

# Elevated temperature linked to signs associated with sea star wasting disease in a keystone European species, Asterias rubens

Smith, S., Kunc, H. P., Hewson, I., & Collins, P. C. (2023). Elevated temperature linked to signs associated with sea star wasting disease in a keystone European species, Asterias rubens. *Marine Ecology Progress Series*, 724, 97-109. https://doi.org/10.3354/meps14451

# Published in:

Marine Ecology Progress Series

**Document Version:** Peer reviewed version

**Queen's University Belfast - Research Portal:** Link to publication record in Queen's University Belfast Research Portal

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# 1 TITLE

2	Elevated temperature linked to signs associated with sea star wasting disease in a keystone
3	European species, Asterias rubens
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6	RUNNING PAGE HEAD
7	Temperature-sensitive SSWD signs in <i>A. rubens</i>
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20 ABSTRACT

21 Sea Star Wasting Disease (SSWD) refers to a suite of gross signs affecting Asteroidea species. 22 These include epidermal lesions, everted viscera, arm autotomy, and ultimately, full body disintegration leading to mortality. The common sea star Asterias rubens is a keystone 23 species in the coastal Northeast Atlantic and may be susceptible to the disease. While the 24 25 precise aetiology of SSWD remains poorly understood, environmental instability, including 26 rising sea temperatures, has been linked to SSWD outbreaks. To investigate this connection, 27 an experiment was conducted to quantify disease sign expression in A. rubens under elevated temperature. We exposed sea stars to either elevated temperature (18°C) or a 28 29 control treatment (12°C) for a 14-day period. We quantified the presence of disease signs 30 associated with SSWD, the progression of signs, and survival of individuals. Elevated 31 temperature induced a greater number of signs consistent with SSWD, and also resulted in mortality for some of those animals. Furthermore, larger individuals were more likely to 32 33 show increased disease signs. Our results provide evidence that signs associated with SSWD 34 increase with elevated temperature.

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37 KEYWORDS

38 Sea Star Wasting Disease, Asterias rubens, Asteroidea, Thermal Stress

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# 42 1. INTRODUCTION

Diseases are important drivers of change in the marine environment, with the potential to 43 44 affect community dynamics and trophic interactions (Burge et al. 2014). These changes can have long-term consequences for ecosystem structuring and function, particularly if 45 46 affecting foundation, keystone, or ecosystem engineer species (Miner et al. 2018). Due to 47 global climate change, occurrences of large and fast temperature fluxes in marine ecosystems may increase in frequency (IPCC 2021). Warmer oceans and increasingly 48 49 frequent temperature anomalies have been linked to disease-induced mortality in many marine species (Harvell et al. 2002, Bruno et al. 2007, Ward et al. 2007, Eisenlord et al. 2016, 50 Harvell et al. 2019). Further, if the species affected by the mass-mortality event is a 51 52 keystone species (sensu Paine 1966), then there may be wider ecological implications (Levitan et al. 2023). 53 54 The common starfish Asterias rubens is a highly abundant asteroid found on the Atlantic

55 coast of Northwest Europe, inhabiting both sub- and intertidal zones (Saier 2001, Gallagher 56 et al. 2008, Agüera et al. 2021). Sea stars in the genus Asterias are typical of many asteroids in that their role as keystone predators provides an important control on prey populations. 57 Asterias rubens is an important predator for bivalve molluscs, notably the blue mussel, 58 Mytilus edulis (Agüera et al. 2020). While Asterias rubens is often considered a pest species 59 60 for mussel cultivators due to predation (Gallagher et al. 2008, Agüera et al. 2020), their 61 controlling effect on bivalves is also a factor in promoting benthic species diversity 62 (Wahltinez et al. 2023). Thus, disease-induced mortality in A. rubens species could therefore have cascading effects on costal benthic ecology. 63

Sea star wasting disease (SSWD) refers to a suite of gross signs affecting Asteroids, and 64 often co-occurs with changes in behaviour (Miner et al. 2018, Hewson 2021). These signs 65 initially manifest to varying degrees as abnormal posture, reluctance to feed, bloating or 66 pinching of arms, and the appearance of epidermal lesions (Bucci et al. 2017, Hewson 2019, 67 Hewson 2021). Progression of these signs can lead to epidermal ulceration resulting in the 68 69 exposure of viscera, including pyloric glands and gonads, followed by disintegration of the 70 epidermis, and then death (Staehli et al. 2009, Eisenlord et al. 2016, Hewson et al. 2014). 71 SSWD was first used to describe disease outbreaks in Asteroids on the Pacific coast of the United States in the 1980's & 1990's (Dungan et al. 1982, Eckert et al. 1999). It has since 72 been used to describe mass mortality events occurring on both Pacific and Atlantic coasts of 73 74 North America (Hewson et al. 2014, Bucci et al. 2017, Hewson et al. 2019). SSWD is now 75 understood to affect over 20 species (Hewson 2021). While a significant mass-mortality event in the Northeast Pacific in 2013/14 brought greater attention to the phenomenon, 76 77 wasting-like signs of disease, or signs consistent with SSWD, have been observed in many 78 species in prior years. Staehli et al. (2009) reports on a mass die-off of the sea star Astropecten jonstoni in the western Mediterranean, while Prestedge (1998) identified a 79 80 condition affecting Patiriella vivipara from Tasmania. Both these cases describe a condition 81 capable of causing severe tissue necrosis which may result in the animals' mortality. As far back as the 19<sup>th</sup> century, Mead (1898) described a necrotic condition affecting the skin, 82 83 before progressing through the rest of the body.

Questions remain, however, on the precise drivers and cause of SSWD, and the exact aetiology is yet to be fully resolved (Miner et al. 2018, Hewson et al. 2019). Researchers identified a densovirus (*Parvoviridae*) as a candidate pathogen for the disease, however

subsequent work has called this into question (Hewson et al. 2014, Hewson et al. 2020,
Jackson et al. 2020a, Jackson et al. 2020b).

While not ruling out a pathogenic aetiology, the role of environmental stressors has also
been proposed (Burge et al. 2014, Hewson et al. 2019). SSWD events have coincided with
increased temperature (Dungan et al. 1982, Staehli et al. 2009, Eisenlord et al. 2016, Harvell
et al. 2019), although correlations with decreased temperature have also been reported
(Menge et al. 2016).

94 More environmental factors have also been investigated, such as an association with 95 precipitation (Hewson et al. 2018), demonstrating that the relationship between SSWD and 96 external stressors is complex. Indeed, it may be that no single aetiology can account for SSWD across both its geographic extent and the species affected (Hewson et al. 2018). 97 98 SSWD as presently understood is not pathognomic (no uniquely identifying features). 99 Rather, it is a constellation of typically observed signs (Hewson et al. 2019), and it is possible 100 that certain signs associated with SSWD are also indicative of additional stressors or other 101 disease. Furthermore, microbial activity at the animal-water interface has been linked with SSWD and, regardless of ultimate aetiology, these microbes are themselves influenced by 102 factors such as organic matter availability, precipitation, and temperature (Aquino et al. 103 2021). 104

Asterias rubens is known to manifest signs indicative of SSWD such as lesions, bloating and
loss of turgor (Menge 1979, Wahltinez et al. 2023). Further, A. rubens shows reduced
feeding rates, energy uptake and growth under elevated temperature (Morón Lugo et al.
2020, Rühmkorff et al. 2023), highlighting sensitivity to thermal change. With SSWD linked
to environmental instability and thermal fluctuation, the susceptibility of A. rubens to SSWD

110	necessitates a deeper understanding of this phenomenon. With mass mortality events
111	reported in various asteroidea species, disease outbreak in this keystone benthic predator
112	may have cascading ecological implications (Harvell et al. 2019, Hewson et al. 2019).
113	The aim of this study was to measure the effect of elevated temperature on the prevalence
114	of signs corresponding to SSWD in A. rubens. We conducted a laboratory experiment to test
115	whether rapid temperature elevation could induce signs associated with SSWD. The
116	presence of disease signs was monitored, as well as survival rates to assess the role that
117	elevated temperature plays in inducing signs consistent with SSWD over time. Disease sign
118	progression at elevated temperature was predicted to increase over the course of the
119	experiment, with prolonged exposure resulting in a greater frequency on gross signs such as
120	lesions, bloating, loss of body turgor and arm autotomy when compared to the control
121	treatment. Further, we hypothesised that the abrupt increase in temperature experienced
122	by sea stars would decrease survival.
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# 131 2. MATERIALS & METHODS

# 132 2.1. Sea Star collection

133 Adult Asterias rubens were collected in October 2022 from Carlingford Lough, Irish Sea, using sea star mops from cultivated mussel beds (sensu Calderwood et al. 2016). Annual sea 134 135 temperatures in Carlingford Lough range between 3°C and 20°C (AFBI 2015), and average 136 water temperature for October 2022 were 13.26°C (SeaTemperatures 2023). Animals were transferred in aerated containers holding chilled seawater to the Queen's University Belfast 137 138 Marine Laboratory in Portaferry within 90 minutes of collection. At the laboratory, specimens were placed in a covered outdoor tank supplied by aerated UV and sand-filtered 139 seawater drawn from the marine Strangford Lough at a rate of approximately 250 Lhr<sup>-1</sup>. Sea 140 star condition was monitored for one week, and deceased or damaged individuals were 141 removed. Animals were fed *Mytilus edulis ad libitum* during this stage. 142 143 Two weeks prior to trial start, sea stars with grossly normal appearance were transferred to 144 an acclimation tank in a temperature-controlled room under a 12hr/12hr light/dark cycle. 145 Grossly normal individuals were identified by the lack of typical gross SSWD signs, *i.e.* no 146 epidermal lesions, turgid body posture, and even taper of arms (Fig. 1). The acclimation tank was supplied by the same flow-through seawater system, and temperature maintained at 147 12°C for the period of acclimation. Halfway through the acclimation phase, sea stars were 148 149 fed again *ad libitum* with *M. edulis*, with uneaten animals and empty shells removed the 150 following day.

151 2.2. Experimental set-up

Thirty-six five litre experimental units (opaque plastic container with translucent lid) were
evenly distributed between four tanks. Each large tank was treated with a 300-Watt

154 aquarium heater below a platform, allowing the large tanks to act as water baths. Prior to the experiment, each unit was sterilised with 70 % ethanol solution and cleaned, before 155 being filled with fresh UV and sand-filtered seawater. Each water bath included a 250 Lhr<sup>-1</sup> 156 157 pump to recirculate water, ensuring even heat distribution. Experimental units were placed 158 inside the water baths, and pressurised air was supplied to each to oxygenate the water 159 inside the units. Experimental units were self-contained in a closed system, therefore 160 limiting cross-contamination between sea stars. To maintain water quality, daily water changes were performed from separate water baths maintained in temperature and salinity 161 162 equilibrium with both temperature treatments.

163 Two temperature treatments were assigned between the four water baths, with a target 164 temperature of 12°C representing a continuation of the acclimatisation temperature 165 regime, and an 18°C target temperature representing a 6°C increase to which sea stars were 166 exposed for a 14-day observation period. Although within the annual temperature range 167 experienced by *A. rubens* at the collection site, animals were subjected to an immediate 168 change in temperature to assess whether an abrupt increase would induce signs of SSWD.

169 2.3. Experimental Procedure

On day 0 of the experiment, 36 sea stars were randomly assigned to individual experimental
units. 18 *A. rubens* were exposed to each temperature regime, with these subdivided
between two baths for each treatment. Sea star radius was measured as the length from
the tip of the longest arm to the mouth at the centre of the oral disc. All animals presented
grossly normal appearance upon induction into the trial.

175 2.4. Data Collection

176 2.4.1. Disease Status and Severity

Sea stars were assessed every 24 hours, and gross signs of disease were recorded for each animal. Overall appearance state for living animals was recorded as a binary assessment of grossly normal/grossly abnormal i.e., those expressing zero signs or those expressing any sign associated with SSWD disease (Fig. 1.).

181 Severity of disease was categorised across seven variables corresponding to commonly 182 reported SSWD signs (Bucci et al. 2017, Hewson 2019, Hewson 2021): loss of turgor, arm autotomy, bloating/pinching on the arms or disc, presence of epidermal lesions, contortion 183 184 of the arms, eversion of viscera (pyloric glands or gonads), and gross disintegration of the 185 epidermis (Fig. 1b-d). Each of these signs was scored out of a maximum with respect to sign characteristics. For signs that affected arms & central disc (bloating/pinching, epidermal 186 lesions, eversion of viscera, gross disintegration) this score ranged from 0 to a maximum of 187 6; 1 point for sign present on each arm, plus the central disc. For signs that only affected 188 arms (contortion, arm autotomy), the maximum score was 5 (Fig. 1e.). Loss of turgor was a 189 190 binary score with 0 being absent, and 1 being present. After each 24-hour period, a sum 191 score was calculated for each sea star based on the presence and extent of each of the signs associated with SSWD, thus approximating disease severity for an individual. To track signs 192 193 through each day of observation, arms were ordered in a clockwise direction around the 194 central disc, with the madreporite allowing for orientation of the start-point at arm no. 1 (Fig. 1a.). 195

Daily temperature and salinity measurements were recorded for each experimental unit.
For the control treatment, the mean temperature for the duration of the study was 12.40°C
(s.d. = 0.14), while for the elevated temperature treatment a mean of 18.24°C (s.d. = 0.13)

was recorded. Mean salinity for all experimental units for the duration of the trial was
36.11ppt (s.d. = 0.14).

201 2.4.2. Disease Category

202 With principal focus on SSWD outbreaks centred on the Pacific coast of North America, several resources have been made available for identification of disease in species local to 203 the region. Guides have been produced for both the ochre sea star (Pisaster ochraceus) and 204 205 the mottled star (Evasterias troschelii). The disease guides (MARINe 2018) utilise a 206 categorisation system with five stages of disease severity applied to both species (Table 1.). 207 Both these species belong to the family Asteriidae and have a similar body plan and 208 ecological niche to Asterias rubens. Assessing disease severity can therefore be conducted utilising disease categorisation as an alternative framework to the effect of temperature on 209 210 total disease sign expression.

A notable difference between the categorical assessment disease and the sum of all recorded disease signs is that the former does not factor in several signs that are commonly recorded. It does not account for the bloating/pinching of arms, nor does it account for loss of body turgor or arm contortion. Further, the categorical approach was modified such that dead animals were recorded separately.

216 2.4.3. Survival

Animals were removed from the trial upon death. Death was determined by total cessation of movement and adhesion of the tube-feet to the surface of the experimental units. In cases where arms had autotomised from the central disc following autotomy, assessment of death was conducted based solely upon the central disc and arms that remained intact.

221 2.5. Statistical analysis

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223 significance level of  $p \le 0.05$  was used. A Kolmogorov-Smirnov test was used to test for 224 normality, and in the case Poisson-distributed data, dispersion tests and tests for zero-225 inflation were applied. The analyses of SSWD in the two temperature treatments utilised a generalised linear mixed 226 227 effects model using the *Ime4* package (Bates et al. 2015). Initial exploration of data displayed a non-gaussian distribution, necessitating the generalised approach. 228 229 To test the relationship between healthy and diseased sea stars over time, a generalised 230 linear mixed effects model was produced using a binomial distribution family. This model 231 also incorporated sea star size (radius/mm) as a predictor in the model outcome. For this analysis, data for day 0 was removed, as all sea stars inducted into the trial were grossly 232 normal in appearance. All data associated with the four sea stars that died were also 233 234 removed from this analysis. To analyse the relationship between disease severity and temperature treatments, a 235 236 generalised linear mixed effects model was used with a Poisson distribution. To account for 237 repeated measures of individuals over the 14-day trial, a random effect of sea star ID and 238 day was incorporated into the model. For this model, data related to day 0 was omitted as 239 all sea stars presented grossly normal appearance upon induction into the trial. Data associated with the four sea stars that died was also omitted. 240 241 To test for the effect of temperature on the disease category, the analysis was performed

using a Poisson distribution. Although dead sea stars were incorporated into the categorical

Statistical analysis was conducted using R (v4.2.2; R Core Team 2022). For each analysis, a

243	assessment of disease severity, the four animals that died in the trial were excluded from
244	this model.
245	To compare the survival of sea stars for the duration of the trial a Kaplan-Meier survival
246	curve was calculated. To compare survival between the two treatments we used a Cox
247	Proportional Hazards model.
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3. RESULTS 262

### **Disease Status** 263 3.1.

264 Sea star radii did not differ significantly between treatments (Control: 92.00 ±14.63 mm,

265 Elevated temperature treatment:  $89.39 \pm 12.24$  mm; *t*-test: *t* = -0.581, *df* = 32.969, *p* =

266 0.565). The proportion of animals expressing any sign associated with SSWD was

267 significantly higher in the elevated temperature treatment than in the control, and the

proportion of SSWD signs increased significantly over the duration of the trial (Fig. 2; Table 268

269 2). Furthermore, signs associated with SSWD were significantly more prevalent among

270 larger individuals (Fig. 3; Table 2).

### 271 3.2. **Disease Severity**

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273 In an initial interactive model temperature treatment showed a significant effect on total 274 disease sign score ( $\beta$  = -0.667, s.e. = 0.187, z = -3.574, p < 0.001), with animals exposed to 275 the elevated temperature treatment displaying more SSWD signs than those in the control 276 (Fig. 4). The effect of day was significant ( $\beta = 0.037$ , s.e. = 0.012, z = 3.019, p = 0.003) and disease signs increased over the duration of the trial. However, there was no significant 277 interaction of treatment and temperature exposure time ( $\beta$  = -0.015, s.e. = 0.017, z = -0.877, 278 279 p = 0.381), showing that the rate of disease sign progression did not differ between 280 treatments. As this interaction was non-significant, subsequent models used an additive effect (Table 3).

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Further, model fit was improved significantly (p < 0.001) when size was incorporated into the 282

283 model as a fixed effect (Table 3). The resulting effects of temperature treatment was

284 significant, as was temperature exposure time. A significant effect of sea star radius was found (Table 3), with greater sea star size associated with increased expression of key SSWD
signs.

287 3.3. Disease category

Sea stars in the elevated temperature treatment showed higher category disease signs than in the control treatment, and disease category increased over the course of the experiment (Fig. 5). However, the effect of sea star radius was not significant (Table 4), therefore differing from the analysis of sum disease signs score (Table 3).

292 3.4. Survival

In total, four animals died during the experiment (Fig. 6). All these individuals were in the
elevated temperature treatment and occurred between Day 6 and Day 8. All displayed signs
consistent with wasting prior to death including loss of body turgor, bloating in the arms and
the appearance of white lesions. In three of these animals, multiple arm autotomies were
recorded prior to death, while one displayed disintegration of the epidermis at the joining
between one arm and the central disc. Viscera was clearly visible protruding through the
epidermis, and death occurred within a day of first observation of these signs.

No mortalities were recorded for the control treatment, thus the Cox Proportional Hazards model resulted in a degenerate estimate. In this case, a log-rank test was applied across the whole distribution, resulting in a significant difference between the two treatments ( $X^2$  = 4.4, df = 1, p < 0.04). Survival probability in the elevated temperature treatment after 14 days of exposure was 0.778 (s.d. = 0.098, 95% CI = 0.608 - 0.996).

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307 4. DISCUSSION

308 We found that exposure to immediate temperature increase is linked to increased SSWD-309 associated signs and their severity. Our experiment shows that rapid change in temperature 310 exacerbates disease expression and survival in A. rubens. Gross signs typically associated 311 with SSWD were found to increase upon exposure to sustained elevated temperature. 312 Mortality only occurred in the raised temperature treatment, highlighting the detrimental effects of rapid temperature increase. Further, size was found to be a significant factor in 313 314 explaining disease expression in our sea stars, with a relationship between increased disease 315 signs and larger individuals. We discuss these results in light to current assumptions on SSWD progression and aetiology. 316

317 4.1. Disease Severity

With elevated temperature influencing SSWD sign expression in A. rubens, our work 318 319 supports the idea that there is a link between SSWD signs and environmental stressors. The first SSWD signs visually occurred after 24 hours, and sea stars in the elevated temperature 320 treatment maintained a higher disease sign score for the duration of the experiment (Fig. 4). 321 322 Prolonged exposure also acted to increase total disease sign score. Although temperature 323 treatments used were within the range reported at the collection site (AFBI 2015), the abrupt and sustained exposure to elevated temperature may not be reflective of field 324 conditions. Therefore, conclusions of wider ecological consequences must be treated with 325 326 caution. It is apparent, however, that rapid and sustained exposure to elevated temperature 327 did induce signs consistent with wasting, therefore supporting the link between environmental stressors and the condition. 328

329 Elevated temperatures result in high cellular oxygen demand due to increased metabolic 330 activity (Rühmkorff et al. 2023). This, combined with lower solubility of oxygen in warmer seawater, leads to reduced concentrations of oxygen in the coelomic fluid and tissues, 331 332 followed by acute stress, tissue damage and mortality (Rühmkorff et al. 2023, Wahltinez et al. 2023). Elevated temperatures endured for longer durations may enhance this effect. 333 334 Furthermore, metabolic stress may limit immune response to opportunistic pathogens (Harvell et al. 2019, Hewson 2021). Coelomocyte count in A. rubens, an indicator of immune 335 336 system function in echinoderms, was found to increase with higher temperatures 337 (Wahltinez et al. 2023), and may indicate that an inflammatory response was occurring. A similar effect was reported for P. ochraceus with active wasting signs (Work et al. 2021), 338 showing an energetic cost. 339 340 Echinoderms have mutable collagenous tissue (MCT) which allows passive changes to the 341 mechanical properties of the body structure (Wilkie 2005). MCT is under direct neural

342 control and allows rapid changes to body rigidity, while also being integral to the process of

arm autotomy (Wilkie 2005, Work et al. 2021). Inflammation of the MCT has been

associated with epidermal ulceration in sea stars (Núñez-Pons et al. 2018, Smith et al. 2022),

and disruption to control of MCT may also be responsible for general lack of body turgor,

the observations of bloating/pinching of arms, and abnormal body posture (Work et al.

347 2021).

Inflammation markers at higher temperatures may relate to disruption of MCT (Wahltinez et
al. 2023), leading to SSWD signs that were observed in *A. rubens*. While questions remain as
to the mechanism behind this inflammatory response, the process appears temperature

351 sensitive. These results also mirror experimental temperature elevation on in *A. johnstoni*352 (Staehli et al. 2009), and *P. ochraceus* (Bates et al. 2009).

353 The addition of size into the model for total disease signs improved the fit, with larger animals trending towards enhanced disease sign expression (Table 3). Elevated temperature 354 355 is related to greater oxygen stress placed on animals with smaller surface-to-volume ratios 356 (Staehli et al. 2009, Eisenlord et al. 2016). Smaller size may act as a refuge, therefore, during periods of environmental instability. An energetically costly inflammatory response as is 357 358 observed under elevated temperatures (Wahltinez et al. 2023) is better sustained by smaller individuals. This may then lead to recovery of early disease signs, for example loss of turgor, 359 as the response is brought under control. 360

Dissolved oxygen (DO) was not monitored in this study as constant airflow was maintained
in all experimental units to maximise oxygen saturation. However, fine-scale hypoxic
conditions may be experienced by some sea stars due to their rugose body structure
(Aquino et al. 2021, Hewson 2021).

365 Microorganisms inhabiting the epidermal tissue may drive hyper-localised hypoxic conditions at the interface between the sea star and the water column, thus reducing DO 366 367 availability (Aquino et al. 2021, Hewson 2021). Localised hypoxic conditions may exacerbate existing disease in animals by microbial colonisation of necrotic tissue at lesion sites 368 (Hewson 2021). The combination of temperature associated inflammation weakened 369 370 immune function, MCT disfunction and progression to lesions and tissue necrosis may all be 371 further confounded by opportunistic microorganisms inducing hypoxia at the boundary layer. Our observations support this mechanism of action, with greater susceptibility to 372 SSWD at higher temperatures, and effects felt more greatly by larger individuals. 373

374 4.2. Disease Category

Overall disease signs were more frequent in sea stars exposed to elevated temperature, but 375 376 we also found a comparable pattern when assessing disease category based on gross body 377 characteristics (Fig. 5). Animals exhibiting Category 1 disease appeared after 24 hours in the 378 elevated temperature treatment, with Category 2 first appearing 48 hours after exposure. 379 Category 3 and Category 4 disease appeared on Day 4 and 5 respectively, and first mortality was observed on Day 6. In contrast, disease in the control treatment never increased above 380 381 Category 1, with animals showing lesions on a single arm or disc at most after five days of 382 exposure.

383 The categorical assessment diverges from disease sign score due to the lack of consideration for abnormal posture i.e., bloating/ pinching; contortion of arms; loss of body turgor. This 384 difference is reflected by the fact that all animals in the control treatment were categorised 385 386 as healthy until Day 5, while disease sign observations began on Day 1 when all signs are 387 counted (Fig. 4-5). This discrepancy is a result of bloating and loss of turgor being observed 388 in some individuals in the control treatment. This may also explain the non-significant effect of size in the categorical assessment of disease, where bloating loss of turgor are not 389 390 considered (Table 4).

As abnormal posture, including bloating, is consistently associated with SSWD in various species (Hewson et al. 2019, Work et al. 2021, Wahltinez et al. 2023), it is questionable as to why a categorisation of disease severity would not account for it. Signs of abnormal posture do, however, appear to be recoverable, placing them clearly at the lower end of disease severity. As the pathway leading to lesion formation begins with inflammation in the MCT (Work et al. 2021), it is possible that early onset of SSWD will cause disruption to MCT

control resulting abnormal posturing of sea stars. The recoverability of varying stages of
disease would therefore be worthwhile investigating, especially given the conflicting results
regarding sea star size.

400 In contrast, asteroids appear to present a limited suite of disease signs, and these may not 401 be consistent with a single aetiology (Hewson 2021). It is therefore possible that a 402 proportion of disease signs observed are driven by other factors. Moving animals from a flow-through system to a closed system with daily water changes may have induced a stress 403 404 response unrelated to temperature. This may account for observations of disease signs in 405 the control treatment (Fig. 2), and that these signs are indicative of stress associated with handling and water quality deteriorations over the intermediary 24 hours between water 406 changes. 407

Limited observations of lesion formation did occur in the control treatment, and may 408 409 suggest that coelomocyte infiltration and inflammation can progress at temperatures often 410 experienced by free-ranging A. rubens (Rühmkorff et al. 2023). Uncertainty remains 411 regarding the precise aetiology of SSWD. Although disease signs and progression were 412 elevated in the high temperature treatment, their presence in the control treatment suggests additional stressors. Reduced immune system functioning derived from alternative 413 factors could be the cause of MCT inflammation, and further investigation is needed to 414 415 disentangle competing environmental stressors, as well as from a potential pathogenic 416 aetiology.

417 4.3. Survival

Exposure to elevated temperature caused greater than 20% mortality in the sea star *A*. *rubens* (Fig. 6). Although a deeper analysis was limited by the small number of cases (4) and

420 no mortality in the control treatment, deaths were all associated with SSWD signs on
421 previous days' recordings.

Under simulated heatwave scenarios with peaks of 26°C, Rühmkorff et al. (2023) reported
100% mortality of *A. rubens*. Similarly, Staehli et al. (2009) reported a significant lethal effect
of elevated temperature in the Mediterranean burrowing sea star (*Astropecten johnstoni*),
with sea stars developing wasting-like signs prior to death. In the latter study, size was also
found to have a significant effect on survival, with larger animals suffering greater mortality
rates.

428 In the Pacific, elevated temperature was linked to increased mortality rates in P. ochraceus 429 (Eisenlord et al. 2016). Laboratory studies conducted by Eisenlord et al. (2016) were paired with field observations, and showed reduced abundance of larger individuals following a 430 431 2014 outbreak of SSWD. As our mortality rates were comparatively low, we were unable to 432 perform an analysis of the effect of size on survival in A. rubens. However, due to the 433 combined effect of increased metabolic activity and reduced oxygen solubility at higher 434 temperatures, smaller animals may have an advantage under warming scenarios due to higher surface-to-volume ratio for diffusion (Rühmkorff et al. 2023). 435

Further, it is notable that first mortality in Rühmkorff et al. (2023) occurred on day 8 after
exposure to 26°C, whereas at 18°C in the present study, first mortality occurred at day 6. It
is possible that sea star size could explain this difference. In Rühmkorff et al. (2023) mean
arm length was 5.5 ± 0.3cm; smaller than the 8.9 ± 1.2cm arm length of those inducted into
this experiment. As discussed, current ideas regarding SSWD progression suggest a
connection between larger individuals and enhanced susceptibility (Staehli et al. 2009,
Eisenlord et al. 2016). However, while the present study found support for size influencing

443 SSWD sign expression, further targeted research is needed to demonstrate the effect of size444 on overall survival and mortality.

445 4.4. Ecological Implications

446 In the face of global climate change, marine disease outbreaks are thought to be becoming more prevalent (Harvell et al. 2002, Eisenlord et al. 2016). Our results support a link 447 environmental stressors and SSWD-associated signs and mortality in A. rubens. As with 448 449 many sea stars, this abundant North Atlantic species acts as a keystone predator, with a controlling effect on the region's bivalve molluscs (Hancock 1955, Saier 2001, Gallagher et 450 al. 2008). The possibility of thermal association of SSWD in A. rubens is concerning given 451 452 projected climate change scenarios. The increasing frequency of extreme climatic events, such as marine heatwaves, is predicted to have widespread and significant impacts on 453 marine ecosystems (Oliver et al. 2019, Rühmkorff et al. 2023). Through predation on 454 455 bivalves, sea stars increase the possibility for settlement and attachment of other 456 organisms. Declines in A. rubens populations would remove this important aspect of benthic 457 ecology and encourage overgrowth of mussels in naturally biodiverse habitat. Further, 458 SSWD has been observed in the common sunstar, *Crossaster papposus*, itself a reported predator of A. rubens, adding greater uncertainty to the potential consequences of SSWD 459 outbreaks in North Atlantic sea stars (Smith et al. 2022). 460

461 4.5. Limitations

While we found that signs associated with SSWD in *A. rubens* are temperature-sensitive, the
underlying mechanism remains elusive, and a pathogenic aetiology is not ruled out.
Regarding hypoxia, enrichment events may themselves be associated with thermal fluxes,
and thus disentangling different environmental stressors as a potential trigger for SSWD

onset may be challenging (Aquino et al. 2021, Hewson 2021). Further, changing thermal
regimes may push natural pathogens towards their thermal optima, thus making a single
pathogenic trigger challenging to disentangle from environmental stressors (Eisenlord et al.
2016, Hewson et al. 2018, Byers 2021). Furthermore, given that some signs of disease were
present in the control treatment, it is possible that additional drivers in disease
manifestation were present.

Additionally, the temperature increase to which the sea stars were exposed was abrupt,
rather than a gradual warming. However, temperature increases on the intertidal can be
rapid and severe (Legrand et al. 2018, Gilson et al. 2021), and *A. rubens* may experience
such extremes at local scales. Projected warming may further increase the magnitude and
spatial scale of such thermal fluxes, and further targeted research is needed to assess the
potential for SSWD outbreaks at the population level.

Finally, the number of sea stars inducted into the study was subject to logistical constraints,
and a greater sample size may add greater validity to the results obtained. Similarly, this
study lacks validation from field observation of wasting *A. rubens*. Anecdotal reports have
provided information about the presence of wasting individuals on the Irish Sea coast,
however this has not been systematically observed to date.

483 4.6. Conclusions

Signs of SSWD in *A. rubens* were induced when exposed to abrupt temperature increase.
Disease signs were more frequent at elevated temperatures and persisted for the duration
of exposure. Further, our work suggests an influence of body size on disease severity, which
aligns with current ideas regarding SSWD aetiology. Although the exact aetiology is yet to be
fully resolved, this work develops our understanding of the condition, and may support the

489	connection of SSWD to environmental stressors. The possible thermal association of SSWD
490	raises concerns for ecological stability and resilience in the face of global climate change.
491	Many sea stars are keystone species, with A. rubens having a significant controlling effect on
492	bivalve molluscs. If the possibility of disease outbreak is influenced by global climate change,
493	it could have cascading ecosystem effects detrimental to benthic diversity in the North
494	Atlantic.
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# 509 ACKNOWLEDGEMENTS

- 510 This work is funded through the Northern Ireland Department for the Economy.
- 511 The Authors would like to acknowledge the crew of the Ex Mare Gratia for assistance in
- 512 obtaining sea stars for this project. Dr Nicholas Baker-Horne, Connie Baker-Horne, and Dr
- 513 Mánus Cunningham provided helpful comments.

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### TABLES

### Table 1. Disease Categories and identification characteristics for Pisaster ochraceus &

### Evasterias troschelii as defined by Pacific Rocky Intertidal.org.

	Identification	Disease Category
	Animal shows grossly normal appearance. Animals above tide line may have deflated appearance due to water loss, yet this is not indicative of SSWD.	0
	Lesions present on one arm or the central disc.	1
	Lesions present on two arms/one arm and the central disc.	2
	Lesions present on most of body. One or Two missing arms.	3
691	Severe Tissue deterioration/ death. Three or more missing arms.	4
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# Table 2. The effect of elevated temperature on disease status in Asterias rubens. A) Displays terms, coefficients, p values for the best fitting model. B) Displays Akaike Information Criterion (AIC) and Log Likelihood for all models testing the effect of temperature treatment on disease signs. Day was included as a random effect, and nested within sea star ID, allowing individual slopes to vary according to individual through time. Significant results (p < 0.05) highlighted in bold.</li>

(a) -Term	Estimate	s.e.	z-value	<i>p</i> -value
Intercept	8.718	2.798	3.116	0.002
Day	-0.238	0.068	-3.483	< 0.001
Sea Star Radius	-0.116	0.032	5.185	< 0.001
Treatment (Control)	4.891	0.943	-3.668	< 0.001
(b) -Model		AIC		Log. Likelihood
Day + Sea Star Radius + Treatment		360.74		-173.37
Day * Treatment		367.61		-176.81
Day + Treatment		371.83		-179.91

# 700 Table 3. The effect of elevated temperature SSWD disease signs score in Asterias rubens.

A) Displays terms, coefficients, p values for the best fitting model. B) Displays Akaike

702 Information Criterion (AIC) and Log Likelihood for all models testing the effect of

- temperature treatment on disease signs. Day was included as a random effect, and nested
- within sea star ID, allowing individual slopes to vary according to individual through time.

Significant results (p < 0.05) highlighted in bold.

(a) -Term	Estimate	s.e.	z-value	<i>p</i> -value
Intercept	-5.747	1.702	-3.376	< 0.001
Day	0.030	0.008	3.506	< 0.001
Log (Sea Star Radius)	1.545	0.380	4.061	< 0.001
Treatment (Control)	-0.854	0.115	-7.438	< 0.001
(b) -Model			_	
			AIC	Log. Likelihood
Day + Log (Sea Star Ra	dius) + Treatm	ent	AIC 1050.5	-518.27
Day + Log (Sea Star Ra Day + Treatment	dius) + Treatm	ent	AIC 1050.5 1062.0	Log. Likelihood -518.27 -525.03

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# 710 TABLE 4. The effect of elevated temperature on SSWD category in Asterias rubens. Table

- 711 displays terms, coefficients, p values for the best fitting model. Day was included as a
- random effect, and nested within sea star ID, allowing individual slopes to vary according to
- individual through time. Significant results (p < 0.05) highlighted in bold.

(a) -Term	Estimate	s.e.	z-value	<i>p</i> -value
Intercept	-25.152	14.329	-1.755	0.079
Day	0.126	0.043	2.928	0.003
Log (Sea Star Radius)	5.131	3.190	1.608	0.108
Treatment (Control)	-3.862	1.018	-3.794	< 0.001

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Figure 1. Specimens of the common starfish Asterias rubens showing key gross signs
associated with sea star wasting disease. Nominally healthy/asymptomatic individual
displaying no gross abnormalities; no epidermal lesions or ulceration, even arm tapering.

730	Arms numbered for tracking of disease signs (a) viscera everted from arm tip (b) epidermal
731	lesion on arm median presenting as bright white patches on epidermis (c) arm displaying
732	bloated/pinched appearance leading to abnormal taper (d) deceased individual displaying
733	multiple arms autotomised from central disc, with associated gross disintegration of
734	epidermal tissue and no movement or adhesion of tube feet (e).
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751	Figure 2. Stacked bar plot displaying the proportion of animals that were grossly normal
752	for each temperature treatment. Plot compares grossly normal animals (dark) against
753	animals expressing any sign of disease (light), with proportions displayed for each day of
754	temperature treatment exposure. Left panel shows control treatment; right panel shows
755	elevated treatment.
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Figure 3. Boxplot of sea star radius and appearance. Grossly normal animals (dark)
compared to animals with any disease sign present (light). Boxplots for each day of
temperature treatment exposure display median and bind 25<sup>th</sup> and 75<sup>th</sup> percentiles.
Whiskers mark upper and lower data bounds, points denote outliers.





771Figure 4. The effect of increasing temperature on disease signs of SSWD. Boxplot of total772number of observed signs for each sea star exposed to differing temperature treatments773over the 14-day exposure period, including day 0. Boxplots describe median values and bind774 $25^{th}$  and  $75^{th}$  percentiles. Whiskers mark upper and lower data bounds, points denote775outliers. Animals that died were removed from data set (n = 4). A target of 12°C represented776the control treatment (n = 18), while a target of 18°C was used for the elevated temperature777treatment (n = 14).





782	Figure 5. Stacked bar plot showing the proportion of animals assigned to discrete disease
783	categories when exposed to different temperature treatments over 14 days. Left panel
784	displays results for the control treatment ( $n = 18$ ); right panel shows the elevated
785	temperature treatment ( $n = 18$ ).
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