IMPACT OF GLOBAL CLIMATE CHANGE ON *Paracentrotus lividus* LARVAE: DEVELOPMENTAL AND MOLECULAR MARKERS OF TOXICITY

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Objective

This study investigates the combined impact of microplastics (MP) and Chlorpyriphos (CPF) on the growth and development of sea urchin (*Paracentrotus lividus*) larvae under the backdrop of ocean warming (OW) and acidification (OA). While the individual toxic effects of these pollutants have been previously reported, their combined effects remain poorly understood.

Introduction

Planet has faced escalating threats from anthropogenic activities, including, the increasing concentration of CO_2 in the atmosphere generating OA and OW. **MP**, defined as plastic fragments smaller than 5 mm, have become pervasive in marine environments. **CPF**, an organophosphate pesticide, is widely used in agriculture to control insect pests and acts as an inhibitor of the enzyme acetylcholinesterase (AChE), leading to the accumulation of acetylcholine and the disruption of nervous system.

Motivation

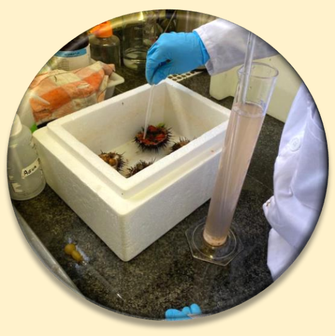
P. lividus is a sentinel species, acting as an indicator of the quality of the ecosystems. This species has a commercial interest since its roe is considered a delicacy in many cultures, being harvested for its high market value. Its role in aquaculture and its potential in pharmaceutical applications further enhance its economic significance. Contaminants and global change negatively impact *P. lividus* by disrupting its reproductive cycles and reducing its populations.

Materials and Methods

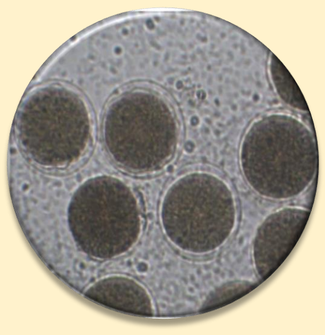
Sea urchin Embryo Test (SET)

Sex determination

Suspension of oocytes and 10 µL of sperm were transferred to 250 mL of FSW, and mixed



Four aliquots of 10 µL were counted under the microscope.



48 h Incubation under different conditions

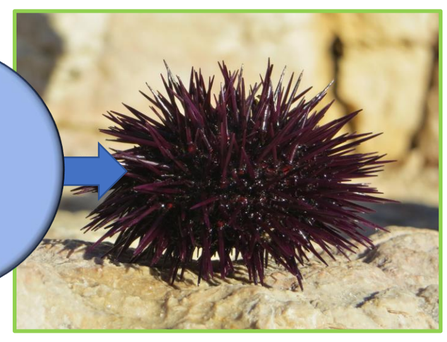
MP= 3000 Particles/mL
CPF EC10 and EC50

pH \ Tª	20 °C	24 °C (OW)
8.1	CTR	CTR+T
	MPCPF	MPCPF+T
OA 7.8	MPCPF-OA	MPCPF-OA+T

One of the goals of my research line is to add molecular and biochemical approaches to the SET, increasing its scope.

SET

Molecular → Biochemical → Biological

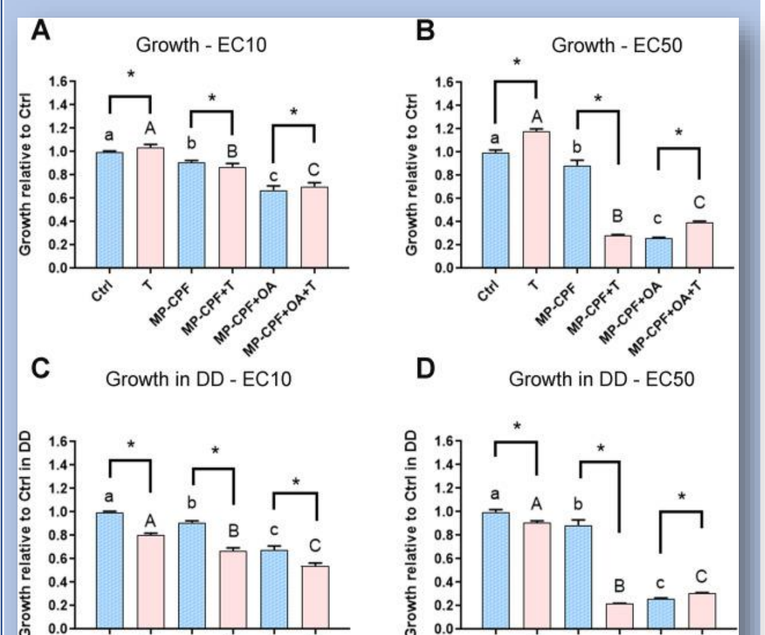


Predictive

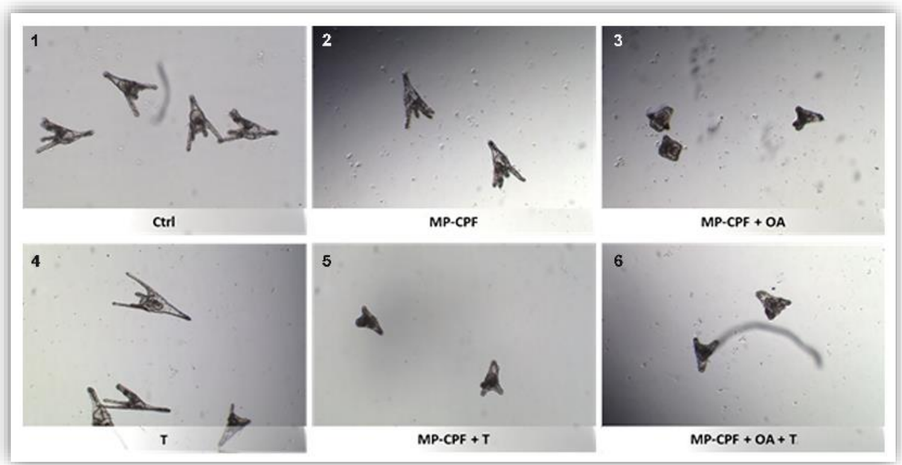
This approach together with IA analysis of data can make SET predictive.

RESULTS

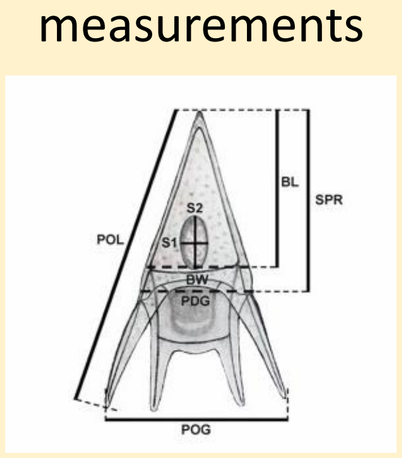
Growth



Growth slightly decrease with MPCPF at 20 ºC and when are co-incubated under OA conditions the growth is even lower. The same trend is observed in the lower part of the picture that shows larvae incubated under OW conditions. When we compare the effect of temperature on each treatment we can see an induction on growth, and an increase in malformations.

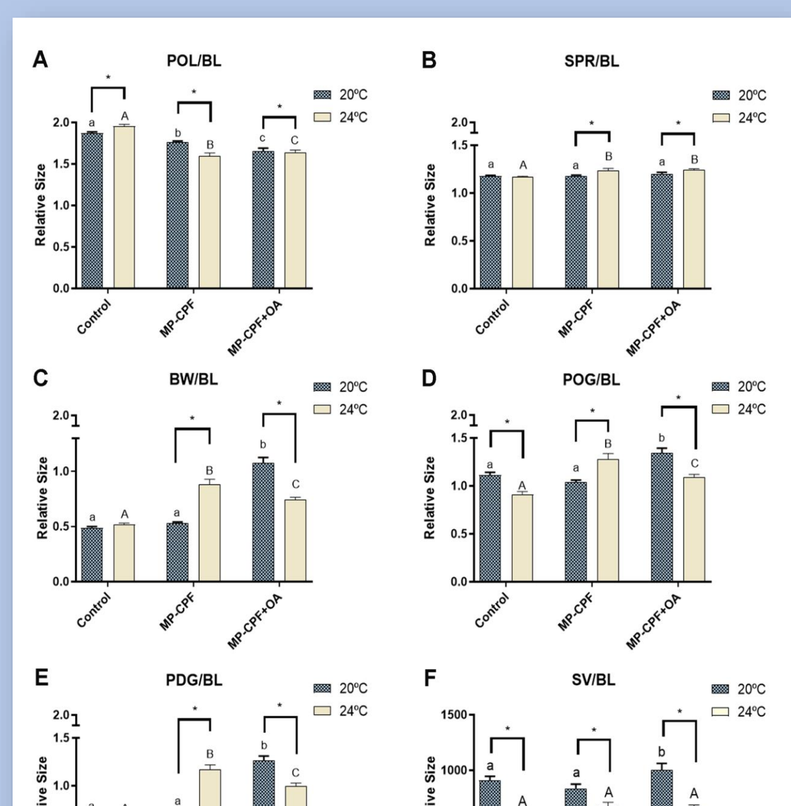


Morphometric measurements

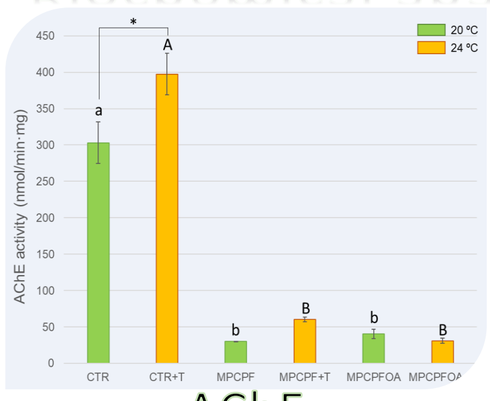


The SV decrease with the increase in temperature for all the treatments. MPCPF under OW conditions produce an increase in the length of the upper side of the larvae and a reduction in the arms length, while when MPCPF are incubated under OA conditions or OA+OW, this produce a reduction in the total length, together with an increase in the width.

Morphology

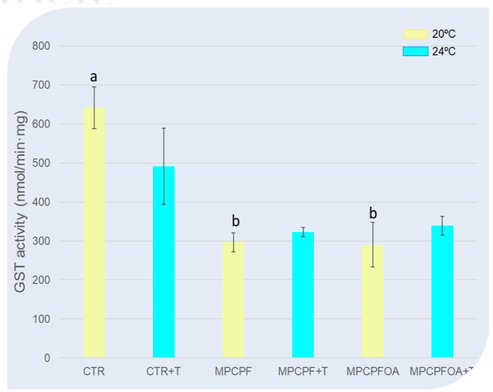


Biochemical analysis



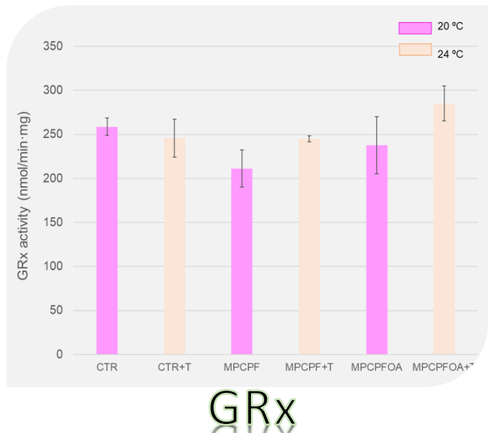
AChE

AChE is essential for regulating cholinergic signaling and ensuring proper neurotransmission in sea urchin larvae.



GST

GST enzymes contribute to detoxifying harmful substances, protecting cells from damage, and aiding in the defense against diseases.



GRx

Glutaredoxins play a role in maintaining cellular redox balance by participating in thiol-disulfide exchange reactions and regulating the activity of various proteins

RNAseq analysis

Enzyme	MP-CPF vs Control		MP-CPF+OA vs Control		MP-CPF+OA vs MP-CPF	
	20 ºC	24 ºC	20 ºC	24 ºC	20 ºC	24 ºC
AChE	Up	2 isf Up/ 2 isf Dw	1 isf Up/ 1 isf Dw	2 isf Up/ 2 isf Dw	ND	ND
GST	Up	Up	1 isf Up/ 1 isf Dw	Up	ND	ND
GRx	ND	Dw	Dw	ND	ND	ND

The gene expression of these enzymes was upregulated showing the necessity of produce more protein in response to pollutants. The different isoforms of each gene (*ache*, *gst*, *grx*) present differences in their expression.

AChE → Inhibition in all the treatments at both temperatures. Temperature induces the activity of AChE in the Ctrl.
GST → Inhibition in all the treatments, with no differences between groups under OW conditions.
GRx → No differences among the treatments were observed.
Antioxidant defences may be **influenced** by short-term exposure of the larvae under stressful circumstances.

Conclusion

- The combination of CPF and MP impacted larvae **morphology**, **development** and the enzyme **activities**. The negative **effect** of MP-CPF on those parameters **increases** under **global climate change** conditions.
- The **body shape** was affected, resulting in wider and more rounded larvae. This would decrease their buoyancy and their capability to ingest food.
- The negative effect of **a decrease in pH** on larval development appears to be **mitigated** by an increase in **temperature**. However, the temperature provokes extra stress.
- Biochemical and molecular markers provided early indications of stress, complementing traditional growth measurements, although molecular markers should be further studied to determine which isoforms better describe the behaviour of the enzymatic activity.

This study underscores the importance of holistic approaches in evaluating impact of environmental stressors on **sea urchin aquaculture**. Comprehending the interplay between pollutants and environmental stressors is essential for devising effective conservation measures. Further investigation should focus on understanding the effects at lower biological levels and examining adaptive responses in sea urchins confronting various stressors.