Preregistration

Preregistration for reproducing Gooding et al. 2009

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Study Information

Title Preregistration for reproducing Gooding et al. 2009: Effects of increasing temperature on sea star growth and feeding rate

Description As ectotherms, marine invertebrates are entirely dependent on their environment to control and regulate their body temperature. Many key physiological processes and rates that dictate the performance and fitness of invertebrates are temperature-dependent, and shifts in thermal regimes, and the associated performances responses by affected species, can lead to population- and community-level changes (Bruno, Carr, & O'Connor, 2015; Vasseur et al., 2014). Human-driven climate change and warming are not only contributing to warmer waters in the worlds oceans, but are also leading to extreme temperature events and heatwaves to occur more frequently and with greater intensity (Oliver et al., 2019). The ochre sea star, *Pisaster ochraceus*, is a keystone predator found in many rocky intertidal zones in the

Northeast Pacific, and has demonstrated behavioural and physiological changes with altered seawater temperatures that have important implications for their impacts on intertidal zone community structure (Gooding, Harley, & Tang, 2009; Paine & Paine, 2008; Sanford, 1999). Testing how *P. ochraceus* responds to a range of seawater temperatures that includes future extremes will allow researchers to better understand how this important predator's physiology, behaviour, and associated community-level effects may change in a warmer world (Kordas, Harley, & O'Connor, 2011). The goal of this project is to conduct a replication study on the impacts of seawater temperature on the growth of *P. ochraceus* and its consumption of a common prey species, the bay mussel *Mytilus trossulus*, originally conducted by Gooding and colleagues as part of a larger study examining the impacts of elevated seawater temperature and CO_2 on the growth, feeding behaviour and calcification rates of *P. ochraceus* (Gooding et al., 2009). By replicating the temperature-specific experiments on *P. ochraceus*, we hope to provide further support to the importance of seawater temperatures, and the implications of climate change, on the physiology and ecology of this keystone species.

Hypotheses Increasing seawater temperatures has had well-documented positive relationships with the physiological rates of a number of marine invertebrates. If seawater temperatures increase incrementally but do not exceed a physiological threshold, we expect to see a positive, linear relationship between seawater temperature and the growth and consumption rates of *P. ochraceus*.

Design Plan

We plan to run growth and feeding trials under different set temperatures for juvenile sea stars *P. ochraceus*.Individuals will be collected and initial wet mass will be determined. Each star will be randomly assigned to temperature treatment between 5 - 21 °C. Sea stars will be acclimated to their tanks without food for approximately 6 days. Individuals will remain in their treatment tank for 8 weeks, being fed ad libitum for the duration.Every second day empty mussel shells will be recorded then discarded and replaced. Individuals will be weighed weekly and final weight will be recorded at the end of the 8 week period. This data will be used to determine relative growth. Feeding rate data will be determine by average daily mussels consumed

per sea star at each temperature.

Study type **Experiment**. A researcher randomly assigns treatments to study subjects, this includes field or lab experiments. This is also known as an intervention experiment and includes randomized controlled trials.

Blinding No blinding is involved in this study.

Study design Juvenile *P. ochraceus* will be reared in the lab, at temperatures ranging from 5 - 21 °C. We will use twenty-four tanks, 246L in volume, with recirculating water to house seastars. Two seastars will be placed inside each tank, contained in their own tupperware with mesh sides and tops to ensure water flow, for a total of 48 seastars. Relative growth of the 2 seastars inside a single tank will be averaged, thus tank is the independent unit in this design.

Randomization Each of the 48 seastars used in this study will be randomly assigned to tanks. We will run a simple linear regression to ensure no bias for initial mass for assigned temperature.

Sampling Plan

We plan to sample 48 individuals, this size complies with our lab space constraints of 24 available tanks. Specimens will be collected in January from Jericho Beach, Vancouver.

Existing data **Registration following simulation of replicable data** As of the date of submission, data has been replicated using the linear regression equation published by Gooding et al. (2009) as a preliminary analysis of its reproducability.The data for this experiment have not yet been collected.

Explanation of existing data	We have simulated data based on the model parameters from the study of which we are replicating. This is to ensure that our results are indeed replicating the results from Gooding et al. (2009).
Data collection procedures	We plan to sample 48 juvenile sea stars between 3 - 7g at initial wet mass. This is specified because juveniles have a larger scope for growth as they are not yet investing into reproductive structures. Specimens will be collected over a 2 week period in January from Jericho Beach, Vancouver. All individuals will have a minimum of 4 weeks in acclimation tanks at &deg13;C prior to experiment start.
Sample size	48 individuals will be sample due to equipment restrictions.As there will be 2 individuals in each tank we will use the average between them for growth and feeding rate to avoid pseudo-replication.
Sample size rationale	Our sample size was determined based on equipment restraints, only 24 available holding tanks.
Stopping rule	We will only collect the number of individuals we have the space for in this experiment, that being 48.

Variables

Manipulated variables	We will be manipulating the water temperature in the 24 tanks from a range of 5 - 21 °C.
Measured variables	We will measure growth rate (change in wet mass / initial mass). Initial wet mass will be taken of each individual by removing them from the tank, lightly patting them dry and then weighing them to the nearest 0.1g. A final wet mass will be taken at the end of the experiment, and then again after acclimated all individuals back to 13 °C. This step is to ensure that difference in water temperature did not affect water retention.

Feeding rate will also be measured. To do this empty mussel shells will be counted every second day, ensuring that no individual runs out of food. A daily average of mussels consumed for each individual will be calculated at the end of the experiment.

Indices The mean between both individuals of a single tank will be taken for growth and feeding rate to avoid pseudo replication as then are subjected to the same manipulated condition.

Analysis Plan

Statistical models A simple linear regression will be used for both growth rate under increasing temperature and feeding rate under increasing temperature. Here the independent variable for both analyses is temperature, which is a manipulated range. Growth rate and feeding rate are the dependent variables.

Transformations Data for feeding rate and relative growth will be log transformed to normalize variance.

Inference criteria For our linear regression analyses we will use a $p < 0.05$ criteria to determine significance of slope, and R^2 values to quantify model fit.

Simulated Data Analysis

Methods We simulated data using results presented in Gooding et al. (2009) to explore the replicability of the analysis. The replication focus was on the relative growth and the feeding rate temperature response linear regression analyses, and we simulated random data using the slope and intercept values from the regression equations from each of these analyses. Linear regression analyses were performed using the simulated data for relative growth as a function of temperature, and for feeding rate as a function of temperature, and the data were plotted with the resulting regression lines.

Results The results from our simulated data closely match the results from Gooding et al. (2009). The summary statistics for the simulated *P. ochraceus* data are shown in Table 1 and Table 2. The relative growth of *P. ochraceus* showed a direct correlation with temperature increase (Figure 1, Table 3). Similarly, increasing temperatures directly correlated with feeding rate (Figure 2, Table 4).

Data exclusion We will visually check for any outliers in the data. If outliers exist, we will conduct a sensitivity analysis by including, and then excluding, outliers in model fits, to determine whether the point(s) have strong leverage on model outputs. If outlier(s) are determined to be the result of measurement error, and not true data, the data will then be excluded from further analysis.

Missing data

Exploratory analyses (optional) No exploratory analyses will be made in this study. We will follow the hypotheses laid out in this pre-registration.

References

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