European flat oysters (*Ostrea edulis*) under anthropogenic pressure:

Assessing the combined effects of increasing microplastic pollution and a warming marine environment

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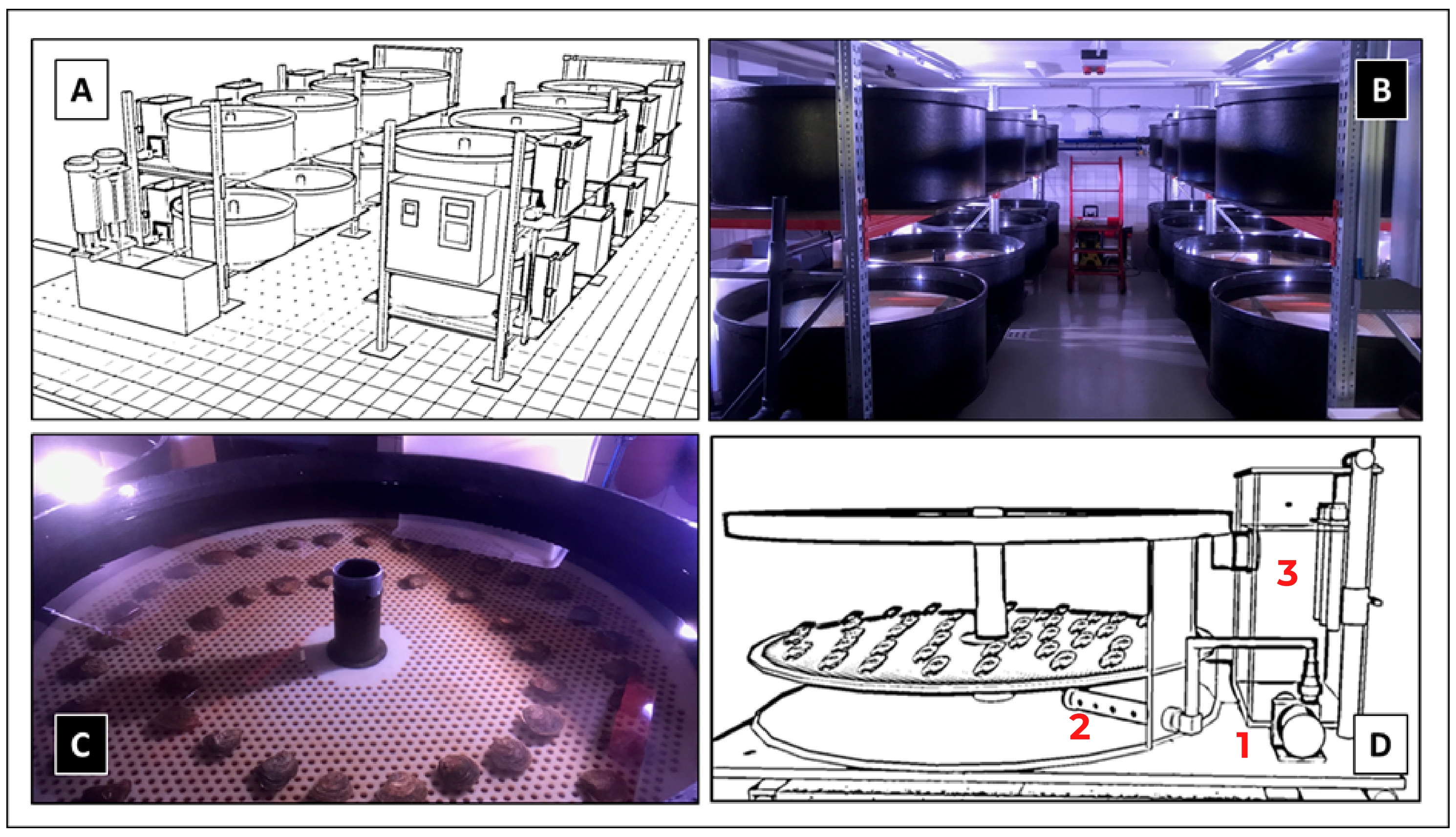
INTRODUCTION

Microplastics (MP) have been identified as an emergent environmental threat, especially for filter-feeding organisms such as bivalves. This project focuses on the chronic multistressor impacts of environmentally relevant MP concentrations under a predicted warming scenario for elucidating potential additive, synergistic, or antagonistic effects on the physiology and performance of the ecologically important European flat oyster (*Ostrea edulis*). Additionally, we will examine changes to MP dynamics between treatments due to potential variability in the filtration, biodeposition and aggregation of MP caused by the oysters themselves.

MATERIALS & METHODS

Sixteen semi-recirculating mesocosms (500 L vol.) were constructed for this experiment at the Alfred Wegener Institute marine station on Helgoland, Germany. Each mesocosm is supplied with running unfiltered seawater (400 L/h), and has one of four treatments applied. Three replicate mesocosms containing 50 *O. edulis* and one control without oysters are assigned to each treatment. Oyster samples from each mesocosm are taken in triplicate every 40 days over 200 days of exposure.

Individual *O. edulis* are assessed and sampled for a broad range of measurements, including clearance rate, growth rate, condition index, and hemolymph microbiome. Ingested MP counts are performed following alkaline digestion (10 % KOH, 50 °C, 48 h) of the soft tissue. Simultaneous MP sampling is conducted for water and sediment from each mesocosm. Additional *O. edulis* from each treatment are assessed for respiration rates and sampled for physiological tissue analysis (oxidative stress, energetics, metabolomics). Weekly water samples are taken for food quality (phytoplankton) assessments using flow cytometry and fluorescence spectroscopy, and further water samples are taken for monitoring MP concentrations. The experimental exposure period began in September 2020 and will continue through to May 2021.



MESOCOSMS

Figure 1
A: Simplified 3D model of the sixteen mesocosm system layout.
B: Photograph of the mesocosm system.
C: Photograph (top view) of an individual mesocosm showing *O edulis* distribution (n = 50) on perforated upwelling shelf.
D: Cutaway 3D model of an individual mesocosm, showing:
1) recirculation pump (5000 L/h).
2) upwelling spray bar.
3) external mixing tank for heating, dosing, and inflow.

FOUR TREATMENTS

- Dosed microplastics with or without raised temperature
- Non-dosed with or without raised temperature

DOSED MICROPLASTICS

Eight mesocosms (heated and unheated) are dosed with fluorescent MP beads (polystyrene; 10 µm) to create an exposure concentration of 50 MP/L (~0.016 µg/L) relative to the inflow rate. Concentrated MP solutions are stored in reservoirs on magnetic stirrers and automatically dosed every 15 minutes into the external mixing tanks of the mesocosms. Wastewater from the mesocosms is filtered to 1 µm to prevent environmental release.

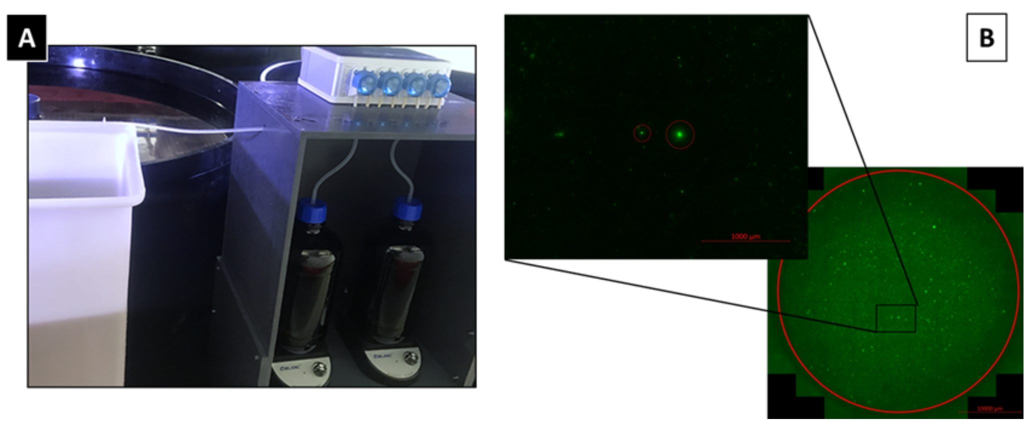


Figure 2
A: Photograph of dosing pumps and reservoirs.
B: Image of water sample on GF/A filter taken with fluorescent microscope (bottom right), and image of identified MP beads after image analysis (top left).

RAISED TEMPERATURE

Eight mesocosms (with and without dosed MP) are warmed to +3.1 °C above the ambient water temperature to simulate Coupled Model Intercomparison Project predicted warming for the North Sea by 2099. Real time monitoring data of the ambient and warmed mesocosm temperatures is relayed to a computerised switching cabinet which turns off corresponding heaters once target temperatures are reached.

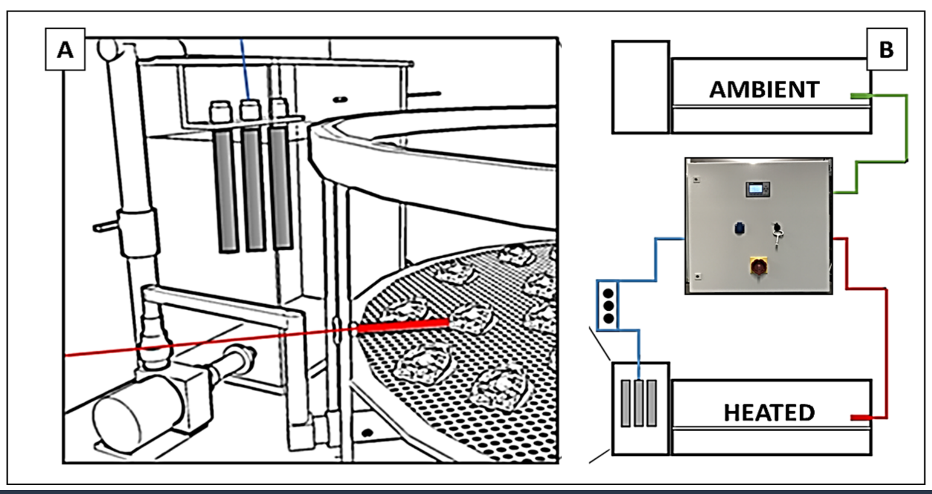


Figure 3
A: Cutaway diagram of heated mesocosm sensor location (Red).
B: Simplified diagram of mesocosm control system.

Ostrea edulis

Linnaeus 1758
Common name: European Flat Oyster or Native Oyster
Depth Range: Littoral fringe - 50 m
Native Distribution: North Atlantic (Norway - Morocco) Mediterranean Sea, Black Sea

Once common in European waters, this species became functionally extinct following overfishing and disease events in the 20th century. Restoration efforts are underway to protect and expand existing populations, and to reintroduce this species to previously inhabited areas.

For more information on *O. edulis* restoration projects please visit https://noraeurope.eu/ or scan the QR code.

Figure 4
Photograph of an adult *Ostrea edulis* showing flat upper valve surface.

RESEARCH OBJECTIVES

- Determine the chronic effects of MP exposure for *O. edulis* under natural seasonal conditions.
- Assess responses to the combined effects of increased temperature and MP exposure from the whole organism to cellular level.
- Model the aquatic distribution of MP in relation to absence/presence of *O. edulis*.
- Identify species-specific vulnerabilities to these anthropogenic pressures for eluding potential ecological consequences.

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