**Cucumber Heat Stress Intro & Methods: Pre-Peer Review Iteration**

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BMSC Directed Studies

The introduction should include a concise summary of relevant background information, as well as and your objectives, research question, and hypotheses. The introduction gives the essential context, justification, or rationale for your research. Make sure to identify the gap in knowledge that you will address in your research and your overall objective or research question. Your hypothesis(/hypotheses) and predictions should also be included.

* Does the paper start relatively broadly?
* Does the beginning of your introduction “hook” the reader by setting the stage for the question(s) your paper answers?
* Does the paper include previous literature that is relevant to the current research question?
* Do you briefly summarize previous work that informs your current question(s)?
* Is the research question clearly stated?
* Are the hypothesis and predictions clearly stated and justified?
* Do you explain and justify your questions(s) instead of just extolling the virtues of your study organism?

**Introduction Ideas**

* Marine heat waves are becoming more common
  + Source: peer reviewed lit?
  + Source: IPCC report
* Cucumber collection is a $10 million dollar industry in BC
* Cucumber farms in Asia are getting hit hard by wasting
  + Xu et al., 2016 (introduction)

**Experiment Framework**

* Cucumbers get affected by marine heatwaves
  + Cucumbers will waste when it’s hot
  + Cucumbers will eat more when it’s hot
  + Cucumbers will die when it’s hot
  + Cucumbers will travel more when it’s hot
  + Cucumbers will travel less when it’s hot
  + Cucumbers will move faster when it’s hot
  + Cucumbers will move slower when it’s hot.

Intro key points ranging from most broad points to most narrow

* Paragraph 1: Start with broad points on climate change and temperature
  + Start broad (IPCC)
  + Narrow to talk about climactic stochastic events (IPCC, peer reviewed lit?)
  + Narrow to talk about marine heat waves (peer reviewed lit)
  + Talk about how marine heat waves affect ecosystems & people
    - can devastate populations, communities, ecosystem goods and services, human food security etc....
  + Bring up mussels, sea star wasting, other examples of organisms getting temperature fucked? (Cavole, 2016).
    - ﻿Subarctic copepods, krill Lack of food reduced population, distribution moved northward.
    - ﻿Dungeness crab and mussels Fishery closed due to toxicity
    - ﻿Salmon Warm temperatures decreased recruitment for some species
    - ﻿Groundfish Potential loss of habitat due to hypoxia
    - ﻿Seabirds, seals, and sea lions Massive die-offs due to lack of food
    - ﻿Baleen whales Expected to decline due to lack of food
  + Talk about importance of understanding impact of MHW on individual organisms (to scale up to understand ecosystem impacts)
  + Transition sentence into direct impacts of MHW
* Paragraph 2: HOW do heat waves DIRECTLY impact marine organisms
  + Lethal: Killing them directly by exceeding their thermal tolerance
    - Find some LT50 studies to back this up?
  + Sublethal: Physiologically reducing their fitness (metabolic effects), and modifying their behaviour, feeding patterns, etc.... in response to heat stress
    - Discuss coral bleaching?
  + Transition sentence into indirect impacts of MHW
* Paragraph 3: Indirect impacts of marine heat waves through disease
  + Discuss sea star wasting syndrome
  + Find other examples of diseases triggered by heat stress
* Paragraph 5: Introduce cukes and Talk about why the heck anyone cares to study giant California sea cucumbers
  + Start this paragraph by stating that it’s important to know how MHW affect cukes and there’s a lack of knowledge here
  + Who cares about cukes?
    - Discuss ecological importance of cukes
    - Discuss economic importance of cukes
  + Transition back to heat waves
* Paragraph 4: Tie the cukes ot heat waves
  + Explain how the effects of heat waves on cucumbers are not well known AT ALL
  + THEN talk about how there is evidence suggesting they may be an example of a system where there is potential for heat waves to have direct effects AND possibly indirect disease effects
  + Discuss how we wonder if cuke wasting (like sea star wasting) could be heat triggered
  + Discuss how there’s evidence for wasting in cucumbers, and how this resembles sea star wasting, and how this MAY or MAY NOT be a contagious thing
  + Transition statement to questions
* Paragraph 6: Statement of intent and specific questions we’re addressing
  + To understand how marine heat waves affect sea cucumbers and potentially also diseases triggered by MHW, we are investigating the following questions by simulating a marine heat wave in a lab environment...
    - Do marine heat waves have direct lethal effects on cucumbers?
    - What are the direct sublethal effects of MHW on cucumbers (movement behaviour, feeding)
    - Is there any evidence (in a lab setting) that MHW induces wasting?
  + Hypotheses:

**Introduction:**

Over the past two centuries, unchecked human development and greenhouse gas emissions have profoundly impacted the biosphere and the climate (1, 2). The rising frequency of extreme climatic and weather events, including marine heatwaves (MHWs), is one of the consequences of our move into the Anthropocene (3). MHWs are occurrences of anomalously warm seawater temperatures for a discrete duration of time in a specific locality (4). Anthropogenic climate change is increasing the intensity, duration, size, and frequency of MHWs with consequences across the globe (3–5).

Marine heatwaves have damaging and potentially catastrophic effects on marine ecosystems and human economies (6). MHWs have bottom-up impacts on ecosystems, driving sudden and large-scale die-offs at all trophic levels from starvation, toxicity, and hypoxia (6–9). For example, the 2014-2015 warm water anomaly (WWA) in the NE Pacific (nicknamed “the Blob”) drastically reduced both the nutritional value and abundance of subarctic copoepods, krill, and sand lace: huge starvation-induced die-offs of species from salmon to sea birds followed (8, 9). The Blob significantly harmed commercial, recreational, and subsistence fisheries and triggered the closures of open water fisheries such as salmon and tuna (6, 9). It also triggered a large harmful algal bloom which resulted in closures of economically important shellfish fisheries (5, 6, 9). The 2014/15 WWA has had multi-year lasting impacts (7); this MHW (and others of comparable severity) has driven sudden and significant change as it has pushed ecosystem resilience past “tipping point” thresholds (8, 10, 11).

At an individual level, MHWs directly impact marine organisms in a myriad of ways. Certain species already close to their thermal tolerances are killed directly {REF}. Thermal stress can also have negative but sub lethal impacts on an organism’s fitness by modifying their behaviour, feeding patterns, and food/nutrient requirements. {SOME EXAMPLES}

Marine heat waves also exert indirect effects on marine organisms, modifying natural processes including disease dynamics. Temperature has been shown to regulate the virulence of marine diseases corals and algae (12). In Australia and Europe, heatwaves have been linked to outbreaks of Pacific Oyster Mortality Syndrome (13). In the NE Pacific, sea star wasting disease (SSWD) epidemics have decimated populations of several sea star species over the last decade. SSWD is an ambiguous set of usually-lethal symptoms including twisted arms, lesions, deflation/loss of turgor, lost arms, lack of grip strength in tube feet, disintegration (14–16). Wasting is assumed (though not proven) to be infectious (17), with epidemics being exacerbated or triggered by environmental factors including heat waves (18, 19). The link between SSWD and temperature is multifactorial and complex; a majority of the literature associates warmer temperatures with wasting (15, 18–22). For example, *Pycnopoidia helianthoides* saw 80-100% population declines across its whole range in 2013-2014; the major die offs occurred during WWA events (19). Recent reports have indicated that wasting may affect more than just sea stars: Giant California Sea Cucumbers (*Apostichopus californicus)* displaying wasting symptoms have been reported in small numbers throughout the Salish Sea and the Northwest Coast of British Columbia and Alaska since 2014 (22, 23).

The direct and indirect effects of MHWs on Giant California Sea Cucumbers is unknown: this is concerning given the potentially devastating impacts of heat-induced disease outbreaks. The most recent sea cucumber wasting (SCW) event occurred in Nanoose Bay, B.C., from August – October 2021 (Em Lim *personal communication*). This event followed several severe regional heat waves (CITE). There is insufficient evidence to confirm that wasting-like symptoms were not caused by direct heat stress, as reported in farmed *Holothuria scabra* (24), or a heat-unrelated viral or bacterial disease as observed in farmed *Apostichopus japonicus* (25, 26). However, wasting symptoms could also be an indirect product of heat stress interactions with disease dynamics, as reported in aforementioned corals, algae, oysters, and sea stars (ALGAE CITE, OLIVER 2017, (21)). Understanding whether Giant California Sea cucumber wasting is linked to MHWs, either directly or indirectly, is important for informing management efforts seeking to protect this important species.

Sea cucumbers, often underappreciated, provide several important ecological and economic services. As benthic detritivores they break down organic tissue and play an important role in nutrient cycling (CITE). Sea cucumbers also maintain sediment health EXPLAIN MORE IN DEPTH (CITE). There is also evidence that they may provide some localized buffering against ocean acidification, because EXPLAIN MORE IN DEPTH (CITE). North America is seeing a growing sea cucumber harvest industry; in British Columbia alone the total industry is worth 10.2 million dollars (27). Recent studies have also been investigating the use of Giant California sea cucumbers for multi-trophic aquaculture (CITE). Given their importance, evaluating how marine heat waves may impact Sea cucumbers is a pressing concern, especially considering recent heat stress events that have devastated subtidal marine invertebrates across the Northeastern Pacific Ocean (CITE).

This paper seeks to assess the direct lethal and sublethal effects of marine heat waves on Giant California sea cucumbers, as well as potential indirect effects of heat-triggered disease presenting as wasting symptoms. We exposed Giant California Sea Cucumbers to a simulated MHW in a controlled laboratory setting and measured direct effects of mortality (lethal), changes to stress, movement, and excretion behaviour (sublethal), and the potentially direct or indirect effects of wasting symptoms. We tested three hypotheses: 1) prolonged exposure to temperatures exceeding the normal range of sea cucumbers will directly cause mortality; 2) sea cucumber stress, movement and defecation will increase with temperature because of higher metabolic activity; and 3) wasting will occur as result of temperature exposure without any previous history of disease or virus in this population. Evaluating how marine heat waves affect Giant California Sea Cucumbers is important to: understand the risks facing the sea cucumber harvesting industry, inform future research into the direct impact of marine heat waves on cucumbers, understand more about *A. californicus* behaviour, and learn about the impact of heat waves on wasting.

**Methods:**

*Study organisms*

We collected 63 *Apostichopus californicus* from in Scott’s Bay and Bamfield Inlet in Bamfield, British Columbia (48°50'02"N, 125°08'45"W) in {MONTH}, 2021. All were gathered from the shallow subtidal, between {NUMBER} and {NUMBER} depth. We place the cucumbers in deep flow-through sea tables at the Bamfield Marine Sciences Centre, which had a constant input of water from 9m depth in Barkley Sound. We also kept abundant supply of kelp harvested from the waters around Bamfield in each sea table, and plankton culture was added to supplement their diet.

The cucumbers remained in the lab for {XXX} days prior to the start of the experiment. Individuals were tagged with several types of tags and monitored in a separate study (Lim et al., unpublished data), the results of which indicated that the tags did not affect the sea cucumbers in any way. One specimen developed injuries around its T-tag, so we removed from our experiment. Studies have shown that body size can affect the thermal tolerance of marine organisms (28, 29), so we kept track of total length from mouthparts to anus, circumference (at widest point), wet weight, and volume (which was measured by placing cucumbers in a container of known water volume and measuring water displacement).

Giant California Sea Cucumbers seasonally lose their internal organs, a process that is poorly understood and may be caused by absorption of internal organs or expulsion by evisceration (30)(CITE SWAN 1967). Independent of the mechanism, the loss of internal organs is hypothesized to be part of a seasonal senescence that could affect their movement or foraging behaviour (30). A pilot study was conducted 20 days before the start of our experiment to determine how many cucumbers had their internal organs. We isolated cucumbers into individual containers, still on the flow-through system, for 24h to determine if they were pooping. We repeated the trial 2 days before the start of our heat trials. Sea tables were then divided by a coarse plastic mesh to allow for plankton and water to flow through, and to prevent gut-having and gutless cucumbers from mixing.

*Experimental Design*

Marine heatwaves have been observed in the Northeast Pacific Ocean year-round with November temperatures at depths of up to 200 meters reaching similar extremes as those recorded in summer months (31). We separated 60 cucumbers into 3 temperature treatments: 8ºC, 16ºC, and 24ºC. 8ºC is the approximate average temperature at depth for Barkley Sound during November (32). 16ºC mimics an above-average bottom temperature (33); 24ºC represents an extreme heat event that could occur under future climate warming scenarios. The sea tables hovered between 10 and 12ºC in the weeks leading up to our heat experiment. During the heat wave simulation, each treatment had 10 bins (with 2 cucumbers in each) sitting in 2 sea tables. The sea tables acted as temperature control water baths, with either a chiller (for the 8ºC treatment) or {XX} heaters per sea table (for the 16ºC and 24ºC treatments). 50% water changes were required at {XX} intervals to keep nitrate and ammonium levels down; water was adjusted to room temperature {XXºC} for the heated treatments to minimize temperature shock to the cucumbers. Cucumbers were exposed to their treatments for {XX} hours, and then monitored for {XX} days afterwards for symptoms.

*Measuring Response to Heat Stress*

We measured several response variables to capture direct lethal sublethal, and indirect (wasting) thermal impacts on Giant California Sea Cucumbers. The first of these was the time until direct mortality. We considered cucumbers to be dead if their tube feet were unresponsive to stimulus and all movement had ceased for over 60 minutes.

We measured the ability of sea cucumbers to stiffen, which is a physiological mechanism used for defence and posture maintenance (34), using two different ordinal scales intended to measure the two aforementioned ecological purposes of stiffening. First, we gently poked the cucumber with one finger and then poked them again 3 seconds later to measure their defense response. We assigned the cucumber a score of 0 if it failed to stiffen when initially poked, a score of 1 if it stiffened but was not still stiff when poked a second time, and a score of 2 if the cucumber got hard and stayed hard. Secondly, we removed each cucumber from their tank and placed them on a 5 cm x 5 cm elevated platform to measure their ability to maintain their posture. We assigned the cucumber a score of 0 if it failed to stiffen at all, a score of 1 if it failed to remain stiff when placed on the platform, a score of 2 if it maintained its posture for less than 2 seconds, and a score of 3 if it maintained its posture for more than 5 seconds. Each stiffness test was performed before, once during, and after the heat wave.

We also recorded several binary variables. We recorded if cucumbers eviscerated, an act where they partially expulse their inner organs, throughout the experiment. We also recorded whether cucumbers spawned, because heat-induced spawning has been reported in other sea cucumber species and may indicate higher levels of physiological stress (35). Finally, we recorded if cucumbers were defecating to measure if they were continuing to feed during the experiment.

To measure movement rates and dispersal distances we removed each cucumber from their aquaria, placed them in a large tank that had been heated to match the temperature of their heat treatment, and then recorded their activity for 30 minutes using a GoPro camera. We used a fancy unknown software to record distance dispersed over 30 minutes. We measured movement before the experiment, on the second of four days of heat treatment, and one day after the experiment.

Finally, we observed cucumbers multiple times each day throughout the experiment and for 7 days following the heat treatments and recorded whether wasting symptoms were occurring.

*Statistical Analyses*

To determine whether survival differed across temperature treatments we used a cox proportional hazards model (36). We included several covariates in the model that we hypothesized could have also affected survival, which were intestinal status (reabsorbed Y/N), initial movement rates, and body volume. We assessed whether our qualitative scales for sublethal responses varied across temperature treatments using ordinal logistic regression, a model designed for use on qualitative scaled data (37). For qualitative values measured as binary response variables we used logistic regression. We determined if movement varied significantly across treatments using a linear regression model with dispersed distance as the response variable and temperature as the predictor variable. Finally, we used a binary logistic regression model to determine if wasting (yes/no) was temperature dependent.

**Methods**:

* Animal Collection:
  + Study organisms (Blah blah about cukes (ecology, physiology)
    - Size range, weight range
    - Evisceration rate
  + Study area (mention where the cucumbers are found)
* Pilot Study (how to determine evisceration)
* Simulating a heat wave (logistics)
  + Talk about temperature trends in Barkley Sound (32) that we’re aiming to replicate & exceed
  + Talk about HOW we’re going to do this
    - Literal aquarium setup
    - # aquariums, # heaters, etc
    - Temperature and treatments: 8, 16, 24ºC.
    - Evisceration as a treatment.
* Measuring cucumber responses to heat (multiple paragraphs)
  + Talk about how we’re measuring each of our response variables, and provide rationale for doing so
    - Stress: firm up in hand, tube feet stick, evisceration, spawning. Make a 1-5 scale of firmness
    - Movement: go pro trials (likely)
    - Wasting: follow sea star
    - Mortality
  + Define a variety of parameters:
    - Mortality, firmness, wasting scale
* Statistical analyses
  + Survival analysis (cox proportional hazard or Kaplan meier)
  + Models to measure behaviour (linear regression?)
  + Models to measure wasting (logistic regression?)

Angles:

* Climate change (heat)
* Guts vs Gutless

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**Sentence RE: heat waves occurring in November: \*\*** although marine heat waves are most prominent during summer months, they have previously occurred as late as November in the Northeast Pacific (31). Our November study is thus a recreation of a biologically plausible heat stress event for *P. californicus*.

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