**Does temperature stress cause wasting symptoms in giant California sea cucumbers?**

by

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# Abstract

Marine heat waves and warming oceans have profound effects on disease dynamics in marine invertebrates. Wasting diseases, potentially worsened by extreme heat events, have devastated sea stars across the Pacific Northwest coast. Recent evidence has emerged of wasting disease symptoms paralleling those in sea stars in giant California sea cucumbers (*Parastichopus californicus*). A severe wasting event in Nanoose Bay, B.C. followed an extreme climatic heat event, and our study aimed to determine whether heat stress could trigger wasting in *P. californicus*. We also aimed to improve the understanding of how two unusual sea cucumber characteristics, stiffening and evisceration, are affected by thermal stress. Under a controlled laboratory setting, we exposed *P. californicus* to an 82-hour simulated marine heat wave of either 17ºC or 22ºC against a 12ºC control. We tracked their stiffness for posture maintenance and antipredator defense, evisceration, mortality, and lesion appearance. Differences between treatments were analyzed using GAIC model fit analysis and ANOVA tests. While we observed both minor and major handling ulcers, no wasting-like symptoms were exhibited in 12ºC ,17ºC or 22ºC treatments. Mortality in the 22ºC treatment demonstrated that *P. californicus* were near their thermal limit and under extreme physiological stress during our experiment. Stiffening behaviour was significantly affected by heat treatments, suggesting that physiological mechanisms behind stiffening were impaired by thermal stress. We also found further evidence in support of seasonal evisceration of digestive tract organs. Our study provides evidence that wasting is not solely triggered by heat stress, leaving the cause of the mass mortality event observed in Nanoose unknown. *P. californicus* are economically important marine detritovores and a multimillion dollar fishing industry. To better understand and predict future catastrophic mortality events, the causal agents of sea cucumber wasting must be identified.

# Keywords

Wasting disease, echinodermata, sea cucumber, skin ulceration syndrome, thermal stress

# Introduction

Anthropogenic climate change is increasing the intensity, duration, size and frequency of marine heat waves across the globe with potentially catastrophic effects on organism fitness, marine ecosystems and human economies (Allan et al., 2021; Frölicher et al., 2018). These extreme climatic events often cause immediate and mass mortality 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 indirectly harmed salmon and tuna by causing mass mortalities in zooplanktonic primary marine consumers and upending marine food webs (Cavole et al., 2016; Di Lorenzo & Mantua, 2016; Frölicher et al., 2018). The warm water anomaly also caused a harmful algal bloom which reduced the abundance of important mid-trophic prey species and caused the starvation of species from salmon to sea birds, and was particularly damaging to shellfish and associated fisheries (Cavole et al., 2016; von Biela et al., 2019). Marine heat waves can also impact marine ecosystems more subtly by impacting biological processes including foraging and movement behaviour (Chiu et al., 2021), reproduction (Shanks et al., 2020), and disease dynamics (Aalto et al., 2020)..

Marine heat waves can worsen marine diseases and epidemics in part because thermal stress can compromise organism’s immune response (Branco et al., 2012; Matozzo et al., 2012). In addition, the virulence of many marine diseases appears to be regulated through temperature effects on pathogen transmission and growth (Marcogliese, 2008; Rubio-Portillo et al., 2016). For example, the appearance and outbreak of Pacific Oyster Mortality Syndrome in Tasmania has been linked to marine heatwaves (Oliver et al., 2017). Many marine bacterial pathogens, including those which affect dominant cover species such as corals and macroalgae, also display temperature-dependent virulence (Case et al., 2011). Anomalously warm temperatures have also been accredited to triggering and exacerbating sea star wasting disease (Aquino et al., 2021; Bates et al., 2009; Eisenlord et al., 2016; Harvell et al., 2019; Hewson et al., 2018). Over the past decade, sea star wasting disease has affected more than 20 sea star species in the Northeast Pacific Ocean (Hewson et al., 2018). Sea star wasting is described as a broad set of symptoms including twisted arms, lesions, deflation/loss of turgor, loss of arms, lack of grip strength in tube feet, and disintegration (Bates et al., 2009; Hewson et al., 2018; Menge et al., 2016). Although wasting-like symptoms are variable and non-specific, recent evidence has emerged that wasting may occur in echinoderms other than sea stars.

Giant California sea cucumbers (*Parastichopus californicus;* Stimpson, 1857) are echinoderms of the class Holuthuroidea and have been reported to display wasting symptoms similar to those of their sea star relatives. These benthic detritivores are found from the Gulf of Mexico to Alaska, and are found from the low intertidal zone to a depth of 250 meters (Purcell et al., 2016). Since 2014, wasting symptoms have been reported in small numbers throughout the Salish Sea and the Northwest Coast of British Columbia and Alaska (Hewson et al., 2020a; Schroeder, 2017). Symptoms of wasting in sea cucumbers are anecdotally reported as similar to those observed in sea stars: non-focal lesions and fissures across the body wall, epidermal tissue sloughing, and rapid liquefaction (Hewson et al., 2020b). A recent wasting event occurred in Nanoose, B.C., Canada, from August – October 2021 (Em Lim, *personal communication*). Reports from citizen scientist divers indicated that up to 75 *P. californicus* exhibited wasting symptoms, and that wasting sea cucumbers were disproportionally found at shallow depths (Em Lim, *personal communication)*. Southwest British Columbia was affected by multiple heat waves in the months leading up to wasting outbreak (Environment & Climate Change Canada, 2021); these extreme air temperatures led to elevated subtidal temperatures in the Strait of Georgia where Nanoose Bay is located (Ocean Networks Canada Data Archive, 2021). As such, there is a potential link between thermal stress and wasting in *P. californicus*, especially given that wasting was mostly observed in the warmer (shallower) part of the water column*.* However, understanding the impact of heat stress on sea cucumber wasting also requires knowledge of how their unique behavioural and physiological characteristics are affected by thermal stress.

Sea cucumbers have several unusual and poorly understood traits that distinguish them from other echinoderms such as sea stars, and may affect the underlying physiological mechanisms behind wasting symptoms. Wasting symptoms in *P. californicus* have been observed to start as small ulcers on the aboral body wall and grow into larger lesions and fissures which can fully cover the organism (Hewson et al., 2020a). The sea cucumber dermis is primarily mutable collagenous tissue that allows them to change mechanical properties of their dermis in response to environmental stimuli (Motokawa & Tsuchi, 2003). Under normal seawater conditions, sea cucumber dermal stiffness can rapidly increase, which is thought to be adaptive for posture maintenance and antipredator defense (Motokawa & Tsuchi, 2003; Yamada et al., 2010). If these lesions extend into the dermis, wasting may affect changes in stiffening behaviour, especially if stiffening is already affected by an altered thermal regime. Another unique behaviour of sea cucumbers is evisceration, the ability to expulse and subsequently regenerate their digestive tract (Fankboner & Cameron, 1985). The mechanisms which trigger evisceration are poorly understood but include seasonality, as a response to the low-productivity winter months (Swan, 1961), and disease stress (Deng et al., 2008). Evisceration may have unknown physiologic consequences, and Hewson et al. (2020a) proposed that seasonal evisceration may be linked to wasting disease because wasting has been reported during the same season when evisceration occurs (Swan, 1961). Therefore, improving the baseline knowledge of how the unique behaviours of *P. californicus* are affected by thermal stress may help predict where and why wasting may occur.

Considering recent marine heat waves and reports of a sea cucumber wasting outbreak in Nanoose Bay, this study assesses whether thermal stress as an isolated factor causes wasting symptoms in *P. californicus*. We also aim to evaluate how *P. californicus* behaviours like stiffening, spawning, and evisceration are affected by extreme temperatures to understand if they may relate to wasting, and more broadly, to sea cucumber thermal stress. We hypothesized that prolonged elevated temperature exposure will cause wasting symptoms, mortality, and behavioural changes due to physiological stress. Based on our hypothesis, we make three predictions: 1) we will observe wasting symptoms only in sea cucumbers in high temperatures; 2) we will observe greater mortality with higher temperatures; and 3) we will observe decreased stiffening, and increased stress spawning and evisceration in temperature treatments.

# Materials and Methods

Study organisms

63*Parastichopus californicus* were collected by divers Em Lim and Siobhan Gray 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 7m and 12m depth. We placed the cucumbers in 19 cm deep flow-through sea tables at the Bamfield Marine Sciences Centre, which had a constant flow of seawater from Barkley Sound. The sea cucumbers were 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 or survival (Lim et al., unpublished data).

We measured sea cucumber size as some studies have shown that body size can affect the thermal tolerance of marine organisms (Di Santo & Lobel, 2017; Kelley et al., 2011). We weighed sea cucumbers twice, 24 hours apart, to get an average wet weight. We conducted a pilot study before the start of our experiment to determine how many cucumbers had their internal organs. Prior to the start of the experiment, we isolated cucumbers into individual containers for 24 hours to determine if they were defecating.

Experimental Design

Our experiment exposed sea cucumbers to 12ºC, 17ºC, and 22ºC temperature treatments. The 12ºC control treatment represents the average seawater temperature 50 meters below surface in Barkley Sound, while 17ºC mimics a high subtidal temperature and 22ºC represents an extreme heat event that is unlikely to occur under natural circumstances (Chen et al., 2021; Pawlowicz, 2017; Xuereb et al., 2018).

We separated the sea cucumbers into three temperature treatments (n22ºC treatment = 18, ncontrol & 17ºC treatments = 19, *N* = 56). Sea cucumbers were separated into 30 bins with 2 cucumbers in each (except 4 bins in which only housed one cucumber). Each treatment consisted of two sea tables with a total of 10 bins. Cucumbers were randomly assigned to bins, and bins were randomly assigned to sea tables. The bins sat in 5cm deep water baths that were either at the temperature of the sea tables’ flow through system (12ºC), standing at ambient (room) temperature (17ºC), or heated with two 800W aquarium heaters to 24ºC, to keep the bins at 22ºC. The temperature treatments lasted from 09:00 on November 09 (Day 1) to 01:00 on November 13 (Day 5) for a total of X hours of heat exposure, and bucket temperature was ramped up over 24h on Day 1 and decreased over 8h on Day 5 to limit temperature shock. In the 22ºC treatment, the water bath was circulated around the sea table with powerhead aquarium pumps to ensure even temperature distribution. We monitored temperature in every bin at least twice daily. 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.  Water changes were done as need to keep nitrate and ammonium levels below 0.5 parts per million. Sea water was heated to the appropriate treatment temperature prior to water changes. We exposed sea cucumbers to treatments for 82 hours and monitored them for 7 days afterwards for mortality and wasting symptoms.

Measuring Sea Cucumber Wasting and Stress

We assessed cucumbers for wasting symptoms on the 4th, 5th, 6th, and 12th days of the experiment. We classified skin ulcers as either minor or major ulcers based on their size and visual appearance (Fig. 1). We considered minor ulcers to be small lesions on the ends of spines, which did not have severe discoloration, where the dermis was not fully removed. Scars and closed ulcers were not counted. We considered major ulcers to be open wounds that were fully through the dermis, where white tissue could be seen beneath. We then determined the maximum number of minor and major ulcers observed on a single cucumber across the experiment’s duration.

We assessed sea cucumber stiffness for antipredator defense and posture maintenance using two different 3-point ordinal scales. First, to assess antipredator defense stiffening we gently palpated the cucumber for 10 seconds to mimic an attempted predation event. We assigned the cucumber a score of 0 if it failed to stiffen and felt like the texture of fat (soft), a score of 1 if it stiffened to the level of un-flexed muscle (standard), and a score of 2 if the cucumber got comparably stiff to a flexed bicep muscle (stiff). Secondly, we removed each cucumber from their bucket and placed them on a 5 cm x 5 cm elevated platform to measure their ability to maintain their structure over 5 seconds. We assigned the cucumber a score of 0 if it failed to stiffen at all (soft), a score of 1 if it failed to remain stiff when placed on the platform (standard), a and a score of 2 if it maintained its structure for the entire 5 seconds (stiff; Fig. 2). Each stiffness test was performed daily.

We checked sea cucumber tanks every 12 hours for evisceration, an act where they partially or fully expulse their inner organs. We also determined if spawning had occurred, since heat-stress induced spawning has been reported in other sea cucumber species (Battaglene et al., 2002). We could only measure spawning per-bucket instead of per-cucumber because the gametes, especially sperm, would flow from one side of the bucket to the other and we were unable to determine which of the cucumbers had spawned *(Nbuckets* = 30)*.*

Statistical Methods

We examined the difference between bucket temperature at each treatment by using a K-W test followed by a Dunn’s K-W test for multiple comparisons.

To assess which variables affected the maximum number of minor ulcers, we used a generalized additive model. Using the function *fitdist* from package GAMLSS (Rigby et al., 2021), we determined that the number of minor ulcers best fit a geometric distribution. We modelled the maximum number of minor ulcers as a function of treatment, weight, evisceration, and defecation status. We included sea table and bucket as random effects. We used backwards selection to determine the combination of variables that best explained the maximum number of ulcers.

To examine how sea cucumber mortality varied across temperature treatments we used a Kruskal-Wallis (K-W) test followed by a Dunn’s Kruskal-Wallis (Dunn’s K-W) test for multiple comparisons. We further examined the covariates that affect the likelihood of mortality with a logistic regression model with cucumber mortality (binary dead/alive) as the response variable and evisceration, defecation status, initial droop and squeeze scores, and initial weight as explanatory variables. We used backwards model selection to investigate which combination of these variables best explained the variation in sea cucumber mortality.

We determined if the two ordinal stiffness metrics (antipredator response and structure maintenance) were correlated using Spearman’s rank correlation coefficient (Schober & Schwarte, 2018). We assumed that these metrics represent two different behaviours, regardless of correlation strength, and therefore constructed separate models with each metric as the response variable. For both response variables, we constructed full ordinal regression models with the following predictor variables: temperature treatment, date (as a discrete variable), and the interaction term between treatment and date. We restricted our measurement period to the days preceding (Day 1), during (Day 2-4) and immediately after the heat treatment (Day 5). We included individual cucumber identity as a random effect to account for repeated measures on the same individuals over time. We also included bucket ID and sea table ID as random effects to account for our paired (two cucumbers per bucket) and blocked (five buckets per sea table) experimental design. We used model selection to determine the most parsimonious models (ΔAIC > 2). From top selected models we calculated 95% confidence intervals and considered effects to be significant if confidence intervals did not overlap zero.

We used a K-W test to compare the number of buckets with spawning cucumbers across temperature treatments. For evisceration, we created a logistic regression model with treatment, weight, and defecation status as explanatory variables in the full model, and sea table as a random effect. We conducted backwards model selection to determine the most explanatory variables.

All statistical analyses were conducted in R version 4.0.3 (CITE). Dunn’s K-W tests were conducted with the *dunnTest* function in package FSA (Derek et al., 2021). Models for minor ulcers, mortality and evisceration were conducted using the *gamlss* function, and model selection was done using the *stepGAIC* function, both from package GAMLSS (Rigby et al., 2021). Ordinal regression models were conducted using the *clmm* function in package ordinal (Christensen, 2019); model selection for these models was conducted using the *dredge* function in package MuMIN (Barton & Barton, 2020). We considered results to be significant at P < 0.05 or if 95 % confidence intervals did not overlap zero.

# Results

The temperature treatments for the experimental heatwaves varied slightly from the target temperatures (Fig. 3). The mean temperature of the 12°C treatment was 12.5°C, but varied from 11.2-14.0°C; the mean of the 17°C treatment was 16.8°C but ranged from 14.9°C to 17.9°C; the mean of the 22°C treatment was 21.9°C but varied from 20.0°C to 23.3°C. Each treatment temperature was significantly different from every other treatment temperature (K-W χ2 = 313.78, df = 2, p < 2.2E-16).

Skin ulcers occurred during the experiment in all three treatments. Minor ulcers were observed on 17 cucumbers in the 12ºC treatment, 15 cucumbers in the 17ºC treatment, and 10 individuals in the 22ºC treatment (Fig. S1). Major ulcers were observed in only the heat treatment: on the final day of the treatment, two cucumbers had 1 and 4 ulcers respectively. Neither of these individuals died, and after the heat treatment stopped their major ulcers appeared to heal and were re-classified as minor ulcers on day 12 of the experiment. The maximum number of minor ulcers per individual was not significantly explained by treatment, weight, evisceration, or defecation status, because all explanatory variables were excluded from the final backward-selected model (Table S1).

Five mortalities occurred in the 22°C treatment on day 3 (n = 2), day 4 (n = 1), day 6 (n = 1) and day 7 (n = 1) of the experiment. These mortalities all occurred in different buckets and in no cases did both sea cucumbers in a shared bucket die. Mortality was significantly different across temperature treatments (K-W χ2 = 11.383, df = 2, p = 0.00337). There was no significant difference between the 12°C and 17°C treatments (Dunn’s K-W Z = 0, p-adj = 1), but the 22°C treatment had significantly higher mortality than the 12°C (Dunn’s K-W Z = 2.935, p-adj = 0.00334) and 17°C (Dunn’s K-W Z = -2.935, p-adj = 0.00334) treatments. Based on backwards selection, our null model was the best fit, indicating that evisceration, defecation status, initial droop and squeeze score, and weight did not significantly predict mortality in the 22°C treatment.

Sea cucumber stiffness, as measured by antipredator response and structure maintenance, was lower in the 22ºC and 17ºC treatments compared to the 12ºC treatment (Fig. 3, 4). Antipredator response and structure maintenance were significantly positively correlated with each other (rs = 0.53, p < 0.001; Fig. S2). Backward-selected models indicated that temperature treatment and day affected both stiffness metrics (Table S2, S3). There was a significantly lower likelihood of high antipredator stiffness values for the 17°C (p = 4.80 x 10-5) and 22°C (p = 5.88 x 10­-9) treatments relative to the control. Sea cucumber antipredator stiffness scores were significantly likely to be lower on day 2 (p = 1.16 x 10­-6), day 3 (p = 1.33 x 10­-7), day 4 (p = 2.06 x 10­-6) and day 5 (p = 5.05 x 10­-8) than on day 1 (Table S2). For structural stiffness, cucumbers were significantly less likely to have high stiffness scores relative to the control treatment in the 17°C (p = 1.99 x 10-7) and 22°C (p = 2.44 x 10­-11) treatments. Structural stiffness values were significantly likely to be lower than day 1 on day 3 (p = 1.37 x 10­-5), day 4 (p = 2.50 x 10­-5) and day 5 (p = 8.66 x 10­-5), but not on day 2 (p = 0.0627; Table S3). In both antipredator and structural stiffness metrics, there were no significant differences in the likelihood of low stiffness values between 17°C and 22°C treatments, as indicated by the high degree of overlap in 95% confidence intervals (Fig. S3).

Over the course of the experiment, evidence of stress spawning (eggs or sperm) occurred in 11 buckets. Nine of these buckets were in the 17ºC and 22ºC treatments. There was no significant difference in per-bucket spawning between temperature treatments (K-W χ2 = 1.94, df = 2, p = 0.379).

We observed *N* = 12 cucumbers eviscerate their digestive organs in the 12 ºC (n = 2), 17ºC (n = 5) and 22ºC (n=5) treatments. Logistic regression results indicated that treatment temperature does not explain a significant amount of the variance in evisceration data because it was excluded from our backwards selected model (Table S5). However, weight (p = 0.0383) and defecation status (p = 0.0163) were included in the top model as significant predictors of evisceration (Table S5). Two cucumbers in the heat treatment eviscerated their respiratory trees in addition to their internal organs (Fig. 5). One cucumber mortality occurred 12 hours after respiratory evisceration, while the other was observed 68 hours after. The latter cucumber was severely impacted by the evisceration of the respiratory tree as stiffness values dropped to zero for the remaining days.

# Discussion

Broad overview

The objective of our study was to determine if heat stress can induce wasting symptoms in *Parastichopus californicus*. Our hypothesis that heat stress would trigger wasting symptoms, mortality, and changes in behaviour was partially supported. Unexpectedly, we observed minor skin ulcers at all treatments and major ulcers in the heat treatment, but neither were characteristic wasting symptoms (Hewson et al., 2020b) (Fig. S1). Although no wasting was observed, we observed mortality in the high temperature treatment, indicating that sea cucumbers were experiencing extreme physiological stress. Further indicating physiological stress, we observed the predicted decrease in stiffness for cucumbers in the elevated temperature treatments (Fig. 4, 5). Spawning and evisceration were not significantly explained by temperature treatments, which was unexpected.

Minor and major ulcers

Neither the minor or major ulcers that we observed matched the wasting symptoms observed in *P. californicus* in Nanoose Bay, B.C., or the isolated wasting events throughout the Pacific coast (Hewson et al., 2020b). Both types of ulcers were very different from the white open lesions and fissures covering the whole aboral body surface of wasting cucumbers in Nanoose (Em Lim, *personal communication*). We also did not see any sloughing of body tissues or liquefaction, as has been anecdotally reported in previous literature (Hewson et al., 2020a; Schroeder, 2017). The minor ulcers we observed on numerous *P. californicus* across treatments were small skin ulcers on the epidermis of the spines. These minor lesions did not break through the epidermal tissue. The major ulcers (N = {NUMBER}) that we observed on two *P. californicus* also did not match the full suite of wasting symptoms, though they bore a closer resemblance in colour, texture, and location (Fig. 1). These major ulcers fully wore through the epidermis, were white and oozing, and some were located on the aboral body wall as opposed to only on the ends of spines. Unlike reports of widespread mortality resulting from wasting in wild *P. californicus*,the major ulcers in both of our specimens healed within the 7-day recovery period. As such, there is no evidence that the sea cucumbers in our experiment were afflicted by the fatal wasting condition that has been previously reported (Hewson et al., 2020a; Schroeder, 2017).

While we are uncertain of the ultimate cause of the lesions that we observed, they may have been the product of intensive daily handling. During the experiment, specimens were handled daily and measured for their posture maintenance. This would have represented a large increase in the potential for abrasion of the epidermal tissue to occur across treatments, and we saw an even distribution of *P. californicus* presenting lesions across all treatments (Fig. S1). Major ulcers may have begun as minor ulcers that had become infected in sea cucumbers that were under high levels of stress in the 22°C treatment.

Mortality

Five *P. californicus* died in our extreme heat treatment and mortality was not explained by any measured variables outside of temperature treatment. We did not observe mortality of both *P. californicus* in a bucket, meaning that bucket water quality or contagious disease presence was unlikely to have affected mortality. Our findings therefore suggest that 22°C exceeds the thermal tolerance of *P. californicus*, but 17ºC does not. Although there are no studies on adult *P. californicus* thermal tolerance, our findings align with previous work on larval life stages. Ren et al. (2018) who found that 22°C but not 16°C or 18°C temperatures limited the survival, specific growth rate, and metamorphosis of larval giant California sea cucumbers. Adult and larval *P, californicus* have very different morphology, behaviour and habitat preferences, but our findings and those of Ren et al. (2018) provide evidence that extreme temperatures are physiologically detrimental to both life stages.

Stiffness

The stiffening behaviours that we quantified were both significantly effected by temperature and were indicative of two physiological processes: longitudinal muscle contractions and dermal stiffening (CITE). Sea cucumbers have a band of longitudinal muscle along each ambulacral zone, and rings of circular muscle throughout the length body which allow them to contract and lengthen. ADD IN DEETS ABOUT HOW HEAT AFFECTS MUSCLE HERE The dermis layer of sea cucumbers is rich in mutable collagenous tissue which allows the dermis to rapidly stiffen as a predator defense mechanism, and to maintain posture (Gao & Yang, 2015). Protein-mediated changes in the attractive forces between macromolecules in the extracellular matrix (the bulk of the cell material in mutable collagenous tissue) drives this stiffening (Takehana et al., 2014). Based on the lack of stiffening observed at higher temperatures, heat stress may increase the production of the de-stiffening protein softenin (Takehana et al., 2014), or denature/decrease the production of the stiffening protein tensilin (Tamori et al., 2016; Yamada et al., 2010). Although the 17°C treatment did not cause any mortality, the sublethal impact of this treatment on stiffening behaviours was comparable to the 22°C treatment, suggesting that the physiological mechanisms behind stiffening are disrupted by heat stress. Even less extreme temperature fluctuations could have adverse effects on sea cucumber stiffening in a broader ecological context.

Spawning

While we did not observe any significant trends in per-bucket spawning; 9 of 11 spawning events occurred in temperature treatments. This trend suggests that *P. californicus* are among Holothuroidea that release gametes in response to thermal stress (Battaglene et al., 2002; Rakaj et al., 2018; Schagerström et al., 2021). Our ability to connect spawning to individual *P. californicus* traits (e.g., size, stiffness, evisceration status) was limited due to the paired bucket experimental design. To better understand spawning, we would recommend keeping specimens in isolated containers to allow researchers to better track and identify spawning individuals.

Evisceration

We did not observe a treatment-related trend in digestive tract evisceration. The lack of a trend could have occurred because all *P. californicus* were overstimulated and stressed from extensive handling during the experiment (Ding et al., 2019). However, both weight and defecation status had significant effects on evisceration (Table S1). In particular, defecation status had a strong predictive power in determining that defecating *P. californicus* were less likely to eviscerate. This indicates that the link between evisceration and defecation likely has biological importance (Nakagawa & Cuthill, 2007). Our findings may provide further evidence of seasonal evisceration because evisceration was significantly more likely when *P. californicus* were not observed to be using their digestive tract (Swan, 1961). Additionally, the *P. californicus* specimens in our experiment may have been undergoing a seasonal senescence, making them more likely to eviscerate their digestive tract in response to handling-induced stress (Ding et al., 2019), as the energetic cost of eviscerating could be lower than sea cucumbers that continued to gather energy from feeding. Our findings in support of seasonal evisceration do not align with the claims of Fankboner and Cameron (1985), who provided evidence of seasonal atrophy, rather than evisceration of *P. californicus* internal organs. We propose that these two explanations may not be mutually exclusive. Instead, intraspecific phenotypic or genetic variation in seasonal organ evisceration or atrophy could maximize fitness in a highly variable environment (Ellis et al., 2006; Smith & Blumstein, 2008).

Respiratory tract evisceration was an unexpected event that only occurred in two individuals in the high temperature treatment. As it was followed by mortality on both occasions, we do not believe there is an adaptive advantage to respiratory evisceration in *P. californicus*. Instead, we postulate that this traumatic event was a physiological response to extreme temperature stress.

Limitations

There are several limitations to our study that are worth noting. First, we did not find any wasting symptoms and thus are unable to refute or support evidence that a link may exist between seasonal evisceration and wasting disease in *P. californicus* (Hewson et al., 2020a). Second, we obtained *P. californicus* from Bamfield, B.C., a population where wasting symptoms have not been reported (Hewson et al., 2020b). While no population genetic studies have specifically examined *P. californicus* in Bamfield, we assume that they are closely related to the Tofino, B.C. subpopulation (Xuereb et al., 2018). The Tofino subpopulation receives significant genetic influx from the *P. californicus* population in the Strait of Georgia where wasting has been reported (Xuereb et al., 2018). Therefore, we do not expect that genetic differentiation could confer differential vulnerability to wasting for *P. californicus* from Bamfield compared to those in Nanoose. Third, seasonality may explain the lack of wasting symptoms, as our experiment was conducted during November 2021 while the wasting event in Nanoose Bay occurred between August and October 2021. However, wasting in *P. californicus* has been previously reported year-round (Hewson et al., 2020b). The final limitation of our study was its short duration. We only exposed sea cucumbers to heat stress for a total of 82 hours and the majority of minor and major ulcers formed in the later days of our experiment. We cannot confirm that they would not have developed into more severe, wasting-like lesions provided with a more extended duration of extreme temperature. However, during our short-term experiment we observed mortality and a significant stiffness-based behavioural response. Despite our findings that *P. californicus* was under extreme thermal stress, wasting did not occur. If wasting was induced by physiological heat stress as an isolated factor, we expect that it would have occurred under these conditions.

Synthesis

White skin ulcerations like those that we observed are a well-documented disease phenomenon in sea cucumbers, described as a Skin Ulceration Disease or Skin Ulceration Syndrome (SUS) (Delroisse et al., 2020). SUS has been documented in commercially farmed sea cucumbers, *Apostichopus japonicus* and *Holothuria scabra,* and has been characterized by white ulcers on the oral and aboral sides of the body wall (Deng et al., 2009; Li et al., 2012; Wang et al., 2007; Zhang et al., 2018). Based on the photographic evidence provided by Zhang et al. (2018) and Deng et al. (2009), minor SUS symptoms in *A. japonicus* and the major ulcers in our *P. californicus* specimens are visually similar. Unlike in SUS reports, we did not see any indication of swelling or discolouration of the peristomes, and we did not see an initial abundance of ulcers around the mouth or cloaca (Becker et al., 2004; Delroisse et al., 2020; Wang et al., 2007). In severe cases, SUS also resembles wasting-like symptoms reported in wild *P. californicus* in Nanoose and elsewhere (CITE HEWSON) (Em Lim, *personal communication*). *A. japonicus* is similar to *P. californicus* as it is a temperate species with a similar life history strategy (CITE), but because of their geographic and morphological differences it is difficult to fully apply knowledge about SUS to skin ulceration in *P. californicus*. However, severe cases of SUS have high transmissibility and mortality (CITE), and similar to wasting outbreaks in *P. californicus* the cause remains uncertain. In SUS, experiments have identified bacterial pathogenetic origins through sampling infected tissue and injection experiments, but SUS has not been linked to any specific bacteria (Delroisse et al., 2020; Liu et al., 2010a; Zhang et al., 2018). Viruses have also been considered as a potential causal agent based on affected tissue samples, but this has not been corroborated with injection experiments (Deng et al., 2008; Hewson et al., 2020b; Liu et al., 2010a; Wang et al., 2007).

We observed major ulcers only in 2 of 18 sea cucumbers in the 22ºC treatment, and the lack of similarity between these ulcers and wasting symptoms further suggests that the wasting event in Nanoose was not triggered solely by thermal stress. Future research on sea cucumber wasting should investigate the potential for shared pathology with SUS, given the symptomatic similarities between *P. californicus* wasting and SUS in *A. japonicus*. Furthermore, studies should investigate the potential for a pathogenic causal agent, as this has been previously linked to wasting-like symptoms both in other sea cucumbers (Deng et al., 2008, 2009; Liu et al., 2010b) and sea stars (Hewson et al., 2014, 2018; Work et al., 2021). Infection experiments examining vectors of varying size fraction (parasitic, bacterial, viral) will be an essential part of this future research. Abiotic factors that may act alone or in combination with pathogenic agents to trigger wasting in *P. californicus* should also be further investigated. Studies conducted in locations where wasting has not previously occurred (e.g., Bamfield) could provide insight into whether wasting can occur in populations without pre-existing vulnerability to wasting. In the face of widespread environmental degradation and climate change (Allan et al., 2021; Marcogliese, 2008), multifactorial stressors will likely increase the severity of wasting across the Pacific Northeast Ocean.

In this study we have demonstrated that *P. californicus* stiffening behaviour degrades under extreme thermal stress, but we found no evidence that wasting is triggered by temperature stress in the absence of pathogenic agents. Determining the factors that cause and exacerbate wasting in *P. californicus* will be essential for preventing the mass mortality of these ecologically important echinoderms, as seen in sea star wasting (Hewson et al., 2014, 2018; Work et al., 2021) and SUS (Choo, 2008). Sea cucumbers, often underappreciated, provide several important ecological and economic services. As benthic detritivores, sea cucumbers break down organic matter, recycle nutrients and maintain sediment health (Purcell et al., 2016; Wheeling et al., 2007). In North America, sea cucumber harvest is a growing industry (Hannah et al., 2013; van Dam-Bates et al., 2016), that is worth 10.2 million dollars in British Columbia alone (Fisheries and Oceans Canada, 2021). Efforts to protect, manage and harvest giant California sea cucumbers in the face of global climate change and environmental degradation will require a comprehensive understanding of sea cucumber wasting.

# Acknowledgments

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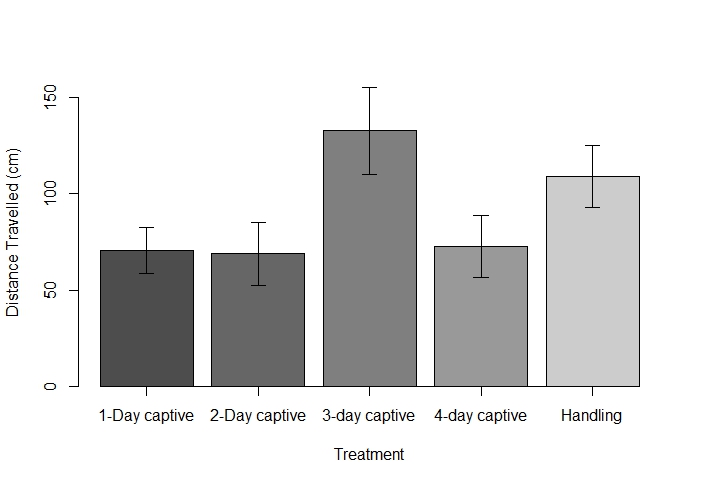
# References

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# Tables and Figures

**Table 1.** Lorem ipsum tale senserit appellantur ex pro. Rebum consequuntur ut mel, qui brute congue inermis in.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | H (AV) | A(AV) | B(AV) | C(AV) | D(AV) |
| Body counts | 21.88 | 11.13 | 11.15 | 20.72 | 12.89 |
| Dis. tr.(mm) | 1090.61 | 706.06 | 688.18 | 1331.14 | 845.56 |
| STD | 17.27 | 10.36 | 15.63 | 19.96 | 16.58 |

**Fig. 1.** Lorem ipsum tale senserit appellantur ex pro. Rebum consequuntur ut mel, qui brute congue inermis in.