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 ochre sea star *Pisaster ochraceus* 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

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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 sea stars. Two sea stars 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 sea stars. Relative growth of the 2 sea stars 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 can run a simple linear regression to ensure no bias for initial mass for assigned temperature.

Sampling Plan

We plan to sample 48 individuals, which 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 linear equations from Gooding *et al.* (2009) for the purposes of this preregistration and to reproduce their statistical outputs. These data, and the original data from Gooding *et al* (2009), will not be included in the final analysis.
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, the original collection site from Gooding *et al.* (2009).All individuals will have a minimum of 4 weeks in acclimation tanks at &deg13;C prior to the start of the experiment.
Sample size	To maintain consistency across studies, 48 individuals will be used in our replication experiment. As there will be 2 individuals in each tank, we will use the averaged growth and feeding rate values within each tank to avoid pseudo-replication in the same manner as the original study.
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. Because we plan to follow the linear regression design from Gooding *et al.* (2009), there will not be replication at any single temperature.

Measured variables	For the first replication experiment, we will measure the relative growth (change in wet mass / initial mass * 100) of *P. ochraceus* across the range of tank temperatures. Initial wet mass will be taken by removing each individual from their 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 all individuals are re-acclimated to 13 °C seawater, in order to ensure that differences 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 in order to avoid pseudoreplication and maintain methodological consistency between our study and Gooding *et al.* (2009).

Analysis Plan

Statistical models	A simple linear regression will be used for both relative growth under increasing temperature and feeding rate under increasing temperature. Here the independent variable for both analyses is temperature, which is manipulated between 5 - 21 °C. For this study, relative growth 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 the significance of the linear relationship, 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 reproducibility 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. The relative growth of *P. ochraceus* showed a direct correlation with temperature increase (Figure 1, Table 2). Similarly, increasing temperatures directly correlated with feeding rate (Figure 2, Table 3).

Table 1: Summary statistics for simulated data set

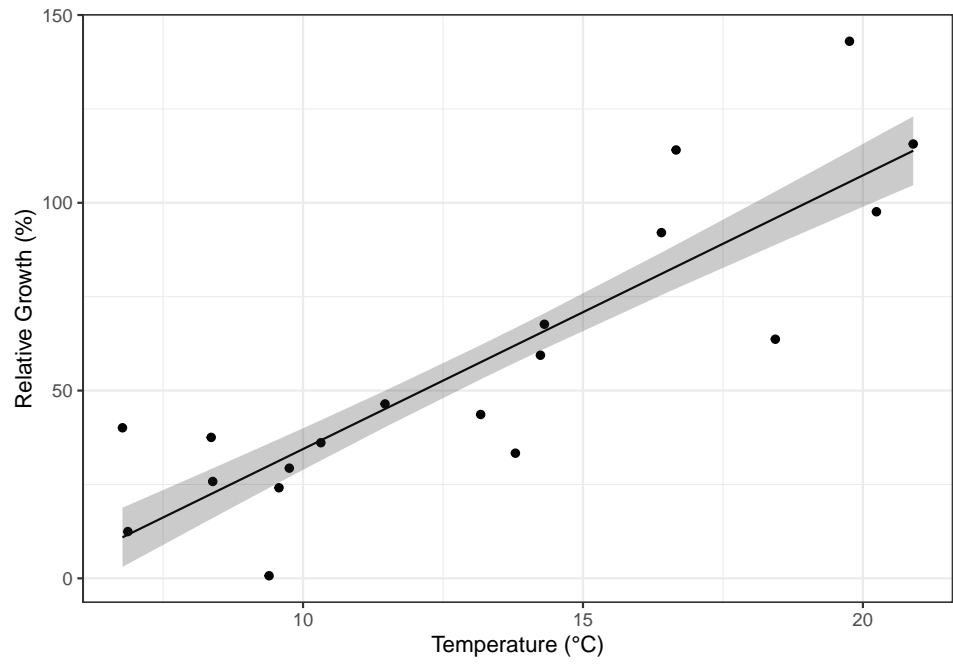| Temperature	Relative Growth	Feeding Rate
Min. : 6.775	Min. : 0.6902	Min. :0.3126
1st Qu.: 9.482	1st Qu.: 31.3329	1st Qu.:0.9467
Median :13.173	Median : 43.6248	Median :1.1857
Mean :13.096	Mean : 56.9834	Mean :1.2428
3rd Qu.:16.532	3rd Qu.: 79.8569	3rd Qu.:1.5520
Max. :20.899	Max. :143.0124	Max. :2.3528

Figure 1: Linear regression of realtive growth of *P. ochraceus* as a function of temperature

Table 2: Linear regression results for relative growth of *P. ochraceus* as a function of temperature

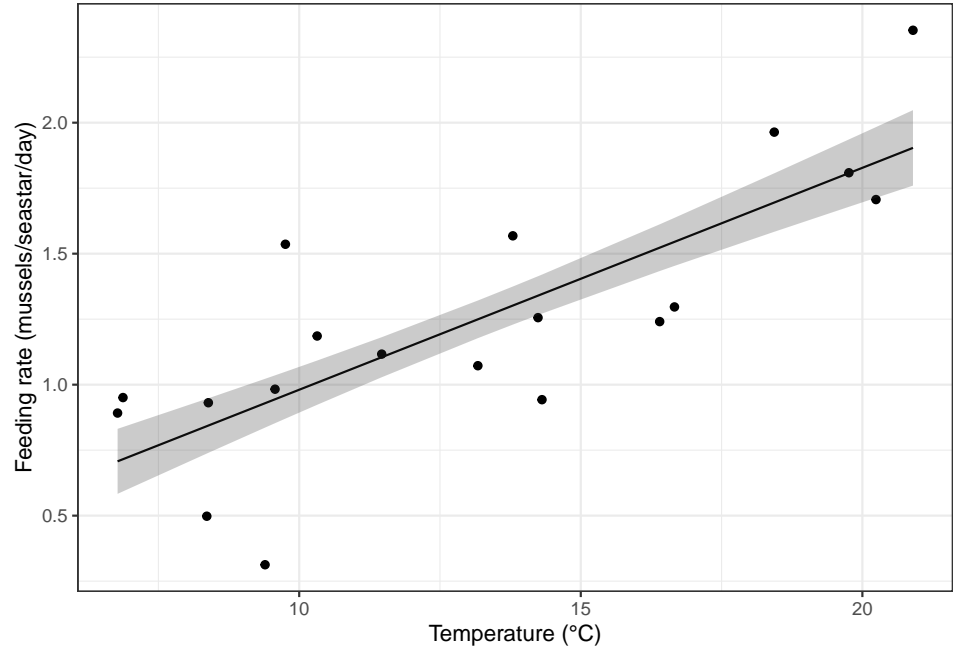|	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-38.463187	14.081628	-2.731445	0.0142097
temperature	7.288427	1.017203	7.165161	0.0000016

Figure 2: Linear regression of feeding rate of *P. ochraceus* as a function of temperature

Table 3: Linear regression results for feeding rate of *P. ochraceus* as a function of temperature

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.1334245	0.221593	0.6021151	0.5550441
temperature	0.0847146	0.016007	5.2923375	0.0000597

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.

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Missing data The only foreseeable source of missing data would be the result of sea star death. If a sea star dies during the experiment the data from the other sea star in the tank will not be included in the study because we will not be able to calculate the mean of both individuals in the tank.

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

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