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

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ABSTRACT: Sea star wasting disease (SSWD) refers to a suite of gross signs affecting Asteroidea species. 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 species in the coastal Northeast Atlantic and may be susceptible to the disease. While the precise aetiology of SSWD remains poorly understood, environmental instability, including rising sea temperatures, has been linked to SSWD outbreaks. To investigate this connection, 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 control treatment (12°C) for a 14 d period. We quantified the presence of disease signs associated with SSWD, the progression of signs, and survival of individuals. Elevated 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 show increased signs of disease. Our results provide evidence that signs associated with SSWD increase with elevated temperature.

KEY WORDS: Sea star wasting disease · *Asterias rubens* · Asteroidea · Thermal stress

1. INTRODUCTION

Diseases are important drivers of change in the marine environment, with the potential to affect community dynamics and trophic interactions (Burge et al. 2014). These changes can have long-term consequences for ecosystem structure and function, particularly if they affect foundation, keystone, or ecosystem-engineer species (Miner et al. 2018). Due to global climate change, occurrences of large and fast temperature fluxes in marine ecosystems may increase in frequency (IPCC 2021). Warmer oceans and increasingly frequent temperature anomalies have been linked to disease-induced mortality in many marine species (Harvell et al. 2002, 2019, Bruno et al. 2007, Ward et al. 2007, Eisenlord et al. 2016). Furthermore, if the species affected by the mass-mortality event is a keystone species (sensu Paine 1966), then there may be wider ecological implications (Levitan et al. 2023).

The common starfish *Asterias rubens* is a highly abundant asteroid found on the Atlantic coast of northwest Europe, inhabiting both sub- and intertidal zones (Saier 2001, Gallagher 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. *A. rubens* is an important predator for bivalve molluscs, notably the blue mussel *Mytilus edulis* (Agüera et al. 2020). While *A. rubens* is often considered a pest species for mussel cultivators due to predation (Gallagher et al. 2008, Agüera et al. 2020), their controlling

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effect on bivalves is also a factor in promoting benthic species diversity (Wahltinez et al. 2023). Thus, disease-induced mortality in *A. rubens* species could have cascading effects on coastal benthic ecology.

Sea star wasting disease (SSWD) refers to a suite of gross signs affecting asteroids and often co-occurs with changes in behaviour (Miner et al. 2018, Hewson 2021). These signs initially manifest to varying degrees as abnormal posture, reluctance to feed, bloating or pinching of arms, and the appearance of epidermal lesions (Bucci et al. 2017, Hewson et al. 2019, Hewson 2021). Progression of these signs can lead to epidermal ulceration, resulting in the exposure of viscera, including pyloric glands and gonads, followed by disintegration of the epidermis and then death (Staehli et al. 2009, Hewson et al. 2014, Eisenlord et al. 2016).

SSWD was first used to describe disease outbreaks in asteroids on the Pacific coast of the USA in the 1980s and 1990s (Dungan et al. 1982, Eckert et al. 2000). It has since been used to describe mass-mortality events occurring on both the Pacific and Atlantic coasts of North America (Hewson et al. 2014, 2019, Bucci et al. 2017). SSWD is now understood to affect over 20 species (Hewson 2021). While a significant mass-mortality event in the Northeast Pacific in 2013–2014 brought greater attention to the phenomenon, wasting-like signs of disease, or signs consistent with SSWD, have been observed in many species in prior years. Staehli et al. (2009) reported on a mass die-off of the sea star *Astropecten jonstoni* in the western Mediterranean, while Prestedge (1998) identified a condition affecting *Patiriella vivipara* from Tasmania. Both of these cases described a condition capable of causing severe tissue necrosis which may result in the animals' mortality. As far back as the 19th century, Mead (1898) described a necrotic condition affecting the skin before progressing through the rest of the body.

Questions remain, however, about 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 have identified a densovirus (*Parvoviridae*) as a candidate pathogen for the disease (Hewson et al. 2014); however, subsequent work has called this into question (Hewson et al. 2020, Jackson et al. 2020a,b). 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). Other environmental factors have also been investigated, such as an association with precipitation (Hewson et al. 2018), demonstrating that the relationship between SSWD and 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). SSWD as presently understood is not pathognomic (no uniquely identifying features). Rather, it is a constellation of typically observed signs (Hewson et al. 2019), and it is possible that certain signs associated with SSWD are also indicative of additional stressors or other diseases. Furthermore, microbial activity at the animal–water interface has been linked with SSWD and, regardless of ultimate aetiology, these microbes are themselves influenced by factors such as organic matter availability, precipitation, and temperature (Aquino et al. 2021).

Asterias rubens is known to manifest signs indicative of SSWD such as lesions, bloating, and loss of turgor (Menge 1979, Wahltinez et al. 2023). Furthermore, *A. rubens* shows reduced feeding rates, energy uptake, and growth under elevated temperatures (Morón Lugo et al. 2020, Rühmkorff et al. 2023), highlighting sensitivity to thermal change. With SSWD having been linked to environmental instability and thermal fluctuation, the susceptibility of *A. rubens* to SSWD necessitates a deeper understanding of this phenomenon. With mass-mortality events reported in various Asteroidea species, disease outbreaks in this keystone benthic predator may have cascading ecological implications (Harvell et al. 2019, Hewson et al. 2019).

The aim of this study was to measure the effect of elevated temperature on the prevalence of signs corresponding to SSWD in *A. rubens*. We conducted a laboratory experiment to test whether rapid temperature elevation could induce signs associated with SSWD. The presence of disease signs was monitored, as well as survival rates, to assess the role that elevated temperature plays in inducing signs consistent with SSWD over time. Disease sign progression at elevated temperature was predicted to increase over the course of the experiment, with prolonged exposure resulting in a greater frequency of gross signs such as lesions, arm autotomy, bloating, and loss of body turgor compared to the control treatment. Furthermore, we hypothesised that the abrupt increase in temperature experienced by sea stars would decrease survival.

2. MATERIALS AND METHODS

2.1. Sea star collection

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 temperatures in Carlingford Lough range between 3° and 20°C (AFBI 2015), and the average water temperature for October 2022 was 13.26°C (SeaTemperatures 2023). Animals were transferred in aerated containers holding chilled seawater to the Queen's University Belfast Marine Laboratory in Portaferry, UK, within 90 min of collection. At the laboratory, specimens were placed in a covered outdoor tank supplied by aerated UV- and sand-filtered seawater drawn from the marine Strangford Lough at a rate of approximately 250 l h^{-1}. Sea star condition was monitored for 1 wk, and deceased or damaged individuals were removed. Animals were fed *Mytilus edulis* ad libitum during this stage.

Two weeks prior to trial start, sea stars with grossly normal appearance were transferred to an acclimation tank in a temperature-controlled room under a 12 h light:12 h dark cycle. Grossly normal individuals were identified by the lack of typical SSWD signs, i.e. no epidermal lesions, turgid body posture, and even taper of arms (Fig. 1a). The acclimation tank was supplied by the same flow-through seawater system, and temperature was maintained at 12°C for the period of acclimation. Halfway through the acclimation phase, sea stars were again fed *M. edulis* ad libitum, with uneaten animals and empty shells removed the following day.

2.2. Experimental setup

A total of 36 experimental units (5 l opaque plastic containers with translucent lids) were evenly distributed among 4 tanks. Each large tank was treated with a 300 W aquarium heater below a platform, allowing the large tanks to act as water baths. Prior to the experiment, each unit was sterilised with a 70% ethanol solution and cleaned, before being filled with fresh UV- and sand-filtered seawater. Each water bath included a 250 l h^{-1} pump to recirculate water, ensuring even heat distribution. Experimental units were placed inside the water baths, and pressurised air was supplied to each unit to oxygenate the water inside the units. Experimental units were self-contained in a closed system, thus limiting cross-contamination between sea stars. To maintain water quality, daily water changes were performed from separate tanks maintained in temperature and salinity equilibrium with both temperature treatments.

Two temperature treatments were assigned between the 4 water baths, with a target temperature of 12°C representing a continuation of the acclimation temperature regime, and an 18°C target temperature representing a 6°C increase to which sea stars were exposed for a 14 d observation period. Although within the annual temperature range experienced by *A. rubens* at the collection site, animals were subjected to an immediate change in temperature to assess whether an abrupt increase would induce signs of SSWD.

2.3. Experimental procedure

On Day 0 of the experiment, 36 sea stars were randomly assigned to individual experimental units. A total of 18 *A. rubens* were exposed to each temperature regime, with these subdivided between 2 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.

2.4. Data collection

2.4.1. Disease status and severity

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

Severity of disease was scored across 7 variables corresponding to commonly reported SSWD signs (Bucci et al. 2017, Hewson et al. 2019, Hewson 2021): loss of turgor, arm autotomy, bloating and/or pinching on the arms or disc, presence of epidermal lesions, contortion of the arms, eversion of viscera (pyloric glands or gonads), and gross disintegration of the epidermis (Fig. 1b–d). Each of these signs was scored out of a maximum with respect to sign characteristics. For signs that affected arms and the central disc (bloating and/or pinching, epidermal lesions, eversion of viscera, gross disintegration), this score

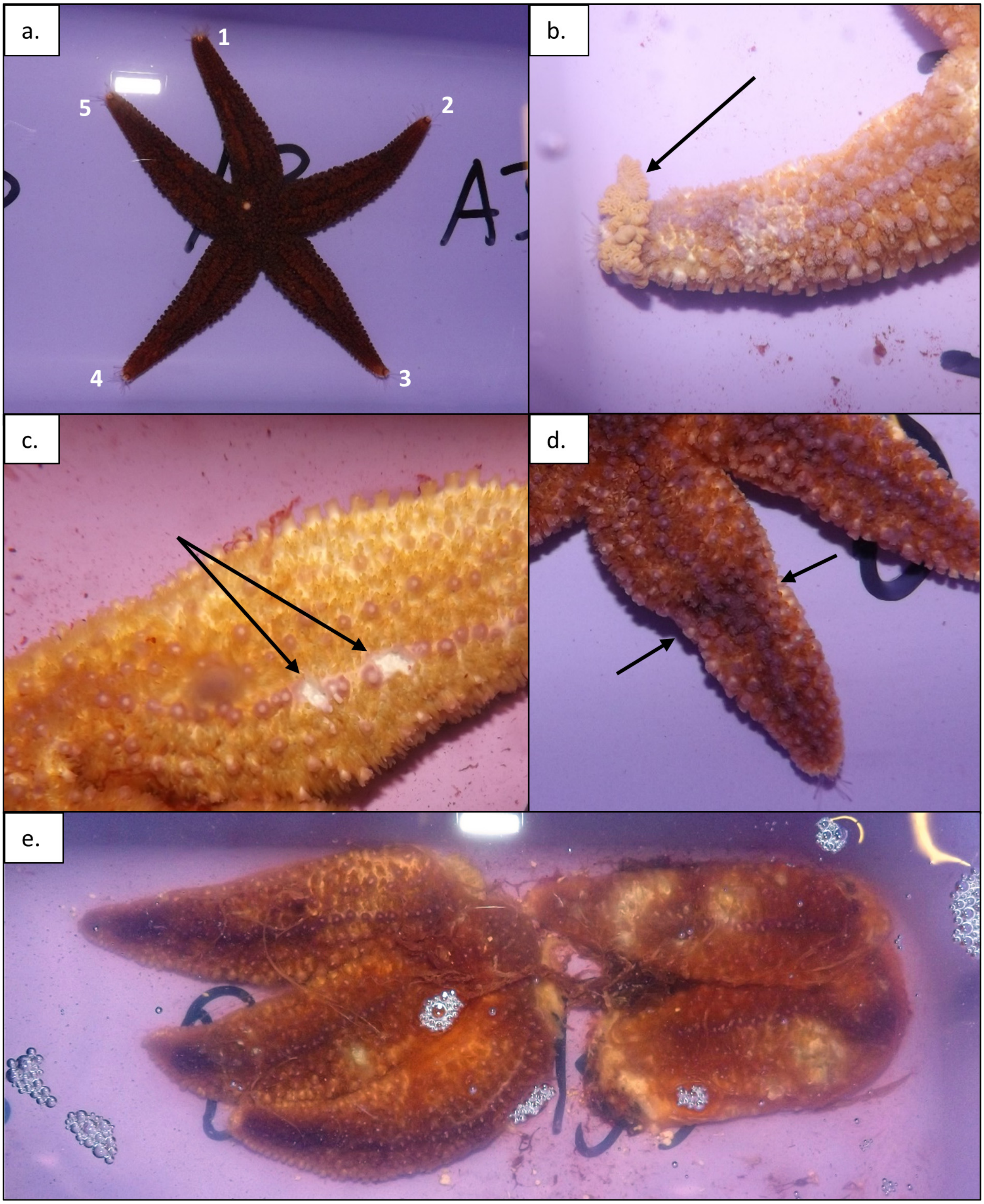

Fig. 1. Specimens of the common starfish *Asterias rubens* showing (a) a nominally healthy or asymptomatic individual displaying no gross abnormalities; no epidermal lesions or ulceration, and with even arm tapering. Arms numbered for tracking of disease signs and (b–e) key gross signs associated with sea star wasting disease. (b) Viscera everted from arm tip; (c) epidermal lesion on arm median presenting as bright white patches on epidermis; (d) arm displaying bloated and/or pinched appearance leading to abnormal taper; (e) deceased individual displaying multiple arms autotomised from central disc, with associated gross disintegration of epidermal tissue and no movement or adhesion of tube feet

ranged from 0 to a maximum of 6; one point for sign present on each arm, plus the central disc. For signs that only affected arms (contortion, arm autotomy), the maximum score was 5 (Fig. 1e). Loss of turgor was a binary score, with 0 being absent and 1 being present. After each 24 h period, a sum 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 through each day of observation, arms were ordered in a clockwise direction around the central disc, with the madreporite allowing for orientation of the start-point at arm no. 1 (Fig. 1a).

Daily temperature and salinity measurements were recorded for each experimental unit. For the control treatment, the mean (±SD) temperature for the duration of the study was 12.40 ± 0.14°C, while for the elevated temperature treatment, a mean of 18.24 ± 0.13°C was recorded. Mean salinity for all experimental units for the duration of the trial was 36.11 ± 0.14 ppt.

2.4.2. Disease category

With a principal focus on SSWD outbreaks centred on the Pacific coast of North America, several resources have been made available for the identification of disease in species local to the region. Guides have been produced for both the ochre sea star *Pisaster ochraceus* and the mottled star *Evasterias troschelii*. The disease guides (MARINe 2018) utilise a categorisation system with 5 stages of disease severity applied to both species (Table 1). Both these species belong to the Family Asteriidae and have a body plan and ecological niche similar to *A. rubens*. Assessing disease severity was therefore conducted by utilising disease categorisation as an alternative framework to the effect of temperature on total disease sign expression.

A notable difference between the categorical assessment of 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 and/or pinching of arms, nor does it account for loss of body turgor or arm contortion. Furthermore, the categorical approach was modified such that dead animals were recorded separately.

Table 1. Disease categories and identification characteristics for *Pisaster ochraceus* and *Evasterias troschelii* as defined by the Multi-Agency Rocky Intertidal Network (MARINe 2018). SSWD: sea star wasting disease

Identification	Disease category
Animal shows grossly normal appearance. Animals above tide line may have a 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 1 or 2 arms and the central disc	2
Lesions present on most of body. One or 2 missing arms	3
Severe tissue deterioration and death. Three or more missing arms	4

2.4.3. Survival

Animals were removed from the trial upon death. Death was determined by total cessation of movement and loss of 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.

2.5. Statistical analysis

Statistical analysis was conducted using R v.4.2.2 (R Core Team 2022). For each analysis, a significance threshold of $\alpha = 0.05$ was used. A Kolmogorov-Smirnov test was used to test for normality, and in the case of Poisson-distributed data, dispersion tests and tests for zero-inflation were applied.

The analyses of SSWD in the 2 temperature treatments utilised a generalised linear mixed effects model, using the R package 'lme4' (Bates et al. 2015). Initial exploration of the data displayed a non-Gaussian distribution, necessitating the generalised approach.

To test the relationship between healthy and diseased sea stars over time, a generalised linear mixed effects model was produced using a binomial distribution family. This model also incorporated sea star size (radius, in 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 normal in appearance. All data associated with the 4 sea stars that died were also removed from this analysis.

To analyse the relationship between disease severity and temperature treatments, a generalised linear mixed effects model was used with a Poisson distribution. To account for repeated measures of individuals over the 14 d trial, a random effect of sea star ID and day was incorporated into the model. For this model, data related to Day 0 was omitted, as all sea stars presented grossly normal appearance upon induction into the trial. Data associated with the 4 sea stars that died was also omitted.

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 assessment of disease severity, the 4 animals that died during the trial were excluded from this model.

To compare the survival of sea stars over the duration of the trial, a Kaplan-Meier survival curve was calculated. To compare survival between the 2 treatments, we used a Cox proportional hazards model.

3. RESULTS

3.1. Disease status

Sea star radii did not differ significantly between treatments ([mean ± SD] control: 92.00 ± 14.63 mm; elevated temperature treatment: 89.39 ± 12.24 mm; *t*-test: $t = -0.581$, df = 32.969, $p = 0.565$). The proportion of animals expressing any sign associated with SSWD was 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 2). Furthermore, signs associated with SSWD were significantly more prevalent among larger individuals (Fig. 3, Table 2).

Table 2. Effect of elevated temperature on disease status in *Asterias rubens*. (a) Terms, coefficients, and p-values for the best fitting model; (b) Akaike's 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 ($p < 0.05$) are highlighted in **bold**

(a) Terms	Estimate	SE	*z*	p
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

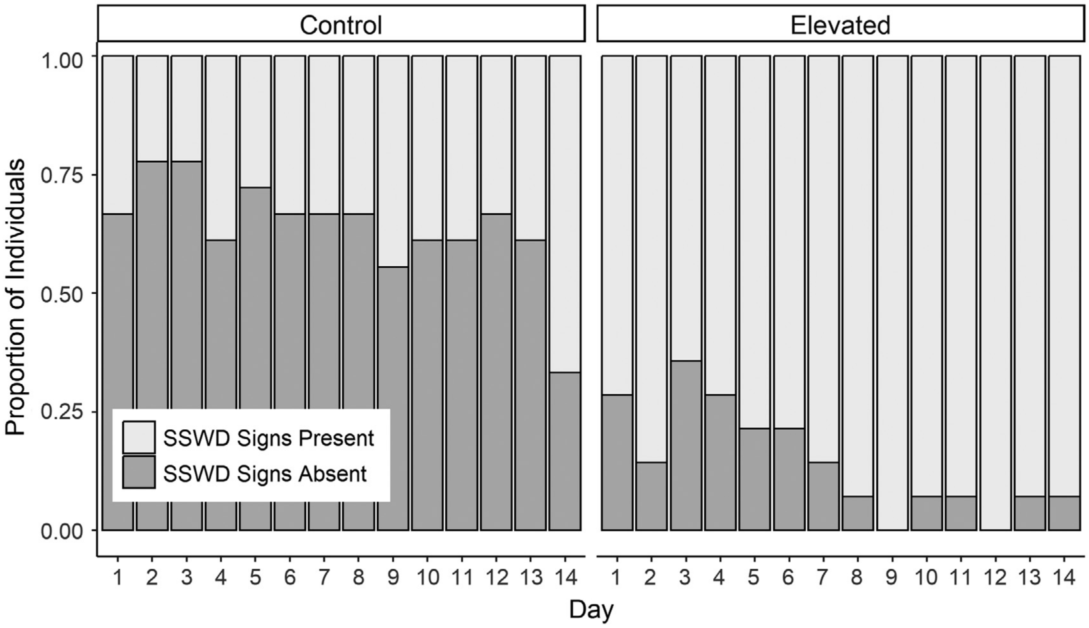

Fig. 2. Proportion of *Asterias rubens* that were grossly normal versus those expressing any sign of sea star wasting disease (SSWD) for each day of temperature treatment exposure (control and elevated)

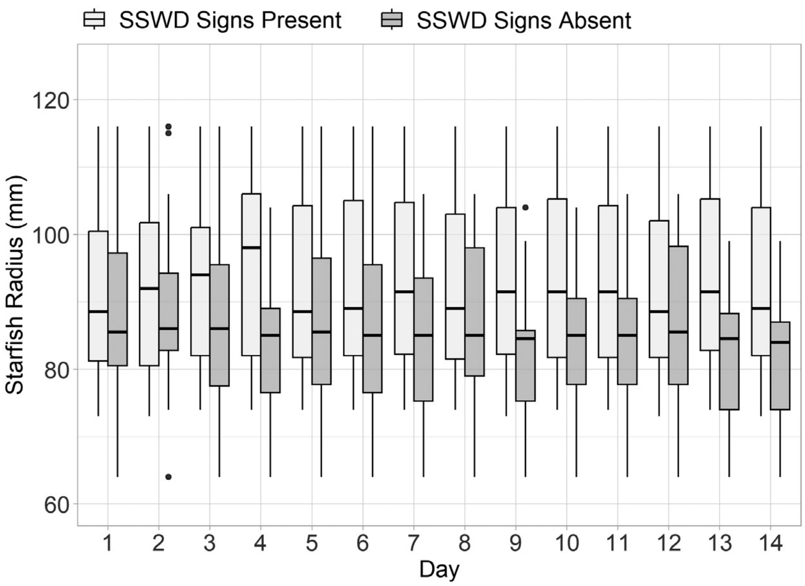

Fig. 3. Sea star radius in grossly normal *Asterias rubens* compared to animals with any sea star wasting disease (SSWD) sign present over the 14 d temperature exposure experiment. Boxplots: median and 25th and 75th percentiles; whiskers: upper and lower data bounds; points: outliers

3.2. Disease severity

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

Model fit was improved significantly ($p < 0.001$) when size was incorporated into the model as a fixed effect (Table 3). The resulting effect of the temperature treatment was 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.

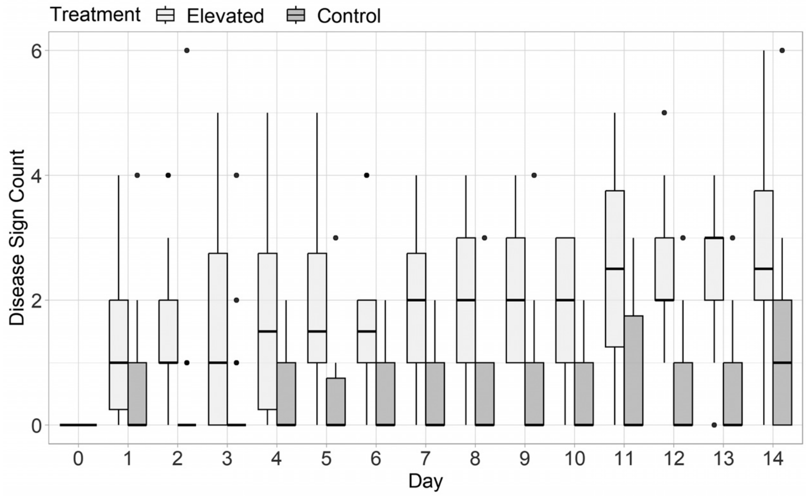

Fig. 4. Number of observed signs of sea star wasting disease (SSWD) for each sea star exposed to differing temperature treatments over the 14 d exposure period, including Day 0. Boxplot parameters as in Fig. 3. Animals that died were removed from the data set (n = 4). Control treatment: 12°C, n = 18; elevated temperature treatment: 18°C, n = 14

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), thus differing from the analysis of the sum of disease signs score (Table 3).

3.4. Survival

In total, 4 animals died during the experiment (Fig. 6). All these individuals were in the elevated temperature treatment and the deaths occurred between Day 6 and Day 8. All individuals displayed signs consistent with SSWD prior to death, including loss of body turgor, bloating in the arms, and the appearance of white lesions. In 3 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 1 d of the first observation of these signs.

Table 3. Effect of elevated temperature on sea star wasting disease sign scores in *Asterias rubens*. (a) Terms, coefficients, and p-values for the best fitting model; (b) Akaike's 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 ($p < 0.05$) are highlighted in **bold**

(a) Terms	Estimate	SE	*z*	p
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 + sea star radius + treatment	1050.5	−518.27
Day × treatment	1062.0	−525.03
Day × treatment	1063.3	−524.65

No mortalities were recorded in the control treatment; therefore, 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 2 treatments ($\chi^2 = 4.4$, df = 1, $p < 0.04$). Survival probability in the elevated temperature treatment after 14 d of exposure was 0.778 ± 0.098, 95 % CI = 0.608–0.996.

Table 4. Effect of elevated temperature on sea star wasting disease category in *Asterias rubens*. Table displays terms, coefficients, and 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 ($p < 0.05$) are highlighted in **bold**

Term	Estimate	SE	*z*	p
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**

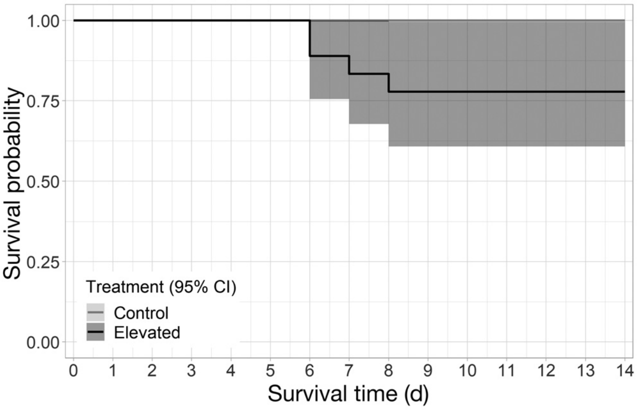

Fig. 6. Kaplan-Meier survival plot of *Asterias rubens* subjected to different temperature treatments over a 2 wk period. Control treatment: 12°C, n = 18; elevated temperature treatment: 18°C, n = 18. Shaded area: 95 % CI

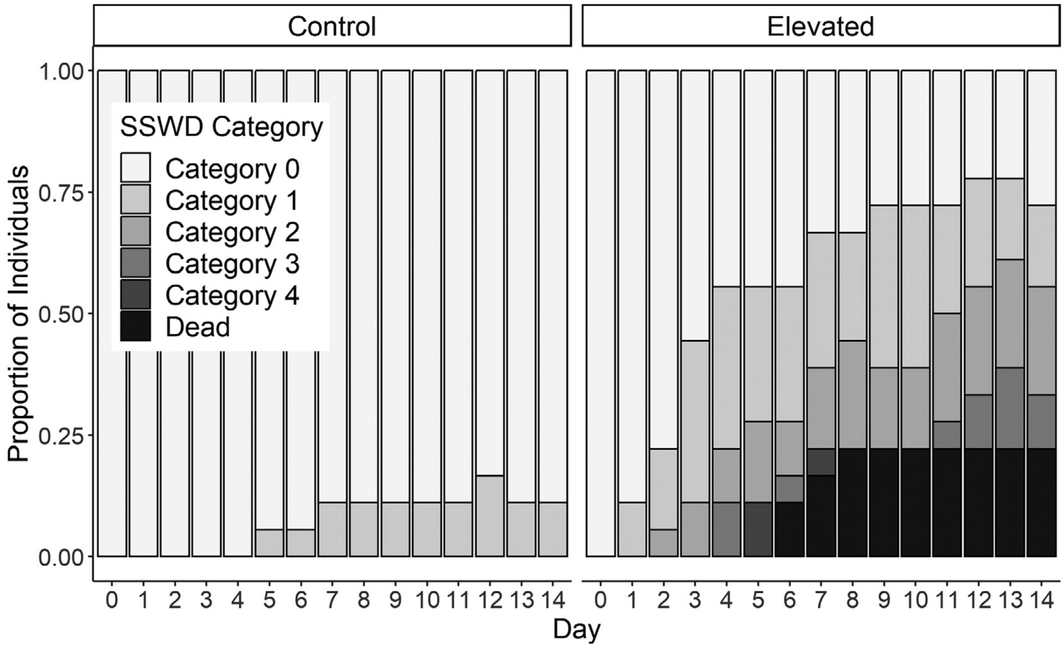

Fig. 5. Proportion of *Asterias rubens* assigned to discrete sea star wasting disease (SSWD) categories when exposed to different temperature treatments over 14 d. Control treatment: n = 18; elevated temperature treatment: n = 18

4. DISCUSSION

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

4.1. Disease severity

With elevated temperature influencing SSWD sign expression in *A. rubens*, our work supports the idea that there is a link between SSWD signs and environmental stressors. The first SSWD signs visually occurred after 24 h, and sea stars in the elevated temperature treatment maintained a higher disease sign score for the duration of the experiment (Fig. 4). Prolonged exposure also acted to increase total disease sign score. Although the temperature treatments that we 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 conditions. Therefore, conclusions of wider ecological consequences must be treated with caution. It is apparent, however, that rapid and sustained exposure to elevated temperature did induce signs consistent with SSWD, thus supporting the link between environmental stressors and the condition.

Elevated temperatures result in high cellular oxygen demand due to increased metabolic 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, 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.

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 system function in echinoderms, was found to increase with higher temperatures (Wahltinez et al. 2023) and may indicate that an inflammatory response was occurring. A similar effect was reported for *Pisaster ochraceus* with active wasting signs (Work et al. 2021), showing an energetic cost.

Echinoderms have mutable collagenous tissue (MCT), which allows passive changes to the mechanical properties of the body structure (Wilkie 2005). MCT is under direct neural 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, L. C. Smith et al. 2022), and disruption to control of MCT may also be responsible for the general lack of body turgor, observations of bloating and/or pinching of arms, and abnormal body posture (Work et al. 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 to be temperature-sensitive. These results also mirror those found during experimental temperature elevation with *Astropecten jonstoni* (Staehli et al. 2009) and *P. ochraceus* (Bates et al. 2009).

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 is related to greater oxygen stress placed on animals with smaller surface-to-volume ratios (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 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, as the response is brought under control.

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). 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 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 (Hewson 2021). The combination of temperature-associated inflammation-weakened immune function, MCT dysfunction, and progression to lesions and tissue necrosis may all be further confounded by opportunistic microorganisms inducing hypoxia at the boundary layer. Our observations support this mechanism of action, with greater susceptibility to SSWD at higher temperatures and effects felt more greatly by larger individuals.

4.2. Disease category

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

The categorical assessment diverges from disease sign score due to the lack of consideration of abnormal posture i.e. bloating and/or pinching, contortion of arms, and loss of body turgor. This difference is reflected by the fact that all animals in the control treatment were categorised as healthy until Day 5, while disease sign observations began on Day 1 when all signs are counted (Figs. 4 & 5). This discrepancy is a result of bloating and loss of turgor being observed in some individuals in the control treatment. This may also explain the non-significant effect of size in the categorical assessment of disease, in which bloating loss of turgor are not 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 this condition. 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.

By contrast, asteroids appear to present a limited suite of disease signs, and these may not be consistent with a single aetiology (Hewson 2021). Therefore, it is possible that a 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 response unrelated to temperature. This may account for observations of disease signs in the control treatment (Fig. 2), and that these signs are indicative of stress associated with handling and water quality deteriorations over the intermediary 24 h between water changes.

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

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 no mortality occurring in the control treatment, deaths were all associated with SSWD signs on 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 *A. jonstoni*, 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.

In the Pacific, elevated temperature was linked to increased mortality rates in *P. ochraceus* (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 2014 outbreak of SSWD. As our mortality rates were comparatively low, we were unable to perform an analysis of the effect of size on survival in *A. rubens*. However, due to the combined effect of increased metabolic activity and reduced oxygen solubility at higher temperatures, smaller animals may have an advantage under warming scenarios due to higher surface-to-volume ratio for diffusion (Rühmkorff et al. 2023).

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 ± 0.3 cm; smaller than the 8.9 ± 1.2 cm 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 SSWD sign expression, further targeted research is needed to demonstrate the effect of size on overall survival and mortality.

4.4. Ecological implications

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 between environmental stressors and SSWD-associated signs and mortality in *A. rubens*. As with 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 al. 2008). The possibility of thermal association of SSWD in *A. rubens* is concerning given projected climate change scenarios. The increasing frequency of extreme climatic events, such as marine heatwaves, is predicted to have widespread and significant impacts on marine ecosystems (Oliver et al. 2019, Rühmkorff et al. 2023). Through predation on bivalves, sea stars increase the possibility for settlement and attachment of other organisms. Declines in *A. rubens* populations would remove this important aspect of benthic ecology and encourage overgrowth of mussels in naturally biodiverse habitat. Furthermore, 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 outbreaks in North Atlantic sea stars (S. Smith et al. 2022).

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 has not been ruled out. Regarding hypoxia, enrichment events may themselves be associated with thermal fluxes, and therefore disentangling different environmental stressors as a potential trigger for SSWD onset may be challenging (Aquino et al. 2021, Hewson 2021). Furthermore, 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 of 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, but this has not been systematically observed to date.

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

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