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

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Abstract

Oceanic heatwaves have significant impacts on disease dynamics in marine ecosystems. Following an extreme heatwave in Nanoose Bay, British Columbia, Canada, a severe sea cucumber wasting event occurred that resulted in the mass mortality of *Apostichopus californicus*. Here, we sought to determine if heat stress in isolation could trigger wasting symptoms in *A. californicus*. We exposed sea cucumbers to i) a simulated marine heatwave (22ºC), ii) an elevated temperature treatment (17ºC), or iii) control conditions (12ºC). We measured the presence of skin lesions, mortality, posture maintenance, antipredator defences, spawning, and organ evisceration during the 79-hour thermal exposure, as well as 7-days post-exposure. Both the 22°C and 17°C treatments elicited stress responses where individuals exhibited a reduced ability to maintain posture and an increase in stress spawning. The 22ºC

41 heatwave was particularly stressful, as it was the only treatment where mortality was observed.
42 However, none of the treatments induced wasting symptoms as observed in the Nanoose Bay
43 event. This study provides evidence that sea cucumber wasting may not be triggered by heat
44 stress in isolation, leaving the cause of the mass mortality event observed in Nanoose unknown.
45

46 **Introduction**

47 Climate change is increasing the intensity, duration, range, and frequency of marine heatwaves
48 across the globe with potentially catastrophic effects on organism fitness, ecosystems, and
49 human economies (Frölicher, Fischer & Gruber, 2018; Allan et al., 2021). In marine ecosystems
50 these extreme climatic events often cause immediate and mass mortality at all trophic levels from
51 thermal stress, starvation, toxicity, and hypoxia (Cavole et al., 2016; Di Lorenzo & Mantua,
52 2016; von Biela et al., 2019; Suryan et al., 2021). Marine heatwaves may exacerbate disease
53 because thermal stress can compromise the immune response, and warmer temperatures can
54 increase the virulence of pathogens (Harvell et al., 1999; Marcogliese, 2008; Branco et al., 2012;
55 Matozzo et al., 2012; Oliver et al., 2017). This temperature-associated increase in virulence and
56 infectivity has been associated with stimulating and intensifying sea star wasting disease to
57 devastating effects (Harvell et al., 1999; Bates, Hilton & Harley, 2009; Eisenlord et al., 2016;
58 Hewson et al., 2018; Aquino et al., 2021). A notorious example of the impacts of a wasting
59 disease outbreak was the 2013-current sea star wasting epidemic, which has affected more than
60 20 sea star species in the Northeast Pacific Ocean over the last decade (Hewson et al., 2018). Sea
61 star wasting disease encompasses a broad set of symptoms including twisted arms, lesions,
62 deflation/loss of turgor, loss of arms, lack of grip strength in tube feet, and liquefaction (Bates,
63 Hilton & Harley, 2009; Menge et al., 2016; Hewson et al., 2018). In most sea star species, the
64 driving cause of wasting disease remains largely uncertain. It has often been assumed that
65 wasting in echinoderms is driven by infectious agents (Hewson et al., 2014; Bucci et al., 2017;
66 Miner et al., 2018; Hewson, Johnson & Tibbetts, 2020). Warm temperatures have also been
67 linked to several mass mortality events (Bates, Hilton & Harley, 2009; Eisenlord et al., 2016;
68 Harvell et al., 2019). Because of the population-level impacts on ecologically important species,
69 understanding the pathogenic and environmental drivers of sea star wasting remains an area of
70 active research (Aalto et al., 2020; Aquino et al., 2021; Hewson, 2021).
71

72   Wasting is not limited to sea stars and is an emerging concern across closely related taxa. For

73   example, sea urchins have faced a variety of disease-linked mass mortality events and epizootics

74   (Feehan & Scheibling, 2014). Several of these bacterial and amoebic diseases have been linked

75   to warm water anomalies, from climate events or storms (Sweet, 2020). Red sea urchins

76   (*Mesocentrotus franciscanus*) and purple sea urchins (*Strongylocentrotus purpuratus*) have

77   suffered from "bald sea urchin disease" and "sea urchin wasting disease" along the Pacific coast

78   of British Columbia (B.C.). Pathology of these diseases includes lesions to the body wall and a

79   shortening or loss of spines, and both have been associated with mass mortality events (Feehan

80   & Scheibling, 2014; Sweet, 2020). While these diseases are believed to have bacterial origins, a

81   single bacterial strain has not been identified as the cause (Sweet, 2020). Disease-mediated mass

82   mortality events in urchins and sea stars have caused trophic cascades and dramatic ecosystem

83   shifts, highlighting the importance of understanding these ecological phenomena (Schultz,

84   Cloutier & Côté, 2016; Suryan et al., 2021; Traiger et al., 2022). Recent evidence has emerged

85   that wasting may occur in sea cucumbers as well, promoting further concerns about the impacts

86   of marine diseases in shallow, near-shore ecosystems.

87

88   Since 2014, there have been reports of giant California sea cucumbers (*Apostichopus*

89   *californicus*) with wasting symptoms similar to those of sea stars throughout their range in the

90   northeast Pacific Ocean (Hewson, Johnson & Tibbetts, 2020). Sea cucumber wasting disease is

91   poorly defined, with symptoms typically including non-focal lesions and fissures across the body

92   wall, epidermal tissue sloughing, and rapid liquefaction (Hewson, Johnson & Tibbetts, 2020;

93   Fig. 1C). Much like in sea stars, there are numerous potential causal agents that could elicit this

94   suite of symptoms. Heat stress may be particularly relevant given that after a series of heatwaves

95   from June 25 to July 07, 2021 (Environment & Climate Change Canada, 2021; Ocean Networks

96   Canada) a wasting event with very high mortality occurred in the Strait of Georgia near Nanoose,

97   B.C., from late August to October 2021 (Fig. 1D; Lim, *pers comm*). At its peak, up to 94% of

98   observed *A. californicus* at a single site showed evidence of wasting. On average, across all

99   affected sites (n = 7), 50% of observed *A. californicus* exhibited wasting symptoms. The six to

100 ten week delay between peak near-surface sea temperatures and widespread mortality may be

101 indicative of a potential link between thermal stress and wasting in *A. californicus*, perhaps

102 mediated by the progression of disease symptoms and immune failure. Temperature is an

103   especially compelling mechanism considering that healthy individuals were abundant below

104   depths of 19 metres where the water was cooler (Lim, *pers comm*). The effect of temperature-

105   related stressors on wasting in sea cucumbers has yet to be tested.

106

107   Here, we assess whether acute heat stress rapidly induces wasting in *A. californicus*. We also

108   evaluate behavioural stress response signatures through stiffening, spawning, and evisceration to

109   explore how they are affected by extreme elevated temperature. Sea cucumbers are important

110   benthic detritivores that play an important role in organic matter decomposition and sediment

111   aeration (Purcell, Conand & Byrne, 2016). Evidence of a connection between temperature and

112   mass wasting events in sea stars (Bates, Hilton & Harley, 2009; Eisenlord et al., 2016; Harvell et

113   al., 2019) necessitates a proactive assessment of whether heat stress can induce similar

114   symptoms in sea cucumbers.

115

116   **Materials & Methods**

117

118   Collection and Acclimation

119   We collected *Apostichopus californicus* via SCUBA from a depth of 7-12 m in July 2021 from

120   Scott's Bay and Bamfield Inlet in Barkley Sound, British Columbia (48°50'02"N, 125°08'45"W).

121   The sea cucumbers were initially collected and used in an unrelated short term tagging

122   experiment comparing T-bar and coded wire tags at the end of July 2021, after which they were

123   all tagged with coded wire tags. Tagging had no measurable impact on sea cucumber behaviour

124   or physiology in the short or long term (Leedham et al., unpublished data; Cieciel, Pyper &

125   Eckert, 2009). After this experiment sea cucumbers were then maintained in an open flow-

126   through system at the Bamfield Marine Sciences Centre, with inflow from Bamfield Inlet for

127   four months prior to the start of the thermal stress experiment.

128

129   Experimental Design

130   To determine an average wet weight of each individual, sea cucumbers were weighed twice at

131   the beginning of the experiment. Individuals ($N = 56$) were randomly divided into one of three

132   temperature treatments: control (12ºC, $n = 19$), warm (17ºC, $n = 19$), or heatwave (22ºC, $n = 18$).

133   Our experimental temperatures reflect average summer temperatures and those recorded in the

134 Strait of Georgia when wasting of *A. californicus* was observed (Fig. 1D). Specifically, the

135 average temperature across July and August 2020 and 2021 was 17.5ºC at 5 m depth and 11.9ºC

136 at 20 m depth (Fig. S1). In the summer 2021 heatwave, a maximum temperature at 5 m depth of

137 21.6ºC was reached on August 04, 2021 (Pawlowicz, 2017; Xuereb et al., 2018; Chen et al.,

138 2021; Ocean Networks Canada). Our control temperature (12ºC) was that of the Bamfield Inlet at

139 the time of the temperature experiment and was the temperature the cucumbers were acclimated

140 to.

141

142 We separated the sea cucumbers into 27 isolated experimental containers (61 x 41 x 22.2 cm)

143 with two individuals per container, aside from 2 smaller containers (33 x 45.7 x 11.4 cm) that

144 housed one individual each. A plastic mesh divider was used to separate sea

145 cucumbers within each container to allow for tracking individuals throughout the experiment.

146 The containers were randomly placed into sea tables which acted as water baths. The target water

147 bath temperatures were achieved by having a constant flow of cooler inlet sea water to act as the

148 control (12ºC), allowed to reach ambient room temperature for the warm treatment (17ºC), or

149 heated using two 800W aquarium heaters for the heatwave treatment (22ºC). The 22ºC treatment

150 temperature was gradually increased over a 24-hour period in the sea table water baths (day 1;

151 Fig. S2). During the temperature treatment period, there was no seawater flow to the

152 experimental containers in which sea cucumbers were housed for any of the temperature

153 treatments. To prevent stagnation and maintain high oxygen levels, all containers were

154 constantly aerated with airstones. The water remained at target temperatures for 79 hours, after

155 which they were lowered back to the control temperature of 12ºC over 9-hours. Days 5 through

156 12 were a recovery period where all sea cucumber holding containers were maintained on the

157 flow through system. Throughout the experiment, the sea cucumbers were closely monitored. To

158 avoid water fouling, water changes were completed in individual containers as needed to

159 maintain nitrate and ammonium levels below 0.5 ppm, and fresh sea water was heated to the

160 appropriate treatment temperature prior to water changes. Water quality was measured using

161 API® saltwater ammonium and nitrate kits, and food was withheld during the thermal stress

162 window. We monitored if sea cucumbers were defecating three times each day throughout the

163 experiment, based on the presence of fecal pellets, as cessation of defecation is considered to be

164 indicative of a loss or atrophy of digestive organs (Swan, 1961; Fankboner & Cameron, 1985).

165

166   Phenotyping Sea Cucumber Stress

167   We assessed sea cucumbers for wasting symptoms throughout the experiment and counted skin

168   lesions on days 5, 6, 7, and 12. Epidermal damage was classified as either minor lesions or major

169   ulcers based on their size and appearance. We defined minor lesions as regions of epidermal

170   damage at the spine tips without signs of considerable discoloration, where the dermis was not

171   damaged across its full thickness (Fig. 2B). We classified major ulcers as epidermal ulceration

172   exposing underlying white mutable connective tissues beneath (Fig. 2D).

173

174   We assessed body stiffness using a 3-point ordinal scale to measure heat-stress induced

175   deviations from normal behaviour. To do so, first we removed each individual from their bin and

176   gently palpated them for 10 seconds to encourage them to stiffen. Then we placed them on an

177   elevated platform to measure their ability to maintain their posture over 5 seconds. The stiffness

178   testing platform was constructed by cutting a piece of plastic pipe into an 8 cm long, 8 cm in

179   diameter, and 4 cm tall section, which we glued onto an 8 cm tall block. We assigned a score of

180   0 if the organism failed to stiffen at all (full droop), a score of 1 if it did not remain stiff for the

181   full 5 seconds when placed on the platform (partial droop), and a score of 2 if it maintained its

182   posture for the entire 5 seconds (no droop). Stiffness was measured on days 1-5 of the

183   experiment (as a baseline and throughout the treatment), on day 7 (48 hours after experimental

184   endpoint) and day 12 (7 days after experimental endpoint). Frequency of handling individuals

185   occurred equally across treatments.

186

187   As some sea cucumbers spawned, we recorded whether there was any evidence of spawning

188   every 12 hours. Spawning was assigned to each container rather than individual, since some

189   individuals were co-housed ($N_{bin} = 30$). We also evaluated whether specimens had eviscerated

190   based on the presence or absence of ejected internal organs during these routine checks.

191

192   Statistical Analyses

193   All statistical analyses were conducted in R (v4.0.3, R Core Team, 2020). We tested the

194   distribution of the number of lesions using the *fitDist* function (Rigby & Stasinopoulos, 2005)

195   and determined that minor lesion count best fit a geometric distribution. We modelled the

196   maximum number of minor lesions as a function of treatment, weight, evisceration (binary), and
197   defecation status (binary). We included the sea table and bin as random effects. We then used
198   backwards selection with the *stepGAIC* function from GAMLSS (Rigby & Stasinopoulos, 2005)
199   to determine the most parsimonious combination of variables that best explained the maximum
200   number of lesions.
201
202   We examined the covariates that affected the likelihood of mortality with a logistic regression
203   model. Sea cucumber mortality was a binary measure, with treatment, evisceration, defecation
204   status, initial droop score, and initial weight as explanatory variables. Terms for the final model
205   were selected via backwards model selection using *stepGAIC* (Rigby & Stasinopoulos, 2005) to
206   produce the most parsimonious model of variation in sea cucumber mortality.
207
208   To assess changes in stiffness, we constructed full ordinal regression models using the *clmm*
209   function (Christensen, 2019) with temperature treatment, date, and the interaction between
210   treatment and date as explanatory variables. We restricted stiffness measurements to those taken
211   before the heat treatment began (day 1), during the treatment (days 2-4) and immediately after
212   the heat treatment (day 5). We included individual identity as a random effect to account for
213   repeated measures on the same individuals over time. We also included bin and sea table as
214   random effects to account for the paired (co-housing) and blocked (five bins per sea table)
215   experimental design. We used backwards model selection to determine the most parsimonious
216   models using *dredge* (Bartoń, 2020).
217
218   To assess the drivers of evisceration, we constructed a logistic regression model with treatment,
219   weight, and defecation status as explanatory variables, along with sea table as a random effect.
220   We determined the most parsimonious model through backwards selection. To compare the
221   incidence of stress spawning across temperature treatments, we conducted a Dunn's Kruskal-
222   Wallace (K-W) test using the *dunnTest* function from the FSA package (Ogle et al., 2021).
223
224   All data and annotated code for the analyses described above are publicly available at
225   https://github.com/declan-taylor/sea_cucumber_wasting (DOI: 10.5281/zenodo.7259325) .
226

227   **Results**

228

229   The temperature treatments varied slightly from the target temperatures during the experimental

230   heatwaves. During the 79h treatment, the mean temperature of the control treatment was 12.4°C

231   and varied from 10.8 - 14.0°C; the mean of the warm treatment was 16.6°C and ranged from

232   14.8°C to 17.9°C; the mean of the heatwave treatment was 21.7°C and varied from 19.6°C to

233   23.3°C. Temperature treatments were significantly different from each other (K-W $\chi2$ = 463.32,

234   df = 2, p < 2.2e-16).

235

236   Minor skin lesions occurred during the experiment and were not statistically different between

237   treatments ($n_{control}$ = 17, n_{warm} = 15, $n_{heatwave}$ = 10; Fig. 2). Major ulcers were only observed in the

238   warm (n = 1) and heatwave treatments (n = 2). These major ulcers appeared to heal throughout

239   the recovery period and were re-classified as minor lesions on day 12. The maximum number of

240   minor lesions per individual was not significantly explained by treatment, weight, evisceration,

241   or defecation status.

242

243   Mortality was only observed in the 22°C heatwave treatment (Fig 3; n = 5), while there were no

244   mortalities observed in the control or warm treatments. Based on backwards model selection,

245   mortality was driven by treatment and weight (Table S1). Body stiffness was lower in the warm

246   and heatwave treatments compared to the control treatment (Fig. 3). Backwards-selected models

247   indicated that temperature treatment and day affected stiffness (Table S2). Sea cucumbers were

248   significantly less likely to have high stiffness scores relative to the control treatment in the warm

249   (p = 1.99e-7) and heatwave (p = 2.44e-11) treatments. Structural stiffness values were

250   significantly likely to be lower than day 1 on day 3 (p = 1.37e-5), day 4 (p = 2.50e-5) and day 5

251   (p = 8.66e-5), but not on day 2 (p = 0.0627; Table S2).

252

253   Stress spawning was observed during the temperature treatments (n = 11 bins), with the majority

254   of the stress spawning occurring in the 17°C warm (n = 5 bins) and the 22°C heatwave (n = 4

255   bins) treatments. However, there was no significant difference in spawning between temperature

256   treatments (K-W $\chi2$ = 1.94, df = 2, p = 0.379).

257

258 Evisceration was observed across all treatments ($n_{control}$ = 2, n_{warm} = 5, $n_{heatwave}$ = 5). Treatment

259 temperature did not explain a significant amount of the variance in the occurrence of evisceration

260 (Table S3), however, weight (p = 0.0383) and defecation status (p = 0.0163) were significant

261 drivers increasing likelihood of evisceration (Table S3). Along with evisceration of internal

262 organs, we also observed the expulsion of the respiratory tree in two individuals. Both

263 individuals were in the 22ºC heatwave treatment and mortality shortly followed the expulsion of

264 the respiratory tree.

265

Discussion

267 The objective of our study was to determine if heat stress can induce wasting symptoms in

268 *Apostichopus californicus*. While we saw minor skin lesions at all treatment levels, and major

269 ulcers in the warm and heatwave treatments, these were not characteristic of wasting symptoms

270 observed previously (Hewson et al., 2020; Fig. 1). Neither the minor lesions nor major ulcers

271 that we observed matched the wasting symptoms reported in *A. californicus* in Nanoose Bay,

272 B.C. (Lim, pers comm), or the isolated wasting events reported along the Pacific coast (Hewson,

273 Johnson & Tibbetts, 2020). We also did not see any sloughing of body tissues or liquefaction, as

274 has been anecdotally reported in previous wasting events (Schroeder, 2017; Hewson, Johnson &

275 Tibbetts, 2020). Despite the major ulcers sharing some resemblance in colour, texture, and

276 location to wasting disease symptoms, the sea cucumbers did not exhibit the full suite of

277 symptoms that is typical from a wasting cucumber, and within the treatment period these lesions

278 did not develop into fissures covering the dorsal body wall as seen in the wild (Fig. 1C). In

279 Nanoose, wasting symptoms developed into a mass outbreak and mortality event over several

280 months. However, unlike in reports of widespread wasting-driven mortality in wild *A.*

281 *californicus*, the major ulcers in our specimens healed within the 7-day recovery period, and

282 there was no evidence of these symptoms persisting or spreading to co-housed individuals. As

283 such, we could not conclude that the sea cucumbers in our experiment were afflicted by the fatal

284 wasting disease that has been previously reported (Schroeder, 2017; Hewson, Johnson &

285 Tibbetts, 2020). Future experiments could address the multi-week delay between the heat dome

286 and maximum die-off through prolonged exposure to elevated temperatures and utilise

287 histopathology to further investigate the etiology of epidermal ulceration.

288

289   While we are uncertain of the ultimate cause of the lesions that we observed, it is likely they

290   were associated with handling while assessing their posture maintenance, which increased the

291   frequency of potential abrasions on the epidermal surface tissue. Major ulcers may have begun as

292   minor lesions that were then exacerbated by the high physiological stress caused by the 22°C

293   treatment. White skin ulcerations, like the major ulcers we observed, are a recognized condition

294   in other Holothuroidea, described as a Skin Ulceration Disease or Skin Ulceration Syndrome

295   (SUS; Delroisse et al., 2020). SUS has been documented in commercially farmed *Apostichopus*

296   *japonicus* and *Holothuria scabra*, and has been characterized by white ulcers on both sides of the

297   body wall (Wang et al., 2007; Deng et al., 2009; Li et al., 2012; Zhang et al., 2018). Minor SUS

298   symptoms in commercially farmed *A. japonicus* and the major ulcers in our *A. californicus*

299   specimens are visually similar (Deng et al., 2009; Zhang et al., 2018). Unlike the SUS symptoms

300   reported in *A. japonicus*, we did not see any indication of swelling or discolouration of the

301   peristomes, and we did not see an initial abundance of ulcers around the mouth or cloaca (Becker

302   et al., 2004; Wang et al., 2007; Delroisse et al., 2020). Extreme cases of SUS in farmed *A.*

303   *japonicus* also bear resemblance to the wasting symptoms in wild *A. californicus*, raising further

304   questions about the causes of skin ulceration in Holothuroidea (Delroisse et al., 2020).

305

306   Despite not seeing evidence of wasting, we observed compelling evidence of stress symptoms in

307   response to thermal stress. We observed five mortality events in the 22°C heatwave treatment.

308   None of these mortalities were co-housed together, suggesting that it is unlikely a fatal disease

309   spread between cohoused individuals. Therefore, we suggest that the heatwave treatment is close

310   to the upper critical thermal tolerance of *A. californicus*, but the warm treatment temperature

311   does not cause sufficient stress for mortality to occur. Our findings align with previous work on

312   larval life stages, where Ren *et al.* (2018) found that at 22°C, larval *A. californicus* experienced

313   reduced survival which was not observed at 16°C or 18°C.

314

315   Beyond lethal effects, we observed reduced stiffening behaviour associated with both the 22°C

316   heatwave and 17°C warm water treatment. A temperature-induced loss in stiffness may have

317   implications for sea cucumber fitness under warming sea temperatures, as a limited ability to

318   stiffen may inhibit their ability to avoid predation or maintain posture while feeding and

319   spawning. Thermal stress may have reduced stiffness by causing muscular fatigue and relaxation

320   (Dowd & Somero, 2013) in the circular and longitudinal-ambulacral muscles (Gao & Yang,

321   2015). Stiffening is also caused by protein-mediated changes in mutable collagenous tissue

322   within the dermis of sea cucumbers (Takehana et al., 2014). Therefore, heat stress may have

323   reduced stiffening by denaturing or decreasing the production of tensilin, a stiffening protein, or

324   increasing the production of the de-stiffening protein softenin (Yamada et al., 2010; Takehana et

325   al., 2014; Tamori et al., 2016).

326

327   Unlike stiffening behaviour, we did not observe any significant trends in spawning behaviour or

328   evisceration. Of the 11 spawning events, 9 occurred in elevated-temperature treatments (warm or

329   heatwave) and this trend, although statistically insignificant, was expected as stress spawning has

330   been previously reported in other sea cucumber species (Battaglene et al., 2002; Rakaj et al.,

331   2018; Schagerström et al., 2021). We were unable to associate spawning with individual

332   phenotypes due to the co-housing experimental design. Evisceration appeared random across

333   treatments, potentially because *A. californicus* in all treatments may have been reacting to

334   handling stimulation and stress during the experiment (Ding et al., 2019). However, there is

335   evidence that biological mechanisms, weight and defecation status, partially explain the non-

336   treatment related variation in evisceration (Table S1). Defecation status in particular showed that

337   *A. californicus* that were not defecating were more likely to eviscerate. This may have occurred

338   because the energetic cost of eviscerating digestive organs would be lower for *A. californicus*

339   that had already ceased using their organs, either because they were preparing to eviscerate

340   (Swan, 1961) or undergoing viscera atrophy (Fankboner & Cameron, 1985). As such, when

341   stressed by handling, *A. californicus* that had already begun seasonal reductions in digestive

342   function were more likely to eviscerate from stress and overstimulation. Unlike digestive tract

343   evisceration, we do not believe that the expulsion of the respiratory tree in the 22°C heatwave

344   treatment was linked to seasonal senescence. For both individuals, respiratory evisceration was

345   followed by mortality, suggesting that this is an indication of extreme physiological distress from

346   thermal exposure.

347

348   Since we observed skin lesions under thermal stress that were likely unassociated with wasting,

349   other factors are likely causing recent wasting outbreaks in *A. californicus*. Like wasting, severe

350   cases of SUS are highly transmissible and result in mortality (Delroisse et al., 2020); bacterial

351   and viral pathogenic causal agents have been previously linked to SUS and wasting-like

352   symptoms, both in other sea cucumbers (Deng et al., 2008, 2009; Liu et al., 2010; Zhang et al.,

353   2018; Delroisse et al., 2020) and sea stars (Hewson et al., 2014, 2018; Work et al., 2021). A

354   study examining a single wasting *A. californicus* specimen found a high viral load but was

355   unable to identify a specific pathogen causing wasting symptoms (Hewson et al., 2020). Avenues

356   for future research on wasting diseases in *A. californicus* could address the potential for shared

357   etiology with SUS, given the symptomatic similarities, as well as the possibility of pathogenic

358   origins. Such investigations would benefit from histopathological examinations of potentially

359   diseased tissue to reveal more about the symptom's pathogenesis. Infectious factors (viral,

360   bacterial) and non-infectious factors (chemical pollution, hypoxia, eutrophication) should both be

361   investigated because widespread environmental degradation and anthropogenic climate change

362   are shifting pathogenic dynamics globally (Marcogliese, 2008; Allan et al., 2021). These

363   investigations would add valuable insight to the field of wasting diseases, given the scarcity of

364   published information on these events in echinoderms.

365

366   In this study, we exposed *A. californicus* to extreme thermal stress as measured by mortality,

367   degraded stiffening behaviour, and the development of skin lesions. Despite this, we found no

368   evidence that wasting is induced by temperature stress alone. Therefore, the August 2021 mass

369   wasting event in Nanoose, British Columbia, was likely not triggered solely by the anomalous

370   heatwave. Determining the factors that cause and exacerbate wasting in *A. californicus* is

371   essential for predicting and managing mass mortality events. Sea cucumbers are ecologically

372   important benthic detritivores, which break down organic matter, recycle nutrients, and maintain

373   sediment health (Wheeling, Verde & Nestler, 2007; Purcell, Conand & Byrne, 2016). Efforts to

374   protect, manage, and sustainably harvest *A. californicus* in the face of global environmental

375   change will require a comprehensive understanding of their stress responses, disease dynamics,

376   and the novel threat of sea cucumber wasting.

377

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386

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603

604 **Figure 1: Temperature anomalies and sea cucumber wasting.** A) Map of western North
605 America with B) an inset of Vancouver Island showing the data collection locations of Bamfield
606 Inlet (green), Nanoose Bay (purple). C) *A. californicus* in Nanoose Bay exhibiting wasting
607 symptoms. D) 8-day rolling average seasonal sea surface temperatures in Nanoose Bay from
608 2010 to 2020 (black line with grey SE ribbon) and 2021 (colour gradient line), with experimental
609 temperature treatments highlighted.

610

611 **Figure 2: Skin ulcers observed in *Apostichopus californicus* across treatments**. A) Minor
612 ulcer count; note ill-defined area of integument pallor (arrow: B). C) Major ulcer count, note
613 complete absence of epidermis revealing underlying white mutable connective tissue (arrow: D).

614

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

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.
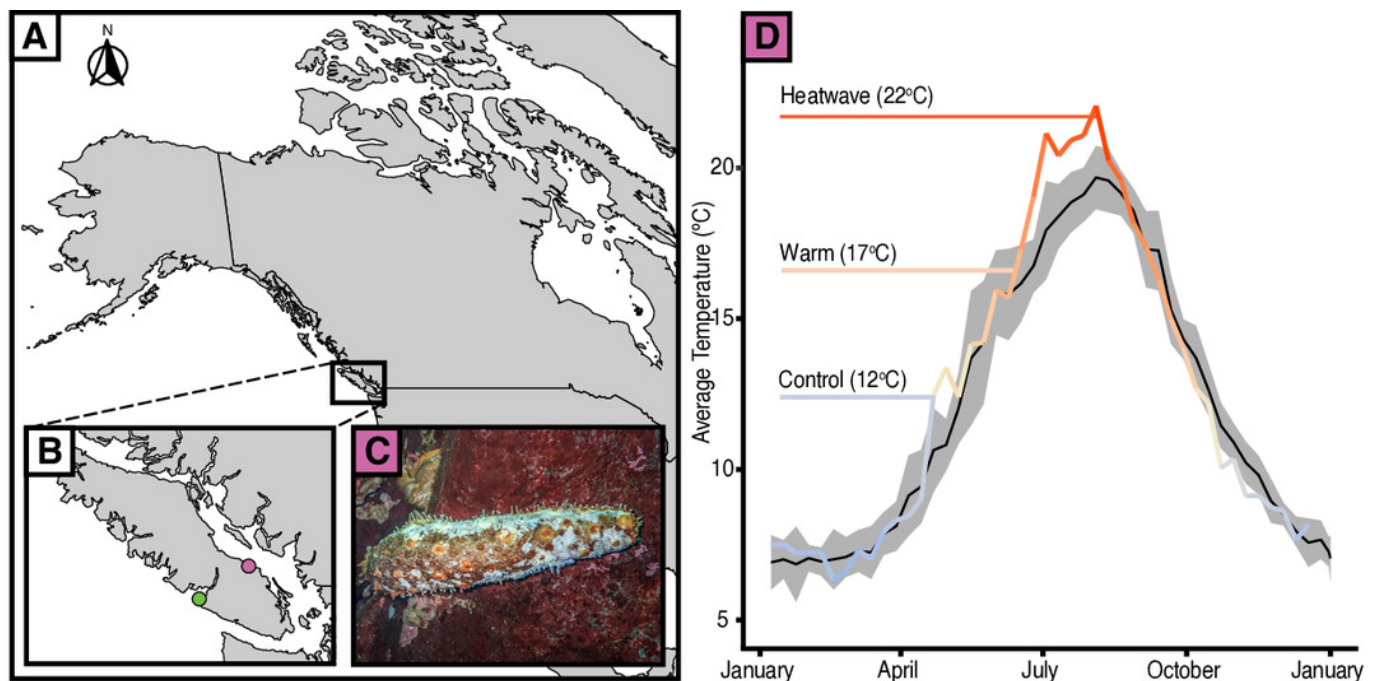
Figure 2

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).
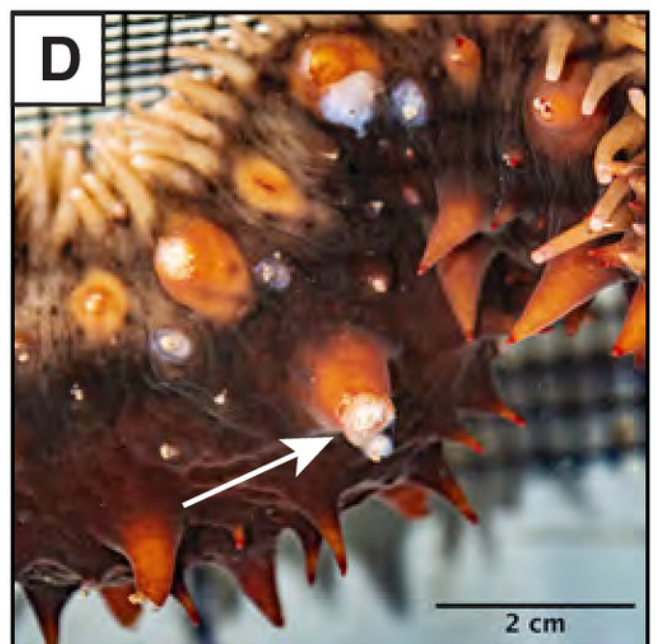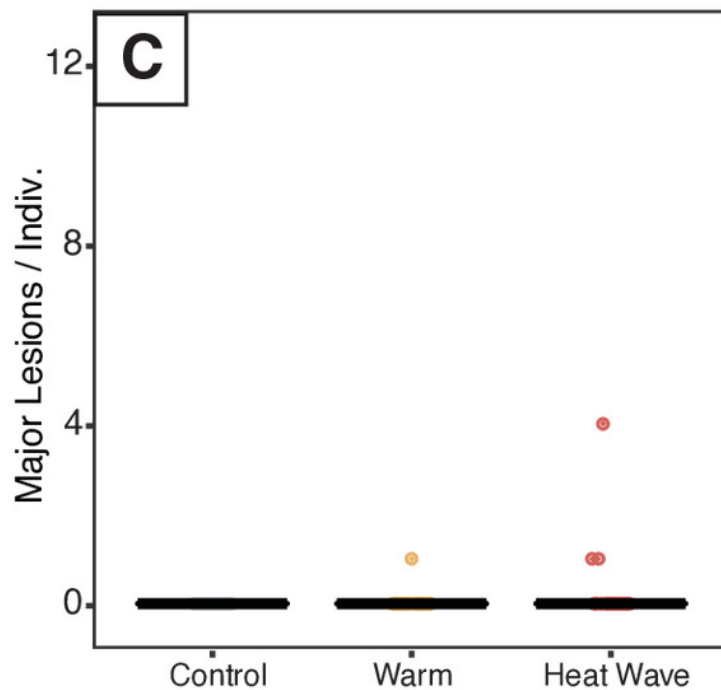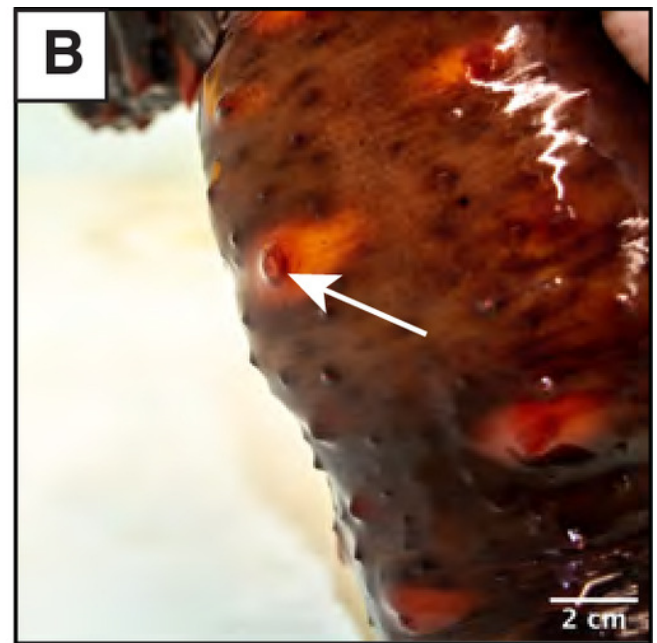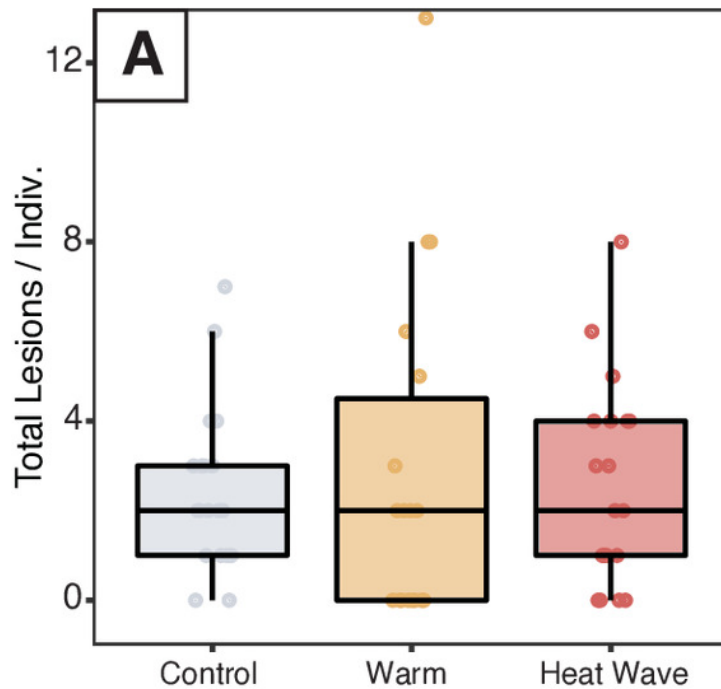
Figure 3

Stiffness and mortality of *Apostichopus californicus.*

Heat ramp (day 1), during temperature stress (days 2 - 4), and recovery (days 5 - 7) from the temperature treatment.