nature ecology & evolution

Article

https://doi.org/10.1038/s41559-025-02797-2

Vibrio pectenicida strain FHCF-3 is a causative agent of sea star wasting disease

Received: 17 January 2025

Accepted: 18 June 2025

Published online: 04 August 2025

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More than 10 years following the onset of the sea star wasting disease (SSWD) epidemic, affecting over 20 asteroid species from Mexico to Alaska, the causative agent has been elusive. SSWD killed billions of the most susceptible species, sunflower sea stars (*Pycnopodia helianthoides*), initiating a trophic cascade involving unchecked urchin population growth and the widespread loss of kelp forests. Identifying the causative agent underpins the development of recovery strategies. Here we induced disease and subsequent mortality in exposure experiments using tissue extracts, coelomic fluid and effluent water from wasting sunflower sea stars, with no mortality in controls. Deep sequencing of diseased sea star coelomic fluid samples from experiments and field outbreaks revealed a dominant proportion of reads assigned to the bacterium *Vibrio pectenicida*. Fulfilling Koch's postulates, *V. pectenicida* strain FHCF-3, cultured from the coelomic fluid of a diseased sunflower sea star, caused disease and mortality in exposed sunflower sea stars, demonstrating that it is a causative agent of SSWD. This discovery will enable recovery efforts for sea stars and the ecosystems affected by their decline by facilitating culture-based experimental research and broad-scale screening for pathogen presence and abundance in the laboratory and field.

In 2013, sea star wasting disease (SSWD) emerged along the Pacific Coast of North America, quickly becoming the largest documented marine epidemic recorded in a non-commercial species[1,2]. Of the more than two dozen asteroid species affected by SSWD[3], the sunflower sea star (*Pycnopodia helianthoides*) is the most susceptible to disease[4]. Once ranging in often high densities from Baja California, Mexico, to Alaska, USA, *P. helianthoides* is now considered functionally extinct throughout much of its southern range in the continental USA, with losses exceeding 87% in the northern refuges where it still persists[5]. As a result, *P. helianthoides* has been listed as critically endangered by the International Union for Conservation of Nature[6] and is being recommended for threatened status under the US Endangered Species Act[7]. In turn, the rapid disappearance of sunflower sea stars has been linked to a trophic cascade involving unchecked population growth of urchins, and subsequent overgrazing and widespread loss of kelp forests along the Northeast Pacific Coast[8]. There is a need to identify the causative agent of SSWD to enable broad-scale research on disease dynamics in this multihost system and to facilitate the development of mitigation strategies for the sunflower sea star and the ecosystems they support[9].

Over a decade of research on SSWD has suggested a range of proposed aetiological agents. Early experimental work identified a new densovirus as a possible causative agent[2]; however, inconsistencies in experimental results and molecular evidence have since refuted this finding[10–13]. Subsequent hypotheses have been advanced[14,15]; however,

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no definitive candidate(s) have been identified, and histological examination of diseased tissue indicated tissue damage but no causative agent[2]. Here we use controlled exposure experiments, genetic datasets and field observations to demonstrate that the bacterium, *Vibrio pectenicida* strain FHCF-3, is a causative agent of SSWD.

Results

Controlled exposure experiments

Between 2021 and 2024, we conducted seven controlled exposure experiments on grossly normal (that is, absent of visible disease signs) sunflower sea stars collected subtidally in WA, USA, or captive-bred at Friday Harbor Laboratories, WA, USA (Supplementary Tables 1 and 2). We conducted experiments at the US Geological Survey (USGS) Marrowstone Marine Field Station, where we housed all individuals in isolation at all times to prevent disease transmission. In the laboratory, grossly normal stars were subjected to a minimum 2-week quarantine to account for potential prior disease exposure, and we classified individuals as 'apparently healthy' if they showed no disease signs during this period (grossly normal upon collection and following a 2-week quarantine).

Before the identification of a candidate causative agent, all experiments relied on the collection of diseased sunflower sea stars from the field to provide tissues for exposures. We tested several exposure modalities on apparently healthy stars, including immersion in seawater effluent from diseased star tanks and injection with inoculum made of tissue homogenate or coelomic fluid from diseased star(s). We also used direct contact (cohabitating a diseased star with an apparently healthy star) to maintain disease in the laboratory. The most common method of exposure was injection, with experiments following the same basic methodology: (1) isolation of tissues from a diseased star, (2) preparation of inoculum for exposed and control treatment groups, (3) injection of apparently healthy stars and (4) experiment monitoring and sampling (Fig. 1).

All exposure modalities resulted in transmission, with disease signs and mortalities occurring in 46 of 50 (92%) previously apparently healthy individuals. The characteristic pattern of disease signs started with arm twisting (also referred to as limb curling or ray dysplasia[2,10,11]), followed by arm autotomy and then mortality (Fig. 2 and Extended Data Fig. 1). Mortality occurred as early as 6 days post exposure (DPE) and stars died in less than 2 weeks (mean = 11.6 ± 3.3 days). The time from visible disease signs to mortality occurred within as little as 3 days and typically stars died within a week following first signs of disease (mean = 5.6 ± 2.3 days).

Each experiment included negative controls, which involved either injection with heat-treated versions of the same tissue homogenate (n = 36) or coelomic fluid (n = 18) inocula used for the exposed treatment groups or exposure to effluent seawater originating from a tank containing a star injected with heat-treated coelomic fluid inoculum (n = 6). Within these control groups, no arm autotomy occurred, and all individuals survived until the end of the experiments (Supplementary Table 2). Arm twisting was occasionally observed in controls; however, significantly more arm twisting was observed in exposed individuals across all experiments, regardless of exposure method used (Extended Data Fig. 2 and Supplementary Table 3). To confirm that injection with coelomic fluid alone does not cause disease signs, we assessed the response of stars exposed to coelomic fluid taken from an apparently healthy star, which did not cause disease signs or mortality in any individuals (n = 11).

Disease was not induced in any individuals injected with 0.22-µm filtrate (19 individuals across two independent experiments) or heat-treated inocula (54 individuals across four independent experiments), but was transmitted to most individuals injected with unfiltered tissue homogenate (24 of 26 individuals across two independent experiments) or coelomic fluid inocula (16 of 18 individuals across two independent experiments) sourced from diseased stars, suggesting that the pathogen was cellular (Supplementary Table 2). Given these results, we performed an exposure experiment whereby we injected unfiltered coelomic fluid from a diseased star or its corresponding heat-treated control into apparently healthy stars (n = 8 stars per treatment group). Individuals were monitored for 3 weeks, during which all exposed stars exhibited disease signs and died. Arm twisting occurred 3–12 DPE, followed by arm autotomy 5–13 DPE and mortality 6–13 DPE (Fig. 2a). No mortality occurred in control stars. Throughout the experiment, we collected coelomic fluid for sequencing to compare the microbial communities in stars exhibiting disease signs to those from apparently healthy controls. Samples were collected from all stars before injection (n = 16), from control stars 5 days post injection (n = 8) and from exposed stars coincident with disease signs, including when >25% of an individual's arms were twisted (n = 8) and following each individual's first autotomized arm (n = 7). A sample of the inoculum was also collected for sequencing. From these samples we co-extracted RNA and DNA to generate both metatranscriptomic and 16S ribosomal RNA gene amplicon sequencing datasets, respectively (Supplementary Table 4).

Metatranscriptomic and 16S rRNA gene amplicon sequencing

We first used metatranscriptomic sequencing to profile the suite of microbes (bacteria, protists, RNA viruses and DNA viruses) associated with diseased and apparently healthy stars (n = 28, Supplementary Table 4). We trimmed reads, aligned them to the host (*P. helianthoides*) genome[16] (National Center for Biotechnology Information (NCBI) accession ASM3215829v1) and assembled unmapped (non-host) reads into contigs, which we annotated against the NCBI non-redundant nucleotide database using the BLASTx function of DIAMOND (v.2.1.8.162)[17]. Results were filtered to retain only annotations of high quality, resulting in 58,629 annotations largely dominated by bacteria (n = 55,257 contigs), in comparison to eukaryotes (n = 2,521), archaea (n = 475) and viruses (n = 376) (Supplementary Data 1). A principal coordinate analysis (PCoA) on a normalized contig count matrix explained 49% of the variation in the dataset and created two clusters of samples: samples from (1) stars showing disease signs and (2) apparently healthy stars (Fig. 3). The vectors explaining the most variation separating the two groups ($r^2 \geq 0.90$) included sequences assigned to bacteria in the family Vibrionaceae, the genus *Vibrio* and the species *V. pectenicida*, which were all associated with samples from diseased stars.

Across all annotated metatranscriptomic contigs, only three microbial taxa exhibited the classic presence/absence pattern strongly indicative of a disease agent (that is, present in all samples from diseased stars (n = 7) and absent from apparently healthy star samples (n = 21)). Of the 8,618 contigs assigned to these annotations, 95.7% belonged to the species *V. pectenicida*, 3.4% to *V. hepatarius* and 0.9% to *V. aquimaris* (Supplementary Data 1). The high proportion of sequences assigned to *V. pectenicida* in samples from diseased stars, and their absence from apparently healthy star samples, suggested *V. pectenicida* as a strong candidate causative agent of SSWD.

In addition to metatranscriptomic libraries, we generated a dataset of 16S rRNA gene sequences on the same samples (n = 34, Supplementary Table 4), to target the bacterial community. We analysed these data using QIIME 2 (ref. 18), with taxonomy assigned by a naive Bayes classifier trained on the Greengenes 2 16S V4 marker gene reference database[19,20]. Analysis of compositions of microbiomes with bias correction (ANCOM-BC; ref. 21) identified a single species, *V. pectenicida*, as significantly enriched in diseased star samples (log(FC) = 8.72, s.e. = 0.60, $q = 1.12 \times 10^{-44}$). Although we detected reads assigned to *V. pectenicida* in 62.5% of apparently healthy star samples (n = 24), its relative abundance averaged only 23 reads, accounting for <1% of all assigned reads. In comparison, we detected reads assigned to *V. pectenicida* in all samples from diseased stars (n = 9) with an average of 93,401 reads, accounting for 23–99% of all assigned reads (Fig. 4, Supplementary Table 5 and Supplementary Data 2).

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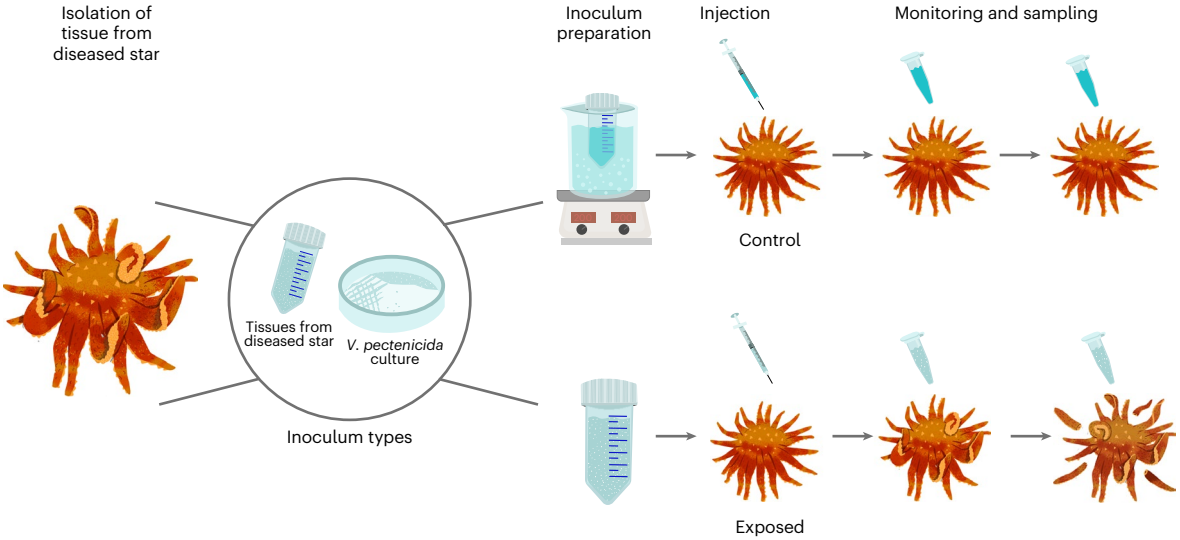

Fig. 1 | A conceptual overview of exposure experiments used to identify the causative agent of SSWD. For initial investigations, inoculum was prepared from the tissues of a diseased star collected from the field. Inoculum was prepared from tissue homogenate or coelomic fluid for exposed (untreated inoculum; bottom row) and control (heat-treated inoculum prepared by submerging inoculum vial in boiling water on a heat block; top row) treatment groups. We injected several individuals and monitored stars for 2–4 weeks. Exposed and control stars were subsampled throughout, generally corresponding to the timing of disease signs in exposed stars (arm twisting and arm autotomy). Following preliminary identification of *V. pectenicida* as a candidate pathogen for SSWD, subsequent exposure experiments used inoculum made from a pure culture of *V. pectenicida* (strain FHCF-3 or isolate FHCF-5) that we isolated from the coelomic fluid of a diseased sunflower sea star. Inoculum preparation, injection, monitoring and sampling remained the same. Details for each of the seven independent experiments outlined in this work (for example, treatment groups, inoculum type, replicate numbers and experimental results) are provided in Supplementary Table 2.

Combined, the metatranscriptomic and 16S rRNA gene sequencing results converged on an association of *V. pectenicida* with SSWD. Notably, the 16S rRNA gene data were more sensitive to low-level detection of *V. pectenicida* in apparently healthy star samples (0–72 reads annotated as *V. pectenicida* accounting for <1% relative abundance), which we did not observe in the metatranscriptomic contig sequences assembled from these samples (Supplementary Table 6). The presence of *V. pectenicida* in apparently healthy star samples is not surprising given that the stars used in these experiments were probably exposed to SSWD at some point before collection; SSWD outbreaks remain ongoing, and the geographic distribution of this epidemic reaches all populations sampled for this work. Our data suggest that low levels of *V. pectenicida* can be tolerated under favourable conditions, which could turn into outbreaks with a change in environmental conditions that favour the pathogen and/or immunocompromise the host (for example, temperature[4,5]). Indeed, *Vibrio* spp. have been coined 'the microbial barometer of climate change', because of the increasing prevalence of pathogenic species associated with warming water temperatures[22]. Given that existing evidence indicates a relationship between increasing seawater temperature and SSWD incidence[5,23] (but see ref. 24), an important next phase of research will be to empirically define this relationship, a goal now possible as a result of the identification of a causative agent.

Culturing and exposure experiments with *V. pectenicida*

Following identification of *V. pectenicida* as a strong candidate causative agent of SSWD, we collected coelomic fluid from two stars exhibiting disease signs (arm twisting) in Friday Harbor. We isolated bacterial colonies by spreading coelomic fluid onto marine luminescent bacteria (MLB) agar media[25–27] (0.05% w/v each of casamino acids, peptone and yeast extract, 0.3% v/v glycerol, in a natural seawater base; solidified with 1.2% w/v agar) and incubating at 21 °C for 5–7 days. Axenic clonal cultures were created by selecting and restreaking individual colonies onto new plates. We confirmed pure cultures of *V. pectenicida* by Sanger sequencing of polymerase chain reaction (PCR)-amplified 16S rRNA gene fragments from several isolates (Supplementary Table 7).

To establish a causal relationship between *V. pectenicida* and SSWD, we exposed apparently healthy stars to an inoculum prepared from a pure culture (Fig. 1). In the first experiment, we tested two isolates, *V. pectenicida* FHCF-3 and FHCF-5, for pathogenicity. This experiment comprised four treatment groups ($n = 3$ stars per treatment group); stars were injected with ~10^7 colony-forming units (c.f.u.) of *V. pectenicida* isolate FHCF-3, FHCF-5 or their heat-treated controls. All the stars exposed to live bacteria ($n = 6$) developed disease signs and died, while all control stars, exposed to heat-treated bacteria ($n = 6$) survived the 4-week experiment. For the exposed stars, disease signs and trajectory did not differ between isolates FHCF-3 and FHCF-5; arm twisting started 2–7 DPE followed by arm autotomy 5–7 DPE and mortality 5–7 DPE (Fig. 2b). From this experiment we re-isolated bacteria from the coelomic fluid of diseased stars and confirmed its identity as *V. pectenicida* by Sanger sequencing of PCR-amplified 16S rRNA gene fragments (Supplementary Table 7).

Given that both *V. pectenicida* isolates caused disease, experiments proceeded with a single isolate, FHCF-3. To further confirm the species and strain identity of this isolate, we obtained complete 16S rRNA gene sequences from whole-genome sequencing[28]. *V. pectenicida* isolate FHCF-3 has nine copies of the 16S rRNA gene, with nucleotide similarity among copies varying between 99.12% and 99.94% (NCBI accessions PQ700178 and PQ763222–PQ763229). We constructed a phylogenetic tree including 147 *Vibrio* spp. 16S rRNA gene sequences, which clustered isolate FHCF-3 with other known *V. pectenicida* strains (NCBI accessions Y13830, NR_118241 and NZ_VTXC01000112) in a clade with high bootstrap support (100/100; Extended Data Fig. 3). Notably, the 16S rRNA gene sequence of strain FHCF-3 has 99.29% average nucleotide identity (ANI) to the great scallop (*Pecten maximus*) pathogen, *V. pectenicida* strain Ifremer A365 (NCBI accession Y13830)[29]. In addition

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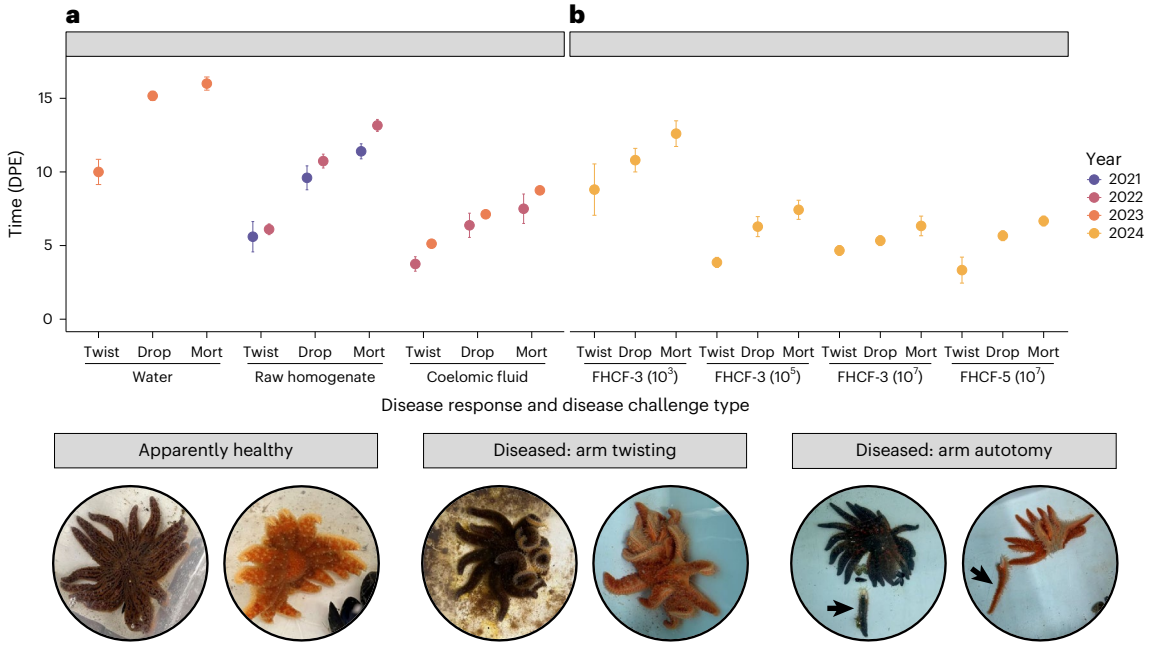

Fig. 2 | The disease signs and trajectory of *P. helianthoides* exposed to SSWD. **a**,**b**, Top: disease trajectory of sunflower sea stars exposed to SSWD. Data represent 65 individual mortalities (excluding five individuals that were exposed but did not die), summarized by exposure method and year of experiment. Responses are grouped into three types, arm twisting (twist), arm autotomy (drop) and mortality (mort). Symbols indicate the average number of DPE when each response was observed. Exposure methods include immersion in effluent water from the vicinity of diseased stars (water) in 2023 (**a**, $n = 6$), injection with raw homogenate made from diseased star tissues in 2021–2022 (**a**, 2021 $n = 5$ and 2022 $n = 19$), injection with coelomic fluid taken from diseased stars in 2022–2023 (**a**, 2022 $n = 8$ and 2023 $n = 8$) and injection with cultured *V. pectenicida* in 2024 (**b**, FHCF-3 10^3 $n = 6$, FHCF-3 10^5 $n = 7$, FHCF-3 10^7 $n = 3$ and FHCF-5 10^7 $n = 3$). Cultured *V. pectenicida* is indicated by the isolate ID (FHCF-3 or FHCF-5) and the approximate number of c.f.u. injected. The error bars indicate 1 s.e. Control groups were included in all experiments, for which no arm autotomy or mortality was observed. The presence of *V. pectenicida* was confirmed in all inocula by amplifying and sequencing 16S rRNA gene fragments (Supplementary Table 11). Bottom: examples of apparently healthy (no disease signs observed upon collection or during a 2-week quarantine) and diseased (showing disease signs following exposure to SSWD) sunflower sea stars. Disease signs include arm twisting and arm autotomy. Autotomized arms are indicated by black arrows.

to phylogenetic analysis, we calculated ANI using whole-genome sequences, which ranged from 97.7% to 98.0% between strain FHCF-3 and the three published genomes of *V. pectenicida* strains available on NCBI (Supplementary Table 8). In comparison, ANI between strain FHCF-3 and other phylogenetically closely related *Vibrio* species (*V. ostreicida*, *V. tapetis* and *V. penaeicida*) ranged between 71.1% and 73.5% (Supplementary Table 8). Combined, phylogenetic analysis of the gene coding 16S rRNA, as well as comparative analysis of the complete genome are consistent with strain FHCF-3 belonging to the species *V. pectenicida*.

Following induction of SSWD with cultured *V. pectenicida*, we conducted a second exposure experiment testing two lower doses of *V. pectenicida* strain FHCF-3. The experiment comprised four treatment groups ($n = 7$ stars per treatment group), with stars injected with either a high (~10^5 c.f.u.) or low (~10^3 c.f.u.) dose of *V. pectenicida* strain FHCF-3 or their respective heat-treated controls. For stars exposed to live bacteria, 13 of 14 individuals developed disease signs and died. Disease trajectory proceeded faster for stars exposed to the higher dose, where arm twisting started 3–10 DPE, arm autotomy 5–11 DPE and mortality 6–11 DPE (Fig. 2b). In comparison, arm twisting started 5–15 DPE, arm autotomy 10-15 DPE and mortality 11–16 DPE in the low-dose treatment group (Fig. 2b). One star in the low-dose treatment group survived to the end of the experiment, with a single arm twist 12 DPE, but otherwise showing no disease signs. A water temperature spike (maximum 13.9 °C) occurred between experimental days 0 and 2 from seasonal warming in the region and during this time period 43% of controls showed transient signs of arm twisting in one or two arms maximum. Despite this, there was no arm autotomy or mortality in any control stars throughout the 3-week period of observation.

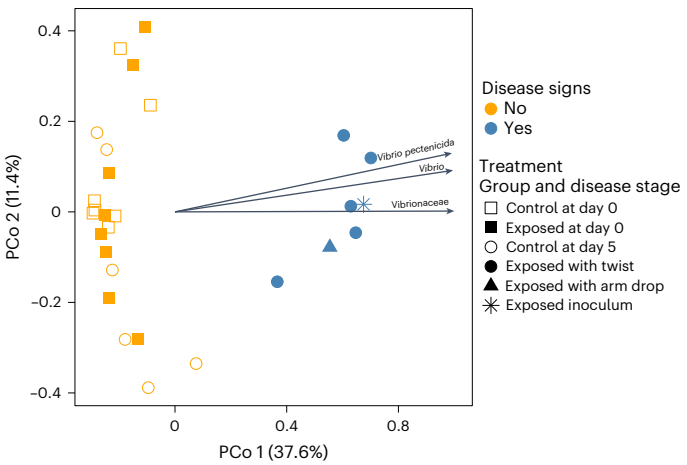

Fig. 3 | PCoA plot of a normalized contig count matrix of microbial species identified from metatranscriptomic sequencing data generated from controlled experimental exposures of *P. helianthoides* to SSWD. Exposed stars were injected with coelomic fluid inoculum taken from four diseased sunflower sea stars. Control stars were injected with the same dose of the coelomic fluid inoculum, which was heat-treated. Day 0 represents samples taken from stars before injection. Twist is a disease stage characterized by stars twisting >25% of their arms (observed 3–12 DPE). Arm drop is a disease stage characterized by stars autotomizing at least one arm (observed 5–13 DPE). A sample of the inoculum used to start the experiment is also included (*). Microbial species were fit onto the ordination using two-sided multiple regression testing (envfit) and species plotted as vectors were chosen on the basis of the coefficient of determination ($r^2 \geq 0.90$).

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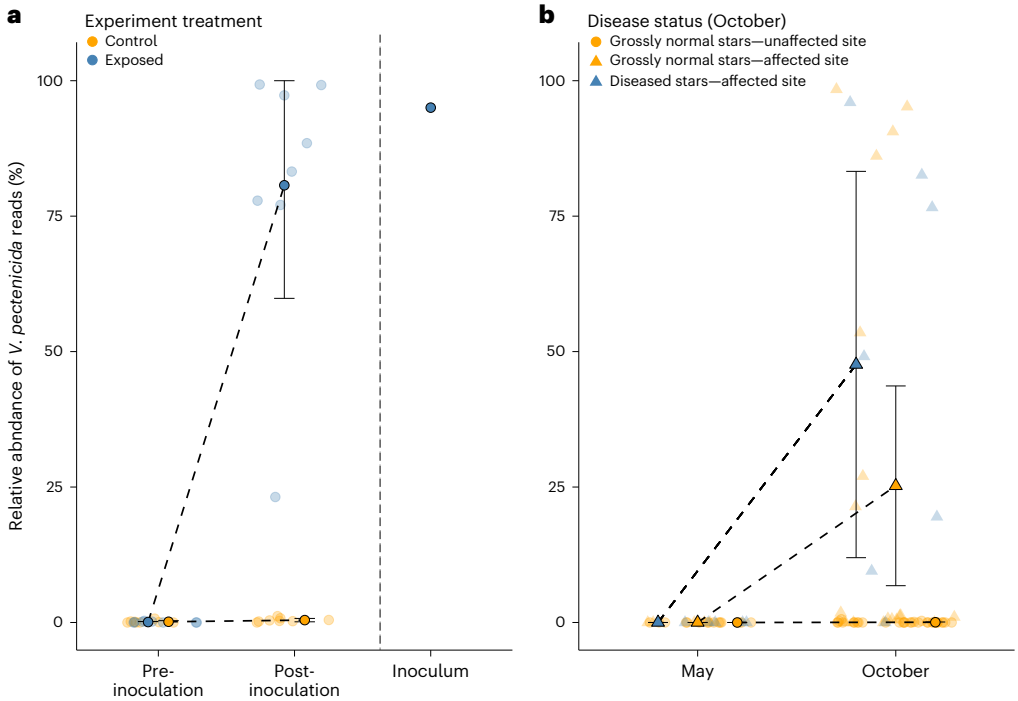

Fig. 4 | Relative read abundance of *V. pectenicida* in 16S V4 rRNA gene sequencing datasets. a,**b**, Datasets were generated from a controlled exposure experiment to (**a**) and a field outbreak of (**b**) SSWD in *P. helianthoides*. Raw data are shown as semitransparent symbols with averages (±95% confidence interval) shown by solid symbols outlined in black. **a**, Apparently healthy (no visible disease signs following 2-week quarantine) control stars before injection (pre-inoculation, $n = 8$) and after injection (post-inoculation, $n = 8$) with heat-treated coelomic fluid inoculum (orange), and exposed stars before injection (pre-inoculation/apparently healthy, $n = 8$) and after injection (post-inoculation/showing disease signs, $n = 8$) with coelomic fluid inoculum (blue). Relative abundance of *V. pectenicida* in the coelomic fluid inoculum used to expose stars is also shown as a solid symbol (not an average). Disease signs and mortality were observed in all exposed individuals and all controls survived. Pre-inoculation samples were collected before injection. Post-inoculation samples were collected 5 d post-inoculation (controls) or coincident with the observation of disease signs (exposed; 3–12 d and 5–13 d post-inoculation for arm twisting and arm autotomy, respectively). **b**, Grossly normal stars sampled in May (when no SSWD outbreaks were observed, $n = 25$) and October (when two of five sites had SSWD outbreaks, $n = 57$). Colours represent the disease status of stars sampled in October; grossly normal (no visible disease signs at sampling, orange, $n = 50$ of 57 samples) and diseased (blue, $n = 7$ of 57 samples). Shapes correspond to site-level data, representing sites with (affected, triangle) and without (unaffected, circle) SSWD outbreaks in October.

Screening for *V. pectenicida* in field samples

In addition to controlled exposure experiments, we generated 16S rRNA gene amplicon libraries from sunflower sea star coelomic fluid samples collected from five sites in the fjords of British Columbia, Canada, in 2023 (Supplementary Table 9). We sampled each site at two time points. In May, none of the five sites showed evidence of SSWD (unaffected); however, by October, two of the five sites contained sunflower sea stars showing disease signs (affected). From the affected sites, we collected samples from grossly normal stars and stars showing early disease signs (arm twisting but no arm autotomy).

From these data, we confirmed the presence of *V. pectenicida* in a field outbreak of SSWD. In May, when SSWD was not observed, no sequences were classified as *V. pectenicida* in any star samples ($n = 25$). In contrast, in October, when SSWD was observed in two populations, *V. pectenicida* sequences were detected in ~16% ($n = 31$) of stars sampled from apparently unaffected sites, ~74% ($n = 19$) of grossly normal stars sampled from affected sites and ~86% ($n = 7$) of diseased stars sampled from affected sites. Similarly, the relative abundance of *V. pectenicida* sequences was substantially lower in stars from unaffected sites (<1% of all assigned reads), then stars from affected sites (~25% and ~48% of all assigned reads in grossly normal and diseased stars, respectively) (Fig. 4, Supplementary Table 10 and Supplementary Data 3). An ANCOM-BC analysis on samples from October identified *V. pectenicida* as the only significantly enriched taxa in stars sampled from SSWD affected sites ($\log(FC) = 5.95$, s.e. = 0.84, $q = 3.36 \times 10^{-10}$).

To further explore the presence of *V. pectenicida* in field outbreaks of SSWD, we re-analysed the 16S rRNA gene amplicon data of ref. 30 collected in 2016 from wild populations of sunflower sea stars in southeast Alaska, when the SSWD epidemic was first observed in this area. From these data we identified 67 sequences classified as *Vibrio* spp., 11 of which we assigned to *V. pectenicida* (percentage identity >99%) based on a BLASTn search of the NCBI 16S rRNA database. Across samples, we identified sequences assigned to *V. pectenicida* in all groups, but with higher prevalence in diseased (~67%, $n = 18$) and grossly normal (~60%, $n = 20$) star samples from affected sites, in comparison to those from an unaffected site (~6%, $n = 47$). These data indicate that *V. pectenicida* was present during the original spread of the SSWD epidemic into sunflower sea star populations in southeastern Alaska.

Discussion

An isolate of *V. pectenicida* was first obtained in an aquaculture hatchery in France from moribund scallop larvae[29,31] and phylogenetically designated a new species (type strain A365)[29]. Exposure studies demonstrated pathogenicity of *V. pectenicida* to both great scallop and oyster (*Ostrea edulis* and *Crassostrea (Magallana) gigas*) larvae[32–34], by inhibiting the chemiluminescent activity of haemocytes within hours of infection[32,35]. The pathogenic mechanism has been attributed to a heat-stable toxin, named vibrio hemocyte-killer toxin[36]. Histological examination of exposed scallop larvae revealed no evidence of *V. pectenicida* cell walls, despite positive immunostaining confirming infection of host cells[33]. The failure to detect *V. pectenicida* in histological samples from infected scallop larvae could help to explain the absence of visible bacteria across hundreds of diseased sea star tissue samples examined early in the epizootic[2]. Given that *V. pectenicida* strain FHCF-3

causes SSWD, future research could explore the mechanism(s) of disease in sea stars and whether it is toxin mediated.

Outside Europe, *V. pectenicida* has been isolated from sick geoduck (*Panopea generosa*) seed in an aquaculture hatchery in WA, USA, in 2000[37]; this strain (99-46-Y) is the closest known relative of FHCF-3, with an ANI of 98.04%, supporting high genetic similarity between the two characterized North American strains of *V. pectenicida* (Supplementary Table 8). Within the Global Biodiversity Information Facility database, occurrence records for *V. pectenicida* collected between 2009 and 2019 span a broad geographic extent, including Australia, Asia, Europe and the USA[38]. Given that these records are primarily from seawater samples, and thus not associated with hosts, their pathogenic potential broadly and affiliation with SSWD more specifically, is unknown. Further characterizing the known distribution, strain diversity and pathogenicity mechanisms of this pathogen are important potential future research directions. For strains with confirmed associations to SSWD, elucidating the routes of transmission, both across hypothesized asteroid hosts as well as the potential for transmission from bivalve prey will be critical for effective management.

The challenge of identifying a causative agent for SSWD could in part stem from the fact that *V. pectenicida* can be detected in samples from some grossly normal stars. For example, several samples from our field collections of grossly normal individuals contained high relative abundances of *V. pectenicida* sequences, particularly those sampled nearby ongoing SSWD outbreaks. In contrast, controlled laboratory exposure experiments allow for sampling of individuals with low-to-no recent exposure, as stars can be quarantined and monitored over time for disease signs. Given the speed at which SSWD progresses in sunflower sea stars (Fig. 2) there is a limited window for observing outbreaks in the field and, if SSWD is observed within a population, it is likely that some grossly normal individuals are also exposed. This highlights the importance of quarantine and controlled exposure experiments for the discovery of causative agents of disease. Despite these challenges, we retained a clear association of *V. pectenicida* with diseased stars in both field and experimental datasets when we sampled coelomic fluid. The high relative abundance of *V. pectenicida* sequences in 16S rRNA gene amplicon data from the coelomic fluid of diseased stars suggests that coelomocytes (the immune cells of echinoderms[39]) may be critical for disease development. If so, sampling alternate tissue types (for example, dermal biopsies collected in ref. 30) could be less effective for detecting *V. pectenicida*.

Since the inception of the SSWD epidemic, the clear identification of what constitutes a SSWD outbreak has been challenging. Asteroids respond to many environmental insults (for example, increased water temperature) with similar physiological behaviours (for example, limb twisting and arm autotomy), and as such, the development of a case definition for SSWD without a known pathogen has not been possible[11]. Our results provide an avenue through which samples can be tested for pathogen presence. The association of *V. pectenicida* with diseased sea star samples throughout 4 years of controlled exposure studies as well as field samples from a broad geographic (Alaska and Washington, USA, and British Columbia, Canada) and temporal (2016 and 2021–2024) range, indicates that it is the causative agent of SSWD, which we can now investigate further with broad-scale testing. Interrogation of samples across environments and host asteroid species will vastly improve our understanding of, and response to, SSWD outbreaks as we work to conserve coastal marine ecosystems affected by this disease.

Methods

Sea star collections and experimental setup

Between 2021 and 2024, we conducted seven controlled exposure experiments on grossly normal (absent of visible disease signs) sunflower sea stars (*P. helianthoides*). We used sunflower sea stars in these experiments because of their critical conservation need[6,7,9] and as their apparent high susceptibility to SSWD allowed for clear interpretation of results (most stars exposed to disease died in a consistent and predictable manner). Although research with sea stars is not explicitly regulated in the USA, we followed the ethical principal of reduction in all experiments, using the minimum number of required individuals per experiment to retain statistical power. Facilities housing the animals involved were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and inspected regularly by the Institutional Animal Use and Care Committee at the USGS, Western Fisheries Research Center.

We collected grossly normal sunflower sea stars subtidally in WA (Supplementary Table 1), with diving support from the Washington Department of Fish and Wildlife (WDFW) and community volunteer divers, under scientific collection permits issued by the WDFW (permit nos. HARVELL 21-1172 R, 22-175, 23-087, 24-053). We transported stars to the USGS Marrowstone Marine Field Station on Marrowstone Island, WA for experiments. Juvenile and adult sunflower sea stars measured between 5–20 cm and >40 cm diameter, respectively. In 2023 and 2024, we also included captive-bred sunflower sea stars in experiments, which were born from wild broodstock locally collected in 2019[40]. We received captive-bred sunflower sea stars from Friday Harbor Laboratories, WA, and transported them to the USGS Marrowstone Marine Field Station under WDFW shellfish transfer permits (permit nos. 23-1249, 24-1249). Experimental comparison of disease response showed no difference in disease signs or trajectory between wild versus captive-bred juvenile sunflower sea stars (Extended Data Fig. 1)[41].

Within the USGS Marrowstone Marine Field Station, we housed each star in its own 'tank', containing an airstone and inflowing water line. We maintained tanks inside large seawater tables, keeping each individual in physical isolation from all others at all times to prevent disease transmission. We partially flooded the seawater tables to create a water bath that maintained consistent temperature. Tanks were supplied with single-pass processed seawater, which was treated with sand filtration, particle filtration to 10–25 µm and double ultraviolet irradiation. We monitored and maintained seawater temperature below 13 °C whenever possible, and cooled seawater with an inline chiller when temperature exceeded 13 °C. We fed stars with locally collected mussels or clams three times a week. We froze all food for a minimum of 24 h before feeding to reduce the introduction of live microbes into the tanks.

As SSWD is endemic on the Pacific coast and we have no prior knowledge of the exposure history of stars collected from the field; we subjected all incoming stars to a minimum 2-week quarantine before they were used in an experiment. Given the rapid and predictable decline of sunflower sea stars when exposed to disease (<2 weeks to mortality from exposure, Fig. 2), a 2-week quarantine period was sufficient to confidently eliminate individuals that may be exposed but not yet showing disease signs from experiments. During quarantine, we checked stars at least once daily for disease signs and general condition. We classified individuals as apparently healthy if they showed no disease signs during this period (grossly normal upon collection and after a 2-week quarantine).

Challenge experiment design and sample collection

Before the identification of a candidate causative agent, all experiments relied on the collection of a diseased sunflower sea star from the field to provide tissues for exposures. For the first three years of experiments, we collected adult sunflower sea stars in late spring, a time of year with relatively low levels of disease incidence[1,42]. This timeline ensured that we could collect enough unexposed stars for experiments. Despite the low levels of disease prevalence in the field in spring, in all years at least one individual collected showed disease signs during the quarantine period and thus could be used as the source of disease for experimental exposures. Given that disease progresses quickly to death in sunflower sea stars, we established a disease line each summer to maintain a consistent source of diseased individuals for experiments, whereby

we serially cohabitated a star exhibiting disease signs with an apparently healthy star for 24–48 h to transmit and maintain disease within the laboratory for several months. Within each summer, this involved passage of disease by cohabitation about eight to ten times. Because sunflower sea stars produce similar physiological behaviours to many environmental insults (for example, increased water temperature), we conducted an initial transmission (via cohabitation) from the primary apparently diseased star collected in the field to ensure that we were working with a transmissible disease source. Once we successfully transmitted disease from the primary apparently diseased star to the second star in the disease line and quarantined all other stars, experiments began.

Initial exposure experiments emulated early strategies for transmitting SSWD[2,43] using tissue homogenate prepared from diseased sunflower sea star(s) as a source of inoculum. We prepared tissue homogenate inoculum by blending mixtures of all tissue types from diseased stars with tank water from affected stars using a Tissue Tearor Homogenizer (220/240 VAC, 0.6 A, Cole Palmer, UX-04750-55). We centrifuged homogenized tissue mixtures for 10 min at 190*g* in a Nuaire Awel centrifuge (CF 48-R) fitted with a swing rotor (SL-400 RFID) to pellet large tissue pieces and removed the supernatant to use as inoculum. While we were able to transmit disease using inoculum made from the unfiltered tissue homogenate, inoculum that was passed through a 0.22-µm filter (vacuum filtered through a 0.22-µm pore-sized polyethersulfone (PES) Millipore Steriflip Vacuum Tube Top Filter) did not elicit disease signs (Supplementary Table 2), thus conflicting with early experiments that suggested the causative agent of SSWD was viral[2]. Subsequently, we collected coelomic fluid from a diseased sunflower sea star and used it as inoculum, given that its consistency (like seawater) makes it easier to filter than tissue homogenates which often clogged filters. As with the raw tissue homogenate, injection of apparently healthy stars with unfiltered coelomic fluid from a diseased star successfully transmitted disease; however, 0.22-µm filtration of the coelomic fluid (syringe filtered through a swinnex filter holder fitted with a 1.0-µm pore-sized polycarbonate track-etched membrane filter (Sterlitech) followed by a 0.22-µm pore-size polyvinylidene difluoride filter (MilliporeSigma Millex GVWP)) consistently failed to recreate disease signs, providing additional evidence that the SSWD agent is unlikely to be viral (Supplementary Table 2).

Given that raw homogenate and coelomic fluid 0.22-µm filtrates consistently failed to recapitulate disease, we conducted an exposure experiment whereby we injected unfiltered coelomic fluid from a diseased star or its corresponding heat-treated control into apparently healthy stars (n = 8 stars per treatment group). To begin the experiment, we drew ~12.5 ml of coelomic fluid from four diseased adult sunflower sea stars (twisting of 2–11 arms) by inserting a syringe fitted with a 26G 1-inch needle into the coelomic cavity through the armpit (intersection at the base of two arms) of each star. We divided the coelomic fluid into fractions to prepare inoculum for each treatment group; we placed one fraction on ice (for exposed injections) and heat-treated another (for control injections) by immersing the tube in boiling water for 10 min to kill or inactivate the microbes present. We cooled heat-treated inoculum on ice before performing injections. To initiate the experiment, we injected a single 150-µl dose of either live or heat-treated inoculum into the coelomic cavity through the armpit of each star using a 0.3-ml syringe fitted with a 29G 1-inch needle. We monitored individuals twice daily for disease signs for a period of 3 weeks to develop a disease trajectory and took photographs of each individual once per day, with subsequent photographs taken when individuals were exhibiting disease signs. We sampled coelomic fluid from stars throughout the experiment using a 0.3-ml syringe fitted with a 29G 1-inch needle inserted into the coelomic cavity through the armpit of each star. Following retrieval, we deposited coelomic fluid samples into 1.5-ml tubes and stored them at −80 °C until the time of DNA and RNA extraction. Importantly, performing injections and sampling coelomic fluid never induced disease signs in any apparently healthy stars.

Statistical analysis of disease response

Of the observed disease responses (arm twisting, arm autotomy and mortality), only arm twisting was observed in both control and exposed treatment groups. Thus, we evaluated the effect of treatment group (control versus exposed) and exposure method (tissue homogenate injection, coelomic fluid injection, *V. pectenicida* culture injection and immersion in effluent water) on the number of arms observed twisting in the days following exposure[41].

To test the influence of each of the exposure methods on host arm-twisting response, we fit all experiments in a single generalized linear mixed effects model (glmmTMB)[44]. We fit a model with arms twisted as the response variable, and the fixed variables included exposure method, total number of arms per star and the interaction between DPE and treatment group. We included total number of arms per star to account for the fact that individuals with more arms can have a higher number of arms twisting at any given time. The random variables included experiment ID (to account for replicate experiments) and sea star ID (to account for replicate stars within experiments) and fit the model with a Poisson distribution. Model predictions were visualized using plot_model in sjPlot[45].

There was a significant difference in the number of arms twisted by treatment group (exposed versus control) and DPE (Supplementary Table 3 and Extended Data Fig. 2). Exposure method on its own did not have a significant effect on the number of arms observed twisting (Supplementary Table 3). Thus, while arm twisting is a behaviour observed in control individuals, the amount and extent of arm twisting is higher in individuals that are exposed to SSWD.

Field sampling of 2023 SSWD outbreak

In addition to controlled laboratory exposure experiments, we collected coelomic fluid samples from sunflower sea stars at five sites in the fjords on the central coast of British Columbia in 2023, under scientific collection permit XMCFR 18 2023 issued by Fisheries and Oceans Canada. The Heiltsuk, Wuikinuxv and Nuxalk First Nations hold Indigenous rights to their territories, where all samples were collected. This work was done with formal agreements and collaboration with each Nation. We sampled each site at two time points. In May, none of the five sites showed evidence of SSWD (unaffected); however, by October, two of the five sites contained sunflower sea stars showing disease signs (affected). From the affected sites, we collected samples from grossly normal stars and stars showing early disease signs (arm twisting but no arm autotomy).

At both time points, we collected coelomic fluid samples from several sunflower sea stars (n = 4–14 per site; Supplementary Table 9) by bringing individuals to the surface and withdrawing up to 1 ml of coelomic fluid using a 3-ml syringe fitted with a 25G 1.5-inch needle inserted into the coelomic cavity through the armpit of the star. Once collected, we dispensed the coelomic fluid into 1.5-ml tubes, which were frozen in liquid nitrogen before being transferred to a −80 °C freezer for storage until the time of DNA and RNA extraction. Following sample collection, we returned stars to their original location on the seafloor. Collection and processing of any stars showing disease signs was conducted in isolation from grossly normal stars.

DNA/RNA extraction and quantification

DNA and RNA were co-extracted from the following samples: (1) inoculum and coelomic fluid samples collected during the coelomic fluid exposure experiment described above (n = 34; Supplementary Table 4), (2) field samples collected from fjord populations of *P. helianthoides* off the central coast of British Columbia (n = 82; Supplementary Table 9) and (3) samples of inocula (either tissue homogenate or coelomic fluid) used to start the remaining four controlled exposure experiments

conducted between 2021 and 2023 described in this study (to confirm the presence of the candidate pathogen, *V. pectenicida* in the inocula of all experiments for which phenotypic data were summarized; $n = 5$, Supplementary Tables 2 and 11).

We extracted DNA and RNA using the ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research, catalogue no. R2002), according to a modified version of the manufacturer's protocol. First, we added up to 750 µl of coelomic fluid to a BashingBead Lysis Tube containing 750 µl of DNA/RNA shield. We processed all samples individually with the exception of samples taken from diseased stars with autotomized arms; given the low volume of coelomic fluid often retrieved at this late stage of disease, the likelihood of obtaining genetic material from each sample individually was low, thus we combined these samples across stars ($n = 7$) and processed it as a single sample representing all diseased stars with autotomized arms. We performed mechanical homogenization of the samples by shaking tubes in a high-speed vortex for three intervals of 5 min separated by 2 min of cooling between intervals, during which time we placed the samples on ice. Following centrifugation, we transferred up to 700 µl of supernatant from the BashingBead tube for downstream processing, which followed the manufacturer's protocol. For RNA purification we replaced the kits Zymo-Spin IIICG columns with Zymo-Spin IC columns (catalogue no. C1004) for low yield samples. We removed the Zymo-Spin III-HRC filter steps at the end of the manufacturer's protocol.

We quantified DNA and RNA using a Qubit 4 Fluorometer, and the dsDNA Broad Range (Invitrogen, catalogue no. Q32850) and RNA High Sensitivity kit (Invitrogen, catalogue no. Q32852) for DNA and RNA samples, respectively. For 2022 exposure experiment samples, average DNA and RNA concentrations were 6.0 and 15.4 ng μl^{-1}, respectively. For field samples, average DNA concentration was 45.5 ng μl^{-1}. For the remaining inocula samples, average DNA concentration was 16.0 ng μl^{-1}.

Metatranscriptomic sequencing

We generated a metatranscriptomic sequencing dataset from the inoculum and coelomic fluid samples collected from the coelomic fluid exposure experiment to profile the suite of microbes (bacteria, protists, RNA viruses and DNA viruses) associated with diseased and apparently healthy sunflower sea stars ($n = 28$; Supplementary Table 4). Library preparation and sequencing was conducted by Centre d'expertise et de services Génome Québec. Briefly, total RNA was quantified and assessed for integrity using the 5K/RNA/Charge Variant Assay LabChip and RNA Assay Reagent Kit (Perkin Elmer). Ribosomal RNA was depleted from 125 ng of total RNA using the -5S/16S/23S and QIASelect -Globin Kits (Qiagen). Complementary DNA synthesis was performed using the NEBNext RNA First Strand Synthesis NEBNext Ultra Directional RNA Second Strand Synthesis Modules (New England Biolabs). The remainder of the library preparations were achieved using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs), with adaptors and PR primers purchased from New England Biolabs. Prepared libraries were quantified using the KAPA Library Quantification Kits—Complete Kit (Universal, Kapa Biosystems), and average library size was estimated using a Fragment Analyzer 5300 (Agilent) instrument. Libraries were normalized, pooled, denatured in 0.02 N NaOH and neutralized using HT1 buffer. A PhiX library was mixed with sample libraries at 1% as a control. The final library was loaded at 175 pM on a single S4 lane of an Illumina NovaSeq 6000 using the Xp protocol as per the manufacturer's recommendations for paired-end 2 × 150 base pair (bp) sequencing. Raw reads are available in the NCBI Short Read Archive database (BioProject no. PRJNA1195080).

16S rRNA gene amplicon sequencing

We generated 16S rRNA gene amplicon datasets for the following samples: (1) inoculum and coelomic fluid samples collected during the coelomic fluid exposure experiment ($n = 34$; Supplementary Table 4), (2) field samples collected from fjord populations of *P. helianthoides* off the central coast of British Columbia ($n = 82$; Supplementary Table 9) and (3) samples of inocula (tissue homogenate or coelomic fluid) used to start the remaining four exposure experiments conducted between 2021 and 2023 described in this study (to confirm the presence of the candidate pathogen, *V. pectenicida* in the inocula of all experiments for which phenotypic data was summarized; $n = 5$, Supplementary Tables 2 and 11). As additional procedural controls, we generated 16S rRNA gene amplicon datasets on DNA extracted from samples of inflowing seawater (5 l) and tap water (1 l) from the facility and did not identify any reads annotated at *V. pectenicida* in either sample.

We amplified the 16S V4 rRNA gene region on each sample in triplicate, using the primers 515F and 806R[46] amended with Illumina sequencing indexes. The 25 µl of reaction volume contained 2× Taq FroggaMix (FroggaBio, catalogue no. FBTAQM), 50 µg of bovine serum albumin (New England Biolabs, catalogue no. B9000), 0.2 µM of each primer (Integrated DNA Technologies) and 10–20 ng of DNA. The thermocycler programme began with a 3-min denaturation period at 94 °C followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 60 s and elongation at 72 °C for 90 s and ending with a final elongation period of 10 min at 72 °C. We pooled triplicate amplifications of each sample and purified the PCR products using SPRIselect beads (Beckman Coulter, catalogue no. B23317) in a bead to product ratio of 0.8:1 to remove fragment sizes smaller than 200 bp. We quantified libraries using a Quant-IT dsDNA assay kit, High Sensitivity (Invitrogen, catalogue no. Q33130) on a microplate reader and pooled samples in equimolar amounts. We quantified the final pooled library using a Qubit dsDNA high sensitivity kit (Invitrogen, catalogue no. Q32851) on a Qubit 4 fluorometer (Thermo Fisher Scientific) and used the NEBNext Library Quant Kit for Illumina (NEB, catalogue no. E7630S) to precisely estimate the library molarity using a qPCR instrument (CFX-96, BioRad). Before sequencing, we conducted a final quality-control assessment of the library using a Qiaxcel instrument (Qiagen) to check for the presence of a single fragment or presence of bead carryover. We conducted paired-end 300 bp sequencing on an Illumina MiSeq platform using a 600 cycle V3 kit (Illumina, catalogue no. MS-102-3003). Raw reads are available in the NCBI Short Read Archive database (BioProject no. PRJNA1195080).

Bioinformatics on metatranscriptomic datasets

Metatranscriptomic sequencing datasets contained an average of ~64 million reads per sample (range ~54–79 million reads per sample; Supplementary Data 1). Base calling of sequenced libraries was performed by Genome Quebec with RTA (v.3) and libraries were demultiplexed using bcl2fastq2 (v.2.20, Illumina). We trimmed raw reads using Trimmomatic (v.0.36)[47], removing bases from the start and end of reads with quality scores below 5 and trimming when the average quality of 4-bp sliding windows fell below 15. We also removed reads shorter than 50 bp from the dataset at this time. On average, we retained 98% of paired reads in trimmed datasets (Supplementary Data 1), which we subsequently quality assessed using FastQC (v.0.11.7)[48] and summarized with MultiQC (v.1.0)[49]. We aligned reads to the host (*P. helianthoides*) genome (NCBI accession ASM3215829v1)[16], using BWA-MEM (v.0.7.17-r1188)[50], retrieved unmapped reads (non-host reads, averaging ~3 million reads (or 5%) of each samples dataset; Supplementary Data 1) using Samtools (v.1.9)[51] and assembled unmapped reads into contigs for each sample using rnaSPAdes (v.3.11.1)[52]. The number of assembled contigs per sample ranged from 3,162 to 31,062 (average of 13,576 contigs per sample; Supplementary Data 1). On average, ~6% of the contig dataset of each sample contained contigs >1,000 bp in length and the average contig N50 length was 424 bp (Supplementary Data 1). We annotated assembled contigs against the NCBI non-redundant nucleotide database (as of December 2023) using the BLASTx function of DIAMOND v.2.1.8.162 (ref. 17). Annotation success varied across samples, ranging from 31% to 62%, with an average of 44% of contigs annotated per sample, totalling 169,156 annotated contigs across samples (Supplementary Data 1).

To contrast the microbial communities in samples from apparently healthy and diseased stars, we imported annotated contig datasets for each sample into MEGAN6 (v.6.25.10)[53] and filtered the data to retain only high-quality annotations using the following filtering parameters: minimum score of 80.0, maximum expected of 1.0×10^{-10}, minimum percentage identity of 85.0, minimum read length of 200, contaminant filter as metazoa. Filtered datasets contained 72–7,158 high-quality annotated contigs (average 2,094; Supplementary Data 1). We compared high-quality annotated contigs across all samples to create an absolute count matrix, from which, we identified taxa present in all diseased star samples ($n = 7$) and absent from apparently healthy star samples ($n = 21$). We normalized the absolute contig count matrix by subsampling to the sample with the smallest dataset, and used the normalized contig count matrix to construct a PCoA biplot[41] in R (v.4.4.1)[54], with the packages vegan (v.2.6-8)[55], ggplot2 (v.3.5.1)[56] and glue (v.1.8.0)[57].

Bioinformatics on 16S rRNA gene amplicon datasets

Amplicon sequencing generated an average of 76,491 reads per sample (Supplementary Table 5) for the inoculum and coelomic fluid samples collected during the coelomic fluid exposure experiment, and 145,500 reads per sample (Supplementary Table 10) for the field samples collected from fjord populations of *P. helianthoides* off the central coast of British Columbia, Canada. We analysed all 16S rRNA gene amplicon datasets with QIIME 2 (v.2023.9)[18] using the same approach. First, we removed primers using the cutadapt function[58], and quality trimmed, denoised and merged reads with DADA2 to produce a feature table of amplicon sequence variants (ASVs)[59]. We selected trimming parameters using the interactive quality plot generated by the demux summarize function; forward and reverse reads were truncated at the base where the 25th percentile quality score dropped below 25. The trimmed and filtered read datasets contained an average of 45,111 reads per sample for the exposure experiment samples (Supplementary Table 5) and 32,726 reads per sample for the field samples (Supplementary Table 10). We filtered the resulting ASV feature tables to remove rare sequences (identified as ASVs with frequencies <0.1% of the mean sample read depth) that may be attributed to bleed-through between MiSeq runs. We conducted sample filtering by examining a rarefaction curve to identify the read depth at which species richness plateaus, and removed samples with a read depth below this threshold. To retain as many samples as possible, we chose a sequencing depth threshold of 1,000 reads for sample filtering, resulting in the removal of one diseased star sample from the exposure study dataset. We assigned taxonomy to ASVs using the classify-sklearn feature classifier function with a naive Bayes classifier trained on the Greengenes 216S V4 marker gene reference database (gg_2022_10_backbone.v4.nb.qza)[19,20]. We further filtered annotated ASVs to remove any sequences identified as potential contaminants using the frequency method implemented in the R package decontam (v.1.26.0)[60], and merged ASVs with the same annotation using the taxa collapse function of QIIME 2. The final filtered datasets contained 1,146 ASVs (representing 549 unique taxa) for the exposure study samples (Supplementary Data 2) and 2,442 ASVs (representing 1,098 unique taxa) for the field samples (Supplementary Data 3). We plotted the relative read abundance of *V. pectenicida* reads[41] and conducted an analysis of compositions of microbiomes with bias correction (ANCOM-BC; ref. 21) to identify differentially abundant taxa in diseased versus apparently healthy star samples using a significance threshold of 0.001.

Isolation and strain identification of *V. pectenicida* FHCF-3

Following identification of *V. pectenicida* as a candidate causative agent of SSWD, we collected coelomic fluid samples from two captive-bred sunflower sea stars exhibiting disease signs (arm twisting) at University of Washington Friday Harbor Laboratories in February 2024. These stars were housed in aquaria with flow through 20-µm filtered seawater. They were both exposed to a single star in January 2024 that subsequently died from apparent SSWD. We sampled ~10 ml of coelomic fluid from each star using a syringe fitted with a 26G 1-inch needle inserted into the coelomic cavity through the armpit of each star. We deposited the samples into several 5-ml tubes, which we transported on ice to the University of British Columbia and processed within 8 h of collection. Both sampled stars autotomized arms within 48 h of sample collection and died 6 and 8 d following sampling.

To isolate viable *V. pectenicida* from the coelomic fluid samples we used a spread plate approach. As the bacteria titre was unknown, we prepared serial tenfold dilutions of the samples to be assayed. We diluted the samples with autoclaved 0.22-µm filtered seawater with a salinity of 24 practical salinity units (p.s.u.) and pipetted 100 µl of undiluted, 10× and 100× diluted samples onto the surface of MLB agar plates (0.05% w/v Difco casamino acids, 0.05% w/v Difco peptone, 0.05% w/v Difco yeast extract, 0.3% v/v glycerol; 1.2% w/v Fisher Scientific purified agar, 24 p.s.u. natural seawater)[25–27]. We used sterile plastic spreaders (VWR 76208-438) to evenly distribute the sample over the agar surface, sealed the plates with parafilm and examined colony growth daily. After incubating at room temperature (~21 °C) for 5–7 d, the plate inoculated with 100 µl of undiluted coelomic fluid appeared to be a monoculture of approximately 300 small (~1 mm diameter) non-pigmented bacteria colonies. To isolate and purify potential candidates for *V. pectenicida*, we aseptically picked a well-separated non-pigmented bacteria colony using a sterile plastic 1-µl inoculating loop, and restreaked it onto an MLB plate to obtain single well-separated colonies. We repeated this process three to four times, picking a single well-separated colony each time to obtain axenic clonal cultures. If single well-separated colonies were not attained, the process was repeated. This process resulted in the purification of ten clonal isolates, eight of which we identified as *V. pectenicida* using Sanger and whole-genome sequencing (described below). We routinely grew *V. pectenicida* isolates in MLB or Zobell (ZOB) broth (0.5% w/v Difco peptone, 0.1% w/v Difco yeast extract; 24 p.s.u. seawater base) and on MLB or ZOB agar plates (MLB or ZOB broth solidified with 1.2% w/v agar A, Biobasic) at 18 °C and 21 °C, respectively. We preserved stock cultures in 20% (v/v) glycerol, stored at −80 °C.

To confirm pure cultures of *V. pectenicida*, we first sequenced the V4 region of the 16S rRNA gene. We extracted DNA from ten candidate isolates using a Qiagen DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's protocol. We quantified extracted DNA using a Qubit 4 Fluorometer and the dsDNA Broad Range kit (Invitrogen) and amplified the 16S V4 rRNA gene region using the same primers as used in the 16S rRNA gene amplicon libraries with a slightly modified PCR master mix: the 25 µl of reaction volume contained 1× PCR buffer, 4 mM $MgCl_2$, 50 µg of recombinant albumin (New England Biolabs), 0.2 mM dNTPs, 0.2 µM of each primer (Invitrogen), 0.5 U of Q5 high-fidelity DNA polymerase (New England Biolabs) and 5 ng of DNA. Thermocycler parameters were the same as those used for the 16S rRNA gene library amplifications. We cleaned the PCR product using the Monarch PCR and DNA Cleanup Kit (New England Biolabs, catalogue no. T1030), following the manufacturer's protocol and submitted the samples for Sanger sequencing to the University of British Columbia Sequencing and Bioinformatics Consortium. We aligned forward and reverse sequenced reads and trimmed the alignments for poor-quality bases using Geneious Prime (v.2024.0.5). We assigned taxonomy to each sample as the top hit from a BLASTn search of each consensus sequence against the NCBI 16S rRNA database. Of the eight isolates identified as *V. pectenicida*, we identified two unique sequences, differing by a single nucleotide polymorphism (Supplementary Table 7).

Subsequent to preliminary identification of *V. pectenicida* in culture using Sanger sequencing, we performed whole-genome sequencing of one isolate, FHCF-3. We prepared samples from 3-day-old cultures grown in ZOB broth at 18 °C. We harvested cells by spinning down 1.5-ml cultures for 5 min at 11,000*g* and 10 °C in a Beckman Allegra X-22R centrifuge fitted with a fixed-angle rotor

(F2402H). We submitted the cell pellets to Seqcenter (Pittsburgh, PA) for genomic DNA extraction (Zymo fungal/bacterial DNA miniprep kit; Zymo Research) and hybrid assembly sequencing (medium nanopore/Illumina combo package). Briefly, the Illumina sample was prepared using the Illumina DNA library prep kit (Illumina) and sequenced on an Illumina NextSeq2000 instrument with 151-bp paired-end chemistry. The nanopore sample was prepared using the Oxford Nanopore Technologies (ONT) ligation sequencing kit and sequenced on a MinION instrument using an R9 flow cell (R9.4.1), with base calling performed using ONT Guppy (v.4.2.2).

To assemble the genome of our isolate, adaptors and low-quality reads were trimmed using bcl2fastq (v.2.19.0, Illumina 2019) and porechop (v.0.2)[61] for Illumina and ONT sequences, respectively. Hybrid assembly with Illumina and ONT reads was performed using Unicycler (v.0.5)[62]. The integrity of the bacterial genome was checked using CheckM (v.1.0.18)[63]. We extracted the 16S rRNA gene sequences from the genome of strain FHCF-3 following annotation using MetaErg (v.1.2.3)[64] with default parameters (NCBI accessions PQ700178 and PQ763222–PQ763229). To determine the phylogenetic placement and taxonomic identity of isolate FHCF-3, we conducted phylogenetic analysis using its complete 16S rRNA gene sequence (NCBI accession PQ700178) alongside 147 sequences of *Vibrio* spp. from the SILVA rRNA gene database (v.138.1)[65] and the NCBI nr database (as of 14 November 2024), rooted with sequences from two strains of *Escherichia coli* (NCBI accessions CP033092 and MT215717) to serve as an outgroup (Extended Data Fig. 3). We aligned sequences using MAFFT (v.7)[66] with default parameters and trimmed the multiple alignment using Clipkit (v.1.4.1, parameters: -m kpic-smart-gap)[67]. We constructed a maximum-likelihood phylogeny using IQ-TREE (v.2.2.0.3)[68], using the optimal model and gamma-distributed substitution rates determined and implemented by IQ-TREE, with 1,000 bootstrap replicates (parameters: -m MFP -B 1000 -bnni). From the phylogeny we identified four *Vibrio* species that clustered closely with our isolate (*V. pectenicida*, *V. ostreicida*, *V. tapetis* and *V. penaeicida*) and calculated ANI between our isolate and all available genomes for the aforementioned species available on NCBI, using the OrthoANIu method implemented on the EzBioCloud server[69].

Culturing and exposure experiments with *V. pectenicida* strains

To establish a causal relationship between *V. pectenicida* and SSWD we exposed apparently healthy stars to an inoculum prepared from a pure culture (Fig. 1). In the first experiment, we tested two isolates, *V. pectenicida* FHCF-3 and FHCF-5, for pathogenicity. This experiment comprised four treatment groups ($n = 3$ stars per treatment group); stars were injected with ~10^7 c.f.u. of *V. pectenicida* isolates FHCF-3 or FHCF-5 or their heat-treated controls.

To prepare inoculum from *V. pectenicida* cultures, we first established a seed culture by inoculating 2–3 ml of ZOB broth with a loopful of colonies from a week-old plated culture. We allowed the seed culture to incubate for 1–2 d before using it to prepare inoculum, for which 50–100 µl of seed culture was transferred to 5 ml of ZOB broth and grown overnight. We incubated all liquid cultures at 18 °C in loosely capped 50-ml Falcon tubes without shaking. We estimated the dose of each inoculum as the total number of viable cells, calculated using the total viable count spread plate method. Briefly, we prepared several serial tenfold dilutions for each culture in triplicate, using sterile 0.22-µm filtered (Nalgene Nalgene Rapid-Flow Filter Units, PES membrane) seawater (24 p.s.u.) as the diluent. We spread 100 µl of each dilution onto ZOB agar plates, incubated the plates for 1–2 d at room temperature (21 °C) and counted the number of c.f.u. We assayed 100 µl of the sterile 0.22-µm filtered seawater diluent as a negative control.

To prepare inoculum for exposed and control treatment groups, we divided liquid cultures of each isolate into two fractions; we placed live fractions for exposed injections at 4 °C while we heat-treated additional fractions for control injections by placing ~1 ml of culture in a dry heat block set at 95 °C for 30 min. We cooled heat-treated cultures on ice before performing injections. We injected a single 100-µl dose of either live or heat-treated culture into the coelomic cavity through the armpit of each star using a 0.3-ml syringe fitted with a 29G 1-inch needle. Following injections, we monitored individuals twice daily for a period of 4 weeks. During this period, we took photographs of each individual once per day, with subsequent photographs taken when individuals were exhibiting disease signs. We collected samples of coelomic fluid from stars exhibiting disease signs from this experiment, which we used to re-isolate *V. pectenicida*. Culturing methodology was consistent with previously described isolations. We confirmed the identity of *V. pectenicida* cultures by Sanger sequencing of the V4 region of the 16S rRNA gene as described above. All isolates identified as *V. pectenicida* had sequences identical to those of isolates FHCF-3 and FHCF-5 (Supplementary Table 7).

Given that both *V. pectenicida* isolates caused disease, experiments proceeded with a single isolate, strain FHCF-3, which had been genome sequenced. Our second exposure experiment tested two lower doses of *V. pectenicida* strain FHCF-3. The experiment comprised four treatment groups, each containing seven randomly selected apparently healthy juvenile sunflower sea stars (three from the wild, three from the Friday Harbor Laboratories captive-breeding programme and a seventh, randomly selected star from either group). The treatment groups included sunflower sea stars exposed to either a high (~10^5 c.f.u.) or low (~10^3 c.f.u.) dose of *V. pectenicida* strain FHCF-3 or their respective heat-treated controls. We prepared the inoculum and injected stars in the same manner as described for the first culture-based exposure experiment and likewise monitored individuals twice daily for 3 weeks. During this period, we took photographs of each individual once per day, with subsequent photographs taken when individuals were exhibiting disease signs.

Re-analysis of 16S rRNA gene amplicon data

To further explore the presence of *V. pectenicida* in field outbreaks of SSWD, we downloaded and re-analysed the 16S rRNA gene amplicon dataset of ref. 30 to examine samples collected in 2016 from wild populations of sunflower sea stars in southeast Alaska, when the SSWD epidemic was first observed in this area. The authors sampled dermal tissue from both grossly normal and diseased sunflower sea stars from affected sites (sites with an ongoing SSWD outbreak) in addition to grossly normal stars from apparently unaffected sites (no prior evidence of a SSWD outbreak). The authors reported high prevalence of *Vibrio* spp. associated with both diseased and grossly normal star samples from affected sites but without evidence for one specific causative agent. Our objective was to mine this dataset for evidence of *V. pectenicida* sequences in these samples to identify whether it was present in the initial spread of the SSWD epidemic.

We downloaded the raw dataset from the SRA archive (BioProject PRJNA931596) and processed the data using the same bioinformatic pipeline described above for the challenge experiment and field samples. We set trimming parameters to trim at 16 bp and 280 bp for forward reads and 0 bp and 220 bp for reverse reads. Trimmed and quality filtered datasets contained an average of 92,589 reads per sample (range of 19,374–157,463 reads per sample). Given the high read depth of all samples, we did not remove any samples from the analysis. We identified a total of 1,628 ASVs across samples, of which, 67 were classified to the genus *Vibrio*. We retrieved the sequences for all *Vibrio* spp. ASVs and searched them against the NCBI 16S rRNA database using BLASTn. We filtered the results to 100% query cover and 98–100% identity to determine the most likely *Vibrio* species of each sequence. We screened all samples for the presence of any *V. pectenicida* ASVs and summarized the results as the proportion of samples from diseased and grossly normal stars from affected and unaffected sites that contained sequences identified as *V. pectenicida*.

Biological materials availability
All unique materials used (*V. pectenicida* strain FHCF-3 culture) are available by request from the authors.

Reporting summary
Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability
Metatranscriptomic and 16S rRNA gene sequence datasets are archived in the NCBI Short Read Archive (BioProject no. PRJNA1195080). The whole-genome of *V. pectenicida* strain FHCF-3 is available from the NCBI GenBank Repository (accession no. JBLZMR000000000), with raw sequence reads archived in the NCBI Short Read Archive (BioProject no. PRJNA1232168). The complete 16S rRNA gene sequences of *V. pectenicida* strain FHCF-3 are deposited in the NCBI GenBank Repository (accessions PQ700178 and PQ763222–PQ763229). Source data are provided with this paper.

Code availability
Code generated in this study is available via Dryad at https://doi.org/10.5061/dryad.5mkkwh7g9 (ref. 41).

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Acknowledgements

B. Blake and N. Siu helped to acquire permits for this work. K. Bachen, H. Carson, K. Collins, D. Currie-Olsen, T. Frierson, T. Froese, J. Kocian, E. Loose, Z. Monteith, O. Pontier, G. Sadlier-Brown, A. Schmill, K. Sowul, B. Stevick and D. VanMaanen assisted with field collections. F. Curliss, A. Kalytiak-Davis, C. Schwab and V. Valdez supported sea star transfers from and sample collections at Friday Harbor Laboratories. D. Rogers provided local shellfish to feed experimental stars. J. Beal, C. Grady, J. Gregg, A. MacKenzie and W. Richards provided facilities and logistical support at the USGS Marrowstone Marine Field Station and H. Kuttenkeuler, K. Rolheiser and M. Winningham aided experiment monitoring and sampling. Y. Gouin and A. Nimmon assisted with entering and collating data.
C. L. J. Huang assisted with preparation of culture media. C. Burge, C. Conway, J. Hansen, A. Hawthorn, J. Lovy, A. Roberts and Q. Yang provided advice. Graphic illustrations are credited to M. Minck. We are grateful for the support from E. Peterson, C. Munck, N. Eddy and J. Wilson. Funding was provided by The Nature Conservancy of California (C.D.H. and A.-L.M.G.), the Tula Foundation (A.-L.M.G. and C.A.S.), the Natural Sciences and Engineering Research Council of Canada Discovery grant no. RPGIN-2020-06515 (C.A.S.), the Canadian Foundation for Innovation and British Columbia Knowledge

Development Fund Infrastructure award no. 25412 (C.A.S.), the University of British Columbia, the USGS Biological Threats Research Program, Ecosystems Mission Area (P.K.H.) and the Quantitative and Evolutionary STEM Traineeship NRT-1735316 (A.M.). Any use of trade, firm or product names is for descriptive purposes only and does not imply endorsement by the US government.

Author contributions

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Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41559-025-02797-2.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41559-025-02797-2.

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Peer review information *Nature Ecology & Evolution* thanks Kevin Lafferty and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

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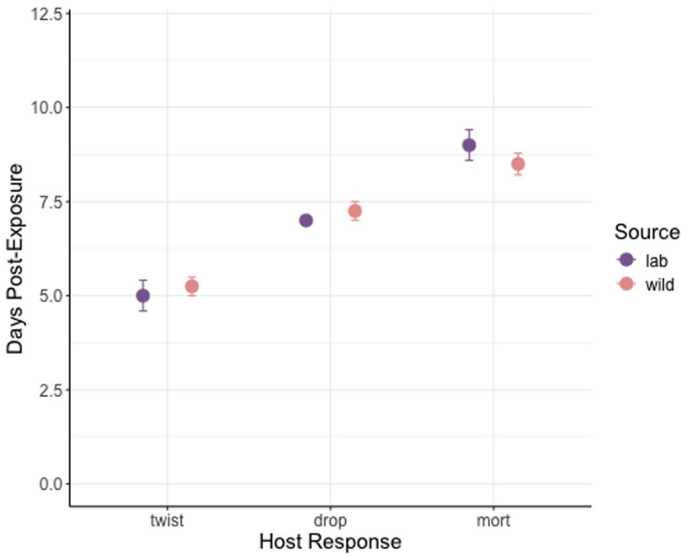
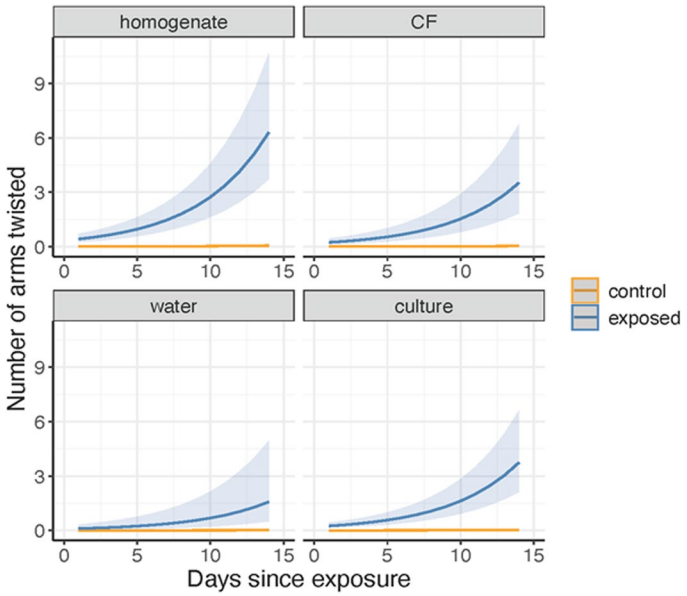
https://doi.org/10.1038/s41559-025-02797-2



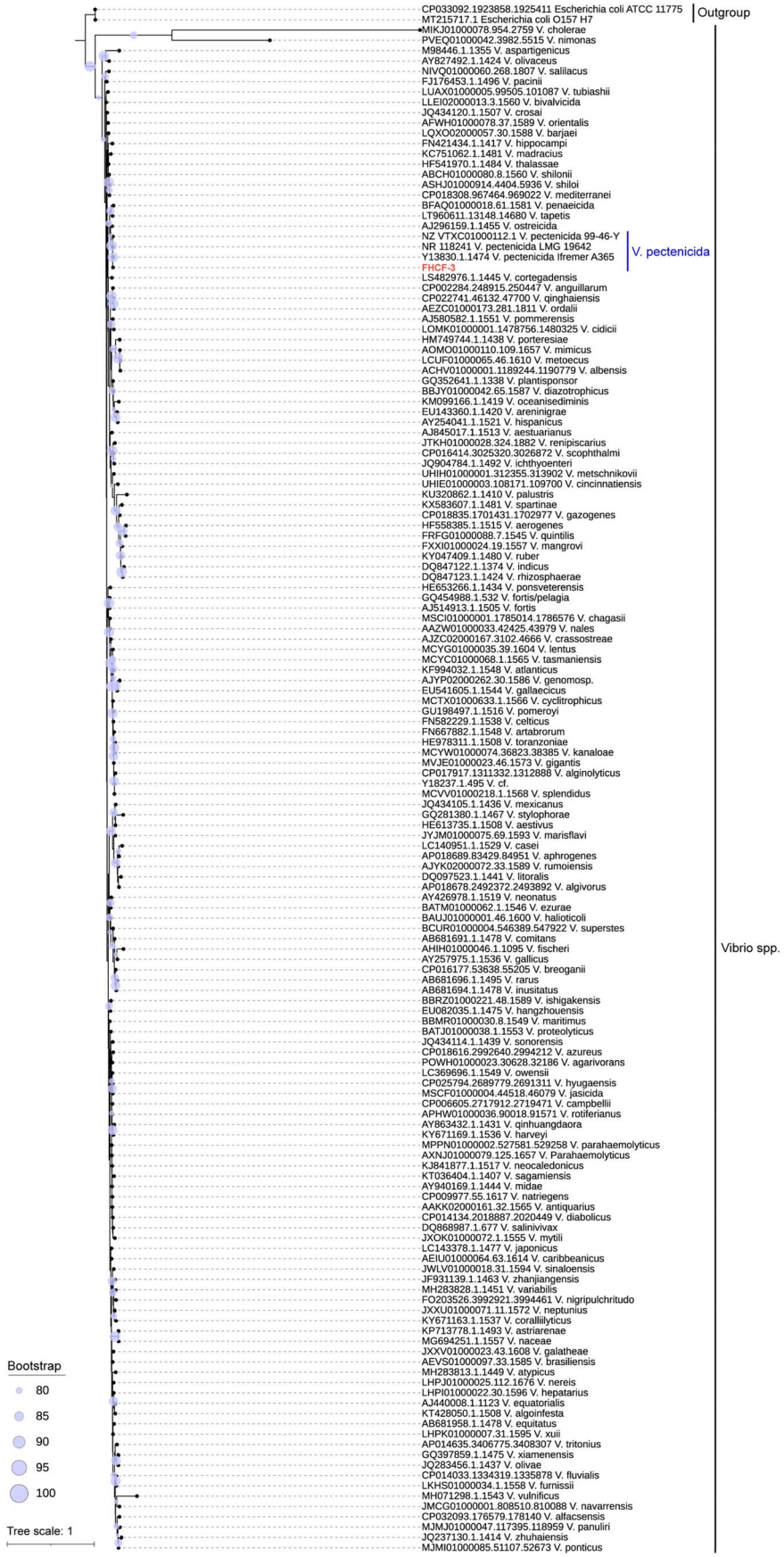
Extended Data Fig. 1 | The disease trajectory of captive-bred ("lab", n = 4) and wild ("wild", n = 4) sunflower sea stars (*Pycnopodia helianthoides*) following injection with coelomic fluid from a diseased sunflower sea star. Data represents 8 individual mortalities observed in sunflower sea stars exposed to sea star wasting disease (excluding 2 individuals that were exposed but did not die). Host responses are grouped into three types, arm twisting ('twist'), arm autotomy ('drop') and mortality ('mort'). Symbols indicate the average number of days post exposure when disease responses were observed. The error bars indicate 1 s.e. We confirmed the presence of *V. pectenicida* in the inoculum used to start this experiment using 16S rRNA gene amplicon sequencing (Supplementary Table 11).

https://doi.org/10.1038/s41559-025-02797-2



Extended Data Fig. 2 | Model predicted values of the number of arms twisted in sunflower sea stars (*Pycnopodia helianthoides*) following controlled exposure to sea star wasting disease by exposure method. Lines indicate adjusted predicted values for stars within exposed (blue) and control (orange) treatment groups with 95% confidence intervals. Exposure methods include stars injected with coelomic fluid ('CF'), tissue homogenate ('homogenate'), or *Vibrio pectenicida* culture ('culture'), and stars exposed to effluent water from a wasting sea stars tank ('water'). Statistical results can be found in Supplementary Table 3.

https://doi.org/10.1038/s41559-025-02797-2



Extended Data Fig. 3 | See next page for caption.

https://doi.org/10.1038/s41559-025-02797-2

Extended Data Fig. 3 | Phylogenetic relationship of 16S rRNA gene sequence of *V. pectenicida* strain FHCF-3 (this study) to other *Vibrio* spp. *Vibrio* spp. 16S rRNA gene sequences were retrieved from the SILVA rRNA gene database (version 138.1) and the NCBI nr database (as of 14 November 2024). The maximum-likelihood phylogeny was built using 1,000 replicates, and rooted with sequences from two strains of *Escherichia coli* (NCBI Accessions CP033092 and MT215717) as an outgroup. Values on the nodes of the phylogeny represent bootstrap values. *V. pectenicida* strain FHCF-3 (isolated and sequenced in this study) is highlighted in red.

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☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No commercial, open source or custom code was used to collect the data in this study.
Data analysis	For metatranscriptomic data we used the following software: -RTA (v3) for basecalling -bclfastq2 (v2.20) for demultiplexing -Trimmomatic (v0.36) for trimming raw reads -FastQC (v0.11.7) and multiQC (v1.0) for quality assessment of trimmed data -BWA-MEM (v0.7.17-r1188) for alignment of trimmed reads to host genome -Samtools (v1.9) for extracting unmapped reads from alignment -rnaSPAdes (v3.11.1) for assembling unmapped reads into contigs -BLASTx function of DIAMOND (v2.1.8.162) for annotating contigs against the NCBI non-redundant nucleotide database -MEGAN6 (v6.25.10) for filtering annotated contig dataset and exporting absolute and normalized contig count matrices -R (v4.4.1) with packages vegan (v2.6-8), ggplot2 (v3.5.1), and glue (v1.8.0) for constructing PCoA biplot from normalized contig count matrix* For 16S data, we used QIIME2 (v2023.9) and its -associated functions/plug-ins, including: -cutadapt (v4.5) for trimming primers -DADA2 (v1.26.0) for quality filtering and to produce a table of Amplicon Sequence Variants (ASVs) -classify-sklearn (v2023.9) to assign taxonomy to ASVs -R (v4.4.1) with package decontam (v1.26.0) to identify and remove putative contaminant ASVs -taxa collapse (v2023.9) to merge ASV table -ANCOM-BC (v2.0.1) to identify differentially abundant taxa

-R (v4.4.1) with package ggplot2 (v3.5.1) for constructing relative abundance plot*

For editing and analysis of Sanger sequencing data we used Geneious Prime (v 2024.0.5).

For phylogenetic analysis of Vibrio pectenicida complete 16S sequences we used the following software:
-ONT Guppy (v4.2.2) for basecalling
-bcl2fastq (v2.19.0) for adapter removal and read filtering of Illumina reads
-porechop (v0.2) for adapter removal of ONT reads
-Unicycler (v0.5) for hybrid assembly of Illumina and ONT reads
-CheckM (v1.0.18) for genome assembly checking
-MetaErg (v1.2.3) for annotation of 16S sequences
-MAFFT (v7) for sequence alignments of 16S sequences
-Clipkit (v1.4.1) for trimming of multiple sequence alignment
-IQ-TREE (v2.2.0.3) for construction of maximum likelihood phylogeny

For figures describing the experimental data (Figure 2, Extended Data Figure 1, Extended Data Figure 2), we used R (v4.4.2) with packages dplyr (v1.1.4), tidyr (v1.3.1), ggplot2 (v3.5.1), sjPlot (v2.8.17), PNWColors (v0.1.0), emmeans (v1.11.0), and ggh4x (v0.2.8).*

*Custom code is available on Dryad (https://doi.org/10.5061/dryad.5mkkwh7g9)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Metatranscriptomic and 16S rRNA gene sequence datasets are archived in the National Center for Biotechnology Information (NCBI) Short Read Archive (BioProject Number PRJNA1195080). The whole genome of V. pectenicida strain FHCF-3 is available from the NCBI GenBank Repository (Accession Number JBLZMR000000000), with raw sequence reads archived in the NCBI Short Read Archive (BioProject Number PRJNA1232168). The complete 16S rRNA gene sequences of V. pectenicida strain FHCF-3 are deposited in the NCBI GenBank Repository (Accessions PQ700178, PQ763222-PQ763229).

This manuscript also re-analyzed the 16S rRNA gene amplicon dataset of McCracken et al. 2023. The raw data for this study can be found in NCBI Short Read Archive (BioProject Number PRJNA931596).

Several large publicly available databases were used for analyses presented in this paper, including:
The Greengenes 2 16S V4 marker gene reference database (https://ftp.microbio.me/greengenes_release/)
The NCBI 16S rRNA database (https://www.ncbi.nlm.nih.gov/)
The NCBI non-redundant nucleotide database (https://www.ncbi.nlm.nih.gov/)
The SILVA rRNA gene database (https://www.arb-silva.de/)
The Global Biodiversity Information Facility database (https://www.gbif.org/)

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	No research was performed involving human participants, their data, or biological material.
Reporting on race, ethnicity, or other socially relevant groupings	No research was performed involving human participants, their data, or biological material.
Population characteristics	No research was performed involving human participants, their data, or biological material.
Recruitment	No research was performed involving human participants, their data, or biological material.
Ethics oversight	No research was performed involving human participants, their data, or biological material.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

Between 2021-2024, we conducted seven controlled exposure experiments on grossly normal (i.e. absent of visible disease signs) sunflower sea stars (Pycnopodia helianthoides). Briefly, each experiment involved exposure of sunflower sea stars to either material sourced from wasting sunflower sea stars (2021-2023) or a pure culture of the bacteria Vibrio pectenicida (2024). The relevant details of each experiment are summarized in Supplementary Table 2. This table includes information on the year that the experiment was conducted, a description of treatment groups within the experiment (including controls), the subjects of the experiment (including age and source of experimental animals), the number of independent experimental animals in each treatment group (N), and the results of the experiment (number of individuals in each treatment group that died after exposure to specified treatment).

This study also involved collection and analysis of samples from the field at two time points (May and October of 2023), which are summarized in Supplementary Table 11.

Research sample

We used sunflower sea stars in our experiments due to their critical conservation need, and as their apparent high susceptibility to SSWD allowed for clear interpretation of results (i.e., most stars exposed to disease died in a consistent and predictable manner).

For experiments, we collected both juvenile and adult sunflower sea stars subtidally from multiple sites in Washington, USA. Juvenile and adult sunflower sea stars measured between 5-20 cm and >40 cm diameter, respectively. We also received captive-bred sunflower sea stars from Friday Harbor Labs, WA, USA, which were born from wild broodstock locally collected in 2019.

In addition to controlled laboratory exposure experiments, we collected coelomic fluid samples from sunflower sea stars at five sites in two fjords on the central coast of British Columbia, Canada, in 2023.

Following identification of Vibrio pectenicida as a strong candidate causative agent of SSWD, we collected coelomic fluid from two captive-bred adult sunflower sea stars exhibiting disease signs (i.e., arm twisting) in Friday Harbor, Washington, USA. These samples were used to create pure cultures of Vibrio pectenicida for subsequent challenge experiments.

This paper also includes a re-analysis of the 16S rRNA gene amplicon dataset of McCracken et al. (2023) to examine samples collected in 2016 from wild populations of sunflower sea stars in southeast Alaska, when the SSWD epidemic was first observed in this area. We downloaded the raw dataset from the SRA archive (BioProject PRJNA931596), and processed the data using the same bioinformatic pipeline described for our own experimental and field samples.

Sampling strategy

For experimental sampling, we sampled coelomic fluid from stars throughout the experiment using a 0.3 mL syringe fitted with a 29 G one inch needle inserted into the coelomic cavity through the armpit of each star. Following retrieval, we deposited coelomic fluid samples into 1.5 mL tubes and stored them at -80°C until the time of DNA and RNA extraction.

Following identification of V. pectenicida as a candidate causative agent of SSWD, we collected coelomic fluid samples from two captive-bred sunflower sea stars exhibiting disease signs (i.e., arm twisting) at University of Washington Friday Harbor Laboratories in February, 2024. We sampled ~10 mL of coelomic fluid from each star using a syringe fitted with a 26 G one inch needle inserted into the coelomic cavity through the armpit of each star. We deposited the samples into multiple 5 mL tubes, which we transported on ice to The University of British Columbia and processed for culturing within eight hours of collection.

For field sampling, we sampled each field site at two time points, May and October, 2023. We collected coelomic fluid samples from sunflower sea stars (N=4-14/site, Supplementary Table 11) by bringing individuals to the surface and withdrawing up to 1 mL of coelomic fluid using a 3 mL syringe fitted with a 25 G 1.5 inch needle inserted into the coelomic cavity through the armpit of the star. Once collected, we dispensed the coelomic fluid into 1.5 mL tubes, which were frozen in liquid nitrogen before being transferred to a -80°C freezer for storage until the time of DNA and RNA extraction. Following sample collection, we returned stars to their original location on the seafloor. Collection and processing of any stars showing disease signs was conducted in isolation from grossly normal stars.

Individuals and/or samples of sunflower sea stars were collected from a species currently listed by the IUCN as critically endangered. Populations are severely depleted and ongoing SSWD outbreaks often result in the temporary or persistent loss of populations. Thus, the sample sizes used in our experiments and of samples collected from the field reflect what was available at the time the work was completed. We always attempted to reach a balance between obtaining sufficient sample sizes for our work and limiting the impact of our collections on local populations. For this reason, we also supplemented our experiments with lab-reared sunflower sea stars (which we experimentally demonstrated did not differ in response in comparison to wild sunflower stars, Extended Data Figure 1). However, lab-rearing of sunflower sea stars is a time- and effort-intensive process and thus our use of lab-reared stars for experiments was also limited.

Data collection

Data in the field and lab was collected on paper and transcribed into a Google Drive document by the authors and individuals listed in the acknowledgments.

Timing and spatial scale

Between 2021-2024, we conducted seven controlled exposure experiments, which were conducted during spring/summer months for several reasons. Prior to the identification of a candidate causative agent, all experiments relied on the collection of a diseased sunflower sea star from the field to provide tissues for exposures. For the first three years of experiments, we collected adult sunflower sea stars in late spring, a time of year with relatively low levels of disease incidence. This timeline ensured that we could collect sufficient numbers of unexposed stars for experiments. Despite the low levels of disease prevalence in the field in spring, in all years at least one individual collected showed disease signs during the quarantine period and thus could be used as the source of

disease for experimental exposures.

Experimental data collection dates encompass collection of individuals from the field used in experiments (all conducted in Washington, USA), and collection of samples throughout the course of experiments. Sampling frequency during experiments corresponded to observation of disease signs. In all experiments, a sample from each individual was taken prior to the start of the experiment (referred to as "Day 0" or "Pre-inoculation") to be used as a baseline. Experimental dates for each year are as follows:
2021: data collection between 2021-07-29 to 2021-10-18
2022: data collection between 2022-05-25 to 2022-10-02
2023: data collection between 2023-06-07 to 2023-09-21
2024: data collection between 2024-06-03 to 2024-08-10

In addition to experimental data, we collected samples from a field outbreak of SSWD in 2023 (in British Columbia, Canada) in May (2023-05-13 to 2023-05-15), and October (2023-10-08 to 2023-10-09).

The samples of coelomic fluid used to obtain a pure culture of Vibrio pecenticida were collected from two lab-reared, adult wasting sunflower sea stars in Friday Harbor, Washington, USA on February 09, 2024.

Data exclusions

For our metatranscriptomic dataset, six samples were excluded from sequencing due to failure to meet the thresholds required for sequencing (unsatisfactory RNA quality and/or quantity).

For our 16S datasets, no samples were removed prior to sequencing. During analysis of the dataset, we conducted sample filtering by examining a rarefaction curve to identify the read depth at which species richness plateaus, and removed samples with a read depth below this threshold. To retain as many samples as possible, we chose a sequencing depth threshold of 1,000 reads for sample filtering, resulting in the removal of one diseased star sample from the experimental challenge study dataset. No samples were removed during the analysis of the field outbreak dataset.

Reproducibility

Throughout the course of this work we took several measures to ensure the reproducibility of our results. These include:
-We used replication (within our means) within experiments, with equivalent weight given to control and exposed treatment groups
-We used replication across multiple experiments and years to demonstrate consistency in our ability to cause disease
-We used multiple types of genomic datasets to investigate a range of putative pathogens; we first used metatranscriptomic sequencing to profile the largest suite of microbes (bacteria, protists, RNA viruses and DNA viruses), followed by 16S rRNA gene amplicon sequencing to target the bacterial community at greater depth
-We reproduced our experimental findings in a 16S dataset sequenced from samples collected from a field outbreak of SSWD to show consistency of our genomic results in the lab and field
-We conducted two independent challenge experiments with cultured Vibrio pecetnicida to demonstrate reproducibility of our results in satisfying Koch's postulates
-Across experiments and datasets, all attempts to repeat the experiment were successful, with high levels of mortality in exposed treatment groups (92% mortality across experiments) and no mortality in control treatment groups
-Across genomic datasets we obtained clear associations between Vibrio pectenicida and diseased sunflower sea stars in the lab and field

Randomization

For each experiment, we assigned individual sunflower sea stars to treatment groups randomly, using a random number generator. We then assessed the size distribution within each treatment group to ensure that treatment and control groups were size comparable.

When we started to include lab-reared sunflower sea stars in our experiments, we first experimentally demonstrated that there was no difference in response to exposure between the two sources (i.e. lab-reared and wild) of sunflower sea stars (Extended Data Figure 1). For following experiments which included both lab-reared and wild individuals, we included a minimum of 3 of each type to enable downstream evaluation of any potential differences if desired.

Blinding

Blinding was not possible during our experiments for several reasons. Given the limitations we faced in the number of individuals we had for our experiments, and prior knowledge that SSWD is water transmissible, we were necessarily cautious to avoid cross-contamination and potential exposure of control treatment groups. Thus, experiments were conducted with exposed and control individuals in separate water tables to prevent cross-contamination. Further, care-taking and sampling was conducted independently, where exposed sea stars were handled and sampled by one individual and controls a separate individual.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions

Environmental conditions were not recorded at the time of field collections as no environmental covariates were included in this work.

Location

Supplementary Table 1 provides a list of the 26 field sites (and associated coordinates) in Washington, USA and British Columbia, Canada, from which individuals or samples were collected.

In Washington, individuals were collected between ~5-10 m and ~18 m depth for juvenile and adult sunflower sea stars, respectively.

In British Columbia, individuals were collected for sampling between ~5-10 m depth and returned to their original collection location following sampling.

Access & import/export

For experiments, we collected sunflower sea stars subtidally in Washington, USA, with diving support from the Washington

Access & import/export	Department of Fish and Wildlife (WDFW) and community volunteer divers, under scientific collection permits issued by the WDFW (permit no. HARVELL 21-1172R, 22-175, 23-087, 24-053). We also received captive-bred sunflower sea stars from Friday Harbor Labs, WA, USA, and transported them to the USGS Marrowstone Marine Field Station under WDFW shellfish transfer permits (permit no. 23-1249, 24-1249). In addition to controlled laboratory exposure experiments, we collected coelomic fluid samples from sunflower sea stars at five sites in the fjords on the central coast of British Columbia, Canada, in 2023, under scientific collection permit XMCFR 18 2023 issued by Fisheries and Oceans Canada. All experiments were conducted at the USGS Marrowstone Marine Field station which is well suited and equipped for marine disease experimental work. Facilities housing the animals involved were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAA-LAC) and inspected regularly by the Institutional Animal use and Care Committee (IACUC) at the U.S. Geological Survey, Western Fisheries Research Center. Within the USGS Marrowstone Marine Field Station, we housed each star in its own "tank," containing an airstone and inflowing water line. We maintained tanks inside large seawater tables, keeping each individual in physical isolation from all others at all times to prevent disease transmission. We partially flooded the seawater tables to create a water bath that maintained consistent temperature. Tanks were supplied with single-pass processed seawater that was treated with sand filtration, particle filtration to 10-25 µm, and double UV irradiation.
Disturbance	No disturbance was caused by the collection of animals from our field sites, aside from the removal of individuals from sites in Washington, USA to be used in experiments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Lab-reated sunflower sea stars (Pycnopodia helianthoides) between 1-3 years in age were used in our experiments.
Wild animals	Wild sunflower sea stars (Pycnopodia helianthoides) were used in our experiments or sampled from the field. Estimating age of sunflower sea stars is challenging, thus for the purpose of this work we categorize individuals into two groups: adults (>40 cm diameter) and juveniles (5-20 cm diameter). We collected grossly normal sunflower sea stars subtidally in Washington, USA, with diving support from the Washington Department of Fish and Wildlife (WDFW) and community volunteer divers, under scientific collection permits issued by the WDFW (permit no. HARVELL 21-1172R, 22-175, 23-087, 24-053). We transported stars to the United States Geological Survey (USGS) Marrowstone Marine Field Station on Marrowstone Island, WA, USA, for experiments. For transport, each sea star was individually wrapped in seawater soaked sheets and placed in a cooler containing ice. During experiments, 92% of individuals exposed to disease agents died. At the end of each experimental year, individuals that did not die (including controls) were unsuitable for release back into the wild due to risks of disease introduction to wild populations and were thus euthanized. Euthanasia was conducted using the method of Wahltinez et al. (2021): Practical Euthanasia Method for Common Sea Stars (Asterias rubens) That Allows for High-Quality RNA Sampling (published in Animals, 11(7):1847). When individuals were no longer reactive they were frozen for a minimum of 24 hours, and then autoclaved before being disposed.
Reporting on sex	Sex was not considered in this study as it is not possible to assign sex to a sunflower sea star using external features.
Field-collected samples	Within the USGS Marrowstone Marine Field Station, we housed each star in its own "tank," containing an airstone and inflowing water line. We maintained tanks inside large seawater tables, keeping each individual in physical isolation from all others at all times to prevent disease transmission. We partially flooded the seawater tables to create a water bath that maintained consistent temperature. Tanks were supplied with single-pass processed seawater that was treated with sand filtration, particle filtration to 10-25 µm, and double UV irradiation. We monitored and maintained seawater temperature below 13°C whenever possible, and

cooled seawater with an inline chiller when temperature exceeded 13°C. Photoperiod was consistent with the natural photoperiod cycle during the course of experiments. We fed stars locally collected mussels or clams three times a week. We froze all food for a minimum of 24 hours prior to feeding to reduce the introduction of live microbes into the tanks.

All equipment used throughout the course of our experiments was decontaminated by soaking in (1) a 10% bleach solution, (2) freshwater, and (3) a 10% povidone-iodine (betdine) solution.

All unused food or products thereof were decontaminated by soaking in a 10% bleach solution for a minimum of 24 hrs., followed by autoclave treatment and disposal.

During experiments, 92% of individuals exposed to disease agents died. At the end of each experimental year, individuals that did not die (including controls) were unsuitable for release back into the wild due to risks of disease introduction to wild populations and were thus euthanized. Euthanasia was conducted using the method of Wahltinez et al. (2021): Practical Euthanasia Method for Common Sea Stars (Asterias rubens) That Allows for High-Quality RNA Sampling (published in Animals, 11(7):1847). When individuals were no longer reactive they were frozen for a minimum of 24 hours, and then autoclaved before being disposed.

Ethics oversight

No ethical approval or guidance is required for work on non-cephalopod invertebrates.

Facilities housing the animals involved were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAA-LAC) and inspected regularly by the Institutional Animal use and Care Committee (IACUC) at the U.S. Geological Survey, Western Fisheries Research Center.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A