

**Title:** Heat stress does not induce wasting symptoms in the giant California sea cucumber (*Apostichopus californicus*)

**Authors:** Declan Taylor<sup>1,2,\*</sup>, Jonathan James Farr<sup>2,3,\*</sup>, Em G Lim<sup>4</sup>, Jenna Laurel Fleet<sup>2,7</sup>, Sara Jayne Smith Wuitchik<sup>2,5,6,§</sup>, Daniel Michael Wuitchik<sup>2,5,§</sup>

## Affiliations

<sup>1</sup> Earth, Ocean, and Atmospheric Sciences, University of British Columbia, Vancouver, British Columbia, CAN

<sup>2</sup> Bamfield Marine Sciences Center, Bamfield, British Columbia, CAN

<sup>3</sup> Biological Sciences, University of Alberta, Edmonton, Alberta, CAN

<sup>4</sup> Biological Sciences, Simon Fraser University, Vancouver, British Columbia, CAN

<sup>5</sup> Biology, Boston University, Boston, MA, USA

<sup>6</sup> Informatics Group, Division of Science, Harvard University, Cambridge, MA, USA

<sup>7</sup> Biology, University of Winnipeg, Winnipeg, Manitoba, CAN

\* denotes co-first author

§ denotes equal co-senior author

Corresponding Authors:

Daniel M Wuitchik  
5 Cummington Mall, Boston, MA, 02215, USA  
Email address: [wuitchik@bu.edu](mailto:wuitchik@bu.edu)

Sara JS Wuitchik  
3800 Finnerty Rd, Victoria, BC, V8P 5C2, CAN  
Email address: [sara.wuitchik@gmail.com](mailto:sara.wuitchik@gmail.com)

## 27 **Abstract**

28 Oceanic heat waves have significant impacts on disease dynamics in marine  
 29 ecosystems. A severe sea cucumber wasting event occurred in Nanoose Bay, British  
 30 Columbia, Canada, following an extreme heat wave, resulting in mass mortality of  
 31 *Apostichopus californicus*. Here, we sought to determine if heat stress in isolation  
 32 could trigger wasting symptoms in *A. californicus*. We exposed sea cucumbers to i) a  
 33 simulated marine heat wave (22°C), ii) an elevated temperature treatment (17°C), or  
 34 iii) control conditions (12°C). We measured the presence of skin ulcers, mortality,  
 35 posture maintenance, antipredator defences, spawning, and organ evisceration  
 36 during the 79-hour thermal exposure, as well as 7-days post-exposure. Both the  
 37 22°C and 17°C treatments elicited stress responses where individuals exhibited a  
 38 reduced ability to maintain posture and an increase in stress spawning. The 22°C  
 39 heat wave was particularly stressful, as it was the only treatment where mortality was  
 40 observed. However, none of the treatments induced wasting symptoms as observed  
 41 in the Nanoose Bay event. This study provides evidence that sea cucumber wasting  
 42 is not triggered by heat stress in isolation, leaving the cause of the mass mortality  
 43 event observed in Nanoose unknown.

## 45 **Keywords**

46 Wasting disease, skin ulceration syndrome, sea cucumber, thermal stress,  
 47 echinoderm

## 48 **Introduction**

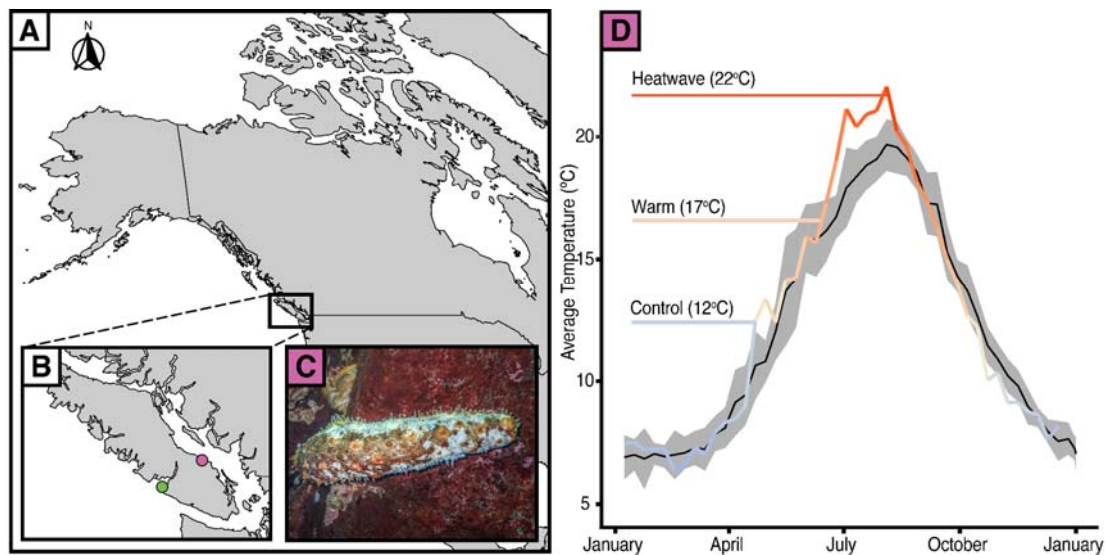
49 Climate change is increasing the intensity, duration, range, and frequency of marine  
 50 heat waves across the globe with potentially catastrophic effects on organism  
 51 fitness, ecosystems, and human economies (Frölicher, Fischer & Gruber, 2018;  
 52 Allan et al., 2021). In marine ecosystems these extreme climatic events often cause  
 53 immediate and mass mortality at all trophic levels from thermal stress, starvation,  
 54 toxicity, and hypoxia (Cavole et al., 2016; Di Lorenzo & Mantua, 2016; von Biela et  
 55 al., 2019; Suryan et al., 2021). Marine heat waves may exacerbate disease because  
 56 thermal stress can compromise the immune response, and warmer temperatures  
 57 can increase the virulence of pathogens (Harvell et al., 1999; Marcogliese, 2008;  
 58 Branco et al., 2012; Matozzo et al., 2012; Oliver et al., 2017). This temperature-  
 59 associated increase in virulence and infectivity has been associated with stimulating  
 60 and intensifying sea star wasting disease to devastating effects (Harvell et al., 1999;  
 61 Bates, Hilton & Harley, 2009; Eisenlord et al., 2016; Hewson et al., 2018; Aquino et  
 62 al., 2021). A notorious example of the impacts of a wasting disease outbreak was the  
 63 2013-current sea star wasting epidemic, which has affected more than 20 sea star

64 species in the Northeast Pacific Ocean over the last decade (Hewson et al., 2018).  
 65 Sea star wasting disease encompasses a broad set of symptoms including twisted  
 66 arms, lesions, deflation/loss of turgor, loss of arms, lack of grip strength in tube  
 67 feet, and liquefaction (Bates, Hilton & Harley, 2009; Menge et al., 2016; Hewson et  
 68 al., 2018). In most sea star species, the driving cause of wasting disease remains  
 69 largely uncertain. It has often been assumed that wasting in echinoderms is driven  
 70 by infectious agents (Hewson et al., 2014; Bucci et al., 2017; Miner et al., 2018;  
 71 Hewson, Johnson & Tibbetts, 2020). Warm temperatures have also been linked to  
 72 several mass mortality events (Bates, Hilton & Harley, 2009; Eisenlord et al., 2016;  
 73 Harvell et al., 2019). Because of the population-level impacts on ecologically  
 74 important species, understanding the pathogenic and environmental drivers of sea  
 75 star wasting remains an area of active research (Aalto et al., 2020; Aquino et al.,  
 76 2021; Hewson, 2021).

77 Wasting is not limited to sea stars and is an emerging concern across closely related  
 78 taxa. For example, sea urchins have faced a variety of disease-linked mass mortality  
 79 events and epizootics (Feehan & Scheibling, 2014). Several of these bacterial and  
 80 amoebic diseases have been linked to warm water anomalies, from climate events  
 81 or storms (Sweet, 2020). Red sea urchins (*Mesocentrotus franciscanus*) and purple  
 82 sea urchins (*Strongylocentrotus purpuratus*), have suffered from “bald sea urchin  
 83 disease” and “sea urchin wasting disease” along the Pacific coast of British  
 84 Columbia (B.C.). Pathology of these diseases includes lesions to the body wall and  
 85 a shortening or loss of spines, and both have been associated with mass mortality  
 86 events (Feehan & Scheibling, 2014; Sweet, 2020). While these diseases are  
 87 believed to have bacterial origins, similarly to that of sea star wasting, a single  
 88 bacterial strain has not been identified as the cause (Sweet, 2020). Disease-  
 89 mediated mass mortality events in urchins and sea stars have caused trophic  
 90 cascades and dramatic ecosystem shifts, highlighting the importance of  
 91 understanding these ecological phenomena. Recent evidence has emerged that  
 92 wasting may occur in sea cucumbers as well, promoting further concerns about the  
 93 impacts of marine diseases in shallow, near-shore ecosystems.

Since 2014, there have been reports of giant California sea cucumbers (*Apostichopus californicus*) with wasting symptoms similar to those of sea stars throughout their range in the northeast Pacific Ocean (Hewson, Johnson & Tibbetts, 2020). Symptoms of wasting in sea cucumbers (Echinodermata: Holothuria) include non-focal lesions and fissures across the body wall, epidermal tissue sloughing, and rapid liquefaction (Hewson, Johnson & Tibbetts, 2020; Fig. 1C). Following a series of heat waves from June 25 to July 07 2021 (Environment & Climate Change Canada, 2021; Ocean Networks Canada) a wasting event with very high mortality occurred in the Strait of Georgia (near Nanoose, B.C.), from late August to October 2021 (Fig. 1D; Lim, *pers comm*). At its peak, up to 94% of observed *A. californicus* at a single site showed evidence of wasting. On average, across all affected sites, 50% of observed *A. californicus* exhibited wasting symptoms. This highlights a potential link between thermal stress and wasting in *A. californicus*, especially given that healthy individuals were found below depths of 19 meters where the water was cooler (Lim, *pers comm*). The effect of temperature-related stressors on wasting in sea cucumbers has yet to be tested.

Here, we assess whether acute heat stress induces wasting in *A. californicus*. We also evaluate behavioural stress response signatures through stiffening, spawning, and evisceration to explore how they are affected by extreme elevated temperature. Sea cucumbers are important benthic detritivores that play an important role in organic matter decomposition and sediment aeration (Purcell, Conand & Byrne, 2016). Evidence of a connection between temperature and mass wasting events in sea stars (Bates, Hilton & Harley, 2009; Eisenlord et al., 2016; Harvell et al., 2019) necessitates a proactive assessment of whether heat stress can induce similar symptoms in sea cucumbers.



**Figure 1: Temperature anomalies and sea cucumber wasting.** A) Map of western North America with B) an inset of Vancouver Island showing the data collection locations of Bamfield Inlet (green), Nanoose Bay (purple). C) *A. californicus* in Nanoose Bay exhibiting wasting symptoms. D) 8-day rolling average seasonal sea surface temperatures in Nanoose Bay from 2010 to 2020 (black line with grey SE ribbon) and 2021 (colour gradient line), with experimental temperature treatments highlighted.

## Materials and Methods

### Collection and Acclimation

We collected *Apostichopus californicus* via SCUBA from a depth of 7-12 m in July 2021 from Scott's Bay and Bamfield Inlet in Barkley Sound, British Columbia (48°50'02"N, 125°08'45"W). The sea cucumbers were initially collected and used in an unrelated short term tagging experiment at the end of July 2021, which had no measurable impact on sea cucumber behaviour or physiology (Leedham et al., unpublished data, Cieciel, Pyper & Eckert, 2009). The sea cucumbers were then maintained in an open flow-through system at the Bamfield Marine Sciences Centre, with inflow from Bamfield Inlet for four months prior to the start of the thermal stress experiment.

### Experimental Design

To determine an average wet weight of each individual, sea cucumbers were weighed and measured twice. We monitored if sea cucumbers were defecating throughout the experiment, as cessation of defecation is considered to be indicative of a loss or atrophy of digestive organs (Swan, 1961; Fankboner & Cameron, 1985). Individuals ( $N = 56$ ) were randomly divided into one of three temperature treatments: control ( $12^{\circ}\text{C}$ ,  $n = 19$ ), warm ( $17^{\circ}\text{C}$ ,  $n = 19$ ), or heat wave ( $22^{\circ}\text{C}$ ,  $n = 18$ ). Our experimental temperatures reflect average summer temperatures and those recorded in the Strait of Georgia when wasting of *A. californicus* was observed (Fig. 1D). Specifically, the average temperature across July and August 2020 and 2021 was  $17.5^{\circ}\text{C}$  at 5m depth and  $11.9^{\circ}\text{C}$  at 20m depth (Fig. S1). In the summer 2021 heat wave, a maximum temperature at 5m depth of  $21.6^{\circ}\text{C}$  was reached on August 04, 2021 (Pawlowicz, 2017; Xuereb et al., 2018; Chen et al., 2021; Ocean Networks Canada). Our control temperature ( $12^{\circ}\text{C}$ ) was that of the Bamfield Inlet at the time of the temperature experiment and was the temperature the cucumbers were acclimated to.

We separated the sea cucumbers into 27 experimental containers (61 x 41 x 22.2 cm) with two individuals per container, aside from 2 smaller containers (33 x 45.7 x 11.4 cm) that housed one individual each. A plastic mesh divider was used to separate sea cucumbers within each container to allow for tracking individuals throughout the experiment. The containers were randomly placed into sea tables which acted as water baths allowing for different treatment temperatures. The target temperatures were achieved by having a constant flow of inlet sea water to the control sea tables ( $12^{\circ}\text{C}$ ), ambient temperature water standing in the sea table (warm;  $17^{\circ}\text{C}$ ), or water heated with two 800W aquarium heaters (heat wave;  $22^{\circ}\text{C}$ ). The  $22^{\circ}\text{C}$  treatment temperature was gradually increased over a 24-hour period in the sea table water baths (day 1; Fig. S1). The water remained at target temperatures for 79 hours, after which they were lowered back to the control temperature of  $12^{\circ}\text{C}$  over 9-hours. Days 5 through 12 were a recovery period where all sea cucumber holding containers were maintained on the flow through system. Throughout the experiment, the sea cucumbers were closely monitored. Water changes were completed as needed to maintain nitrate and ammonium levels below

0.5 ppm, and fresh sea water was heated to the appropriate treatment temperature prior to water changes. Food was withheld during the thermal stress window.

## Phenotyping Sea Cucumber Stress

We assessed sea cucumbers for wasting symptoms throughout the experiment and counted skin ulcers on days 4, 5, 6, and 12. Ulcers were classified as either minor or major based on their size and appearance. We defined minor ulcers as small lesions on the ends of spines, without signs of considerable discoloration, and where the dermis was not fully damaged (Fig. 2B). We classified major ulcers as visibly open wounds exposing white tissue beneath (Fig. 2D).

We assessed body stiffness using a 3-point ordinal scale to measure heat-stress induced deviations from normal behaviour. To do so, first we removed each individual from their bin and gently palpated them for 10 seconds to encourage them to stiffen. Then we placed them on an elevated platform to measure their ability to maintain their posture over 5 seconds. We assigned a score of 0 if the organism failed to stiffen at all (full droop), a score of 1 if it did not remain stiff for the full 5 seconds when placed on the platform (partial droop), and a score of 2 if it maintained its posture for the entire 5 seconds (no droop). Stiffness was measured on days 1-5 of the experiment (as a baseline and throughout the treatment), on day 7 (48 hours after experimental endpoint) and day 12 (7 days after experimental endpoint).

As some sea cucumbers spawned, we recorded whether there was any evidence of spawning every 12 hours. Spawning was assigned to each container rather than individual, since some individuals were co-housed ( $N_{\text{bin}} = 30$ ). We also evaluated whether specimens had eviscerated based on the presence or absence of ejected internal organs during these routine checks (Fig. 4).

## Statistical Analyses

All statistical analyses were conducted in R (v4.0.3, R Core Team, 2020). We tested the distribution of the number of ulcers using the *fitDist* function (Rigby & Stasinopoulos, 2005) and determined that minor ulcer count best fit a geometric



distribution. We modelled the maximum number of minor ulcers as a function of treatment, weight, evisceration (binary), and defecation status (binary). We included the sea table and bin as random effects. We then used backwards selection with the *stepAIC* function from GAMLSS (Rigby & Stasinopoulos, 2005) to determine the most parsimonious combination of variables that best explained the maximum number of ulcers.

We examined the covariates that affected the likelihood of mortality with a logistic regression model. Sea cucumber mortality was a binary measure, with treatment, evisceration, defecation status, initial droop score, and initial weight as explanatory variables. Terms for the final model were selected via backwards model selection using *stepAIC* (Rigby & Stasinopoulos, 2005) to produce the most parsimonious model of variation in sea cucumber mortality.

To assess changes in stiffness, we constructed full ordinal regression models using the *clmm* function (Christensen, 2019) with temperature treatment, date, and the interaction between treatment and date as explanatory variables. We restricted stiffness measurements to those taken before the heat treatment began (day 1), during the treatment (days 2-4) and immediately after the heat treatment (day 5). We included individual identity as a random effect to account for repeated measures on the same individuals over time. We also included bin and sea table as random effects to account for the paired (co-housing) and blocked (five bins per sea table) experimental design. We used backwards model selection to determine the most parsimonious models using *dredge* (Bartoń, 2020).

To assess the drivers of evisceration, we constructed a logistic regression model with treatment, weight, and defecation status as explanatory variables, along with sea table as a random effect. We determined the most parsimonious model through backwards selection. To compare the incidence of stress spawning across temperature treatments, we conducted a Dunn's Kruskal-Wallis (K-W) test using the *dunnTest* function from the FSA package (Ogle et al., 2021).

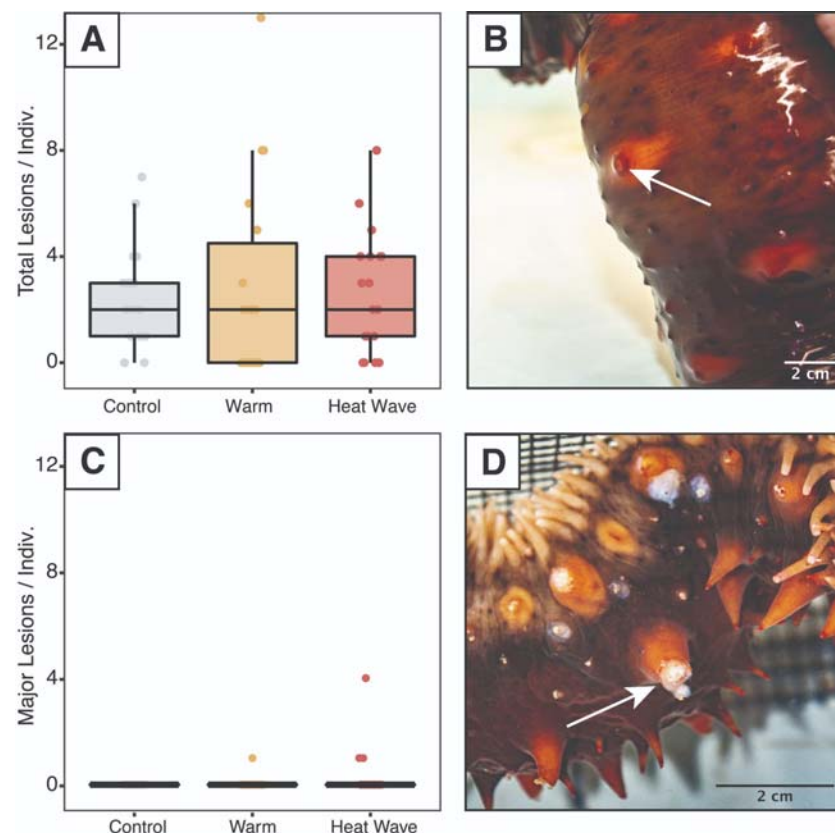


All data and annotated code for the analyses described above are publicly available at [https://github.com/declan-taylor/sea\\_cucumber\\_wasting](https://github.com/declan-taylor/sea_cucumber_wasting).

## Results

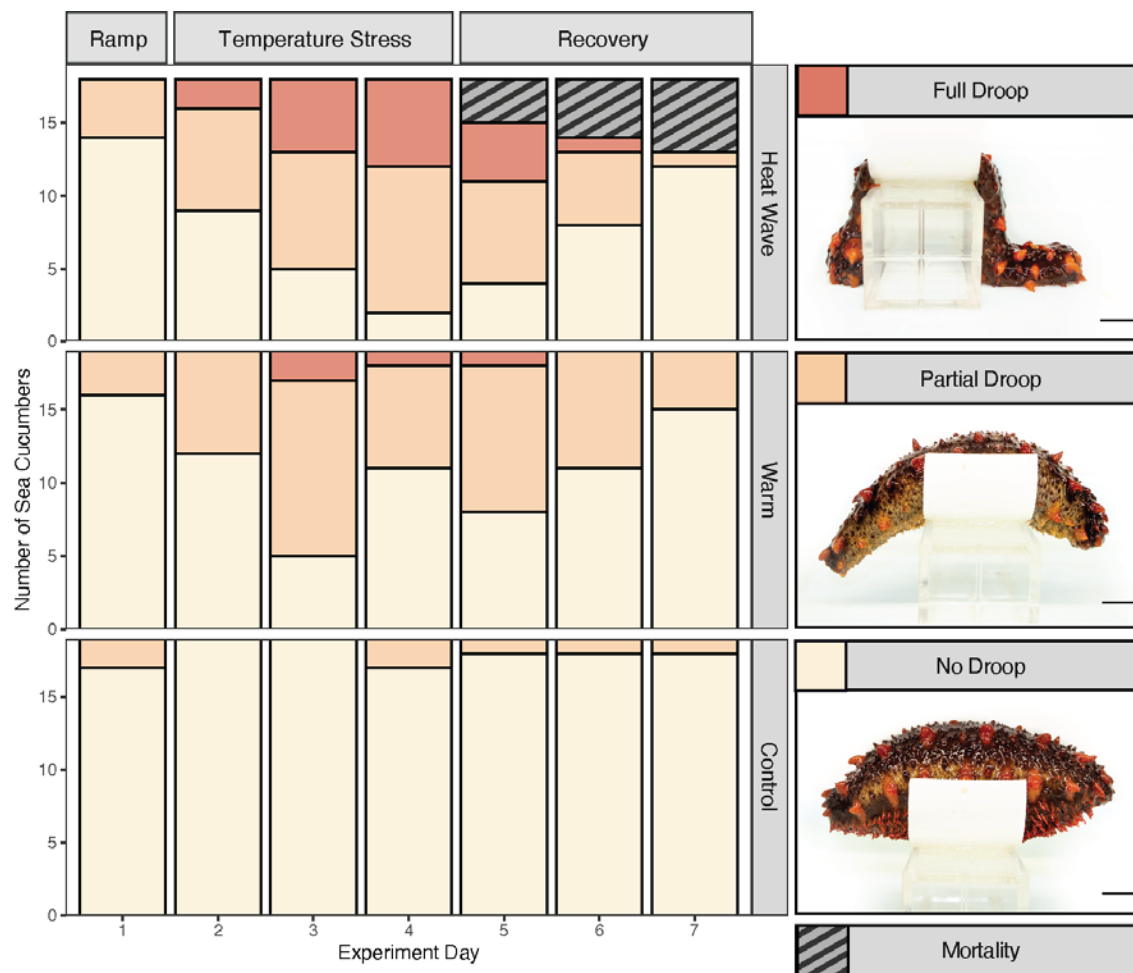
The temperature treatments varied slightly from the target temperatures during the experimental heat waves. During the 79h treatment, the mean temperature of the control treatment was 12.4°C and varied from 10.8 - 14.0°C; the mean of the warm treatment was 16.6°C and ranged from 14.8°C to 17.9°C; the mean of the heat wave treatment was 21.7°C and varied from 19.6°C to 23.3°C. Temperature treatments were significantly different from each other (K-W  $\chi^2 = 463.32$ ,  $df = 2$ ,  $p < 2.2e^{-16}$ ).

Minor skin ulcers occurred during the experiment in all three treatments ( $n_{control} = 17$ ,  $n_{warm} = 15$ ,  $n_{heat\ wave} = 10$ ; Fig. 2). Major ulcers were only observed in the warm ( $n = 1$ ) and heat wave treatments ( $n = 2$ ). These major ulcers appeared to heal throughout the recovery period and were re-classified as minor ulcers on day 12. The maximum number of minor ulcers per individual was not significantly explained by treatment, weight, evisceration, or defecation status.



**Figure 2: Skin ulcers observed in *Apostichopus californicus* across treatments.** A) Minor ulcer count, defined as small lesions on the ends of spines (B). C) Major ulcer count, defined as open wounds that expose white subdermal tissue (D).

Mortality was only observed in the 22°C heat wave treatment (Fig 3;  $n = 5$ ), while there were no mortalities observed in the control or warm treatments. Based on backwards model selection, mortality was driven by treatment and weight (Table S1). Body stiffness was lower in the warm and heatwave treatments compared to the control treatment (Fig. 3). Backwards-selected models indicated that temperature treatment and day affected stiffness (Table S2). Sea cucumbers were significantly less likely to have high stiffness scores relative to the control treatment in the warm ( $p = 1.99e^{-7}$ ) and heat wave ( $p = 2.44e^{-11}$ ) treatments. Structural stiffness values were significantly likely to be lower than day 1 on day 3 ( $p = 1.37e^{-5}$ ), day 4 ( $p = 2.50e^{-5}$ ) and day 5 ( $p = 8.66e^{-5}$ ), but not on day 2 ( $p = 0.0627$ ; Table S2).

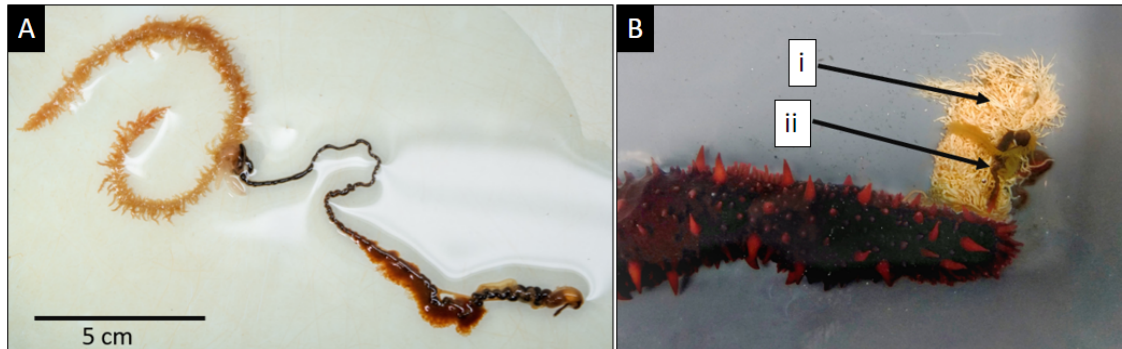


**Figure 3:** *Apostichopus californicus* stiffness and mortality at heat ramp (day 1), during temperature stress (days 2 - 4), and recovery (days 5 - 7) from the temperature treatment.

Stress spawning was observed during the temperature treatments ( $n = 11$  bins), with most of the stress spawning occurring in the 17°C warm ( $n = 5$  bins) and the 22°C heat wave ( $n = 4$  bins) treatments. However, there was no significant difference in spawning between temperature treatments (K-W  $\chi^2 = 1.94$ ,  $df = 2$ ,  $p = 0.379$ ).

Evisceration was observed across all treatments ( $n_{control} = 2$ ,  $n_{warm} = 5$ ,  $n_{heat\ wave} = 5$ ). Treatment temperature did not explain a significant amount of the variance in the occurrence of evisceration (Table S3); however, weight ( $p = 0.0383$ ) and defecation status ( $p = 0.0163$ ) were significant drivers increasing likelihood of evisceration (Table S3). Along with evisceration of internal organs (Fig.

4A), we also observed the expulsion of the respiratory tree in two individuals (Fig. 4B). Both individuals were in the 22°C heat wave treatment and mortality shortly followed the expulsion of the respiratory tree.



**Figure 4.** The eviscerated internal organs and respiratory tree of *Apostichopus californicus*. A) Eviscerated internal organs without respiratory tree expulsion compared to B) where both the i) respiratory tree and ii) internal organs were eviscerated.

## Discussion

The objective of our study was to determine if heat stress can induce wasting symptoms in *Apostichopus californicus*. While we saw minor skin ulcers at all treatment levels, and major ulcers in the warm and heat wave treatments, these are not characteristic wasting symptoms (Hewson et al., 2020; Fig. 1). Neither the minor or major ulcers that we observed matched the wasting symptoms reported in *A. californicus* in Nanoose Bay, B.C. (Lim, *pers comm*), or the isolated wasting events reported throughout the Pacific coast (Hewson, Johnson & Tibbetts, 2020). Both types of ulcers were very different from the white open lesions and fissures covering the dorsal body wall of wasting *A. californicus* in Nanoose (Fig. 1C). We also did not see any sloughing of body tissues or liquefaction, as has been anecdotally reported in previous wasting events (Schroeder, 2017; Hewson, Johnson & Tibbetts, 2020). Despite sharing some resemblance in colour, texture, and location to wasting disease symptoms, the sea cucumbers did not exhibit the full suite of symptoms that is typical from a wasting cucumber. Furthermore, unlike reports of widespread mortality resulting from wasting in wild *A. californicus*, the major ulcers in both of our specimens healed within the 7-day recovery period, and there was no evidence of these symptoms spreading to co-housed individuals. As such, there is no evidence

that the sea cucumbers in our experiment were afflicted by the fatal wasting disease that has been previously reported (Schroeder, 2017; Hewson, Johnson & Tibbetts, 2020).

While we are uncertain of the ultimate cause of the ulcers that we observed, it is possible they were associated with handling. During the experiment, specimens were handled daily and measured for their posture maintenance, increasing the frequency of potential abrasions on the epidermal surface tissue. Major ulcers may have begun as minor ulcers that were then exacerbated by the high physiological stress caused by the 22°C treatment. White skin ulcerations, like the major ulcers we observed, are a recognized condition in other Holothuroidea, described as a Skin Ulceration Disease or Skin Ulceration Syndrome (SUS; Delroisse et al., 2020). SUS has been documented in commercially farmed *Apostichopus japonicus* and *Holothuria scabra*, and has been characterized by white ulcers on both sides of the body wall (Wang et al., 2007; Deng et al., 2009; Li et al., 2012; Zhang et al., 2018). Minor SUS symptoms in commercially farmed *A. japonicus* and the major ulcers in our *A. californicus* specimens are visually similar (Deng et al., 2009; Zhang et al., 2018). Unlike the SUS symptoms reported in *A. japonicus*, 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; Wang et al., 2007; Delroisse et al., 2020). Extreme cases of SUS in farmed *A. japonicus* also bear resemblance to the wasting symptoms in wild *A. californicus*, raising further questions about the causes of skin ulceration in Holothuroidea (Delroisse et al., 2020).

Despite not seeing evidence of wasting, we saw strong evidence of whole organism and behavioural response to thermal stress. We observed five mortality events in the 22°C heat wave treatment. Though all mortalities occurred in the 22°C heat wave treatment, none of the mortalities were co-housed together, suggesting that the cause was unlikely to be a contagious disease or due to poor water quality. Therefore, we suggest that the heat wave treatment is close to the upper critical thermal tolerance of *A. californicus*, but the warm treatment temperature does not cause sufficient harm for mortality to occur. Our findings align with previous work on

larval life stages, where Ren et al. (2018) found that at 22°C, larval *A. californicus* experienced reduced survival which was not observed at 16°C or 18°C.

Beyond lethal effects, we observed reduced stiffening behaviour associated with both the 22°C heat wave and 17°C warm water treatment. A temperature-induced loss in stiffness may have implications for sea cucumber fitness under warming sea temperatures, as a limited ability to stiffen may inhibit their ability to avoid predation or maintain posture while feeding and spawning. Thermal stress may have reduced stiffness by causing muscular fatigue and relaxation (Dowd & Somero, 2013) in the circular and longitudinal-ambulacral muscles (Gao & Yang, 2015). Stiffening is also caused by protein-mediated changes in mutable collagenous tissue within the dermis of sea cucumbers (Takehana et al., 2014). Therefore, heat stress may have reduced stiffening by denaturing or decreasing the production of tensilin, a stiffening protein, or increasing the production of the de-stiffening protein softenin (Yamada et al., 2010; Takehana et al., 2014; Tamori et al., 2016).

Unlike stiffening behaviour, we did not observe any significant trends in spawning behaviour or evisceration. Of the 11 spawning events, 9 occurred in elevated-temperature treatments (warm or heat wave) and this trend, although insignificant, was expected as stress spawning has been previously reported in other sea cucumber species (Battaglione et al., 2002; Rakaj et al., 2018; Schagerström et al., 2021). We were unable to associate spawning with individual phenotypes due to the co-housing experimental design. Evisceration appeared random across treatments, potentially because *A. californicus* in all treatments may have been reacting to handling stimulation and stress during the experiment (Ding et al., 2019). However, there is evidence that biological mechanisms, weight and defecation status, partially explain the non-treatment related variation in evisceration (Table S1). Defecation status showed that *A. californicus* that were not defecating were more likely to eviscerate. This may have occurred because the energetic cost of eviscerating digestive organs would be lower for *A. californicus* that had already ceased using their organs, either because they were preparing to eviscerate (Swan, 1961) or undergoing viscera atrophy (Fankboner & Cameron, 1985). As such, when stressed by handling, *A. californicus* that had already begun seasonal reductions in digestive



function were more likely to eviscerate from stress and overstimulation. Unlike digestive tract evisceration, we do not believe that the expulsion of the respiratory tree in the 22°C heat wave treatment was linked to seasonal senescence (Fig. 4B). For both individuals, respiratory evisceration was followed by mortality, suggesting that this is an indication of extreme physiological distress from thermal exposure.

Since we observed skin ulcers under thermal stress that were not associated with wasting, other factors are likely causing recent wasting outbreaks in *A. californicus*. Like wasting, severe cases of SUS are highly transmissible and result in mortality (Delroisse et al., 2020); bacterial and viral pathogenic causal agents have been previously linked to SUS and wasting-like symptoms, both in other sea cucumbers (Deng et al., 2008, 2009; Liu et al., 2010; Zhang et al., 2018; Delroisse et al., 2020) and sea stars (Hewson et al., 2014, 2018; Work et al., 2021). A study examining a single wasting *A. californicus* specimen found a high viral load, but was unable to identify a specific pathogen causing wasting symptoms (Hewson et al., 2020). Avenues for future research on wasting diseases in *A. californicus* could address the potential for shared pathology with SUS, given the symptomatic similarities, as well as the possibility of pathogenic origins. These investigations would add valuable insight to the field of wasting diseases, given the scarcity of published information on these events in echinoderms.

Studies conducted on historically asymptomatic populations that are isolated from wasting outbreaks could provide insight into whether the causal agents of wasting are naturally present in the *A. californicus* virome and/or microbiome. The specimens used here were from an asymptomatic *A. californicus* population that is genetically distinct from the Strait of Georgia populations. However, this population still receives substantial genetic influx (Xuereb et al., 2018), so we do not expect genetic diversity to confer differential vulnerability to wasting in our specimens compared to those in the Nanoose. Biotic factors (viral, bacterial) and abiotic factors (chemical pollution, hypoxia, eutrophication) should both be investigated because widespread environmental degradation and anthropogenic climate change are shifting pathogenic dynamics globally (Marcogliese, 2008; Allan et al., 2021).



In this study, we exposed *A. californicus* to extreme thermal stress as measured by mortality, degraded stiffening behaviour, and the development of skin ulcers. Despite this, we found no evidence that wasting is induced by temperature stress alone. Therefore, the August 2021 mass wasting event in Nanoose, British Columbia, was likely not triggered solely by the anomalous heat wave. Determining the factors that cause and exacerbate wasting in *A. californicus* is essential for predicting and managing mass mortality events. Sea cucumbers are ecologically important benthic detritivores, which break down organic matter, recycle nutrients, and maintain sediment health (Wheeling, Verde & Nestler, 2007; Purcell, Conand & Byrne, 2016). Efforts to protect, manage, and sustainably harvest *A. californicus* in the face of global environmental change will require a comprehensive understanding of their stress responses, disease dynamics, and the novel threat of sea cucumber wasting.

## Acknowledgments

The species collections and experiments took place on the traditional territories of the Huu-ay-aht First Nations, a Nuuchah-nulth Nation and signatory to the Maa-nulth First Nations Final Agreement, and we are grateful for the opportunity to conduct research in protected and sacred areas. We would like to thank the Bamfield Marine Sciences Centre for the resources and support required to conduct this research; Chloe Curry, Arya Horon, and Julianne Jones for their time providing lab assistance; Payton Arthur, Mike Chung, Gabrielle Languedoc, Sammie Foley, Julianne Jones, and Carter Burtlake for feedback on early versions of the manuscript.

## Literature Cited

Aalto EA, Lafferty KD, Sokolow SH, Grewelle RE, Ben-Horin T, Boch CA, Raimondi PT, Bograd SJ, Hazen EL, Jacox MG, Micheli F, De Leo GA. 2020. Models with environmental drivers offer a plausible mechanism for the rapid spread of infectious disease outbreaks in marine organisms. *Scientific Reports* 10:5975. DOI: 10.1038/s41598-020-62118-4.

Allan RP, Arias PA, Berger S, Canadell JG, Cassou C, Chen D, Cherchi A, Connors

415 SL, Coppola E, Cruz FA, Diongue-Niang A, Doblas-Reyes FJ, Douville H,  
416 Driouech F, Edwards TL, Engelbrecht F, Eyring V, Fischer E, Flato GM,  
417 Forster P, Fox-Kemper B, Fuglestedt JS, Fyfe JC, Gillett NP, Gomis MI,  
418 Gulev SK, Gutiérrez JM, Hamdi R, Harold J, Hauser M, Hawkins E, Hewitt  
419 HT, Johansen TG, Jones C, Jones RG, Kaufman DS, Klimont Z, Kopp RE,  
420 Koven C, Krinner G, Lee J-Y, Lorenzoni I, Marotzke J, Masson-Delmotte V,  
421 Maycock TK, Meinshausen M, Monteiro PMS, Morelli A, Naik V, Notz D, Otto  
422 F, Palmer MD, Pinto I, Pirani A, Plattner G-K, Raghavan K, Ranasinghe R,  
423 Rogelj J, Rojas M, Ruane AC, Sallée J-B, Samset BH, Seneviratne SI,  
424 Sillmann J, Sörensson AA, Stephenson TS, Storelvmo T, Szopa S, Thorne  
425 PW, Trewin B, Vautard R, Vera C, Yassaa N, Zaehle S, Zhai P, Zhang X,  
426 Zickfeld K. 2021. IPCC: Climate Change 2021: The Physical Science Basis.  
427 *Cambridge University Press. In Press.:42.*

428 Aquino CA, Besemer RM, DeRito CM, Kocian J, Porter IR, Raimondi PT, Rede JE,  
429 Schiebelhut LM, Sparks JP, Wares JP, Hewson I. 2021. Evidence That  
430 Microorganisms at the Animal-Water Interface Drive Sea Star Wasting  
431 Disease. *Frontiers in Microbiology* 11.

432 Bartoń K. 2020. *MuMIn: Multi-Model Inference*.

433 Bates AE, Hilton BJ, Harley CDG. 2009. Effects of temperature, season and locality  
434 on wasting disease in the keystone predatory sea star *Pisaster ochraceus*.  
435 *Diseases of Aquatic Organisms* 86:245–251. DOI: 10.3354/dao02125.

436 Battaglione SC, Seymour JE, Ramofafia C, Lane I. 2002. Spawning induction of three  
437 tropical sea cucumbers, *Holothuria scabra*, *H. fuscogilva* and *Actinopyga*  
438 *mauritiana*. *Aquaculture* 207:29–47. DOI: 10.1016/S0044-8486(01)00725-6.

439 Becker P, Gillan D, Lanterbecq D, Jangoux M, Rasolofonirina R, Rakotovao J,

440 Eeckhaut I. 2004. The skin ulceration disease in cultivated juveniles of  
 441 *Holothuria scabra* (Holothuroidea, Echinodermata). *Aquaculture* 242:13–30.  
 442 DOI: 10.1016/j.aquaculture.2003.11.018.

443 von Biela V, Arimitsu M, Piatt J, Heflin B, Schoen SK Trowbridge J, Clawson C.  
 444 2019. Extreme reduction in nutritional value of a key forage fish during the  
 445 Pacific marine heatwave of 2014-2016. *Marine Ecology Progress Series*  
 446 613:171–182. DOI: 10.3354/meps12891.

447 Branco PC, Pressinotti LN, Borges JCS, Iunes RS, Kfoury JR, da Silva MO,  
 448 Gonzalez M, dos Santos MF, Peck LS, Cooper EL, da Silva JRMC. 2012.  
 449 Cellular biomarkers to elucidate global warming effects on Antarctic sea  
 450 urchin *Sterechinus neumayeri*. *Polar Biology* 35:221–229. DOI:  
 451 10.1007/s00300-011-1063-5.

452 Bucci C, Francoeur M, McGreal J, Smolowitz R, Zazueta-Novoa V, Wessel GM,  
 453 Gomez-Chiarri M. 2017. Sea Star Wasting Disease in *Asterias forbesi* along  
 454 the Atlantic Coast of North America. *PLOS ONE* 12:e0188523. DOI:  
 455 10.1371/journal.pone.0188523.

456 Cavole L, Demko A, Diner R, Giddings A, Koester I, Pagniello C, Paulsen M-L,  
 457 Ramirez-Valdez A, Schwenck S, Yen N, Zill M, Franks P. 2016. Biological  
 458 Impacts of the 2013–2015 Warm-Water Anomaly in the Northeast Pacific:  
 459 Winners, Losers, and the Future. *Oceanography* 29. DOI:  
 460 10.5670/oceanog.2016.32.

461 Chen Z, Shi J, Liu Q, Chen H, Li C. 2021. A Persistent and Intense Marine Heatwave  
 462 in the Northeast Pacific During 2019–2020. *Geophysical Research Letters*  
 463 48:e2021GL093239. DOI: 10.1029/2021GL093239.

464 Christensen RHB. 2019. ordinal—Regression Models for Ordinal Data.

- 465 Ciecziel K, Pyper B, Eckert G. 2009. Tag Retention and Effects of Tagging on  
466 Movement of the Giant Red Sea Cucumber *Parastichopus californicus*. *North*  
467 *American Journal of Fisheries Management* - NORTH AM J FISH MANAGE  
468 29:288–294. DOI: 10.1577/M07-194.1.
- 469 Delroisse J, Van Wayneberghe K, Flammang P, Gillan D, Gerbaux P, Opina N,  
470 Todinanahary GGB, Eeckhaut I. 2020. Epidemiology of a SKin Ulceration  
471 Disease (SKUD) in the sea cucumber *Holothuria scabra* with a review on the  
472 SKUDs in Holothuroidea (Echinodermata). *Scientific Reports* 10:22150. DOI:  
473 10.1038/s41598-020-78876-0.
- 474 Deng H, He C, Zhou Z, Liu C, Tan K, Wang N, Jiang B, Gao X, Liu W. 2009.  
475 Isolation and pathogenicity of pathogens from skin ulceration disease and  
476 viscera ejection syndrome of the sea cucumber *Apostichopus japonicus*.  
477 *Aquaculture* 287:18–27. DOI: 10.1016/j.aquaculture.2008.10.015.
- 478 Deng H, Zhou Z, Wang N, Liu C. 2008. The syndrome of sea cucumber  
479 (*Apostichopus japonicus*) infected by virus and bacteria. *Virologica Sinica*  
480 23:63–67. DOI: 10.1007/s12250-008-2863-9.
- 481 Di Lorenzo E, Mantua N. 2016. Multi-year persistence of the 2014/15 North Pacific  
482 marine heatwave. *Nature Climate Change* 6:1042–1047. DOI:  
483 10.1038/nclimate3082.
- 484 Ding K, Zhang L, Sun L, Lin C, Feng Q, Zhang S, Yang H, Brinkman R, Lin G,  
485 Huang Z. 2019. Transcriptome analysis provides insights into the molecular  
486 mechanisms responsible for evisceration behavior in the sea cucumber  
487 *Apostichopus japonicus*. *Comparative Biochemistry and Physiology Part D:*  
488 *Genomics and Proteomics* 30:143–157. DOI: 10.1016/j.cbd.2019.02.008.
- 489 Dowd WW, Somero GN. 2013. Behavior and survival of *Mytilus* congeners following

490 episodes of elevated body temperature in air and seawater. *Journal of*  
491 *Experimental Biology* 216:502–514. DOI: 10.1242/jeb.076620.

492 Eisenlord ME, Groner ML, Yoshioka RM, Elliott J, Maynard J, Fradkin S, Turner M,  
493 Pyne K, Rivlin N, van Hooideonk R, Harvell CD. 2016. Ochre star mortality  
494 during the 2014 wasting disease epizootic: role of population size structure  
495 and temperature. *Philosophical Transactions of the Royal Society B:*  
496 *Biological Sciences* 371:20150212. DOI: 10.1098/rstb.2015.0212.

497 Environment and Climate Change Canada warns western Canadians about  
498 dangerous record-high temperatures. 2021.

499 Fankboner PV, Cameron JL. 1985. Seasonal atrophy of the visceral organs in a sea  
500 cucumber. *Canadian Journal of Zoology* 63:2888–2892. DOI: 10.1139/z85-  
501 432.

502 Feehan CJ, Scheibling RE. 2014. Effects of sea urchin disease on coastal marine  
503 ecosystems. *Marine Biology* 161:1467–1485. DOI: 10.1007/s00227-014-  
504 2452-4.

505 Frölicher TL, Fischer EM, Gruber N. 2018. Marine heatwaves under global warming.  
506 *Nature* 560:360–364. DOI: 10.1038/s41586-018-0383-9.

507 Gao F, Yang H. 2015. Chapter 4 - Anatomy. In: Yang H, Hamel J-F, Mercier A eds.  
508 *Developments in Aquaculture and Fisheries Science*. Elsevier, 53–76. DOI:  
509 10.1016/B978-0-12-799953-1.00004-0.

510 Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DJ, Hofmann  
511 EE, Lipp EK, Osterhaus ADME, Overstreet RM, Porter JW, Smith GW, Vasta  
512 GR. 1999. Emerging Marine Diseases--Climate Links and Anthropogenic  
513 Factors. *Science* 285:1505–1510. DOI: 10.1126/science.285.5433.1505.

514 Harvell CD, Montecino-Latorre D, Caldwell JM, Burt JM, Bosley K, Keller A, Heron

515 SF, Salomon AK, Lee L, Pontier O, Pattengill-Semmens C, Gaydos JK. 2019.  
516 Disease epidemic and a marine heat wave are associated with the  
517 continental-scale collapse of a pivotal predator (*Pycnopodia helianthoides*).  
518 *Science Advances* 5:eaau7042. DOI: 10.1126/sciadv.aau7042.

519 Hewson I. 2021. Microbial respiration in the asteroid diffusive boundary layer  
520 influenced sea star wasting disease during the 2013-2014 northeast Pacific  
521 Ocean mass mortality event. *Marine Ecology Progress Series* 668:231–237.  
522 DOI: 10.3354/meps13735.

523 Hewson I, Bistolas KSI, Quijano Cardé EM, Button JB, Foster PJ, Flanzenbaum JM,  
524 Kocian J, Lewis CK. 2018. Investigating the Complex Association Between  
525 Viral Ecology, Environment, and Northeast Pacific Sea Star Wasting.  
526 *Frontiers in Marine Science* 5.

527 Hewson I, Button JB, Gudenkauf BM, Miner B, Newton AL, Gaydos JK, Wynne J,  
528 Groves CL, Hendler G, Murray M, Fradkin S, Breitbart M, Fahsbender E,  
529 Lafferty KD, Kilpatrick AM, Miner CM, Raimondi P, Lahner L, Friedman CS,  
530 Daniels S, Haulena M, Marliave J, Burge CA, Eisenlord ME, Harvell CD.  
531 2014. Densovirus associated with sea-star wasting disease and mass  
532 mortality. *Proceedings of the National Academy of Sciences* 111:17278–  
533 17283. DOI: 10.1073/pnas.1416625111.

534 Hewson I, Johnson MR, Tibbetts IR. 2020. An Unconventional Flavivirus and Other  
535 RNA Viruses in the Sea Cucumber (Holothuroidea; Echinodermata) Virome.  
536 *Viruses* 12:1057. DOI: 10.3390/v12091057.

537 Li C, Feng W, Qiu L, Xia C, Su X, Jin C, Zhou T, Zeng Y, Li T. 2012.  
538 Characterization of skin ulceration syndrome associated microRNAs in sea  
539 cucumber *Apostichopus japonicus* by deep sequencing. *Fish & Shellfish*

540 *Immunology* 33:436–441. DOI: 10.1016/j.fsi.2012.04.013.

541 Liu H, Zheng F, Sun X, Hong X, Dong S, Wang B, Tang X, Wang Y. 2010.

542 Identification of the pathogens associated with skin ulceration and peristome

543 tumescence in cultured sea cucumbers *Apostichopus japonicus* (Selenka).

544 *Journal of Invertebrate Pathology* 105:236–242. DOI:

545 10.1016/j.jip.2010.05.016.

546 Marcogliese D. 2008. The impact of climate change on the parasites and infectious

547 diseases of aquatic animals. 27:467. DOI: 10.20506/rst.27.2.1820.

548 Matozzo V, Chinellato A, Munari M, Finos L, Bressan M, Marin MG. 2012. First

549 Evidence of Immunomodulation in Bivalves under Seawater Acidification and

550 Increased Temperature. *PLoS ONE* 7:e33820. DOI:

551 10.1371/journal.pone.0033820.

552 Menge BA, Cerny-Chipman EB, Johnson A, Sullivan J, Gravem S, Chan F. 2016.

553 Sea Star Wasting Disease in the Keystone Predator *Pisaster ochraceus* in

554 Oregon: Insights into Differential Population Impacts, Recovery, Predation

555 Rate, and Temperature Effects from Long-Term Research. *PLOS ONE*

556 11:e0153994. DOI: 10.1371/journal.pone.0153994.

557 Miner CM, Burnaford JL, Ambrose RF, Antrim L, Bohlmann H, Blanchette CA, Engle

558 JM, Fradkin SC, Gaddam R, Harley CDG, Miner BG, Murray SN, Smith JR,

559 Whitaker SG, Raimondi PT. 2018. Large-scale impacts of sea star wasting

560 disease (SSWD) on intertidal sea stars and implications for recovery. *PLOS*

561 *ONE* 13:e0192870. DOI: 10.1371/journal.pone.0192870.

562 Ocean Networks Canada. Temperature data from 14 Jun 2019 - 1 Jan 2022.

563 Ogle DH, Doll JC, Wheeler P, Dinno A. 2021. *FSA: Fisheries Stock Analysis*.

564 Oliver ECJ, Benthuyssen JA, Bindoff NL, Hobday AJ, Holbrook NJ, Mundy CN,



565 Perkins-Kirkpatrick SE. 2017. The unprecedented 2015/16 Tasman Sea  
566 marine heatwave. *Nature Communications* 8:16101. DOI:  
567 10.1038/ncomms16101.

568 Pawlowicz R. 2017. Seasonal Cycles, Hypoxia, and Renewal in a Coastal Fjord  
569 (Barkley Sound, British Columbia). *Atmosphere-Ocean* 55:264–283. DOI:  
570 10.1080/07055900.2017.1374240.

571 Purcell SW, Conand C, Byrne SU& M. 2016. Ecological Roles of Exploited Sea  
572 Cucumbers. In: *Oceanography and Marine Biology*. CRC Press,.

573 R Core Team. 2020. *R: A Language and Environment for Statistical Computing*.  
574 Vienna, Austria: R Foundation for Statistical Computing.

575 Rakaj A, Fianchini A, Boncagni P, Lovatelli A, Scardi M, Cataudella S. 2018.  
576 Spawning and rearing of *Holothuria tubulosa*: A new candidate for  
577 aquaculture in the Mediterranean region. *Aquaculture Research* 49:557–568.  
578 DOI: 10.1111/are.13487.

579 Ren Y, Liu W, Pearce CM. 2018. Effects of stocking density, ration and temperature  
580 on growth, survival and metamorphosis of auricularia larvae of the California  
581 sea cucumber, *Parastichopus californicus*. *Aquaculture Research* 49:517–  
582 525. DOI: 10.1111/are.13482.

583 Rigby RA, Stasinopoulos DM. 2005. Generalized additive models for location, scale  
584 and shape. *Journal of the Royal Statistical Society: Series C (Applied*  
585 *Statistics)* 54:507–554. DOI: 10.1111/j.1467-9876.2005.00510.x.

586 Schagerström E, Christophersen G, Sunde J, Bakke S, Matusse NR, Dupont S,  
587 Sundell KS. 2021. Controlled spawning and rearing of the sea cucumber,  
588 *Parastichopus tremulus*. *Journal of the World Aquaculture Society* 53:224–  
589 240. DOI: 10.1111/jwas.12816.

- 590 Schroeder L. 2017. Wasting-like lesions occurring on California sea cucumbers. *The*  
591 *Dredgings* 57:3.
- 592 Suryan RM, Arimitsu ML, Coletti HA, Hopcroft RR, Lindeberg MR, Barbeaux SJ,  
593 Batten SD, Burt WJ, Bishop MA, Bodkin JL, Brenner R, Campbell RW,  
594 Cushing DA, Danielson SL, Dorn MW, Drummond B, Esler D, Gelatt T,  
595 Hanselman DH, Hatch SA, Haught S, Holderied K, Iken K, Irons DB, Kettle  
596 AB, Kimmel DG, Konar B, Kuletz KJ, Laurel BJ, Maniscalco JM, Matkin C,  
597 McKinstry CAE, Monson DH, Moran JR, Olsen D, Palsson WA, Pegau WS,  
598 Piatt JF, Rogers LA, Rojek NA, Schaefer A, Spies IB, Straley JM, Strom SL,  
599 Sweeney KL, Szymkowiak M, Weitzman BP, Yasumiishi EM, Zador SG. 2021.  
600 Ecosystem response persists after a prolonged marine heatwave. *Scientific*  
601 *Reports* 11:6235. DOI: 10.1038/s41598-021-83818-5.
- 602 Swan EF. 1961. Seasonal Evisceration in the Sea Cucumber, *Parastichopus*  
603 *californicus* (Stimpson). *Science* 133:1078–1079. DOI:  
604 10.1126/science.133.3458.1078.
- 605 Sweet M. 2020. Sea urchin diseases: Effects from individuals to ecosystems. In:  
606 *Developments in Aquaculture and Fisheries Science*. Elsevier, 219–226. DOI:  
607 10.1016/B978-0-12-819570-3.00012-3.
- 608 Takehana Y, Yamada A, Tamori M, Motokawa T. 2014. Softenin, a Novel Protein  
609 That Softens the Connective Tissue of Sea Cucumbers through Inhibiting  
610 Interaction between Collagen Fibrils. *PLOS ONE* 9:e85644. DOI:  
611 10.1371/journal.pone.0085644.
- 612 Tamori M, Ishida K, Matsuura E, Ogasawara K, Hanasaka T, Takehana Y,  
613 Motokawa T, Osawa T. 2016. Ultrastructural Changes Associated with  
614 Reversible Stiffening in Catch Connective Tissue of Sea Cucumbers. *PLOS*

615 ONE 11:e0155673. DOI: 10.1371/journal.pone.0155673.

616 Wang P, Chang Y, Yu J, Li C, Xu G. 2007. Acute peristome edema disease in  
617 juvenile and adult sea cucumbers *Apostichopus japonicus* (Selenka) reared in  
618 North China. *Journal of Invertebrate Pathology* 96:11–17. DOI:  
619 10.1016/j.jip.2007.03.001.

620 Wheeling RJ, Verde EA, Nestler JR. 2007. Diel cycles of activity, metabolism, and  
621 ammonium concentration in tropical holothurians. *Marine Biology* 152:297–  
622 305. DOI: 10.1007/s00227-007-0683-3.

623 Work TM, Weatherby TM, DeRito CM, Besemer RM, Hewson I. 2021. Sea star  
624 wasting disease pathology in *Pisaster ochraceus* shows a basal-to-surface  
625 process affecting color phenotypes differently. *Diseases of Aquatic Organisms*  
626 145:21–33. DOI: 10.3354/dao03598.

627 Xuereb A, Benestan L, Normandeau É, Daigle RM, Curtis JMR, Bernatchez L, Fortin  
628 M-J. 2018. Asymmetric oceanographic processes mediate connectivity and  
629 population genetic structure, as revealed by RADseq, in a highly dispersive  
630 marine invertebrate ( *Parastichopus californicus* ). *Molecular Ecology*  
631 27:2347–2364. DOI: 10.1111/mec.14589.

632 Yamada A, Tamori M, Iketani T, Oiwa K, Motokawa T. 2010. A novel stiffening factor  
633 inducing the stiffest state of holothurian catch connective tissue. *Journal of*  
634 *Experimental Biology* 213:3416–3422. DOI: 10.1242/jeb.044149.

635 Zhang Z, Xing R, Lv Z, Shao Y, Zhang W, Zhao X, Li C. 2018. Analysis of gut  
636 microbiota revealed *Lactococcus garviae* could be an indicative of skin  
637 ulceration syndrome in farmed sea cucumber *Apostichopus japonicus*. *Fish &*  
638 *Shellfish Immunology* 80:148–154. DOI: 10.1016/j.fsi.2018.06.001.

639

640

## Supplementary Information

Table S1 . Results from logistic regression model examining which variables best predicted sea cucumber mortality

Variable	Coefficient	Std. Error	T	p
(intercept)	-21.146	170.49	-0.124	0.902
Treatment: 17C	0.0363	246.59	0	0.999
Treatment: 22C	14.72	170.42	0.086	0.932
Weight	0.0103	0.00553	-1.86	0.0678

\*p < 0.05, \*\*p < 0.01

Table S2. Results of ordinal regression model examining the effect of treatment and experiment day on sea cucumber stiffness.

Variable	Coefficient	Std. Error	T	p
Treatment: 17C	-2.99	0.575	-5.20	1.99e-07**
Treatment: 22C	-4.05	0.606	-6.68	2.44e-11**
Day 2	-0.976	0.524	-1.86	0.0627
Day 3	-2.30	0.528	-4.35	1.37e-05**
Day 4	-2.22	0.527	-4.21	2.50e-05**
Day 5	-2.12	0.541	-3.93	8.66e-05**

\*p < 0.05, \*\*p < 0.01

**Table S3.** Results from a backwards-selected logistic regression model examining the effect of evisceration as a function of defecation status and weight. Asterisks indicate significant effects.

	Coefficient	Std. Error	t-value	p
(Intercept)	1.713	1.128	1.52	0.135
Defecating (yes)	-2.78	1.12	-2.48	0.0163 *
Weight	-0.00431	0.00203	-2.12	0.0383 *

\*p < 0.05, \*\*p < 0.01