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

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BMSC Directed Studies

**Introduction:**

Over the past two centuries, unchecked human development and greenhouse gas emissions have profoundly impacted the biosphere and the climate (Steffen et al., 2018; Walther et al., 2002). The rising frequency of extreme climatic and weather events, including marine heatwaves (MHWs), is one of the consequences of our move into the Anthropocene (Allan et al., 2021). MHWs are occurrences of anomalously warm seawater temperatures for a discrete duration of time in a specific locality (Oliver et al., 2021). Anthropogenic climate change is increasing the intensity, duration, size, and frequency of MHWs with consequences across the globe (Allan et al., 2021; Frölicher et al., 2018; Oliver et al., 2021).

Marine heatwaves have damaging and potentially catastrophic effects on marine ecosystems and human economies (Di Lorenzo & Mantua, 2016). MHWs have bottom-up impacts on ecosystems, driving sudden and large-scale die-offs at all trophic levels from starvation, toxicity, and hypoxia (Cavole et al., 2016; Di Lorenzo & Mantua, 2016; Suryan et al., 2021; von Biela et al., 2019). 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 (Cavole et al., 2016; von Biela et al., 2019). The Blob significantly harmed commercial, recreational, and subsistence fisheries and triggered the closures of open water fisheries such as salmon and tuna (Cavole et al., 2016; Di Lorenzo & Mantua, 2016). It also triggered a large harmful algal bloom which resulted in closures of economically important shellfish fisheries (Cavole et al., 2016; Di Lorenzo & Mantua, 2016; Frölicher et al., 2018). The 2014/15 WWA has had multi-year lasting impacts (Suryan et al., 2021); this MHW (and others of comparable severity) has driven sudden and significant change as it has pushed ecosystem resilience past “tipping point” thresholds (Harley & Paine, 2009; von Biela et al., 2019; Wernberg et al., 2015).

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 (Case et al., 2011). In Australia and Europe, heatwaves have been linked to outbreaks of Pacific Oyster Mortality Syndrome (Oliver et al., 2017). 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 (Bates et al., 2009; Hewson et al., 2018; Menge et al., 2016). Wasting is assumed (though not proven) to be infectious (Work et al., 2021), with epidemics being exacerbated or triggered by environmental factors including heat waves (Aquino et al., 2021; Harvell et al., 2019). The link between SSWD and temperature is multifactorial and complex; a majority of the literature associates warmer temperatures with wasting (Aquino et al., 2021; Bates et al., 2009; Eisenlord et al., 2016; Harvell et al., 2019; Hewson et al., 2018, 2020). For example, *Pycnopoidia helianthoides* saw 80-100% population declines across its whole range in 2013-2014; the major die offs occurred during WWA events (Harvell et al., 2019). 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 (Hewson et al., 2020; Schroeder, 2017).

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* (Delroisse et al., 2020), or a heat-unrelated viral or bacterial disease as observed in farmed *Apostichopus japonicus* (Deng et al., 2008; Liu et al., 2010). 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 (Case et al., 2011; Hewson et al., 2018; Oliver et al., 2017). 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, sea cucumbers break down organic plant and animal tissue and are thus important for nutrient recycling (Wheeling et al., 2007). Sea cucumbers also maintain sediment health for bacteria and other detritivores through bioturbation and sediment cleaning (Purcell et al., 2016). There is also evidence that sea cucumbers may provide localized buffering against ocean acidification by releasing alkaline ammonia compounds that facilitate the calcification of corals and calcareous algae (Schneider et al., 2011). North America is also seeing a growing sea cucumber harvest industry; in British Columbia alone the total industry is worth 10.2 million dollars (Fisheries and Oceans Canada, 2021). Recent studies have also been investigating the use of Giant California sea cucumbers for multi-trophic aquaculture and sea cucumber ranching (Hannah et al., 2013; van Dam-Bates et al., 2016). 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 (Di Santo & Lobel, 2017; Kelley et al., 2011), 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 (Fankboner & Cameron, 1985; Swan, 1961). 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 (Fankboner & Cameron, 1985). 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 (Chen et al., 2021). 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 (Pawlowicz, 2017). 16ºC mimics an above-average bottom temperature (Xuereb et al., 2018); 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 (Motokawa & Tsuchi, 2003), 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 (Battaglene et al., 2002). 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 (Cox, 1972). 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 (McCullagh, 1980). 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.

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