*Anthopleura elegantissima* in a Hot Plastic Ocean

How do the combined stressors of marine microplastic leachate and thermal stress affect the physiology of the temperate coastal anemone Anthopleura elegantissima?

Sarah Tanja

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### Objective

The objective of this study is to examine the combined effects of thermal stress and plastic leachate on photosynthetic efficiency, microbiome community, and gene expression in the aggregating sea anemone *Anthopleura elegantissima*. In essence, this study is both a global change study examining impacts of thermal stress on marine intertidal invertebrates, and an ecotoxicological study that assesses impacts of chemical leachate associated with marine plastic pollution on a cnidarian host and an algal symbiont. We’ve chosen *Anthopleura elegantissima* as our study species because it is a clonal cnidarian that can form symbiotic relationships, can be collected and studied locally in the Pacific Northwest, and has an annotated genome. *Anthopleura elegantissima* can form symbioses with either the chlorophyte *Elliptochloris marina* (Letsch et al. 2009) or the dinoflagellate *Breviolum muscatinei* (LaJeunesse and Trench 2000). In this study, we will preferentially select ‘golden brown morphs’ that are associated with the dinoflagellate *Breviolum muscatinei* in order to reduce variation due to symbiont type, and to assess a symbiont genus commonly found in tropical reef building corals.

### Hypotheses

Symbionts play a critical role in the stress response of Cnidarians to thermal stress

Microplastic leachate exposure changes the microbial community

Microplastic leachate exposure changes the microbial community in such a way that Cnidarians are more susceptible to thermal stress

Microplastic leachate and thermal stress act synergistically to disrupt Cnidarian microbial communities

The microbial community changes differently when exposed to microplastic stress as compared to thermal stress

### Specimen collection

We will collect 84 ‘golden brown’ *Anthopleura elegantissima* from Owen’s Beach at Point Defiance Park in Tacoma in the Salish Sea of Washington State. Individual anemones will be collected from each of 12 clonal aggregates by very gently scraping their pedal disc to separate it from their attached substrate. We will move 5 meters or more to what we can assume is a genetically distinct aggregate and repeat this, until we have 7 individuals from each of 12 clonal mats (a total of 84 individuals). One individual from each aggregate will be immediately flash-frozen in liquid nitrogen to serve as an environmental baseline control for both microbial and gene expression metrics. The remaining 72 anemones will be kept for experimentation.

### Specimen housing & acclimation

The anemones will be transported in sea water from the collection site back to the University of Washington campus where they will be housed individually in labelled glass beakers that will be submerged in a recirculating seawater table and allowed to acclimate for at least 2 weeks to heal their pedal disc.

### Treatment preparation

We will prepare plastic leachate in seawater as a treatment by soaking 250mg of plastic in 250ml of seawater in a glass beaker for 1 week with a stir-bar or shaker table. The 100% leachate that is formed from this soak has a known phthalate acid ester (PAE) concentration of ~10%, equaling 100mg of PAE per 250ml of seawater. This highly concentrated leachate will be diluted 1:1000 in filtered seawater to get to an environmentally relevant concentration of 100ug of PAE per liter of seawater.

### Experiment

The experiment will be setup as a 2x3 factorial design to test thermal stress and plastic leachate. Two seawater tables will be used as temperature baths, one set at an elevated temperature of 20C and another at an ambient temperature of 10C. Temperature will be controlled by an Apex Neptune Aquarium controller coupled to heat exchangers and light level will be standardized using Kessil aquarium lights. 20 specimens (two of each clonal mat) will be randomly placed in one of each water temperature treatment bath. Seatable water volume level will be dropped to just below the lip of the specimen beakers, and specimen beakers will be filled with either filtered seawater or with the dilute plastic seawater leachate treatment. The exposure will last 48 hours, after which anemones will be flash frozen in liquid nitrogen.

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| Figure 2. 2x3 Factorial experimental design in which 1 clonal genotype from each of 10 clonal mats will be exposed to 4 scenarios, thermal stress, plastic leachate pollution, multiple/combined thermal and leachate pollution stresses, and the control of ambient temp and filtered seawater. |

### Sample Processing

Flash frozen samples will be processed using the commercial Zymo Dual DNA/RNA MiniPrep kit to extract microbial DNA and host and symbiont RNA. The microbial DNA data will be used to analyze if there are any discernible shifts in the microbiome related to the treatments. The RNA will help us identify molecular pathways that may be disrupted by heat, leachate, and/or the combined multiple stressors of both in a cnidarian and its algal symbiont.

### Merit

This shotgun of stress response variables will serve to offer a holistic view of cnidarian host and algal symbiont physiological response to temperature and plastic pollution leachate stress. It is well known that ocean heat waves are becoming more frequent and intense. Simultaneously, marine plastic pollution is becoming more prevalent and the chemical additives associated with plastic have become ubiquitous in our environments. As plastics weather mechanically, they also weather chemically by leaching additives not covalently bonded to the plastic polymer resins (Hahladakis et al. 2018). The most common plastic additives are a group of chemicals called plasticizers, also known as phthalates or phthlate acid esters (PAE). They are commonly added to PVC to increase flexibility and durability. However, phthlatates are known to mimic or interfere with hormones that control developmental, reproductive, and immune response in both humans and animals (**maqboolReviewEndocrineDisorders2016?**). Previous research shows that plastic leachates experimentally added to plants, bacteria, and algae impair growth, reduce photosynthesis, and shift microbial communities in lab studies (Capolupo et al. 2020; O’Brien et al. 2022; Tetu et al. 2019). However, little is known about environmental concentrations of plastic leachate in the context of increasing ocean temperatures. Marine heatwaves, plastic manufacturing, and pollution are predicted to increase (**macleodGlobalThreatPlastic2021?**; **oliverMarineHeatwaves2021?**). Therefore, it is urgent that we understand how thermal stress and plastic pollution interact and the effects they may have on coastal marine organisms, which are likely experiencing both the most extreme thermal stress and the highest amount of plastic pollution.

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