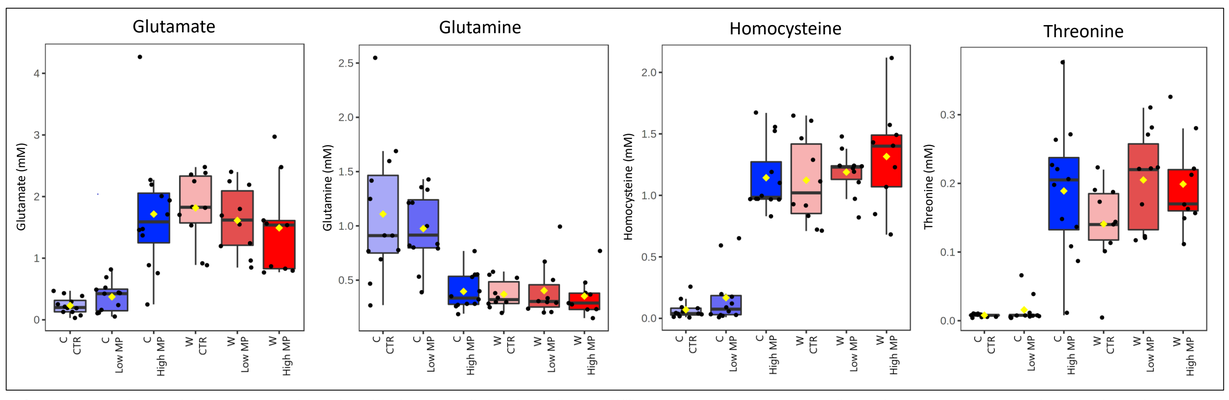


Fig 4 Key amino acid concentrations in gill tissues of *C. gigas* from different MP treatments after air-exposure warming during simulated low tide, compared to T12 as reference for no warming conditions. C: No warming (blue shades of colour); W: Warming (red shades of colour); CTR: Control oysters; Low MP: 0.025 µg/L; High MP: 25 µg/L.

Conclusions & perspectives

Aerial heat stress during simulated low tide and high MP concentrations induced similar metabolic disorders in gill tissue of *C. gigas* (both alone and combined). Apart from **potential energetic effects** (e.g., alanine-aspartate-glutamate metabolism), the increased glutamate levels together with the affected cysteine-methionine metabolism may suggest **ongoing oxidative stress**. Further integrative analyses of energetic parameters, oxidative stress, and antioxidant defense mechanisms will help to clarify the physiological impact of combined MP and warming and the resulting consequences for the overall fitness of intertidal oyster populations.

References
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