**The effect of a simulated marine heat wave on giant California sea cucumbers (*Parastichopus californicus*)**

Introduction and Methods, 29 October 2021

Jonathan Farr and Declan Taylor

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**Introduction:**

Anthropogenic climate change is increasing the intensity, duration, size, and frequency of marine heatwaves (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). They have bottom-up trophic impacts on ecosystems, driving sudden and large-scale die-offs at all trophic levels from thermal stress, 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 in the Northeast Pacific Ocean (nicknamed “the Blob”) drastically reduced both the nutritional value and abundance of subarctic copepods, krill, and sand lace, which caused starvation-induced die-offs of species from salmon to sea birds (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 including 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 Blob has had multi-year lasting impacts (Suryan et al., 2021); this warm water anomaly, and others of comparable severity, have driven sudden and significant change as they can push ecosystem resilience past ecological tipping points (Harley & Paine, 2009; von Biela et al., 2019; Wernberg et al., 2015).

At an individual level, MHWs directly impact marine organisms in a number of ways. Certain species already close to their thermal tolerances are killed directly (Dong et al., 2011; Oliver et al., 2017). Thermal stress can negatively l impact an organism’s fitness by modifying their behaviourand food/nutrient requirements (Kühnhold et al., 2017). Marine heat waves also exert indirect effects on marine organisms, modifying natural processes such as disease dynamics. For example, temperature has been shown to regulate the virulence of marine diseases in corals and algae (Case et al., 2011). In the NE Pacific, sea star wasting diseaseepidemics have decimated populations of several sea star species over the last decade. Wasting is assumed to be infectious (Work et al., 2021), with epidemics being exacerbated or triggered by environmental factors including warm temperatures (Aquino et al., 2021; Bates et al., 2009; Eisenlord et al., 2016; Harvell et al., 2019; Hewson et al., 2018, 2020). Sea star wasting disease 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, and disintegration (Bates et al., 2009; Hewson et al., 2018; Menge et al., 2016). Recent reports have indicated that wasting may affect more than just sea stars: giant California sea cucumbers (*Parastichopus 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).

A recent sea cucumber wasting event occurred in Nanoose Bay, B.C. Canada, from August – October 2021 (Em Lim, *personal communication*). This event followed several severe regional heat waves (Kotyk, 2021). There is insufficient evidence to confirm the cause of the observed symptoms however, warm water anomalies could plausibly play an etiological role through heat stress interactions with disease dynamics, as reported in 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 is important for informing management efforts seeking to protect this important species.

Sea cucumbersprovide several important ecological and economic services. As benthic detritivores, sea cucumbers break down organic matter and are thus important for nutrient recycling (Wheeling et al., 2007). Sea cucumbers maintain sediment health for bacteria and other detritivores through bioturbation and sediment cleaning (Purcell et al., 2016). North America is also seeing a growing sea cucumber harvest industry (Hannah et al., 2013; van Dam-Bates et al., 2016); in British Columbia alone the total industry is worth 10.2 million dollars (Fisheries and Oceans Canada, 2021).Given their importance, evaluating how marine heat waves may impact sea cucumbers is a pressing concern, especially considering recent heat stress events across the NE Pacific Ocean (Harvell et al., 2019).

This study seeks to assess the direct lethal and sublethal effects of marine heat waves on giant California sea cucumbers and to enhance our understanding of the cause etiology of sea cucumber wasting. We hypothesize that prolonged elevated temperature exposure will cause direct mortality, changes in behaviour, and increased wasting symptoms due tophysiological stress. Based on our hypothesis, we prediction: 1) we will observe greater mortality with higher temperatures; 2) we will observe changes in movement rates and stress responses as temperature increases; and 3) we will observe wasting symptoms only in warmer temperature treatments.

**Methods:**

*Study organisms*

We collected 63 *Parastichopus californicus* from in Scott’s Bay and the entrance to Bamfield Inlet in Barkley Sound, British Columbia (48°50'02"N, 125°08'45"W) in July 2021. All were gathered from the shallow subtidal, between {NUMBER} and {NUMBER} depth. We placed the cucumbers in deep flow-through sea tables at the Bamfield Marine Sciences Centre, which had a constant flowof water from Barkley Sound. The sea cucumbers were provided fed with kelp,plankton cultures, and bloodworms. The cucumbers remained in the lab for {NUMBER} days prior to the start of the experiment. As part of a separate study, individuals were tagged with several types of tags and monitored; the results of this study indicated that the tags did not affect the sea cucumbers’ behaviour (Lim et al., unpublished data).

We measured sea cucumber size to account for this potential confound as some studies have shown that body size can affect the thermal tolerance of marine organisms (Di Santo & Lobel, 2017; Kelley et al., 2011). The size measurements collected werewere total length (mouthparts to anus), circumference at widest point, wet weight, and volume (which was measured by measuring volume water displacement). Giant California sea cucumbers seasonally lose their internal organs in a poorly understood process that may be caused by absorption of the internal organs or expulsion by evisceration (Fankboner & Cameron, 1985; Swan, 1961). This loss of internal organs is hypothesized to be part of a seasonal senescence that could affect their behaviour and therefore confound our experiment (Brothers et al., 2015). Prior to the start of the experiment, we isolated cucumbers into individual containers for 24 hours to determine if they were defecating. Sea tables were then divided by a coarse plastic mesh to allow for plankton and water to flow through, and to prevent the mixing of cucumbers with and without internal organs.

*Experimental Design*

We chose temperature treatments to mimic low (8ºC), high (16ºC), and extreme (24ºC) heat events. The 8ºC treatment represents the average seawater temperature 50 meters below surface in Barkley Sound during November (Pawlowicz, 2017). Sixteen degrees Celsius mimics a high, but realistic, subtidal temperature (Xuereb et al., 2018). Twenty-four degrees represents an extreme heat event that is unlikely to occur under natural circumstances (Chen et al., 2021b; Pawlowicz, 2017; Xuereb et al., 2018).

Sea cucumbers (N = 60) were X seperated into the three temperature treatments (NTreatment = 20). Ten bins were placed in different treatment sea tables, which acted as temperature regulatorywater baths with either a chiller (8ºC treatment) or 2 heaters per sea table (16ºC and 24ºC treatments). We used a water permeable divider to separate cucumbers within bins to allow for individual identification throughout the experiment. We did not provide sea cucumbers with food during the experiment. 50% water changes were required at 24h intervals to keep nitrate and ammonium levels low. We acclimatized seawater to room temperature for 6 hours. We exposed sea cucumbers to treatments for four days and monitored them 10 days after treatmentsfor mortality and wasting symptoms.

*Measuring Response to Simulated Marine Heat Wave*

We measured several response variables to capture direct lethal, sublethal, and wasting thermal impacts on giant California sea cucumbers (Table 1). The first of these was the time until mortality. We considered cucumbers to be dead if their tube feet were unresponsive to stimulus and all movement had ceased for over 60 minutes.

Sea cucumbers stiffen as a defense mechanism and for posture maintenance (Motokawa & Tsuchi, 2003). We measured stiffness using two different ordinal scales. First, we gently poked the cucumber with one finger and then poked them again after 3 seconds 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.

Cucumbers were observed once every 12 hours for signs of spawning, evisceration, or sea cucumber wasting symptoms. . We also checked for symptoms of wasting and whether cucumbers spawned, since heat-stress induced spawning has been reported in other sea cucumber species (Battaglene et al., 2002). Finally, we recorded if cucumbers were defecating to determine if there was a change in the status of their organ reabsorption throughout the course of 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. To analyze movement, we replayed the video and laid 4x5 grid over the screen. We recorded the number of times the cucumber crossed a gridline, and the duration of time it took for a cucumber to reach the sidewall of the container. We measured movement before the experiment, on the second of four days of the heat treatment, and one day after the heat treatment.

*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: intestinal status (reabsorbed Y/N), initial movement rates, and body size metrics. We assessed whether our qualitative scales (stiffness 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 dispersal distance or time until reaching the container wall as the response variables and temperature as the explanatory variable. Finally, we used a binary logistic regression model to determine if wasting (yes/no) was temperature dependent.

**Table** **1**. The response variables measured to quantify the effect of a marine heat wave on giant California sea cucumbers (*Apostichopus californicus*).

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| --- | --- | --- | --- |
| Category | Response Variables | Measurement Method | Variable Type |
| Direct lethal | Mortality | Time to Death | Continuous |
| Direct sublethal | Stiffening (antipredator defense) | Repeated poke test | Ordinal (3 level) |
| Stiffening (posture maintenance) | Platform test | Ordinal (4 level) |
| Evisceration | Inner organs observed | Binary |
| Spawning | Sperm or eggs observed | Binary |
| Movement | Dispersal distance | Continuous |
| Disease (indirect or direct) | Wasting | Presence of wasting lesions | Binary |

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