

The effect of combined environmental stressors on *Daphnia magna* and *Brachionus calyciflorus*

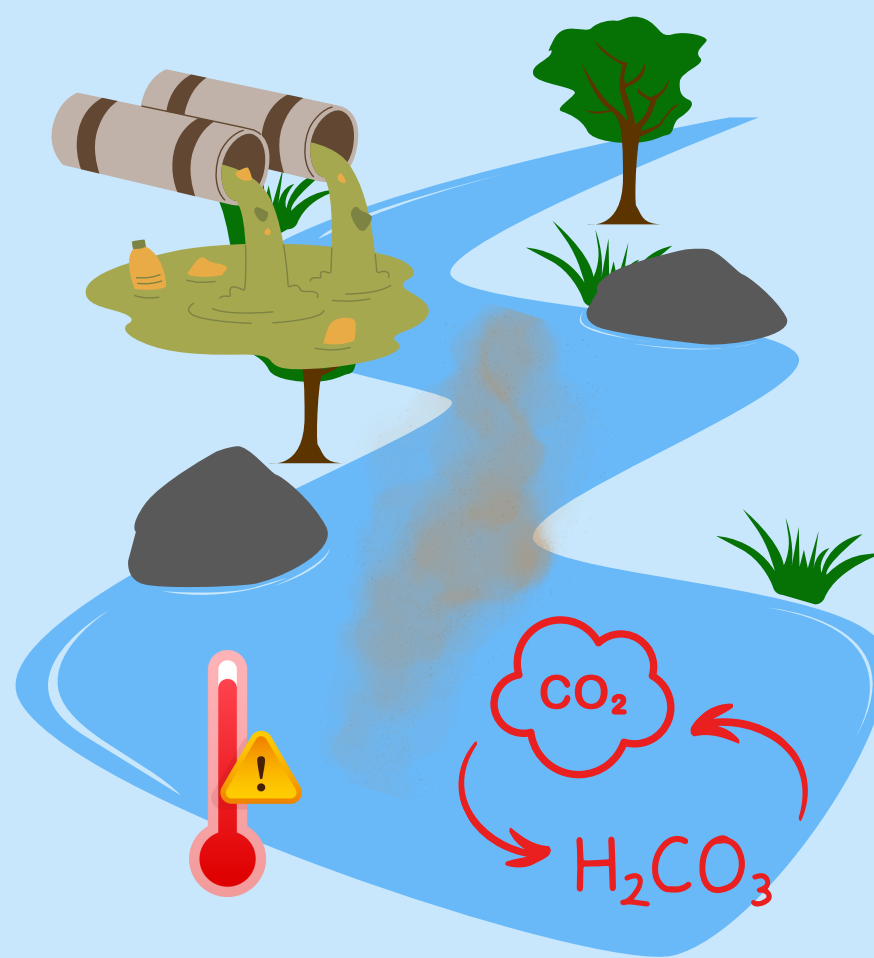
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Introduction

Climate change and human activities simultaneously bombard aquatic ecosystems with a multitude of chemical and non-chemical stressors.

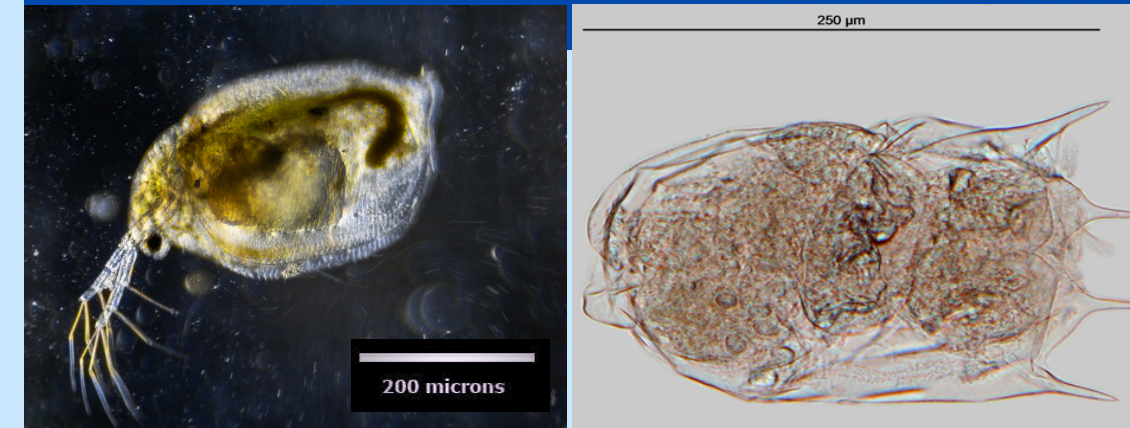
Beyond single threats: Traditional research typically examines stressors in isolation, neglecting how they interact to magnify or downplay their impact. Such an approach hinders our understanding of the biggest threats to these vital ecosystems.

Water's delicate balance: Essential factors such as temperature, pH, and conductivity/ salinity become stressors when they deviate from the optimal range for aquatic life.



Objectives

- To investigate how combinations of non-chemical stressors impact organisms over time during short-term exposures.
- The aim is to shed light on the effects of these stressors under realistic, multi-stressor conditions found in natural ecosystems.



Daphnia magna (Source: <https://www.canadiannaturephotographer.com>) *Brachionus calyciflorus* (Source: <https://www.biolib.cz/en/image/id289297/>)

Methodology

Temperatures °C 15, 20, 25, 30	< 0.5 PSU	0.5 PSU	1 PSU	2.5 PSU	5 PSU	EPA Waters	pH	Conductivity μS/cm
	<1000 μS/cm	1000 μS/cm	2000 μS/cm	4800 μS/cm	9000 μS/cm			
pH 5 MES						Very Soft	6.88	349
pH 6 MES						Soft	7.55	445
pH 7 MOPS						Moderately hard	7.64	457
pH 8 EPPS						Hard	8	774
pH 9 CHES						Very Hard	8.45	968

- Artificially Buffered: Moderately Hard EPA Water + Zwitterionic Good's Buffers + Artificial Sea water.
- EPA Water: Very soft to Very hard with Salinity/Conductivity less than 0.5PSU/~1000 μS/cm.
- 6 Replicates of each treatment combination and 8 individuals exposed in each replicate.
- Water Quality Parameters were monitored at beginning (0hr) and at the end (48hr) of the test.
- Endpoint: Mortality (Observed every 12hr)

Results

Time Series: 20°C

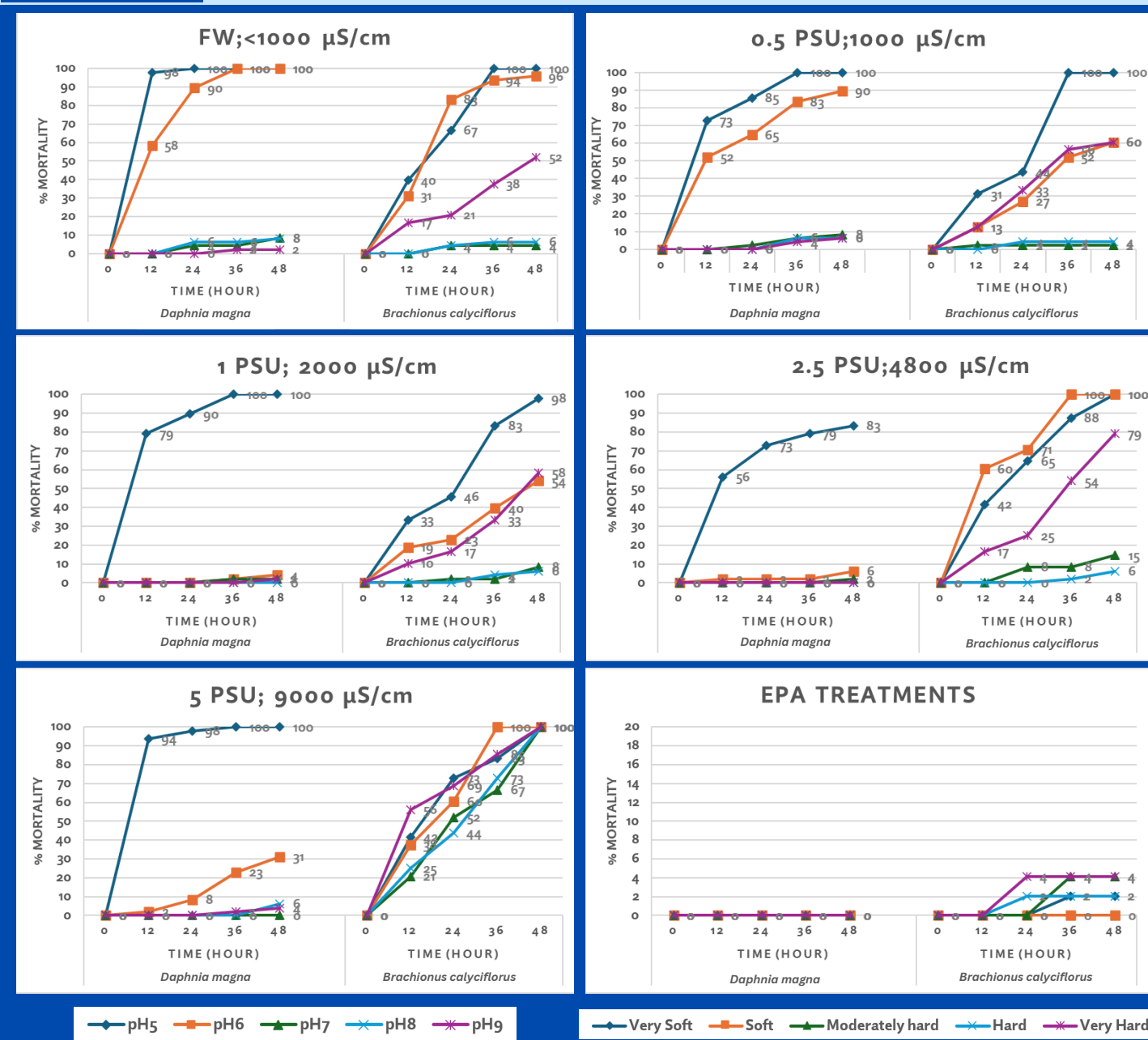


Fig. 1.: % Mortality as function of time for various pH and Salinity/Conductivity treatments and EPA treatments at 20°C showing difference in mortality pattern in buffered solutions

- Data when plotted similar to Fig. 1. for the other temperatures, shows a drastic effect even at shorter time points (12 or 24 hours) with increase in temperature.
- In Fig. 2. we can observe a varying pattern for both the organisms with a change in any of the stressor conditions.
- The EPA solution treatments show % mortality lesser than 10% for temperatures 15°C, 20°C and 25°C and only show higher mortality for a few treatments at 30°C

% Mortality after 48 hours

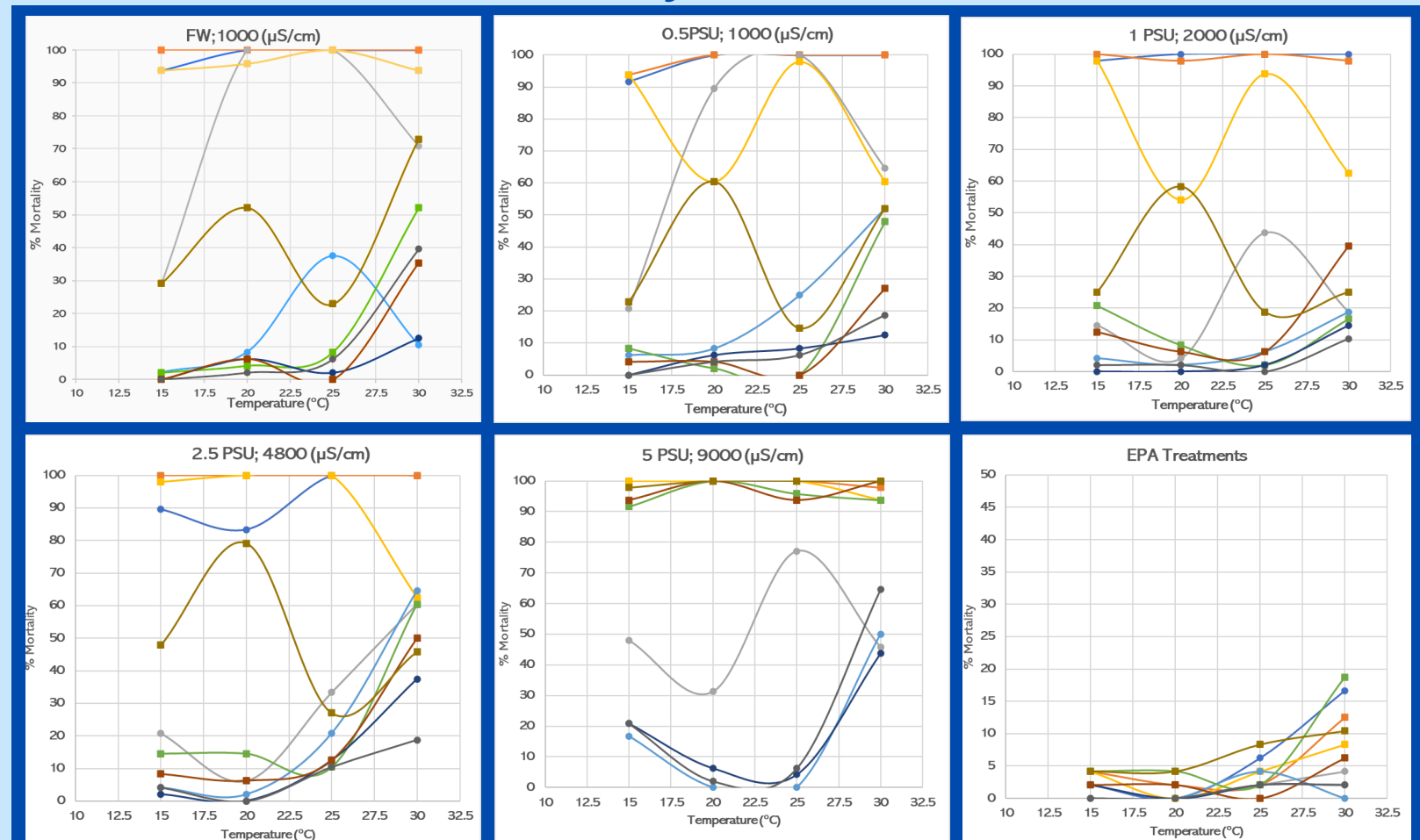


Fig. 2.: % Mortality at 48 hours, as a function of Temperature for different pH and Salinity/Conductivity treatments showing difference in mortality at each temperature.

Legends:

Buffer Treatments		EPA Treatments	
● pH 5; <i>Daphnia magna</i>	● pH 5; <i>Brachionus calyciflorus</i>	● Very Soft; <i>Daphnia magna</i>	● Very Soft; <i>Brachionus calyciflorus</i>
● pH 6; <i>Daphnia magna</i>	● pH 6; <i>Brachionus calyciflorus</i>	● Soft; <i>Daphnia magna</i>	● Soft; <i>Brachionus calyciflorus</i>
● pH 7; <i>Daphnia magna</i>	● pH 7; <i>Brachionus calyciflorus</i>	● Moderately Hard; <i>Daphnia magna</i>	● Moderately Hard; <i>Brachionus calyciflorus</i>
● pH 8; <i>Daphnia magna</i>	● pH 8; <i>Brachionus calyciflorus</i>	● Hard; <i>Daphnia magna</i>	● Hard; <i>Brachionus calyciflorus</i>
● pH 9; <i>Daphnia magna</i>	● pH 9; <i>Brachionus calyciflorus</i>	● Very Hard; <i>Daphnia magna</i>	● Very Hard; <i>Brachionus calyciflorus</i>

Optimal WQ Parameters

Optimal Temperature for:
Daphnia magna: 20°C
Brachionus calyciflorus: 25°C

Optimal pH:
Daphnia magna: 6-9
Brachionus calyciflorus: 6-9

Optimal Conductivity/ Salinity:
Daphnia magna: <4 PSU; <6500 μS/cm
Brachionus calyciflorus: <2 PSU; 3500 μS/cm

Conclusion

- Rotifer species *Brachionus calyciflorus* shows higher sensitivity at most combinations as compared to *Daphnia magna*.
- Zwitterionic buffers themselves exert toxicity towards both organisms
- Mortality seen at temperatures other than suitable ranges could also be due to heat shock treatment.

What next?

- Developing better methodology than Good's buffering system to replicate ecologically relevant scenarios.
- Chronic test for better understanding of organisms' adaptations and response.
- Gradual temperature increase instead of heat shock
- Extend the approach to chronic studies with a suite of organisms, including chemical stressors.