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The long-lived deep-sea bivalve *Acesta excavata* is sensitive to the dual stressors of sediment and warming

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A B S T R A C T

Human influence in the deep-sea is increasing as mining and drilling operations expand, and waters warm because of climate change. Here, we investigate how the long-lived deep-sea bivalve, *Acesta excavata* responds to sediment pollution and/or acute elevated temperatures. *A. excavata* were exposed to suspended sediment, acute warming, and a combination of the two treatments for 40 days. We measured O_2 consumption, NH_4^+ release, Total Organic Carbon (TOC), and lysosomal membrane stability (LMS). We found suspended sediment and warming interacted to decrease O:N ratios, while sediment as a single stressor increased the release of TOC and warming increased NH_4^+ release in *A. excavata*. Warming also increased levels of LMS. We found *A. excavata* used protein catabolism to meet elevated energetic demands indicating a low tolerance to stress. *A. excavata* has limited capacity for physiological responses to the stressors of warming and sediment which may lead to decreased fitness of *A. excavata*.

1. Introduction

Deep sea organisms are sensitive to changes in their environment (Angel, 1992; Haedrich, 1996). Human impacts are expanding in the deep-sea as mining and drilling operations increase in the ocean (Sharma, 2015) and climate change triggers warm water down-welling (Levin et al., 2020). Such human impacts in an otherwise stable environment may challenge deep sea organisms. Mining, and removal of associated waste generates a range of potential environmental impacts that can influence terrestrial (Willig and Walker, 1999), atmospheric (Petavratzi et al., 2005), freshwater (Ryan, 1991) and marine ecosystems (Chansang et al., 1981; Fabricius, 2005). While mining waste (tailings) can be extremely toxic (Duruibe et al., 2007), seemingly benign waste such as natural sediments and crushed rock can also impact ecosystems (Rogers, 1990; Ryan, 1991; Fabricius, 2005; Kutti et al., 2015). The release of inert mine tailings into marine ecosystems through submarine tailings displacement (STD) has been occurring as an alternative to land-based tailings storage for land-based mines for decades, yet, the impacts this may be having on marine ecosystems are only beginning to be understood (Lancellotti and Stotz, 2004; Ramirez-Llodra et al., 2015; Vare et al., 2018).

Deep sea habitats have been impacted by increased suspended sediment loads from drilling activities and the discharge of mine tailings in the water column (Vare et al., 2018). There is also now the potential for deep-sea mining of metallic ores from the seabed (Christiansen et al., 2020; Simon-Lledó et al., 2019). These activities will inevitably affect the deep-sea habitats at the location of mining activities, however, the associated effects of suspended sediment across a much larger area remain unknown. Increased suspended sediment has negative effects on the physiology and ecology of many marine organisms and ecosystems (e.g. Allan, 1995; Lancellotti and Stotz, 2004; Fabricius, 2005; Roberts, 2012; Tjensvoll et al., 2013; Kutti et al., 2015). When concentrated, suspended sediment can homogenise habitats (Berkman and Rabeni, 1987) and eventually bury and smother sessile organisms (Ryan, 1991; Fabricius, 2005). At lower concentrations, suspended sediments can also cause negative effects on marine organisms (Vare et al., 2018), by damaging the gills of fish (Alabaster and Lloyd, 1982; Ryan, 1991) and bivalves (Cheung and Shin, 2005). Filter feeding organisms such as bivalves are especially vulnerable to suspended sediment. Effects of suspended sediment on filter feeders are known to include reduced feeding

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efficiency (Gardner, 1981), elevated metabolic rates (Tjensvoll et al., 2013; Kutti et al., 2015), and increased cellular oxidative damage (Edge et al., 2015).

Deep sea habitats are also expected to warm because of a range of factors including rising sea surface temperatures, and altered hydrodynamic regimes triggering down-welling events where warm surface water is drawn into the deep sea (Collins et al., 2013; Lee et al., 2021). These factors will contribute to coastal warming in future oceans (Collins et al., 2013; Scanes et al., 2020; Lee et al., 2021). This changing climate will place extra physiological demands on some ectothermic organisms (Pörtner and Farrell, 2008). The capacity for ectothermic organisms to withstand these changes will depend on their ability to respond via plastic responses, genetic adaption or relocation. Otherwise, ecosystem dynamic accelerations (Johnston et al., 2022) and/or mass extinctions may occur (Williams et al., 2008; Dawson et al., 2011; Bellard et al., 2012).

The Norwegian fjords contain a diverse array of biota (Mortensen et al., 1995; Kutti et al., 2013, 2014), often dominated by habitat reef building demosponges and cold-water corals (Fosså et al., 2002), and support high abundances of zooplankton and pelagic and benthic fishes (Skjoldal, 2004). In the fjords of Norway, the submarine release of tailings from coastal mines into the marine environment has been common place for decades (Kvassnes and Iversen, 2013). The deep nature (>100 m) of the nearshore environment in fjords has allowed for extensive release of tailings, with what was thought to have minimal environmental impact (Olsgard and Hasle, 1993). It is now known that submarine disposal of inert mine tailings in these areas can change the cellular and metabolic processes of reef forming organisms (Kutti et al., 2015; Edge et al., 2015; Scanes et al., 2018), reduce the biodiversity, and change the structure of deep-sea assemblages up to kilometres away from the discharge site (Olsgard and Hasle, 1993; Bakke et al., 2013; Bianchi et al., 2020). Waste release can elevate suspended sediments in the benthic boundary up to ten times normal concentrations, 1–2 km away from the site of release (Allan, 1995; Bakke et al., 2013; Brooks et al., 2015).

The bivalve *Acesta excavata* (Fabricius 1779) forms vast aggregations in hard bottom habitats along the whole western European continental margin and is abundant in the deep-sea habitats of Norwegian fjords (Correa et al., 2005). *A. excavata* is a long-lived bivalve, with estimates of 50–80 years for a typical life span (Correa et al., 2005). Aggregations of *A. excavata* are known to provide valuable hard substrate for epibionts such as serpulids, bryzoans, sponges and Anomiidae sp. in Norwegian fjords (Correa et al., 2005). The aim of this study was to determine the response of the bivalve *A. excavata* to simulated mine tailings in the form of suspended sediment and/or acute elevated temperature in its environment. Physiological responses of the bivalve *A. excavata* were measured after exposure to suspended sediment, acute warming, and the dual impact of the dual stressors. It was hypothesised that the stressors of chronic suspended sediment from mine tailings, and acute warming would interact to have significant negative effects on the physiology of *A. excavata*. We also hypothesise that these treatments will interact in an, additive or synergistic fashion, rather than antagonistic (Folt et al., 1999).

2. Materials and methods

2.1. Experimental conditions

2.1.1. Collection of animals

Individual *A. excavata* were collected at a depth of 190 m from Nakken Reef in Langenuen Fjord, Norway, (59° 54.951 N, 05° 29.935 E) using a remotely operated vehicle (ROV) operated from the research vessel Håkon Mosby. Organisms were returned to the Austevoll Research Station of the Institute of Marine Research (Hordaland, Norway), and kept in 2000 L flowthrough aquaria for an acclimation period of two months prior to experimental exposure. The flowthrough aquaria used unfiltered seawater drawn from a depth of 160 m from the adjacent Langenuen Fjord (60° 5′16.91″N, 5°16′9.84″E), where it then flowed through tanks at a rate of 70 L h^{-1} $tank^{-1}$. Collected *A. excavata* were of a range of sizes, however, only those with a shell length (interior to anterior margin measurement) of 80–200 mm were used for experiments. Within its natural range in Norwegian fjords, *A. excavata* can be found at depths of 200–800 m and, and have been recorded at temperatures of 6–12 °C, however, most colonies are found at temperatures in the range of 6–8 °C (Correa et al., 2005; Hammer et al., 2011). *A. excavata* did not attach to the tanks via byssal threads so could easily be removed.

2.1.2. Sediment

Crushed granite rock (i.e. simulated inert mine tailings) was collected from a local quarry near the city of Bergen (Fana Stein A/S). Sediment was sieved using stainless steel laboratory sieves to obtain the fine fraction of sediment (<63 μm). The <63 μ fraction of sediment was then eliminated of organic content by combustion at 450 °C for 4 h. Sediment was then stored in airtight containers at room temperature until use.

2.1.3. Experimental treatments

All experiments were conducted at the Austevoll Research Station during November – December 2015. Four individual *A. excavata* were transferred from the acclimation tanks to each 40 L experimental tank fed by a continuous flow of seawater. There was a total of 16 experimental tanks, resulting in 64 individuals of *A. excavata. A. excavata* remained in experimental tanks for a total of 40 days. Seawater in the experimental tanks was unfiltered seawater drawn from a depth of 160 m from the adjacent Langenuen Fjord (60° 5′16.91"N, 5°16′9.84″E), where it then flowed through tanks at a rate of 70 L h^{-1} $tank^{-1}$. Unfiltered seawater at this flow rate was relied upon to supply food. To test the single and combined effects of suspended sediment and elevated temperature on *A. excavata*, there were 4 experimental treatments, each with 4 replicate tanks, and each tank housing four individuals ($n = 4$). Nominal experimental treatments were; control (filtered seawater; 7 °C) suspended sediment (10 mg L^{-1} crushed rock fines; 7 °C), elevated temperature (13 °C), and a combined temperature and sediment treatment (10 mg L^{-1} crushed rock fines; 13 °C).

To simulate a downwelling event in a fjord affected by chronic suspended sediment, organisms in sediment treatments were exposed to suspended sediment from the beginning of experimental exposure. Warming treatments did not commence until 26 days after sediment treatments. To simulate submarine mine tailings release, sediment suspension and exposure was conducted using the methods of Anthony (1999) and Scanes et al. (2018). Briefly; sediment stock solution (pre-ashed crushed rock [< 63 μm]) was delivered to tanks for 12 h in every 24 h to randomly assigned replicate tanks via peristatic dosage pumps (IWAKI (EW/Y)) to give a constant nominal concentration of 10 mg L^{-1} in each replicate suspended sediment treatment tank. Sediment concentrations in replicate tanks were determined daily by measuring the absorbance of a random sample from each tank using UV spectroscopy (Shimadzu UV 160) at 660 nm. Mean (±SE) measured sediment concentrations in the suspended sediment and the combined suspended sediment and elevated temperature treatments over the entire experimental period were 11.33 ± 0.74 mg L^{-1}, and 11.22 ± 0.75 mg L^{-1} respectively.

After 26 days of sediment exposure, the temperature was increased in warming treatments to simulate the acute warming associated with a downwelling event. Heat exchangers were activated and incrementally increased over two days to the desired temperature of 13 °C. Ambient temperatures in the control, and suspended sediment tanks were unaltered from the external environment and remained at 7 ± 1 °C. Elevated temperature treatments were maintained by two heat exchangers warming the incoming seawater to 13 ± 1 °C and then distributing the seawater randomly among replicates in the elevated temperature

treatments. The temperature of 13 °C was selected as the elevated temperature level for this experiment because in 2006 and again in 2008 a downwelling event occurred at Tisler Reef on the Norway coast with temperatures of exceeding 12 °C recorded in the habitats of *A. excavata* (Guihen et al., 2012). In both cases, temperature rose by >4 °C in 24 h and resulted in mortalities of benthic organisms (Guihen et al., 2012). Furthermore, while *A. excavata* has been known to occur at temperatures of 12 °C, this bivalve is widely distributed across the European continental margin and these temperatures are more likely at the southern extremes of its distribution (Correa 2005). 13 °C represents an increase of 5 °C above the temperatures encountered by the populations in Norway, from which we collected our specimens (Correa and Freiwald, 2005). We collected *A. excavata* from a population that would likely have not been acclimated to temperatures above 10 °C. We chose to stagger the onset of experimental treatments to prevent the shock of commencing treatments at the same time for *A. excavata* in the combined suspended sediment and warming treatments.

Individuals were sampled at three points throughout the experiment; 26, 33 and 40 days after commencement of sediment exposure. Therefore, individuals in the elevated temperature treatments had only five days exposure to the elevated temperature at the 33 d, and 12 days at 40 d measurement time.

2.2. Physiological measurements

The metabolic rates of *A. excavata* were determined from the oxygen consumption and ammonia excretion of *A. excavata* measured after 3 h closed incubations following the methods as described by Scanes et al. (2018) and Fang et al. (2018) in detail. All measurements were taken during the 12 h sediment was not dosed into the tanks. At each time point (26, 33, and 40 days), one individual was randomly selected from each replicate tank in all treatments for measurements 500 mL airtight chamber was placed over top of the selected *A. excavata*, capturing seawater to fill the chamber, and forming a seal against the bottom of the tank (Fang et al., 2018). This was done without disturbing the individual. Each chamber was fitted with a fibre-optic O_2 probe (PreSens needle probe NTH-PST1, AS1 Ltd., Regensburg, Germany). The exact incubation time of approximately 3 h was recorded, (during incubation O_2 concentrations were never depleted below 70 %). A "blank" chamber was run concurrently per treatment receiving the same sampling process as all other chambers, only without an organism inside. Before and after the incubation, water samples were taken for ammonia and total organic carbon (TOC) analysis using ashed (400 °C, 4 h) glass pipettes. For each TOC sample, 20 mL of seawater was taken from the incubation chamber and dispensed in a new TOC glass vial and spiked with 250 μL hydrochloric acid and frozen at −20 °C until analysis. Ammonia samples were also placed into a glass vial, 200 μL of chloroform was added and the samples were stored at −20 °C.

Excretion rate was determined by measuring ammonia concentration (μmol) at the end and beginning of incubations using fluorometric determination by direct segmented flow analysis (*Alpkem Flow Solution IV* autoanalyser; Kérouel and Aminot, 1997, Holmes et al., 1999). Concentrations of TOC were measured from samples taken before and after incubation using a high temperature combustion TOC analyser (Teledyne Tekmas Lotix TOC Analyser coupled with the Lotix's TOC TekLink™ software on a PC). For TOC analysis, a standard curve was generated using milliQ ultra-pure water and TOC standards to cover the range of concentrations within samples. Analysis of each sample was done in quadruplicate. Blanks, TOC standards (3.0 ppmC) and samples were injected into the TOC analyser (following the order of the manufacturers protocol) and analysed trough oxidation by catalytic combustion at 680 °C converting the TOC to CO_2 and quantified through a Non-Dispersive Infrared (NDIR) detector. Blank samples were deducted from the samples from chambers containing *A. excavata* as per Eq. (1). See supplementary information for raw TOC data and processing steps.

Individual *A. excavata* were removed from the chambers, opened, and tissue was separated from their shell. Both tissue and shells were weighed using an electronic balance (± 0.001 g). Digestive gland of *A. excavata* was removed for later analysis (see below), and the remaining tissue and shell were dried in an oven at 70 °C for 72 h then weighed using an electronic balance (± 0.001 g). To estimate dry weight of whole *A. excavata* tissue, the relationship between tissue wet weight (after dissection) and dry weight was estimated using linear regression ($y = 0.1193\times + 1.3601$; $R^2 = 0.923$; $P < 0.001$). The equation was then used to estimate dry weights of *A. excavata* tissue from their original whole wet weight.

Excretion of ammonia, TOC and oxygen consumption were standardised to dry weight using Eq. (1) (rate per g^{-1} dry tissue mass; Bayne, 1999). To determine the oxygen consumption to nitrogen (ammonia) excretion ratios, the molar equivalent of O_2 consumption as calculated per Eq. (1) was divided by the rate of nitrogen excretion as calculated by Eq. (1).

$$V_{\text{stand}} = \left(\left(\frac{\text{Vol} \times \Delta V_{meas}}{\Delta}\right) - \text{Blank}\right) \times W_b{}^{-1} \quad (1)$$

Eq. 1. Rate standardisation calculation. Where; V_{stand} is the measured variable normalised g^{-1} of dry tissue mass, Vol is the volume of the respiratory chamber minus the volume of the organism (L), ΔV_{meas} is the change in concentration of the measured variable (μmol or mg), Δt is the whole incubation time (h), Blank is the rate of change in the chamber measured concurrently without an organism, W_b is the dry tissue mass (g).

2.3. Lysosomal stability

To determine the cellular health of *A. excavata*, lysosomal membrane stability assay was done as a proxy for cellular membrane stability. While this assay has never been conducted on *A. excavata*, it has been adapted to a wide range of bivalves (e.g. Ringwood et al., 1998; Regoli et al., 2004; Fang et al., 2008a, 2008b, 2010; Edge et al., 2012). This was then used for the lysosomal membrane stability assay. The lysosomal membrane stability in hepatic cells was quantified with a neutral red retention assay previously described in detail (Ringwood et al., 1998; Ringwood et al., 2004; Edge et al., 2012). Briefly, the digestive gland of *A. excavata* was dissected out of each individual following wet weight measurements. Cells were extracted by tissue disaggregation to form a suspension. After filtering and washing, the cell suspensions were incubated with a neutral red dye for 60 min. The neutral red retention by lysosomes was determined by examining digestive gland cells (6–12 μm) under a light microscope (*Olympus* 400×). Cells with neutral red sequestered in lysosomes were scored as stable, and those with neutral red leaking into the cytoplasm were scored as destabilised. At least 50 cells were scored for each sample, and data were expressed as the percent of cells with neutral red leaking into the cytosol (% membrane destabilisation).

2.4. Data analysis

All data from each independent sampling time were analysed using a two factor Analysis of Variance (ANOVA), where the first factor, "temperature" (nominal 7 °C or 13 °C), was fixed, and the second factor, "sediment" (nominal 0 mg L^{-1} or 10 mg L^{-1}) was also fixed ($n = 4$). Analysis at day 26 only included the factor "sediment" because temperature treatments had not yet been activated. All data were tested for homogeneity of variances prior to analysis using Cochran's test (Cochran's test $P \leq 0.05$), lysosomal data (33 and 40 d) were square root transformed to meet the assumption of homogeneity of variance; TOC data failed Cochran's test due to variability in some treatments, this data must interpreted with caution (Underwood, 1996). Comparisons of variables between sampling times were not made because measurements at each timepoint were not independent of each other, therefore

violating the assumptions of ANOVA. Comparing timepoints was also not an objective of this study. All post hoc tests were conducted using SNK tests when there was a significant interaction (Sokal and Rohlf, 1995). Data analysis was completed using GMAV-5 for Windows (Underwood et al., 2002).

3. Results

3.1. Metabolism

3.1.1. Oxygen consumption

There were no significant effects ($P > 0.5$) of any treatment on the rate of oxygen consumption of *A. excavata* after 26, 33 or 40 days in experimental conditions (Fig. 1A). We observed a non-significant trend for *A. excavata* to consume more oxygen in the control compared to the other three treatments across all time points.

3.1.2. Ammonia excretion

Ammonia excretion varied over the duration of the experiment. At first, there were no significant effects of sediment treatments on the excretion of ammonia after 26 days (warming was not yet a treatment). Following 33 days exposure, there was no significant interaction between the sediment and warming stressors (ANOVA $P = 0.8$), however, there were significant effects of both warming (ANOVA $F_{1,12} = 18.62$, $P = 0.001$) and sediment (ANOVA $F_{1,12} = 6.08$, $P = 0.03$) as individual stressors. Warming increased the rate of excretion of ammonia (NH_3 μmol g^{-1} h^{-1}) by approximately 100 % after 33 days. Sediment decreased the rate that *A. excavata* excreted ammonia by approximately

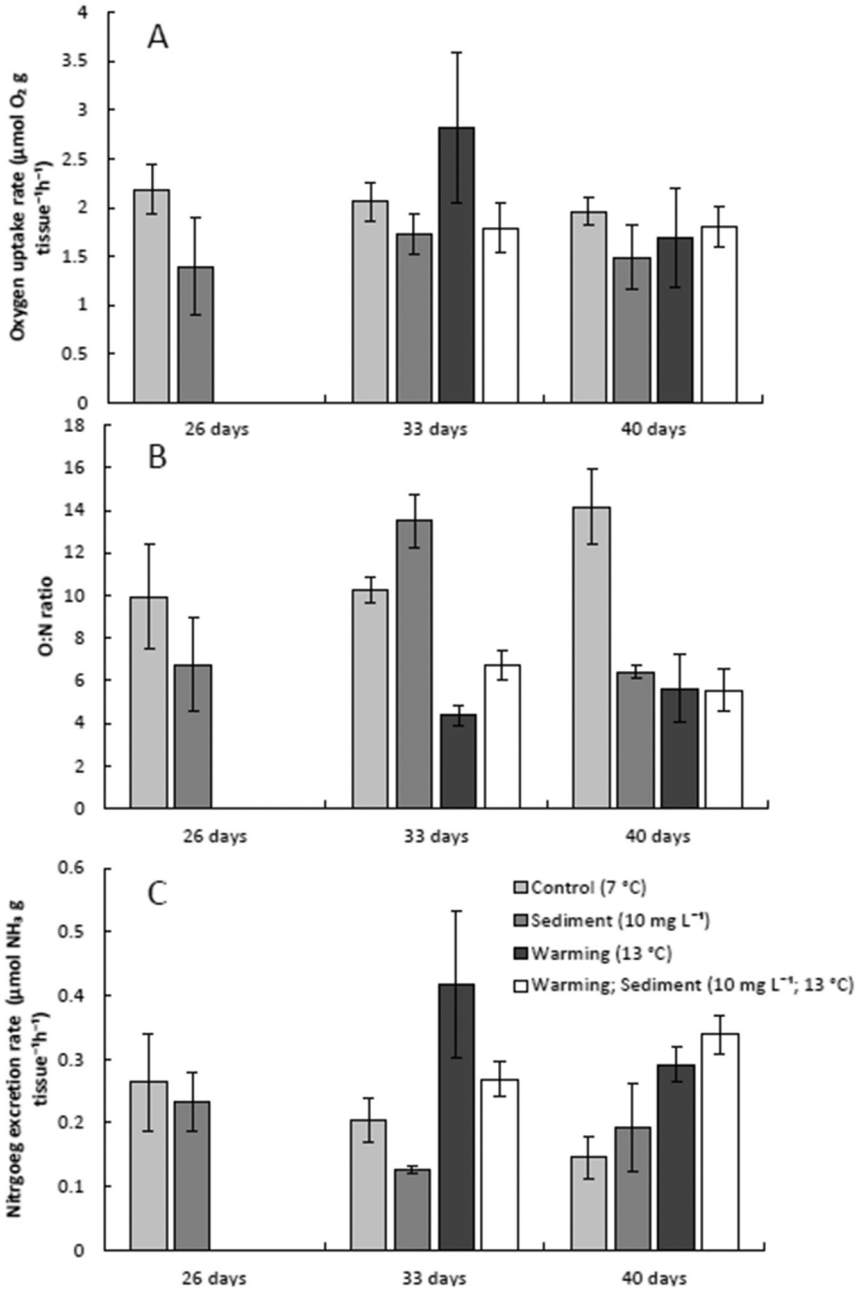

Fig. 1. A, mean (±SE; $n = 4$) oxygen consumption (mg O_2 g^{-1} h^{-1}) after 26, 33 and 40 days respectively; B, nitrogen excretion (μmol NH_3 g^{-1} h^{-1}) after 26, 33 and 40 days respectively; and C, O:N (μmol g^{-1} h^{-1}) ratio of *A. excavata* following 33 days and 40 days exposure to experimental treatments of; control conditions (no manipulation of variables; 7 °C), suspended sediment (10 mg L^{-1}, 7 °C), elevated temperature (13 °C) and combined suspended sediment; elevated temperature (10 mg L^{-1}, 13 °C).

30 %. After 40 days there was no significant interaction between the stressors, and sediment also had no effect on ammonia excretion of *A. excavata*. There was a significant effect of warming as a single stressor, increasing the rate of excreted ammonia of *A. excavata* by approximately 100 % (Fig. 1B; ANOVA $F_{1,12} = 11.72$, $P = 0.005$).

3.1.3. O:N molar ratio

The ratio of molar equivalent oxygen consumed to ammonia excreted (O:N) by *A. excavata* was not significantly affected by sediment after 26 days (ANOVA $P > 0.8$). After 33 days of exposure, there was no significant interaction between the sediment and warming stressors (ANOVA $P = 0.6$), however, there were significant effects of both warming (ANOVA $F_{1,12} = 63.2$, $P \geq 0.001$) and sediment ($F_{1,12} = 12.57$, $P = 0.004$) as single stressors. Warming caused a decline in the ratio of O:N (greater proportion of nitrogen) whereas sediment caused an increase in O:N (greater proportion of oxygen). After 40 days in experimental treatments, there was a significant (ANOVA $F_{1,12} = 8.8$, $P = 0.011$) interaction effect of Temperature x Sediment on the O:N ratio of *A. excavata*. The mean ratios of O:N were almost 3 times greater in the control treatment, compared to the other three treatments (Fig. 1C). The control and combined suspended sediment and warming treatments resulted in a mean (±SE) ratio of 14.1 ± 1.7, and 5.5 ± 0.9 respectively. Post hoc analysis of the Temperature x Sediment interaction indicated the O:N ratio of *A. excavata* after 40 days in experimental treatments was lower when under either suspended sediment or warming when compared to the control. There were no significant differences between either suspended sediment or warming alone, when compared to the combined sediment and temperature treatment.

3.1.4. Total Organic Carbon (TOC)

TOC measurements were made at the 33 day time point. There was no significant interaction between the sediment and warming stressors. We found mesocosms without sediment experienced a small decrease in TOC content during incubations, whereas those treatments containing sediment experienced significant increases in TOC content during incubations (Sediment factor; ANOVA $F_{1,12} = 6.15$, $P < 0.05$). The increases in TOC during incubations were greatest in the suspended sediment alone treatment (Fig. 2).

3.1.5. Lysosomal stability

After 26 days, there was an impact of sediment on the mean percentage of destabilised lysosomes in *A. excavata* (ANOVA $F_{1,12} = 24.15$, $P < 0.001$). After 33 days and 40 days, however, there was no significant interaction between the sediment and warming stressors, or the impact of sediment as a single stressor (ANOVA $P > 0.5$) on the lysosomal stability of *A. excavata*. There was, however, a significant effect of temperature as a single stressor (ANOVA 33d; $F_{1,12} = 34.96$, $P < 0.001$, 40d; $F_{1,12} = 9.8$, $P = 0.008$; Fig. 3). At both these time points there was a greater mean percentage of destabilised lysosomes in *A. excavata* at elevated temperature compared to the control treatment.

4. Discussion

In this study, we tested the hypothesis that exposure to chronic suspended sediment from mine tailings and acute warming would have significant negative effects on the physiology of *A. excavata*. We found that after 40 days of experimental treatment, these stressors interacted to decrease the O:N ratio of *A. excavata*. However, in most cases, sediments and temperature acted as individual stressors, for example, both sediment and temperature increased nitrogen (ammonia) excretion, and lowered the O:N ratio at the 33 day time point. We also saw an increase in destabilised lysosomes at 26 days caused by elevated suspended sediment, and increased destabilised lysosomes at 33 and 40 days caused by warming. While in most cases these stressors did not interact, these results support the hypothesis that either suspended sediment or warming reduce the physiological performance of *A. excavata*. The ability of organisms to retain somatic growth and reproduction under environmental stress is vital to their continued survival (Khoen and Bayne, 1989). Extended exposure to stressors like suspended sediment or warming may reduce the long-term fitness of this long-lived bivalve and potentially result in localised extinction which will affect the biota that relies on the habitat *A. excavata* provides.

We found only once instance of the two stressors, sediment and warming, to significantly interact and affect *A. excavata* physiology. Furthermore, this one instance resulted in an antagonistic interaction (Folt et al., 1999), whereby the O:N ratio in the combined sediment and warming treatment was only in the range of 10 % less than either treatment as a single stressor. Similarly, while not a significant interaction, we saw that TOC production was significantly less in the

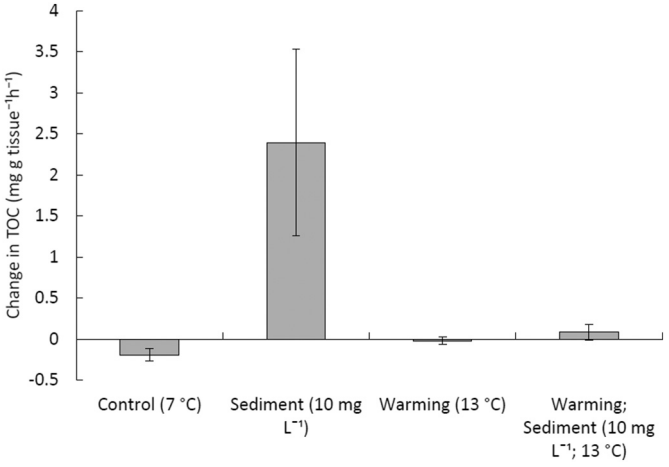

Fig. 2. Mean (±SE; n = 4) Total Organic Carbon (TOC) in seawater from the incubation chambers containing *A. excavata* after 33 days exposure to experimental treatments of; control conditions (no manipulation of variables; 7 °C), suspended sediment (10 mg L^{-1}; 7 °C), elevated temperature (13 °C) and combined suspended sediment; elevated temperature (10 mg L^{-1}; 13 °C).

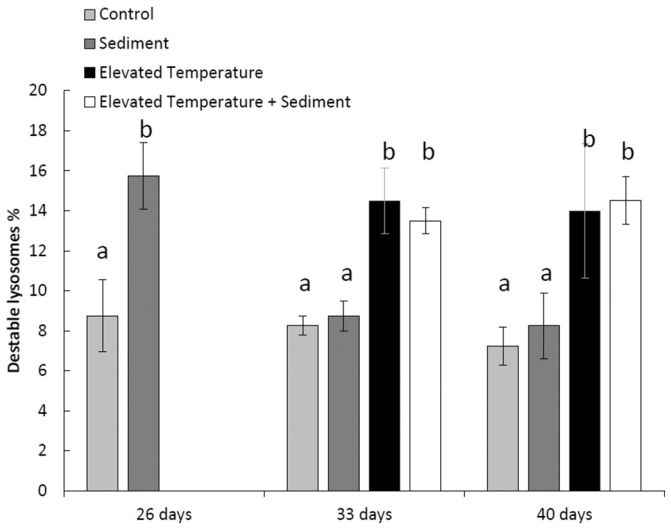

Fig. 3. Mean (±SE; n = 4) lysosmomal membrane destabilisation of cells obtained from *A. excavata* digestive gland flowing 26, 33, and 40 days exposure to experimental treatments of; control conditions (no manipulation of variables; 7 °C), suspended sediment (10 mg L^{-1}; 7 °C), elevated temperature (13 °C) and combined suspended sediment; elevated temperature (10 mg L^{-1}; 13 °C). Letters above columns represent significant differences ($P < 0.05$) among treatments within a sampling time (i.e. 26, 33, and 40 days) as determined by post hoc SNK tests. Comparisons were not made among sampling times.

combined sediment and warming treatment compared to the sediment treatment as a single stressor. These antagonistic interactions went against our hypotheses where we expected the combined stressors to exert significant additive or synergistic interactive effects on the physiology of *A. excavata*.

Environmental changes that force organisms to alter their physiology to optimise fitness have an energy consuming effect (Khoen and Bayne, 1989). Increased suspended sediment can cause cellular damage (Cheung and Shin, 2005). Elevated temperatures can increase the cost of homeostasis (Pörtner, 2001). To meet these increased energetic demands, organisms increase respiration which has a cost on energy budgets. In this study, however, no significant increase in respiration was measured. Suspended sediment and temperature are both known to reduce respiration in bivalves because of the elevated energetic costs associated with these stressors (Aldrige et al., 1987; Grant and Thorpe, 1991; Alexander Jr et al., 1994; Pörtner, 2001). Suspended sediment has also been shown to cause lower clearance rates (Shumway et al., 2003) and can prevent bivalves from absorbing oxygen by creating a film of silt on the surface of their gills (Nishioka et al., 1949; Stevens, 1987). Studies have found clams such as *Mya arenaria* to respire less when exposed to suspended sediment to reduce the amount of sediment they ingest (Grant and Thorpe, 1991). Warming is also known to decrease respiration capacity because of limitations in oxygen supply mechanisms (Pörtner, 2001; Pörtner, 2002), however we found little effect of warming on the respiration rates of *A. excavata*.

Bivalves excrete nitrogen as ammonia, NH_3^+ as a by-product when protein is used as a metabolic substrate (protein catabolism) (Bayne, 1973; Hawkins, 1985; Bayne et al., 1987). Lower O:N ratios indicate a larger portion of the energy budget is met by protein consumption, rather than the consumption of carbohydrates and lipids (Hawkins, 1985; Bayne et al., 1987). Shallow water bivalves that exist in high sediment environments are capable, while feeding, of particle selection to reduce the ingestion of inorganic matter (Kiørboe et al., 1980; Foe and Knight, 1985). Here, a relatively low level of suspended sediment was used (10 mg L^{-1}), yet a physiological shift to protein catabolism was observed. For reference, total suspended matter in fjords from Norwegian Svalbard with no human influence has been recorded at 3–4 mg L^{-1} (Bhaskar et al., 2023), suggesting our treatment levels were roughly double that of normal levels. In contrast, a study investigating the effects of suspended sediment on the clam *M. arenaria* used 100–200 mg L^{-1}, and was also found to increase nitrogen excretion relative to oxygen consumption (Grant and Thorpe, 1991). It was suggested that this occurred because feeding and absorption of *M. arenaria* was inhibited by suspended sediment, causing starvation (Grant and Thorpe, 1991). When *A. excavata* individuals were dissected for LMS analysis, there was a considerable amount (unquantified) of sediment in their gut. We also observed a net increase in the seawater TOC in sediment treatments at the 33d time point. This could be a likely by-product of *A. excavata* using mucus to slough off the sediment from gills in the high sediment treatments. Mucus production is well-established as the mechanism used by bivalves to remove unwanted sediment particles (Beninger and St-Jean, 1997; Jones et al., 2020). However, these findings were only recorded at one timepoint and further investigation is warranted. Interestingly, when warming and suspended sediment were combined, this increase in seawater TOC was not recorded. We also saw a small decrease in TOC for *A. excavata* under control conditions, perhaps, due to their feeding activity. Further research is required to understand the potential impacts of multiple stressors on deep-sea bivalves in their natural habitat.

A. excavata has a large relative area of its gills and known to have a high clearance rate (Järnegren and Altin, 2006). In this study *A. excavata* may be ingesting a high rate of suspended sediment even if timing their feeding to coincide with periods of low sediment concentrations. Ingestion of a high proportion of inorganic matter as part of the diet can result in poor rates of absorption efficiency (Bayne et al., 1987). Furthermore, there is evidence from prior studies to suggest that warming can affect the filtering rate and absorption efficiency of bivalves; especially when stressed at upper thermal limits(Schulte, 1975; Parker et al., 2024). Poor absorption efficiency coupled with a greater demand for energy could lead to a lower scope for growth and an increased reliance on protein catabolism, leading to a lower O:N ratio (Bayne, 1973; Bayne et al., 1987; Sokolova et al., 2012; Parker et al., 2024), as was observed in this study under both warming and sediment. An increased reliance on protein catabolism by *A. excavata* is likely to result in a long-term reduction in growth and reproductive output.

We found oxygen consumption to not be significantly affected by either warming or sediment treatments. Overall, the amount of oxygen consumed by *A. excavata* was found to be relatively low compared to shallow water bivalves. In addition to our findings, Järnegren and Altin (2006) showed that *A. excavata* has one of the lowest oxygen consumption rates of all bivalves (mean = 0.16 mg O_2 g^{-1} h^{-1}), a finding that was consistent in this investigation (mean control [33 and 40 d] = 0.13 ± 0.02 mg O_2 g^{-1} h^{-1}). Deep sea organisms including vertebrates and crustaceans are known to have low rates of oxygen consumption relative to their equivalent shallow living relatives (Childress, 1995). This low rate of metabolism is believed to be an adaption of filter feeding organisms lacking locomotion, to the low supply of food in oligotrophic deep oceanic regions (Childress, 1995; Järnegren and Altin, 2006).

The suspended sediment treatment caused an increase in the number of destabilised lysosomes of *A. excavata* after 26 days but not for any other time point. This result suggests that *A. excavata* has the capacity to acclimate to the effects of sediment. Stickle et al. (1985) found that after 21 days exposure to low salinity, the gastropod *Thais lapillus* showed greater LMS than when exposure times were shorter, also indicating acclimation to this stressor. In contrast, warming decreased lysosomal stability. This indicates the subcellular health of *A. excavata* was reduced by acute warming, rather than chronic exposure to suspended sediments (Ringwood et al., 1998, 2004). Lysosomal membrane stability (LMS) is often employed to determine cellular health of organisms in areas of chemical pollution (e.g. Fang et al., 2010; Edge et al., 2012, 2016) and is an effective tool. The effect size of our treatments on LMS observed here were not at the scale that can be expected when dealing with chronic chemical pollution (Ringwood et al., 1998; Ringwood et al., 2004; Edge et al., 2012). However, LMS can still be a useful tool to detect environmental changes that are not related to contamination, for example, short term effects on LMS in response to changes in the external environment have been observed (Stickle et al., 1985). Exposure to elevated temperatures has been shown to reduce the membrane integrity of bivalve molluscs (Zhang et al., 2006). Furthermore, temperature stress can increase the generation of oxygen free radicals, which contribute to the destabilisation of the cellular membrane (Edge et al., 2015). Increased protein catabolism has also been shown to cause lysosomal destabilisation in digestive cells of mussels (*Mytilus* spp.; Moore and Viarengo, 1987). This finding is consistent with the lower O:N ratios and decreased LMS we observed in elevated temperature treatments.

The ability of an organism to maintain fitness relies on its capacity to maintain a positive energy budget in response to stress (Khoen and Bayne, 1989; Parker et al., 2024). Low available-energy environments such as the deep regions of the ocean require organisms to be adapted to extracting the maximum energy available (Oliver, 1979). Deep sea living organisms such as *A. excavata* may be operating on an energy budget with lower margins for positive energy gain. A lack of available surplus energy may explain why *A. excavata* did not increase aerobic metabolism (an energy consuming process) but there was increased tissue protein catabolism when stressed (indicative of increased maintenance; Hawkins, 1985). Furthermore, we found that TOC was increased when sediment was a stressor on its own, but when combined with elevated temperature, *A. excavata* was unable to increase TOC production. The presence of these antagonistic interactions point towards energetic limitations (Sokolova et al., 2012), and may also explain why no additive or synergistic effects of stressors were observed because *A. excavata* was already operating at a maximum stress response.

Deep sea systems do not experience the seasonal and latitudinal

variability experienced in shallow water environments (Childress, 1995). The stable conditions experienced in the deep sea create a pervasive trend of organisms that have evolved extreme specificity to this environment (Angel, 1992; Haedrich, 1996; Somero, 2010; Byrne et al., 2013). Evolving specific traits to allow for life in the deep sea will likely require trade-offs where variability in some traits is lost in order to conserve energy (Hoffmann and Parsons, 1991; Agrawal, 2001; Burris and Baccarelli, 2014). *A. excavata* may have little flexibility to cope with environmental change. It was found here that there was a limited capacity of *A. excavata* to respond to suspended sediment other than by using protein catabolism. Resorting to protein catabolism is considered to be an extreme metabolic response (Hawkins, 1985). The adaptive capacity to cope with environmental change has been shown to be greatest in those organisms, including bivalves that experience a variable environment (Hofmann and Todgham, 2010). Knowledge on deep-sea biology is limited because these habitats are difficult to explore and replicate in the laboratory. This means any data on organisms from these habitats is valuable (Danovaro et al., 2017). In this study, we took a number of measures to ensure the experimental system was as close to the habitat of *A. excavata* in Norwegian fjords. However, further research may be needed to ensure our observations are representative of undisturbed *A. excavata* in their natural habitat.

Anthropogenic impacts are growing in the deep-sea (Ramirez-Llodra et al., 2015; Vare et al., 2018).The results of this study have shown that the long-lived, deep sea bivalve *A. excavata* is sensitive to environmental change. It was found that exposure to either suspended sediment or warming caused *A. excavata* to use protein catabolism to generate energy. Prolonged exposure of *A. excavata* to either suspended sediment or acute temperature in the wild may result in a loss of condition, decreased reproductive output and even cause mass mortality. The responses of *A. excavata* in this study serve as insight into how other deep-water bivalves may respond to similar stressors.

Ethical standards

The authors have no conflict of interest to declare. Ethics approval was not required by Norwegian law.

CRediT authorship contribution statement

Elliot Scanes: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis. **Tina Kutti:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization. **James K.H. Fang:** Writing – review & editing, Investigation, Conceptualization. **Emma L. Johnston:** Writing – review & editing, Funding acquisition, Conceptualization. **Pauline M. Ross:** Writing – review & editing, Writing – original draft, Conceptualization. **Raymond J. Bannister:** Writing – review & editing, Writing – original draft, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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