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Surviving the depths: metazoan resilience in sulphidic aquaria environments

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Hydrogen sulphide (H_2S) is widely acknowledged as a potent respiratory toxin for eukaryotic cells. However, macrofauna have been observed thriving in environments with elevated H_2S concentrations. Here we report on a saltwater aquarium hosting a community of invertebrates and inhabited by an epibenthic microbial mat. The aquarium was left undisturbed for the duration of the COVID-19 pandemic stay-at-home order, leading to the development of high concentrations of H_2S. Remarkably, the invertebrate community did not collapse. This success offers valuable insights into how invertebrates respond to physiochemical stressors at both individual and community levels. We also observed persistent disequilibrium between H_2S and oxygen (O_2), exhibiting out-of-phase periodic cycles driven by a simulated solar cycle. During daylight, photosynthetic O_2 production increased, resulting in more active behaviour from the metazoan community. Conversely, H_2S production peaked during the dark cycle, causing a moribund animal community. Additionally, over time, overall community diversity in the tank decreased, while macrofaunal abundance appeared largely unaffected. Polychaete worms and cnidarians demonstrated resilience to the high-sulphide conditions for the entire duration of the experiment, whereas others experienced gradual declines in abundance until they perished. These findings challenge conventional expectations of eukaryotic tolerance to H_2S and underscore the significance of behavioural adaptations in withstanding high-sulphide environments. Our findings provide insights into how primitive metazoans may have survived in sulphidic to euxinic Ediacaran seas.
Metazoans, microbial mats, hydrogen sulphide toxicity, Ediacaran

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Hydrogen sulphide (H_2S) is highly toxic to aerobic eukaryotes even in micromolar concentrations, readily permeating cell membranes and ultimately resulting in death (Beauchamp *et al.* 1984; McIlroy *et al.* 2021; Müller et al. 2012; Olson & Straub 2016; Sahoo *et al.* 2016). The mechanism by which H_2S interferes with cellular function is relatively well understood; it binds to cytochrome c oxidase in the mitochondrial respiratory chain, halting ATP production and cellular respiration (Bergstedt & Skov 2023; Mathai *et al.* 2009; Tobler *et al.* 2018). However, all metazoans naturally produce endogenous H_2S when catabolizing sulphur-containing amino acids, and low concentrations of H_2S are involved in various cell signaling pathways. As such, all metazoans possess the fundamental machinery to process H_2S and consequently are capable of detoxifying it to some extent (Kelley *et al.* 2016; McIlroy *et al.* 2021; Tobler *et al.* 2016). Additionally, low concentrations of H_2S can stimulate O_2 uptake (Bergstedt & Skov 2023; Olson & Straub 2016). It is not until concentrations exceed a critical threshold, approximately 0.1 mM (Truong *et al.* 2006), that the toxic effects of H_2S become predominant and result in death. In freshwater environments, 2 μg/L H_2S has been recommended as a safe limit for fishes (Beauchamp *et al.* 1984).

Nonetheless, macrofaunal assemblages (Pogonophoran clams, chimney-dwelling Alvinellid polychaetes, Bythograeid crabs, and various other polychaetes) have been documented in close proximity to deep marine hydrothermal vents with high H_2S concentrations (Felbeck 1983; McMullin *et al.* 2000; Vismann 1991). Furthermore, midge larvae belonging to the family Chironomidae were discovered burrowing beneath photosynthetic algal mats in a lake in Alberta, Canada (Gingras *et al.* 2007). There they mined O_2 from these biomats in order to survive

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in an otherwise low-O_2 and high-H_2S environment (Gingras *et al.* 2007). Similarly, dipteran larvae, coleopteran larvae, and an oligochaete in a hypersaline lagoon in Venezuela were observed to inhabit O_2-enriched environments during daylight hours but faced high-H_2S conditions during the night (Gingras *et al.* 2011). Other studies have focused on the resilience of live-bearing fishes in high-sulphide springs (Kelley *et al.* 2016; Tobler *et al.* 2018), the diversity of communities thriving in H_2S-rich regions of the Namibian shelf (Currie *et al.* 2018), and the emergence of H_2S-rich waters within circulation tanks utilized in fish husbandry (Bergstedt & Skov 2023). Importantly, several annelid worms including the peanut worm, *Sipunculus nudus*, the lugworm *Arenicola marina*, and *Urechis unicinctus* live in environments where they are regularly exposed to elevated concentrations of H_2S. They survive these conditions through a specialized enzyme called sulphide:quinone oxidoreductase (SQR) which converts H_2S into thiosulphate, and in the process, donates electrons to the mitochondrial electron transport chain (Müller et al. 2012; Tobler et al. 2016; Völkel & Grieshaber 1992). Additionally, the 'fat innkeeper worm' *Urechis unicinctus* has been shown to possess the same SQR enzyme (Ma *et al.* 2010; Müller *et al.* 2012) and the mussel *Geukensia demissa* is able to oxidize sulphide in its gills (Doeller *et al.* 1999). These observations clearly highlight certain metazoans develop a tolerance to H_2S, either by physiological adaptations or behavioural strategies.

In this study, we present findings from a marine aquarium containing sediment, diverse cyanobacteria, and several types of invertebrates including polychaetes, starfish, anemones, and amphipods. The aquarium remained undisturbed during the COVID-19 stay-at-home order (>12 months). A single UV light source was used to expose the tank to 8 hours of simulated daylight each day for the duration of the shutdown. When the tank was subsequently opened after more than a year, a distinct sulphidic odour indicated the production of H_2S. Despite this, the invertebrate organisms within the tank not only survived but also reproduced. Consequently, the tank's environment was analysed for both O_2 and H_2S concentrations using two Clark-type amperometric microsensors for O_2 and H_2S.

While the development of sulphidic conditions in the aquarium with a viable invertebrate community was unexpected, the outcomes of the tank experiments are no less noteworthy. There is a natural tendency to avoid unexpected outcomes or treat them as a result of poor planning (Chen *et al.* 2019). However, they often provide valuable insight and drive scientific innovation. Historical examples, such as the discovery of penicillin and cosmic microwave background radiation, illustrate how unexpected results can revolutionize scientific understanding. Moreover, there are numerous smaller examples of so-called 'happy accidents' that have yielded noteworthy scientific implications. One such example is the inadvertent creation of the first terrarium, initially called the 'Wardian Case'. This occurred when Dr Nathaniel Ward placed plant debris that failed to grow in his own garden into a sealed bottle. To his surprise, the ferns and mosses, which struggled to grow in the polluted airs of 19th century London, thrived within the self-contained environment of the bottle. Dr Ward successfully maintained this environment without watering for 18 years (Hershey 1996).

Like the bottle that ultimately became the first terrarium, the aquarium under discussion here was left undisturbed, allowing natural conditions to run their course. Most interestingly, no human-mediated alterations were needed for this experiment to unfold. The tank was not intentionally spiked with sulphide, nor was it inoculated with any sulphate-reducing bacteria. Instead, the conditions evolved organically as the microbial community developed and underwent its metabolic processes. While it was not originally intended to become an experiment on animal resistance to H_2S, the development of such high H_2S concentrations within the aquarium became a perfect opportunity to observe animals contending with extreme conditions in a laboratory setting.

The findings reported here detail a metazoan community that survived for an extended period of time (>12 months) within the H_2S-rich saltwater aquarium. What is particularly surprising is the community's resilience, as it did not succumb to collapse, providing new insights into the tenacity of metazoans in facing harsh environmental conditions.

Material and methods

Tank setup

The 30-gallon tank, part of a complex multi-tank setup designed to house a variety of marine invertebrates used in various experiments, was filled with natural sediment from Arcadia Beach, Oregon, USA. Seawater was simulated using Aquaforest Probiotic Reef Salt. During the COVID-19 shelter-in-place order, invertebrate macrobiota that could be located were removed from the tanks and rehoused in alternative facilities, while smaller individuals or those burrowed in the sediment unknowingly remained. Circulation with other tanks was terminated and the top of the study tank was covered in plastic wrap to minimize evaporation while the tank was left unattended. Upon post-lockdown examination of the tank, and realization that an H_2S-tolerant community had developed, the top was re-sealed with a plexiglass panel and wrapped in Parafilm® to limit diffusive mixing with the ambient atmosphere of the room. Photos of the organisms inhabiting the tank were taken when the H_2S-tolerant community was discovered, representative of the starting diversity in the aquarium. A small (roughly 3x4 inches) sampling window was cut into the plexiglass, which was only opened during measurements and was otherwise left closed. Other than this access hatch, the tank was always sealed. Nothing, including organisms, nutrients, or water, was added to, or removed from the tank for the duration of the experiment.

The tank was set up under a single UV light source to simulate daylight. During the lockdown, the light was connected to a timer which allowed for 8 hours of daylight each day. Once the controlled experiment began, this light-dark timing was continued, and the tank was left undisturbed during dark intervals. After the lockdown ended, and once measurements could begin, the timer was adjusted according to the desired day length for each iteration of the measurements. Day lengths were chosen as an 8-hour day or a 12-hour day, meant to represent variations in the solar day associated with latitude, and the tank was allowed to equilibrate for at least a week between timer adjustments. A third iteration was conducted during which the tank was subjected to a full 24 hours of light or darkness. Measurements were taken one hour before the end of each light or dark cycle, allowing time for the sampling apparatus to be set up and data to be collected before the cycle being measured concluded.

Unisense microsensor

For the purposes of this experiment microsensors, or miniaturized ion-selective probes, were used to collect data on the environmental concentrations of O_2 and H_2S in the water column. This equipment allowed us to detect small-scale chemical differences in the water column with both high precision and high accuracy.

Two Unisense Clark-type microsensors (sensor tips 50 μM), one for O_2 and one for H_2S, were mounted to a wooden dowel, while a jack stand was used to move the microsensors up-and-down through the water column as smoothly as possible whilst avoiding mixing of the water column. The dowel with the attached microsensors was inserted into the tank alongside a metre-stick for tracking measurement depth. A schematic of the sampling setup is provided in Figure 1. The Unisense micromanipulator was not used for this experiment as it did not have a sufficient range of motion in the vertical direction to profile the entirety of the water column. Both the O_2 and H_2S microsensors were calibrated according to manufacturer specifications (Unisense) and were connected to a picoammeter for polarization and signal amplification (PA-2000 and UniAmp X5). Two Unisense programs, Profix and SensorSuite, were used to record data and generate chemical profiles of the tank.

One hour prior to the conclusion of each light or dark cycle, the microsensors were set up and measurements were taken. Beginning at the water surface, measurements were taken down into the water column in one-centimetre increments. Once microsensor signals were stable, the measurement was recorded and the microsensor moved incrementally down one centimetre to the sediment-water interface. Measurements were not taken in the sediment to minimize disruption to the biomat and prevent damage to the microsensor tip. During one of the dark cycles, measurements were taken along a horizontal transect at 6 cm depth to illustrate the fluctuation of H_2S concentrations across the tank at equal depth.

Inductively coupled plasma – mass spectrometry (ICP-MS/MS)

At the conclusion of the experiment, a water sample was collected from 12 cm below the air-water interface using a sterile serological pipette and acidified with 12 μL of 70% nitric acid. The seawater sample was filtered and diluted twenty times using 2% HNO_3 and 0.5% HCl to achieve a TDS under 2300 ppm for analysis. A major element suite was analysed for bulk elemental chemistry to characterize the seawater via inductively coupled plasma mass spectrometry on an Agilent 8800 Triple Quadrupole ICP-MS/MS. A list of all elements analysed can be found in Table 1.

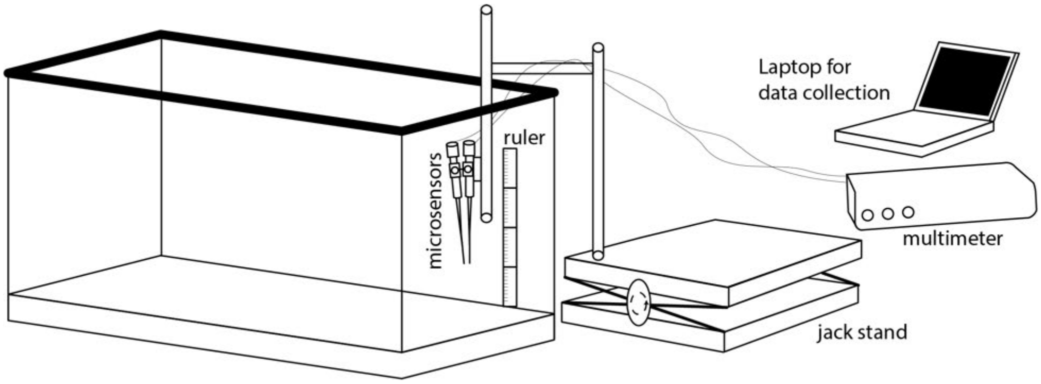

Fig. 1. Schematic of aquarium setup and sampling apparatus.

Table 1. Major element composition of tank water (ppm), as compared to modern seawater (Wright & Colling 1997).

Element	Parameter	Value	modern seawater (ppm, from Wright & Colling,
Si	Conc. (ppm) Detection Limit	36.2 0.575	2
Fe	Conc. (ppm) Detection Limit	BDL 0.044	5.5×10^{-5}
Ca	Conc. (ppm) Detection Limit	795 0.044	412
Al	Conc. (ppm) Detection Limit	BDL 0.044	4×10^{-4}
Ti	Conc. (ppm) Detection Limit	BDL 0.045	1×10^{-3}
Mg	Conc. (ppm) Detection Limit	1202 0.035	1290
Na	Conc. (ppm) Detection Limit	12600 0.044	10770
K	Conc. (ppm) Detection Limit	491 0.011	380
P	Conc. (ppm) Detection Limit	1.02 0.012	0.06
Mn	Conc. (ppm) Detection Limit	BDL 0.044	3×10^{-5}
Ba	Conc. (ppm) Detection Limit	0.184 0.001	0.02
V	Conc. (ppm) Detection Limit	BDL 0.0003	2×10^{-3}
Sr	Conc. (ppm) Detection Limit	7.62 0.0002	8
Co	Conc. (ppm) Detection Limit	BDL 0.00003	-
Cr	Conc. (ppm) Detection Limit	BDL 0.002	3×10^{-4}
Mo	Conc. (ppm) Detection Limit	0.014 0.000002	0.01
Cu	Conc. (ppm) Detection Limit	BDL 0.021	1×10^{-4}
Ni	Conc. (ppm) Detection Limit	0.006 0.0002	4.8×10^{-4}
Zn	Conc. (ppm) Detection Limit	BDL 0.001	5×10^{-4}

Results

Chemical analyses

Chemical profiles of the water column are presented in coupled measurements representing both light and dark segments within each 8-, 12-, or 24-hour cycle. The profiles for each duration are presented in Figure 2. In each case, H_2S concentrations are lowest near the surface, gradually increasing with depth in the water column. Notably, during the 12-hour light cycle, a sharp increase in the concentration of H_2S occurs around 6 cm depth in each profile. In contrast, O_2 concentrations within each of the 12-hour light cycles remain relatively static, fluctuating within a 20% range. An exception occurs during one of the 12-hour daylight segments where a similar sharp increase in O_2 concentration, akin to that observed in H_2S concentrations, is also evident.

The 24-hour cycle shows more stable curves, with less variation in both dissolved gas concentrations. During the dark segment of the cycle, H_2S concentrations remained consistent with depth, while O_2 concentrations displayed only minor fluctuations with depth. Conversely, during the light segment of the cycle, H_2S concentrations were lowest at the water surface, gradually rising with depth. O_2 concentrations steadily increased, with a noticeable uptick directly above the microbial mat at the sediment-water interface. After 24 hours of full light exposure, O_2 concentrations showed greater variability compared to those following 24 hours of darkness.

Across all the measurements, O_2 concentrations fluctuated between 60% to 130% atmospheric saturation, while sulphide concentrations varied more widely, spanning from 0.05 mM to 30 mM. The highest H_2S concentrations were recorded during the 16-hour dark/8-hour light cycle. Across a horizontal transect of the tank, H_2S concentrations all varied within 1 mM (S1). Both O_2 and H_2S concentrations fluctuated throughout the light and dark cycles, maintaining the entire tank environment in a state of disequilibrium and consistent with the kinetically limited rate of sulphide oxidation in oxygenated water noted

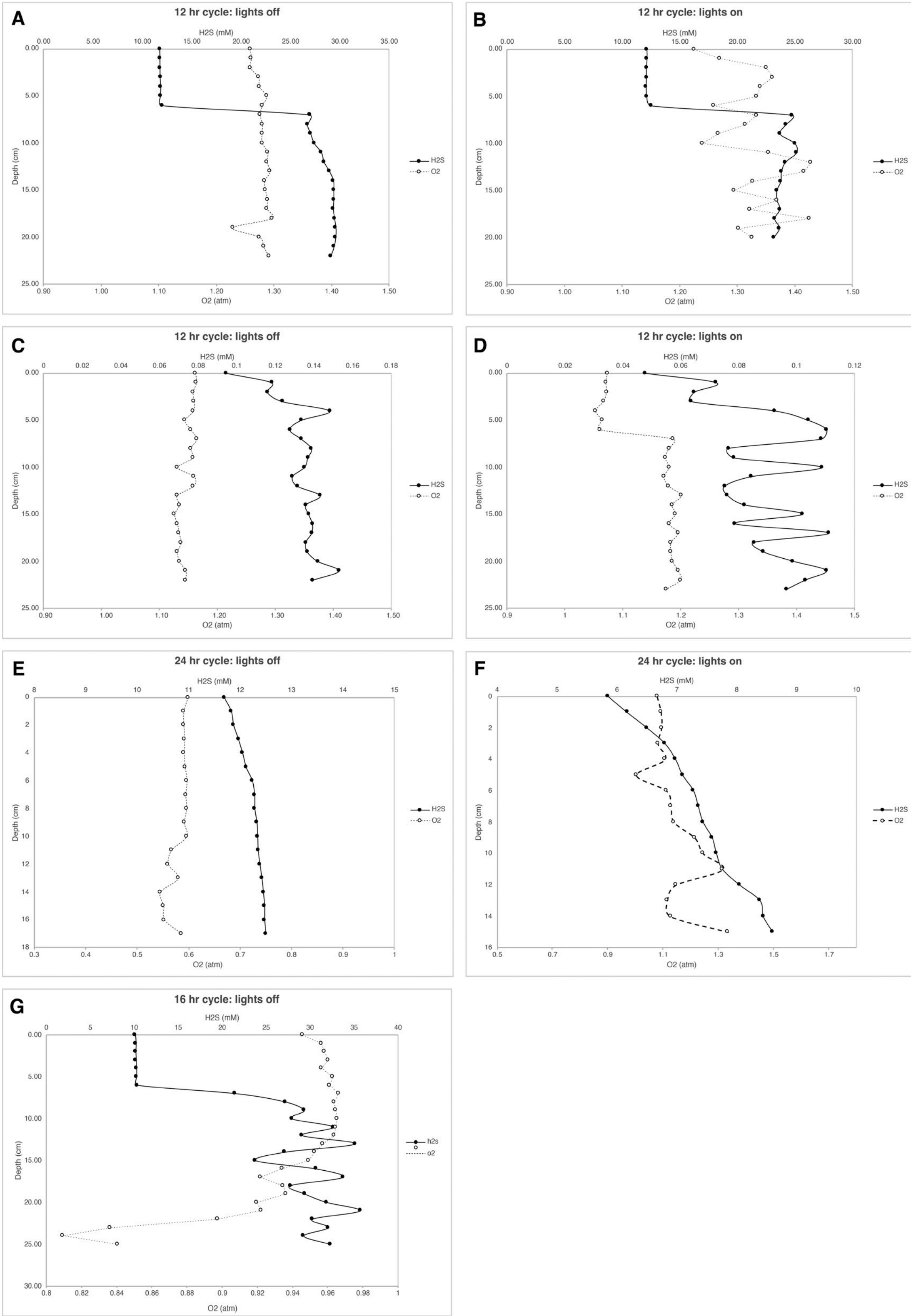

Fig. 2. Microsensor profiles of O_2 and H_2S during varying light and dark cycles. A–D, profiles during 12-hour light and dark cycles. E, F, profiles during 24-hour light and dark cycles. G, profile after 16-hour dark cycle.

by Millero *et al.* 1987 and Luther *et al.* 2011. During light cycles, O_2 was produced, leading to concentrations within the tank above atmospheric levels, while H_2S concentrations decreased. The reverse was true for dark cycles. However, it should be noted that O_2 concentrations within the tank remained relatively stable, rarely dropping below 75% atmospheric concentration, with the lowest point reaching 50% atmospheric concentration only after a complete 24-hour cycle in darkness.

Results from the ICP-MS/MS analysis can be found in Table 1, alongside concentrations of major ions in modern seawater. The seawater in the tank had a consistent pH of 8.48 ± 0.02 throughout the entirety of the water column.

Animal observations

The community living in the aquarium consisted of a type of marine worm (Class Polychaeta), anemone (Order Actiniaria), starfish (Class Asteroidea), and amphipod (Order Amphipoda). Photographs of the community members within the tank are presented in Figure 3. During the early stages of the tank measurement, the animal community was most diverse, containing anemones, polychaetes, starfish, and amphipods. After an indeterminate interval of time, the diversity of the invertebrate community decreased, leaving behind only the anemones and polychaetes. These groups, however, did not appear to suffer. Although overall diversity in the tank decreased, the number of individuals belonging to the surviving groups increased, though community numbers remained relatively small with under a hundred individuals living in the tank. Additionally, all the organisms in the tank were of small body sizes, with the largest being an anemone measuring roughly 2.5 cm but the vast majority being 1 cm or less. Several reproductive episodes were recorded. During the daylight segment of the light cycles, the anemones stood more erect with more upright tentacles. The polychaetes were observed emerging from their burrows and traversing the sediment-water interface. During the dark stages, the anemones were observed drooping at the stalk, while the polychaetes returned to their burrows and no longer moved across the sediment surface.

Interpretation

Microsensors

The microsensor measurements reveal the coexistence of both O_2 and H_2S within the tank's water column. When the lights are on, O_2 is produced and the concentrations slowly diffuse upwards through the water column. This is particularly evident in the 24-hour light cycle, where an increase directly above the microbial mat is observed in the measurements. By contrast, when the lights are off, H_2S is produced by the microbial mat or the sediment below, similarly diffusing upwards through the water column. The two analytes never reach steady-state concentrations, but instead fluctuate in waves. When the lights are on, sufficient photosynthetic O_2 is produced to sustain the metazoan community.

Water composition

As expected, the simulated seawater sample taken from the tank closely resembles that of natural seawater. The only notable exceptions are the concentration of phosphorus, which is 1.02 ppm compared to 0.06 ppm expected in natural seawater, and calcium, which is nearly double the expected concentration in seawater. The increase in phosphorus likely resulted from the degraded biomass of organisms that did not survive the high-H_2S conditions that developed in the tank, while the increase in calcium might be due to the calcium carbonate shells of deceased organisms reacting with the H_2S in the seawater. The concentrations of other major ions, such as sodium, potassium, and magnesium are all less than 1.3 times that of seawater.

Animal observations

The animals in the tank exhibited varying responses to the different environmental conditions, depending on the phase of the 24-hour light-dark cycle. When the lights were on and the O_2 concentrations were at their highest, the animals were the most active – the worms moved about the bottom of the tanks while the anemones stood erect. By contrast, in the dark and when H_2S concentrations increased, the animals became less active, potentially reserving energy. Additionally, all surviving members of the animal community in the tank were small in size. This is counterintuitive, considering larger body sizes result in smaller surface area:volume ratios (Tobler *et al.* 2016). However, it may be the result of the smallest invertebrates of the original tank being left behind, as all the larger members were removed prior to the tank being sealed at the onset of the COVID lockdown. While higher body size results in higher O_2 demands, and low-O_2 conditions have been shown to cause moulting at smaller body sizes in the moth *Manduca sexta* (Callier & Nijhout 2011), O_2 concentrations are unlikely to be solely responsible for the small body

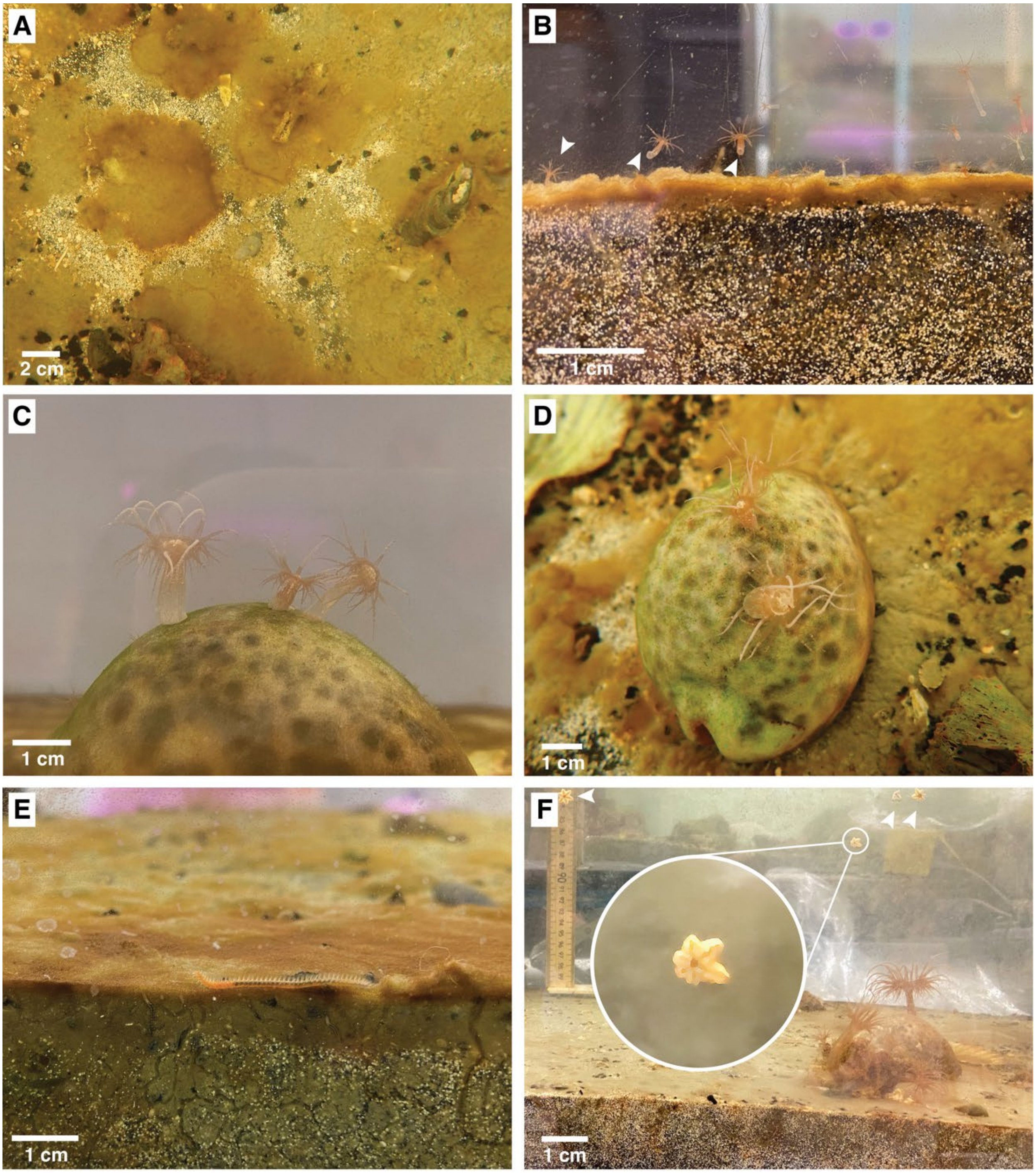

Fig. 3. Organisms living in the tank. A, biomat on the sediment surface. B, juvenile anemones along the sidewall of the tank (highlighted by white arrows). C, D, adult anemones. E, polychaete worms. F, starfish along the sidewall of the tank (highlighted by white arrows).

sizes seen in this community. This is because O_2 concentrations in the tank never reached fully anoxic or dysoxic conditions.

The initial community composition of the tank was more diverse than the final community. Although they survived the first part of the Covid-19 shutdown, it appears that the amphipods and starfish were unable to survive long-term exposure to the diurnally sulphidic conditions in the tank. In contrast, the anemones and polychaetes were more resistant to high-H_2S conditions. The latter taxa not only survived, but also reproduced, throughout the entire duration of the experiment.

Discussion

Microbially-controlled environmental fluctuations

During the daylight phase of the cycle, the microbial mats colonizing the sediment surface within the tank photosynthesized, generating dissolved O_2. When daylight ended and the light was switched off, O_2 production ceased, and its concentration dropped. This pattern repeated diurnally as the tank cycled through light and dark phases. This fluctuation is also reflected in the H_2S concentrations, which reached their peak concentrations during the phase with the longest period of darkness. Many of the organisms within the tank also grew along both the sediment-water interface and on the sidewalls of the aquarium where the colonizing microbial mat was present. The behaviour of these animals mimicked the cyclical changes in O_2 and H_2S concentrations. When the light switched on and the O_2 concentration began to rise, polychaetes emerged from their burrows and traversed the mat-covered sediment surface, while the anemones stood erect with their tentacles outstretched. Conversely, as the day cycle concluded and the O_2 concentrations began to decline, polychaetes retreated to their burrows, and the anemones began to droop, relaxing at the stalk and tentacles.

Similar to the animals observed in Cooking Lake and Venezuela, it appears that the organisms inhabiting the tank utilized the waste O_2 produced by the microbial mat during photosynthesis (Gingras *et al.* 2011; Gingras *et al.* 2007), while also enduring H_2S concentrations that exceeded 30 mM. According to conventional understanding, such concentrations of H_2S would be expected to be fatal to all of the organisms (Bergstedt & Skov 2023; McIlroy *et al.* 2021; Tobler *et al.* 2016). Nevertheless, the community living in the tank remained viable and underwent multiple reproductive episodes. The behaviour of these organisms clearly illustrates that such concentrations of H_2S are not necessarily lethal to their survival, as they were still able to carry out essential life functions, including locomotion and reproduction.

Metazoans are believed to detoxify H_2S to some extent through four mechanisms: (1) minimizing environmental flux via behavioural or integumentary modifications; (2) altering toxicity targets to render them less reactive; (3) enhancing H_2S detoxification capacity or reducing endogenous H_2S production; and/or (4) regulating H_2S concentrations through symbiotic relationships (Felbeck 1983; Kelley *et al.* 2016; McIlroy *et al.* 2021; Roeselers & Newton 2012; Tobler *et al.* 2016). In the context of the aquarium experiments discussed herein, behavioural or symbiotic adaptations appear to be favourable. This is observed by the heightened activity of polychaetes during periods of light exposure when photosynthetic O_2 accumulated in bottom waters. Additionally, the drooping behaviour of the anemones as O_2 levels decreased and H_2S levels increased suggests a potential survival strategy involving reduced metabolic rates (Gingras *et al.* 2011; Kelley *et al.* 2016; Tobler *et al.* 2016). It is also worth considering symbiotic relationships with H_2S-oxidizing bacteria as a mechanism for surviving the high-H_2S conditions present in the aquarium. This method of adaptation has been previously observed in some invertebrates, such as polychaetes and mussels (Currie *et al.* 2018; Kelley *et al.* 2016; Roeselers & Newton 2012).

Interestingly, it has been proposed that eukaryotic mitochondria originated from a facultatively aerobic sulphide-oxidizing alphaproteobacterial endosymbiont, an update to the so-called 'Syntropy hypothesis' of eukaryogenesis (López-García & Moreira 2020). If correct, this model would suggest that tolerance to H_2S, as well as the ability to metabolize H_2S and use that energy in ATP synthesis, is ancestral to eukaryotes more broadly. This model is supported by the similarities between H_2S metabolism in humans and certain bacteria, suggesting a long-standing evolutionary relationship, and the observation that various metazoans can generate ATP through mitochondrial H_2S oxidation, either with the assistance of intracellular bacterial symbionts or with SQR (Doeller *et al.* 1999; Felbeck 1983; Müller *et al.* 2012; Olson & Straub 2016; Völkel & Grieshaber 1992). And, though purely speculative, this also lends the possibility that the organisms surviving in this tank are expressing SQR genes.

Early Earth implications

Euxinic conditions, characterized by anoxic waters enriched in dissolved H_2S, were likely prevalent during the Ediacaran Period, presenting a significant evolutionary hurdle and argued by some to pose a potential extinction threat for early animals (Lyons *et al.* 2014, 2024; Sahoo *et al.* 2016). Despite this, the metazoan clade has its origins rooted in the Ediacaran (Pecoits *et al.* 2012; Peterson *et al.* 2008; Xiao & Laflamme 2009), or earlier in the Cryogenian (dos Reis *et al.* 2015), indicating that early animals must have possessed mechanisms to cope with such harshly fluctuating environmental conditions to survive. The finding presented here suggest that primitive members of the metazoan clade exhibit a higher tolerance to H_2S than is expected from modern members. The low-O_2 conditions of Precambrian oceans would suggest the lineages that arose during that time were well-suited to survive in low-O2, H_2S-rich waters and the ability of modern metazoans living in sulphidic environments to withstand H_2S could instead be seen as vestigial (Müller *et al.* 2012). Further research should consider the implications of this tolerance for early animal evolution and investigate the role of H_2S as either a hindrance or stimulus in evolutionary processes.

Conclusions

This study illustrates that various groups of invertebrates, such as anemones and polychaetes, exhibit resistance to H_2S concentrations higher than those expected to be lethal. Although much of the research on H_2S resistance has centred on vertebrates, some invertebrate groups also exhibit tolerance to H_2S-enriched environments. These organisms play a key role in integrating H_2S into the food web (Currie *et al.* 2018), and thus, they should be considered key to discussions on metazoan diversity and survival in H_2S-rich environments. Moreover, environments characterized by high H_2S and low O_2 create reproductively isolated niches (Tobler *et al.* 2018), with the potential to drive speciation.

Further research should also pay particular attention to the establishment and stability of the environment observed in this aquarium study, along with the animals' responses to these fluctuating and dynamic conditions. While this tank offered a glimpse into the endurance of metazoans in the presence of H_2S, a factor often underestimated, future studies should focus on creating a more controlled environment. Additionally, they should observe the development of anoxia or euxinia to develop a better understanding of how metazoans can tolerate transitions between low-O_2 and high-H_2S environments compared to less physiochemically stressed communities.

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Supplementary Material

S1. H_2S concentrations from a horizontal transect across the tank. All measurements were taken at 6 cm depth.

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