# iAtlantic Deliverable D4.1

# Stressor impacts on planktonic organisms

## Effects of multiple stressors on the deep-sea pelagic jellyfish *Periphylla periphylla*

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# Contents

# List of Acronyms

| Acronym | Full name |
|---|---|
| ANOVA | Analysis of variance |
| CD | Coronal diameter (i.e. diameter bell *Periphylla*) |
| CH | Coronal height (i.e. height bell *Periphylla*) |
| CMIP6 | Coupled Model Intercomparison Project Phase 6 |
| EEZ | Exclusive Economic Zone |
| ETS | Electron Transport System |
| PMS | Polymetallic sulphides |
| RV | Research vessel |
| SD | Standard deviation |
| WW | Wet weight |

# Executive Summary

To investigate the impacts of global warming and sediment plumes released by deep-sea mining on deep planktonic organisms, experiments were conducted with the helmet jellyfish *Peripylla periphylla* (led by GEOMAR; conducted by V.I. Stenvers & H. Hauss and coordinated by H.J.T. Hoving). Travel restrictions due to Covid-19 led to the cancellation of the proposed land-based expedition to Norway and collection using GEOMAR's submersible JAGO. Instead, experiments were performed on shipboard expeditions to Norway. Experiments with *P. periphylla* were carried out during two cruises in 2021 (HE570 and AL568). During incubation experiments, animals were exposed to varying levels of warming (*in situ*, +2°C, +4°C for 7–9 hours) and five abyssal sediment suspension concentrations (0, 16.7, 33.3, 166.7, 333.3 $mg \cdot L^{-1}$ for 24 hours). Both behavioural (posture, mucus sloughing) and physiological responses were measured, including respiration and ammonium excretion rates as well as gene expression. Samples were also obtained for microbiome analyses. *P. periphylla* increased its metabolic activity in response to warming and sediment plume exposure. An increase of 4°C, which is a large increase given projections of future midwater conditions, resulted in an oxygen consumption that is twice as high compared to the *in situ* temperature. Exposure to sediment had a strong physical and metabolic effect on the jellyfish showing production of excess mucus, increasing ammonium production and respiration, and expression of genes related to wound repair and anaerobic respiration. Strikingly, the sediment treatment with the highest load, resulted in a similar respiration increase as the most extreme temperature treatment of 4°C. Sequencing results of the microbiome showed no significant treatment effect on microbial community composition on the outer bell, and mucus production appeared to be an efficient strategy to maintain the microbial community despite sediment smothering. The increase in metabolic rates observed in the sediment experiments will impact the energetics and demand for food in this deep-sea jellyfish that is adapted to a slow metabolic pace in an environment with scarce food sources. Future plans for mining should consider these results since seafloor disturbance could have detrimental effects on deep pelagic communities, if our results are representative for other midwater fauna. These findings are currently in review for publication, and the data have been uploaded to PANGAEA. *Periphylla* was not available on a third cruise to the Arctic (HE605) in August 2022 (during which we had intended to increase our sample size). Instead, pilot experiments were conducted with the amphipod *Themisto abyssorum*, measuring responses of respiration rate to a range of sediment plume concentrations. Data collected for *T. abyssorum* will be processed over the coming months. Thus, with a slight delay caused by the reorganization of fieldwork, all proposed goals were accomplished.

# 1. Introduction

Earth's marine habitats are mostly in the open ocean with more than 90% having depths of greater than 200 m and with an average depth of approximately 4,000 m (Robison, 2009; Levin & Le Bris, 2015). The water column above the seafloor, the pelagic realm, makes up most of the ocean's volume. This environment lacks the physical borders of seafloor habitats since substrate is absent. While deep-sea science is advancing rapidly, with evolving scientific methodologies and approaches (Robison *et al.*, 2017), most deep-sea research is still focused on the seafloor. The deep pelagic ocean, however, is home to an enormous diversity of organisms, and provides rich feeding grounds for commercially relevant fish stocks as well as endangered mammals (Robison, 2004, 2009). The maintenance of this biodiversity, and its associated food webs, is an important ecosystem service of the deep sea. Some pelagic plankton and micronekton aggregate vertically, forming dense scattering layers, and some migrate on a daily basis to the surface to feed and benefit from the enhanced productivity of the epipelagic zone (<200 m depth; Robison, 2009). By migrating down and defaecating and respiring at depth these organisms contribute to the active component of the biological carbon pump. The vertical transport and sequestration of carbon on the seafloor is another important ecosystem service of the deep sea (Luo *et al.*, 2020; Boscolo-Galazzo *et al.*, 2021). We are only beginning to explore the deep ocean and therefore lack biological knowledge for most deep-sea fauna (Robison *et al.*, 2017). As climate change continues to drive rapid ocean change and human uses expand to greater depths we need information on how well deep-sea organisms will be able to cope

The global ocean is changing at an unprecedented rate under the influence of climate change that results in oxygen loss, warming and acidification (Levin & Le Bris, 2015). The upper ocean will warm between approximately 1 and 4.5°C within the 21$^{st}$ century, depending on the rate of ongoing greenhouse gas emissions (Coupled Model Intercomparison Project Phase 6 (CMIP6) model projections, Kwiatkowski *et al.*, 2020). This warming may induce shifts in food webs and species distributions, cause extinctions, and increase the abundance of opportunistic species. Such changes are not restricted to shallow marine ecosystems but are also happening in the deep sea (Levin & Le Bris, 2015). The deep sea will warm at a slower rate, with bottom water temperatures increasing between 0.1 and 0.3 until 2100 (CMIP6 model projections, Kwiatkowski et al. 2020). Oxygen minimum zones may result in the structuring of communities in the water column (e.g. Hoving *et al.*, 2020), such that expansion of such zones will lead to changes in vertical distribution and abundance of organisms, and predator prey interactions. Knowledge of the effects of warming on deep-sea organisms is scarce but the available data suggest that deep-sea organisms have narrow physiological tolerances due to their evolution under stable thermal conditions (Robison, 2009). This is particularly true for sessile (seafloor) and pelagic, non-migrating fauna that live in the cold conditions of the mesopelagic (0.2-1 km), bathypelagic (1–4 km), and abyssal (4–8 km) zones. On the other hand, vertically migrating fauna experience temperature changes throughout the day, suggesting more tolerance towards warming. As an example, *Phronima* amphipods migrate from their daytime depths where the temperature is 8–9 °C up to the surface at night, where it is 25–27 °C. Experiments have shown that they can tolerate the near-surface temperatures for the duration of the night, but longer exposure results in increased expression of heat shock proteins, suggesting stress. As well, higher night-time temperatures resulted in increased mortality (Elder & Seibel, 2015). Elevated temperatures caused by warming oceans and higher levels of carbon dioxide, due to increased uptake from the atmosphere, can increase metabolic rates of pelagic organisms and create an offset of metabolic balances, especially when food is scarce (Boscolo-Galazzo *et al.*, 2021; Oschlies, 2021). However, data on species-specific responses of pelagic animals are limited, as many are difficult to collect from their natural environment and to maintain under exposure to certain stressors (Robison, 2004).

In addition to climate change, the ocean is subject to impacts from human exploitation of resources. Such anthropogenic impacts are also reaching the deep sea. Commercial interest in harvesting minerals from the deep seabed has increased substantially in recent years. Ironically, part of the demand for such elements originates from the production of electric vehicles, wind turbines and solar panels, and other technologies that aim to reduce carbon dioxide emissions. Benthic resources to be targeted include

polymetallic sulphides (PMS), polymetallic nodules and cobalt-rich crusts, which have different global distributions and rates of accumulation (Christiansen *et al.*, 2020). Thus, polymetallic nodules are usually found on abyssal plains in international waters beyond any EEZs, while PMS and cobalt-rich crusts deposits are more frequently found within EEZs of coastal or island nations (Childs, 2020).

Many commercial mining plans focus on the Pacific Ocean (i.e. in the Clarion Clipperton Fracture Zone; Gillard *et al.*, 2019), although the mineral-rich seafloor of the Arctic has also attracted mining interests (Tolvanen *et al.*, 2019). Plans to commercially mine minerals from the deep seabed are well underway, although many questions remain about the impacts on deep-sea ecosystems (Washburn et al., 2019; Christiansen et al., 2020; Drazen et al., 2020). The impacts of mining will include not only the immediate destruction of benthic habitats and the emission of noise, but also the resuspension of fine deep-sea sediments into the water column (Christiansen *et al.*, 2020; Drazen *et al.*, 2020; Williams et al. 2022). As well, minerals collected from the seafloor will need to be cleared of water and sediment, leading to sediment plumes being created near the seafloor or as discharge at the surface (Jones *et al.*, 2017). Ultimately, suspended sediment will be deposited in benthic environments, but the consistency, longevity and potential toxicity of such plumes will depend on the material that is being mined. These processes may cover large areas with sediment burying and smothering benthic fauna, and possibly releasing toxic substances. Recent work on sponges has shown that sedimentation has lasting effects (Durden et al 2023).

Additionally, increased suspended sediment loads in the water can negatively impact respiration and feeding in benthic filter feeding organisms (Erftemeijer *et al.,* 2012; Carreiro-Silva *et al.*, 2022). Deep-sea corals exposed to polymetallic sulphide and suspended quartz show increased toxicological exposure, necrosis, high mortality, increased metabolic costs (enhanced ammonia excretion and oxygen consumption) and also non-lethal responses as evidenced in transcriptome analysis, which suggests immune responses and cellular stress (Carreiro-Silva *et al.*, 2022). Sediment plumes generated during deep-sea mining are suggested to also have negative effects on animals living in the water column (Drazen et al., 2020). Plumes that result from seafloor activities were estimated to disperse an average linear distance of 10 to 20 km, to cover an area of 17 to 150 $km^2$, and to extend more than 800 m upward into the water column (Morato et al 2022), directly impacting the benthopelagic zone and beyond. These unique ecological zones are important for carbon cycling and host fauna different from the midwater (Christiansen *et al.*, 2020). Sedimentation in pelagic environments can smother delicate organisms and clog filter-feeding structures, or cause the release of metals into the water, which can contaminate the surrounding environment and harm pelagic organisms or disrupt the natural carbon cycle. Additional impacts may be reduction of water clarity and compromised bioluminescent communication (Christiansen *et al.*, 2020; Drazen *et al.*, 2020). To date there have been, to our knowledge, no experiments on potential mining impacts on midwater fauna (van der Grient & Drazen, 2022). One of the reasons for this paucity in data is the challenge associated with long-term observation and capture/experimentation on these delicate pelagic species. For integrative understanding of mining impacts, to improve assessment of ecological risks and to guide decision making and management plans, experimental data on abundant taxa are needed.

The midwater is the habitat of a polyphyletic group of gelatinous zooplankton, which share a fragile body form, and a body composition consisting mostly of water. These include the cnidarians (hydromedusae, siphonophores), ctenophores, pelagic tunicates, certain molluscs, and chaetognaths (Haddock, 2004). Some of these taxa, for example *Praya* siphonophores, are among the longest animals in the ocean (Robison, 1995, 2004) and many are too fragile to be captured by nets. The use of *in situ* observational tools has revealed a large diversity of gelatinous zooplankton at mesopelagic depths, and these observations have also shown their role in sustaining the midwater food web as both predators and prey (Robison, 2004; Choy *et al.*, 2017). In coastal marine ecosystems, subject to overexploitation and climate change, it has been argued that gelatinous organisms may be increasing in abundance (Richardson *et al.*, 2009). Part of their success is due to their ability to live under low oxygen conditions, their low metabolic rates and their ability to store oxygen (Hoving *et al.*, 2020). In mesopelagic oxygen minimum zones, gelatinous taxa may indeed be abundant, and in low-oxygen mesoscale eddies gelatinous polychaetes may

comprise up to 95% of the zooplankton abundance (Christiansen et al 2018). Much less is known about the impact of warming on midwater jellies. In addition to being hard to sample, another problem is that it is difficult to keep them alive in the laboratory. To properly assess the impact of climate change and anthropogenic impacts on midwater fauna, representative model organisms are needed that are relatively robust, accessible and widely distributed.

*Periphylla periphylla* (a.k.a. the helmet jellyfish) is a midwater jellyfish, with a circum-global distribution, and it has recently been expanding into the Arctic (Geoffroy *et al.*, 2018). It is a common deep-sea species, with a very broad vertical distribution, occurring from the surface down to 4,000 m (Jacobsen-Stout *et al.*, n.d.). It is extremely long-lived (several decades) with low metabolism and growth rates compared to other scyphomedusae (Jarms *et al.*, 1999). Recent years have shown an increase in helmet jellyfish and it is very abundant in some Norwegian fjords (Fosså, 1992). For example, in Lurefjord biomass estimates reach up to 50,000 t and their carcasses may transport significant amounts of carbon to the seafloor (Sweetman & Chapman, 2011). These characteristics make it a representative model organism that can be used to predict responses of jellyfish to ocean change (Youngbluth & Båmstedt, 2001; Jacobsen-Stout *et al.*, n.d.). Another model organism for oceanic pelagic systems may be the amphipod *Themisto abyssorum*. The genus *Themisto* is abundant in waters deeper than 50 m in the Arctic and *Themisto abyssorum* is a boreal species that is advancing its geographic distribution northwards, making it a potential winner in the changing Arctic ocean(Havermans *et al.*, 2019).

We predict that due to their broad vertical distributions, and their ability to expand their ranges farther north and to greater depths, *P. periphylla* and *T. abyssorum* may be tolerant towards ocean warming. Given the sensitivity of cnidarians to sedimentation, and the absence of sedimentation events in the water column, we hypothesize that *P. periphylla* and *T. abyssorum* are likely to be sensitive to mining induced sedimentation. Using multiple seagoing expeditions, we performed experiments that are among the first to investigate species-specific tolerances towards human-induced stressors in pelagic oceanic fauna. Metabolic rates scale with temperature for ectotherms and there are already comparatively good observations for this scaling within other deep-sea pelagic species. Thus, we can compare the relative impacts of warming and sediment plumes on *P. periphylla* and *T. abyssorum* by measuring their metabolic rates (e.g., oxygen consumption) over ranges of temperature and sediment loadings.

## 2. Materials & Methods

To investigate the impacts of global warming and sediment plumes from deep-sea mining on the mesopelagic jellyfish *P. periphylla*, shipboard experiments were conducted in Norwegian waters. The original idea had been to conduct experiments during a land-based study near Bergen, and to collect jellies using GEOMAR's submersible JAGO. Unfortunately, this plan had to be abandoned because of the Covid pandemic, and it was therefore replaced by ship-based studies (Figure 1) following the successful application for ship time on German medium-sized vessels (H. Hauss co-proponent and co-chief scientist for two cruises). The ship time was in March and November 2021, and jellyfish were collected with nets from the Lurefjord (RV *Heincke* HE570) and Sognefjord (RV *Alkor* AL568), Norway (Figure 1). A third cruise to Norway was planned for June 2021 (AL558), but it had to be cancelled due to technical difficulties encountered by our cruise co-proponents. Moreover, bad weather hampered sampling efforts during the November AL568 cruise. We had hoped to collect additional specimens of *Periphylla* during a fourth cruise to the Arctic (HE605) in August 2022, but we did not catch any *Periphylla*, so we focused on the pelagic amphipod *Themisto abyssorum* instead. The results presented here focus on the experimental data (respiration and excretion) on *Periphylla* as proposed in the grant agreement for D4.1.


**Figure 1**. Sampling locations for exposure experiments on the three cruises AL568, AL558, HE605, to the Northeast Atlantic, and the two vessels used, the sister ships RV *Alkor* and *Heincke* (source for images: AWI/GEOMAR).

We collected hydrographic data, observed and collected jellyfish, and performed a series of *ex situ* experiments on board the research vessels. Using pelagic video transects and a Hydrobios multinet system (0.5 $m^2$ in diameter, nine nets, 2 mm mesh, equipped with flowmeters) we identified where the jellyfish were, as well as their *in-situ* environmental conditions. Net sampling took place after sunset and jellyfish to be used in experiments were collected between 0 and 200 m depth, using conical plankton nets (either WP2 net, 57 cm diameter, 200 µm mesh or WP3 net, 113 cm diameter, 1000 µm mesh) with a non-filtering cod-end, using slow vertical hauls to minimize physical damage. Individuals that were in good condition were selected for the experiments. The jellyfish were acclimated at their *in-situ* temperature (7.5°C) for 7 to 14 hours in large 60-L tanks for the temperature experiments, and in Kreisel tanks (Greve, 1968) for the sediment experiments. Both tanks had continuous water flow and a gentle supply of oxygen supplied by aeration. The water for the experiments was collected at 300 m using a CTD equipped with Niskin bottles. *Periphylla* is sensitive to light (phototoxic, Jarms *et al.*, 2002) and was kept in the dark. Handling during experiments was done under red light.

To determine the effects of warming on the physiological performance of *Periphylla*, we measured metabolic rates (respiration, ammonium excretion) and collected samples to analyse expression of stress related RNA transcripts and changes in associated microbiota. Three temperatures *(in situ*, +2°C, +4°C) were used and experiments were run for 7–9 hours). Before the start of an experiment, single jellyfish were transferred to closed respiration chambers (0.85, 1.15 or 2.5 L depending on the jellyfish size) and which were equipped with calibrated oxygen sensors known as "spots" (PreSens). PreSens Oxy-4 mini fibre optic oxygen sensors, which provide a non-invasive technique for measuring oxygen concentration, were used to continuously record oxygen concentration. Prior to filling the experimental chambers, the seawater was kept at the experimental temperature and saturated with oxygen by aeration. To correct microbial respiration in the seawater, a control chamber without jellyfish was monitored for each temperature treatment. In addition, start- and end-point ammonium concentrations were measured to determine ammonium excretion rates, using the fluorometric method of Holmes *et al*. (1999), on a Turner Trilogy fluorometer fitted with a UV-module. For individual *Periphylla* that were too small for the containers in which respiration was measured (i.e. 2.16–2.35 cm CD) and for which respiration could therefore not be reliably measured, separate measurements of ammonium were made in smaller (0.6 L) air-sealed glass bottles without measuring respiration. Apart from the bottle size, conditions were the

same as those described above.

To determine the effect of sediment on the metabolic rate and well-being of *Periphylla*, five abyssal sediment concentrations (0, 16.7, 33.3, 166.7, 333.3 $mg \cdot L^{-1}$) were tested. The range was chosen to represent different distances from a sediment plume, and the incubations, which lasted 24 hours, and were done at the *in-situ* temperature (7.5 °C). Sediment for the experiments had been previously collected with a box corer from the abyssal plain in the Northeast Atlantic (47.250°N 10.105°W) at 4,427 m depth during cruise SO279 on board RV *Sonne* and had been frozen at -80°C for storage. The abyssal sediment consisted of fine organic and inorganic particles (0.5–120 µm) and organic aggregates (2 – 385 µm). Before the cruise we pre-weighed sediment samples in order to prepare the right concentrations for the on-board experiments.

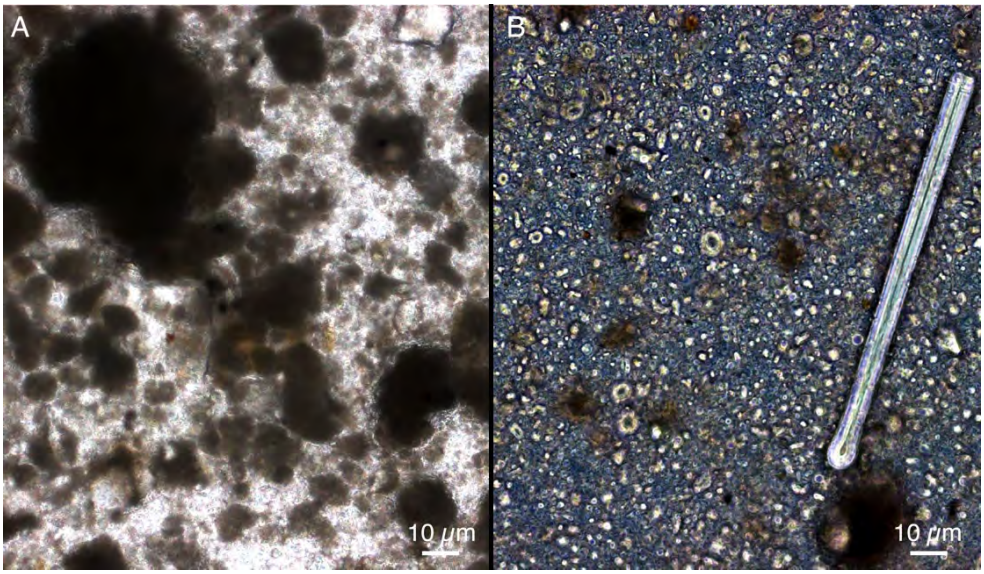


**Figure 2**. Microscopic images (Zeiss Axiovert 200 with a mounted Axiocam camera) of abyssal plain sediment used in the sediment plume experiments, collected at 4,427 m in the North Atlantic (47.250°N 10.105°W). (A) Organic aggregates. (B) Organic and inorganic particles, including diatom shells and sediment grains.

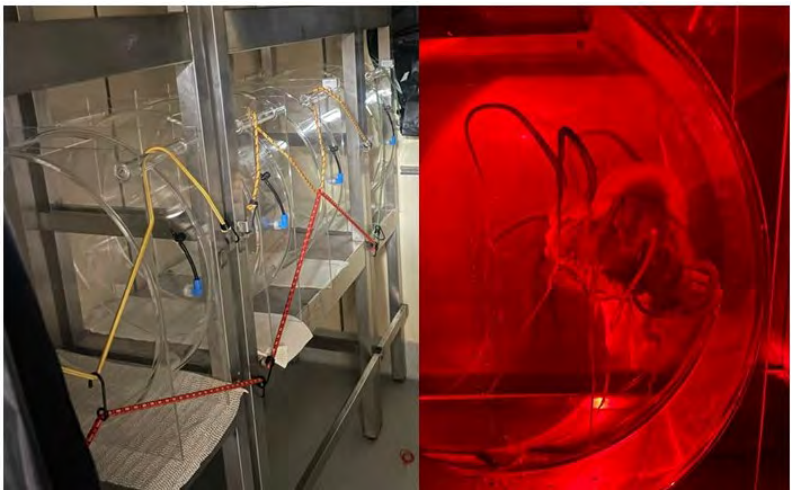

**Figure 3**. Experimental set up with the kreisel tanks on board the RV *Alkor* (A). *Jellyfish Periphylla periphylla* in a Kreisel tank under experimental conditions, mimicking sediment plumes of a mining event (B). The red light conditions for handling and photography prevent damage to *Periphylla* as they are light sensitive (pictures: H.Hauss).

For the experiments we used five 30-L Kreisel tanks, which each contained between 1–4 *Periphylla*, depending on their size (Figure 3). Treatments with sediment were then randomly assigned. The size of the *Periphylla* ranged from 0.83–10.54 cm in coronal diameter (CD). Variables that were monitored during these experiments included physical condition of the jellyfish, temperature of the water and sediment turbidity. The latter was measured with a sensor (Turb® 340 IR). The large size and aeration of the Kreisel tanks prevented direct measurements of oxygen consumption. Therefore, we analysed the activity of the electron transfer system (ETS) of jellyfish tissue in the laboratory after the cruise, which is an indirect measure of respiration. We analysed the behaviour and well-being of the jellyfish by scoring their physical condition. This condition was based on the extent of mucus production and the presence and accumulation of sediment on the bell. Over a 24-hour period, we scored these parameters every 6 hours, on a scale of 1–5 where 5 was perfect physical condition and 1 was the very bad physical condition. The scores were 1: >30% sediment-mucus coverage, substantial sloughing of mucus (i.e. on bell, tentacles and edge of the bell also known as lappets), 2: <30% sediment-mucus coverage, moderate mucus sloughing, 3: Powder-like mucous cover, no mucus sloughing, 4: no coverage of *Periphylla* body (Figure 4). Temperatures of all experimental tanks were monitored throughout the experiments.

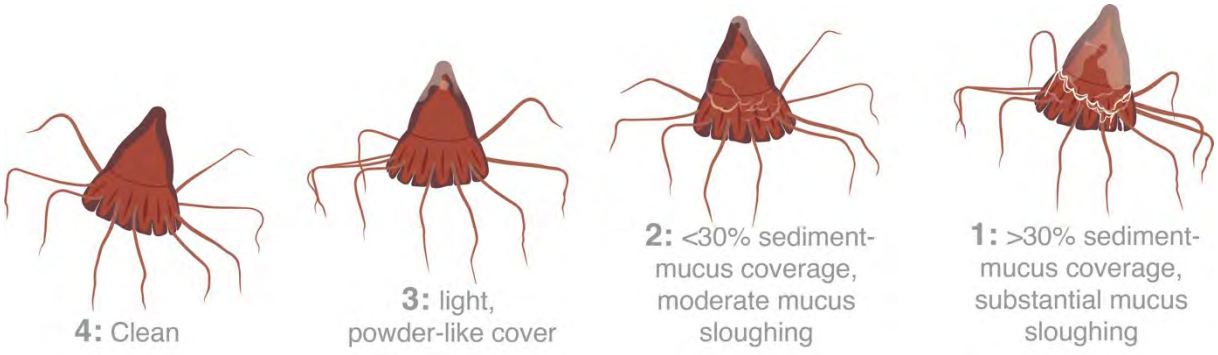


**Figure 4**. Illustrated health rating based on the amount of sediment aggregated on the bell and mucus produced. For scores see text. Illustration by V. Stenvers.

After the termination of the experiments, *Periphylla* were photographed for morphometrics and deep-frozen briefly at -20°C and then transferred to -80°C. The body measurements included the diameter of the corona (CD) and the height of the corona (CH), which were measured in ImageJ v1.52k (Schneider *et al.*, 2012). The size was used to estimate wet weight (WW) using standard equations (Fosså 1992). The short handling time (< 5 minutes) limited handling effects and biases in metabolic measurements. Tissue samples for ETS analysis were also stored at -80°C.

In addition, gene expression of *Periphylla* was characterized from tissue samples (HE570), as well as the microbiome community composition of the jellies in all treatments and in the controls as well as in the surrounding water and the sediment. The results of these analyses will not be further discussed in this report since we are still interpreting the results. These analyses were beyond what we had proposed in the iAtlantic Work Package 4 and are therefore not among the deliverables for this report. We used two one-way Analysis of variance (ANOVA) tests followed by a post-hoc Tukey test (for significant results) to determine if there were statistical differences between respiration rates, ammonium excretion rates and the health score rankings.

Experiments with *T. abyssorum* were conducted during a cruise to the Arctic (RV *Heincke* HE605) to investigate the impacts of sediment plumes (Figure 5A). *Themisto abyssorum* ($n_{individuals}$=142) were collected in fjords around Svalbard and exposed to five different sediment treatments (0, 16.7, 33.3, 166.7, 333.3 mg·$L^{-1}$). For the collection of specimens, we used a multinet (0.5$m^2$ in diameter, nine nets, 2 mm mesh, equipped with flowmeters). Similar to the experiments with *Periphylla*, incubations lasted 24 hours and the same abyssal sediment was used. The consumption of oxygen was measured every six hours in

respiration bottles equipped with oxygen sensor spots attached to a slowly rotating plankton wheel (Figure 6B), containing one to five *T. abyssorum* depending on bottle size (either 50 ml or 100 ml). All experiments were done at 4°C (*in situ* temperature) while bottles were wrapped in aluminium foil to keep the animals dark adapted. We also monitored the behaviour and position of the amphipods during a separate experimental run without wrapping the bottles in foil, which was not included in the respiration analysis due to potential light-exposure bias. These experiments on *Themisto* were beyond what had been proposed in the iAtlantic application but were done to make optimal use of the available ship time (as *Periphylla* was not available on that expedition) and can be considered pilot experiments.

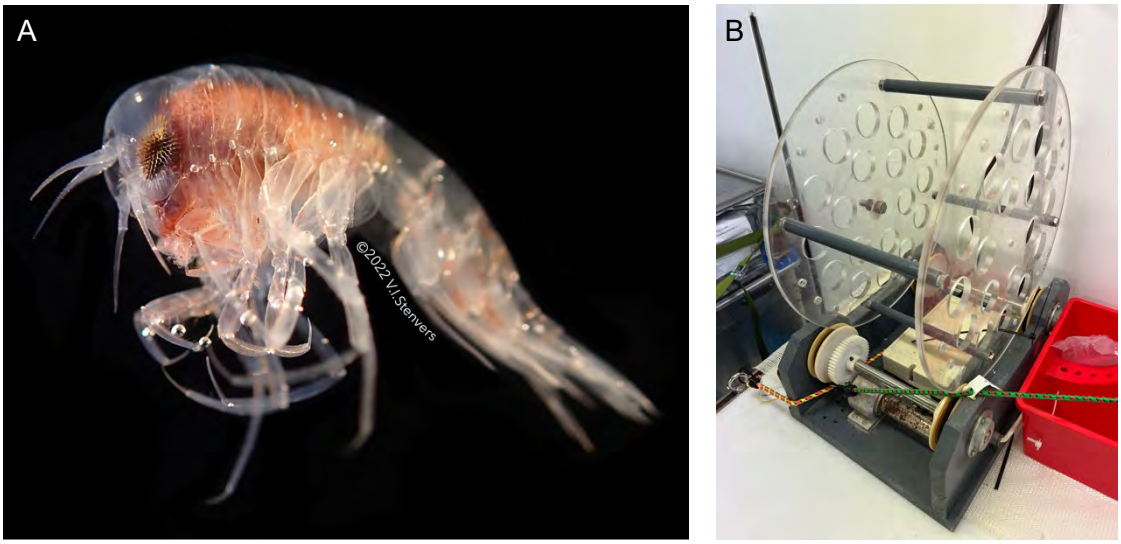


**Figure 5**. (A) microscope photograph of *T. abyssorum*. (B) Plankton wheel used for *Themisto* experiments.

## 3. Results

In total, 206 organisms were collected for our experiments (see Table 1). The data generated for *Periphylla* have been uploaded as required to the PANGAEA repository (Stenvers *et al.*, 2023b, 2023a), and will be made publicly available once the associated manuscript is published. The sequencing data from our gene expression and microbiome analyses will be uploaded to the NCBI GenBank database when submission of the manuscripts for publication is imminent. Data from the *Themisto* experiments will be processed within the following months at GEOMAR (including the determination of dry mass and morphometric dimensions of individual specimens) and uploaded to PANGAEA before the end of 2023. Preliminary results were presented at the Deep-Sea Biology Symposium in Brest in an award-winning talk (Stenvers *et al.*, 2021). Below, we report on the results collected for *Periphylla,* and focus on the effects of temperature and sediment loading on respiration and excretion rates.

**Table 1**. Cruises and Net sampling for Task 4.1

| Cruise | Taxa sampled (n=number of individuals) | Analyses |
|---|---|---|
| HE570 | *Periphylla periphylla* (38, of which 24 for plume and 14 for warming experiments. Within the latter 14, respiration+ ammonium was measured for 11 individuals and ammonium only for 3) | Respiration, Ammonium Excretion, Differential Gene Expression (Transcriptomics), Microbiome characterization (16S Sequencing) |
| AL568 | *Periphylla periphylla* (n=26, of | Respiration, Ammonium |

| Cruise | Taxa sampled (n=number of individuals) | Analyses |
|---|---|---|
| | which 19 for sediment and 7 for temperature experiments. Within the latter 7, respiration+ ammonium was measured for 6 individuals and ammonium only for 1) | Excretion |
| HE605 | *Themisto abyssorum* (n=142, all for sediment plume experiments) | Respiration |

*Periphylla* were collected in spring in Lurefjord (HE570) and in fall/winter in Sognefjord (AL568). In general, primary and secondary productivity were somewhat higher during March in Lurefjord than in November in Sognefjord (pers. obs. H.Hauss). During both cruises, *Periphylla* were distributed mainly below the pycnocline, which was at approximately 100 m depth at both sites (Figure 6A-B). However, *Periphylla* were much more abundant in Lurefjorden, and individuals were larger. Furthermore, they tended to migrate to shallower depths during the night, presumably to forage on *Calanus* copepods in the surface layer. Therefore, we were more successful in obtaining sufficient numbers of individuals in good condition on the first cruise. We had applied to go into Lurefjorden again on AL568 in November, but gale-force winds prevented our transit between fjords.

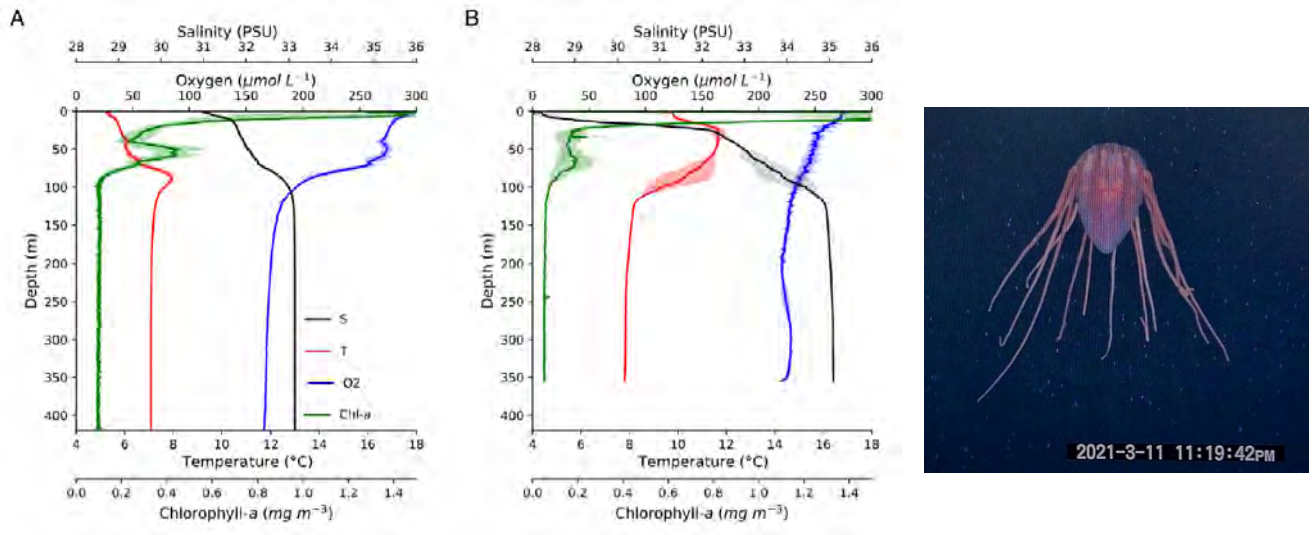


**Figure 6**. Vertical mean (±SD) profiles of salinity (PSU), temperature (°C), oxygen concentration (µmol L-1) and chlorophyll-a (Chl-a; mg m-3) at the *Periphylla* collection locations in the (A) Lurefjord (HE570) and (B) Sognefjord (AL568) in Norway. (C) frame grab of in situ observation video (PELAGIOS) during HE570.

Incubations to test the effect of temperature

Temperature manipulated incubations lasted approximately 7 to 9h. All individuals were still alive and active at the end of the experiments. No handling or heat shock effects were visible in the first two hours of each experiment; therefore oxygen consumption rate was estimated from the slopes of linear regressions through all values from start to end for each individual (Figure 7). Towards the end of each experiment, oxygen concentration was still more than approximately 90% of the start concentration.

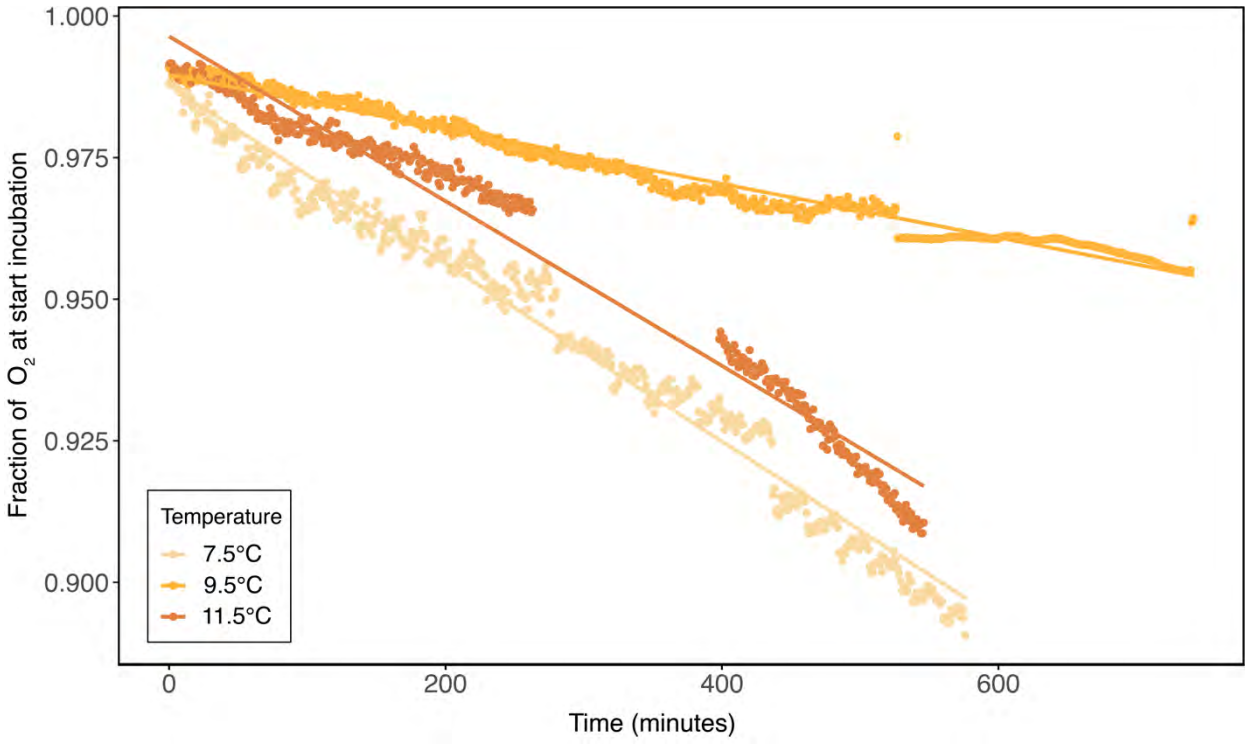


**Figure 7**. Oxygen concentration over time normalized to wet weight for three individual jellyfish kept at three different temperatures, illustrating the fitting of slopes to give rate estimate.

Respiration rates determined in the temperature treatments increased with increasing temperature, with average values nearly doubling in the 11.5°C treatment compared to the in-situ temperature of 7.5°C. However, the differences were not significant (ANOVA F= 1.354 p=0.29, $n_{individuals}$=17). Ammonium excretion was, however, significantly impacted by temperature (ANOVA F= 7.65 p=0.004, $n_{individuals}$=29, Figure 8B), increasing over six-fold in individuals incubated at 11.5°C compared to those at 7.5 and 9.5°C, for which rates were not significantly different (Tukey p-adj. <0.007; i.e. 0.185±0.155 versus 0.030±0.055 and 0.028±0.027 µmol $NH_4$ g $WW^{-1}$ $h^{-1}$ respectively; Fig. 8B). This suggests that there may be a threshold temperature above which nitrogen metabolism significantly increases in *Periphylla*. However, the magnitude of change is difficult to quantify because of the low sample size and large variability among individuals.

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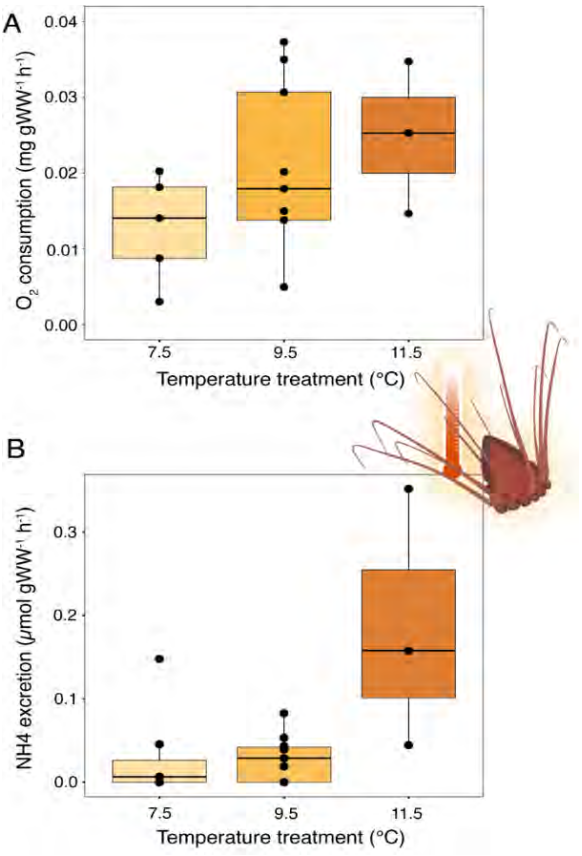


**Figure 8**. Respiration rate (A) and Ammonium excretion rate (B) of *Periphylla* exposed to three different temperatures.

significantly different (Tukey p-adj. <0.007; i.e. 0.185±0.155 versus 0.030±0.055 and 0.028±0.027 µmol $NH_4$ g $WW^{-1}$ $h^{-1}$ respectively; Figure 8B). This suggests that there may be a threshold temperature above which nitrogen metabolism significantly increases in *Periphylla*. However, the magnitude of change is difficult to quantify because of the low sample size and large variability among individuals.

Experiments to test the effects of sediment plumes

Temperatures in Kreisel tanks (sediment plume experiments) remained constant throughout the incubation period (<0.2°C change over time), although the turbidity of the sediment plumes decreased over time despite resuspension being promoted by air flow (Figure 9).

For the gene expression analysis, transcriptomes of 29 medusae were successfully sequenced (11 in the temperature treatments and 18 in the sediment treatments), producing an average 35.7 (±1.1 SD) million read pairs per sample following initial quality filtering steps. For analysis of the microbiomes, 33 medusae were successfully sequenced (12 in the temperature treatments and 21 in the sediment treatments), in addition to 12 seawater and 3 sediment reference samples. This was not part of the original project proposal, and the results are therefore beyond the scope of the deliverables for this report.

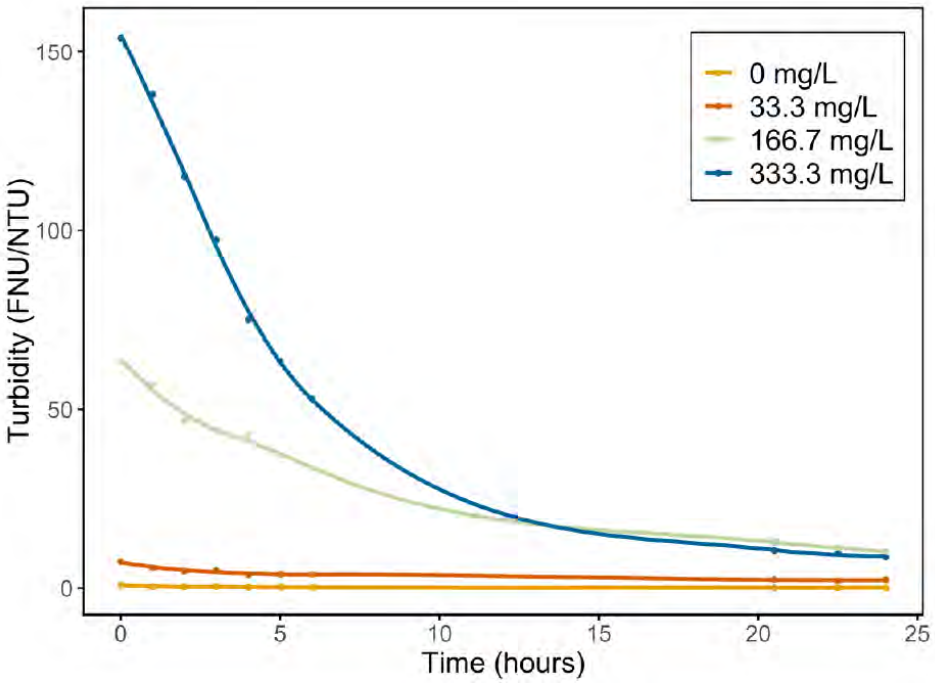


**Figure 9**. Turbidity (FNU/NTU) measured in the Kreisel tanks over 24 hour time period during abyssal sediment incubations. Turbidity was measured with a benchtop turbidity meter (WTW Turb® 340 IR).

Exposure to simulated mining plumes led to marked effects on *Periphylla* condition. ETS-based respiration rates increased continuously with sediment concentration, although the differences among mean values as determined by ANOVA were not significant due to the low sample size (i.e. 0.012 ± 0.006 SD versus 0.028±0.013 mg $O_2$ g $WW^{-1}$ $h^{-1}$ for the control and the highest concentration, respectively; ANOVA F= 0.911, p=0.473, $n_{individuals}$=29; Figure 10A). In terms of observed physical condition, the suspended particles negatively affected *Periphylla* even at the lowest concentration (17 mg·$L^{-1}$), with a deteriorating trend at higher concentrations. In just 1.5 hours, particles began to accumulate on the bell and lappets, and the jellyfish responded by producing excess mucus that slowly sloughed off (Figure 10B). These effects were visible throughout the entire incubation period (even though turbidity decreased markedly, see Figure 9), with *Periphylla* exposed to 33, 167, and 333 mg $L^{-1}$ of suspended sediment exhibiting significantly lower health scores compared to those in the 0 and 17 mg $L^{-1}$ treatments (ANOVA F= 42, p=<0.000, $n_{individuals}$=18;

Tukey p-adj. <0.000; Figure 8B). Moreover, all health ratings that went beyond 6 hours of incubation, except for the control, were significantly lower than those recorded at the beginning of the experiments (ANOVA F= 35.1, p=<0.000, $n_{individuals}$=18; Tukey p-adj. <0.000).

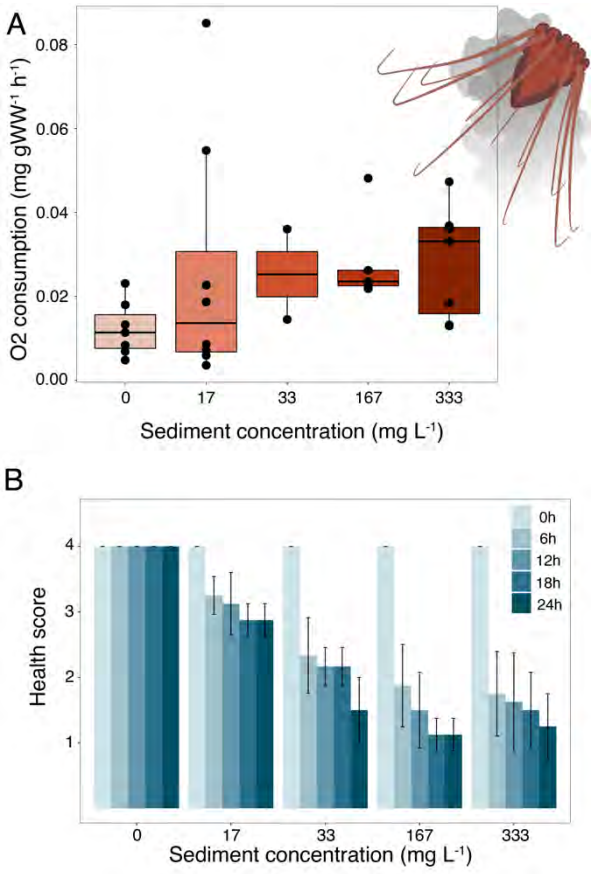


**Figure 10**. Weight-specific respiration rate of *Periphylla* exposed to five different sediment concentrations based upon ETS measurements (A) and physical health scores (mean±SD) determined at five different time points in individuals exposed to different sediment concentrations.

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# 4. Discussion

These experiments are to our knowledge the first to investigate the stress responses of deep pelagic organisms to ocean warming and deep-sea mining induced sediment plumes. The results provide a first insight into the effects of both stressors on deep-sea jellyfish, of which the temperature increase is the better studied stressor (large database of observations including deep sea species exists, see e.g. Ikeda, 2014). Moreover, our efforts will result in the first gene expression dataset and microbial community composition characterization of *Periphylla periphylla*. Work like this has been rare or absent, as pelagic animals are difficult to study given their deep-sea environment. The challenges include sampling, which requires open ocean ship time and complex and often expensive equipment, their generally patchy distribution, which makes it difficult to collect large sample sizes, and lastly because they are easily damaged during capture and often have low survival rates in the laboratory (Robison, 2004, 2009). *P. periphylla* turned out to be a suitable model organism and future efforts should focus on long-term experiments using this species. For example, short-term experiments like ours could be used to test specific sediments from different origins that are being introduced into the water column by mining of polymetallic nodules in the abyssal plain or PMS deposits at off-axis, extinct hydrothermal vents. As well, ecotoxicological assessments could include the determination of harmful substances (e.g., metals), as in our experiments we cannot conclude whether the responses of the jellyfish were entirely due to physical effects, certain compounds in the sediment, or both. This could be done on longer cruises, or via experimental work in shore-based facilities. Additionally, *in situ* sampling with suction sampling devices on a ROV (remotely operated vehicle) or manned submersible could further improve the experiments as these methods can collect animals in better condition. This was also part of the original plan but had to be abandoned since shore-based expeditions were not possible due to the pandemic. Despite the challenges posed by the experimental organisms and the logistical and expedition constraints caused by the covid-19 pandemic, we were able to successfully determine the responses of a deep-water jellyfish to multiple stressors. In addition, we were able to pioneer similar experiments for a pelagic amphipod.

Neither the temperature incubations nor the sediment additions produced significantly different mean respiration rates among treatments, due to the low sample number, overall low respiration rates, and large variability among individuals. Individual *P. periphylla* were also of quite different sizes, and although metabolic rates were standardized to wet weight, this introduced variability. However, in both gradients, an increasing trend was observed in the response variables with temperature and sediment concentration, albeit the uncertainty of these relationships prevents us from concluding the magnitudes of the changes.

Other jellyfish are known to have the capacity to perform aerobic and anaerobic respiration at the same time. *P. periphylla*'s ability to respire anaerobically (Thuesen & Childress, 1994) may also explain the overall low respiration rates, which were within the range of, but slightly higher than, those reported for *P. periphylla* in the North Pacific Ocean (Thuesen & Childress, 1994), and lower than those reported in the Lurefjord, Norway (Youngbluth & Båmstedt, 2001). This physiological adaptation is a widely adopted strategy for midwater taxa living in low oxygen environments, such as mesopelagic oxygen minimum zones (Childress & Seibel, 1998; Hoving *et al.*, 2020). Fauna with such flexible metabolisms also have significantly lower levels of enzyme activity related to aerobic metabolism (e.g. lactate dehydrogenase and citrate synthase)compared to fauna living under conditions with more oxygen available to them (Thuesen *et al.*, 2005).

It appears that *P. periphylla* has a relatively wide temperature tolerance based on our data, which is further substantiated by the fact that *P. periphylla* found in Norwegian fjords can vertically migrate to the surface at night during winter where water temperatures can be 2 to 4°C lower than in the basin waters from which they migrate (Youngbluth & Båmstedt, 2001). Moreover, *P. periphylla* in the open ocean is found across a wide depth range and by inference naturally inhabits a wide temperature range (Jacobsen-Stout *et al.*, n.d.). Our results suggest that global warming within the ranges predicted for the deep sea, i.e. 1°C with 4°C only in extreme scenarios, will impact respiration in *P. periphylla* minimally (Kwiatkowski et al. 2022). Nevertheless, more research is needed to understand potential limitations of *P. periphylla*'s

anaerobic respiration in regard to ongoing ocean deoxygenation.

Similar to the (quite extreme) warming treatments, oxygen consumption seemed to increase with exposure to simulated sediment plumes. Respiration rates under the highest sediment load were twice as high as in the controls, indicating that sediment plumes were causing physiological stress to the jellyfish comparable to a 4°C temperature increase. Greater $O_2$ demand would also necessitate more food intake in order to deal with the stress, and this requirement may be difficult to meet in the deep sea, especially in oligotrophic areas such as the Clarion-Clipperton Zone where polymetallic nodules are likely to be mined. Moreover, *P. periphylla* produced increasing amounts of excess mucus across treatments, that can be linked to increased metabolic costs (Crossland *et al.*, 1980; Wild *et al.*, 2004), which would again require more energy (food) to cope with it. Cnidarians such as corals, anemones and jellyfish are well known to produce mucus in response to physical stress, creating a protective layer against adhering particles or pathogens (Bessell-Browne *et al.*, 2017; Bakshani *et al.*, 2018). Since mucus production was shown to be energetically expensive (Crossland *et al.*, 1980; Wild *et al.*, 2004), *P. periphylla* would need to counterbalance the metabolic costs of mucus production. Our gene expression data will provide insight on physiological compensation strategies. It remains to be seen whether *P. periphylla* and other midwater fauna can recover after long and short-term exposure to suspended sediment. The results obtained here provide a baseline for future experimentation, where exposure duration, sediment load, and full factorial treatments can be further optimized.

Since our experiments were short term, it should be noted that potential (sub)lethal effects may only become visible after longer exposure to sediment loads. Mining plumes are likely to persist much longer than our incubation periods (Rolinski *et al.*, 2001; Spearman *et al.*, 2020; Muñoz-Royo *et al.*, 2021). As, by definition, planktonic organisms drift with the water mass they are in, they cannot avoid prolonged exposure. Analyses of up or downregulated genes could point out sublethal effects and should be looked at in future studies.

Our study provides unique and novel insights into the potential impacts of climate change and anthropogenic activity on deep-sea animals. Although *P. periphylla* may tolerate elevated temperatures, which it also experiences during DVM (diel vertical migration) in the fjords, their metabolic rates scale directly with temperature as in other ectotherms. An increase of 4°C above *in situ* temperature led to a doubling of oxygen consumption, which directly translates into a doubling of nutritional demand, which is hard to meet in the deep sea, especially in oligotrophic areas. This is particularly problematic considering that warmer, more stratified oceans will likely have reduced primary productivity and provide less food to the deep sea (Boscolo-Galazzo *et al.*, 2021). While a warming of the deep-sea of 4°C is currently still an extreme scenario, it must be noted that the highest sediment concentration in our exposure led to an increase in metabolic demand that was comparable to this extreme temperature increase. While the uncertainty of observed values is still high due to the limited sample size, this finding leaves us conclude that in future exploratory or commercial mining operations, great efforts need to be made to minimize sediment plumes throughout the pelagic ocean, as these will affect a huge volume of the world's oceans, likely persist over long-time scales, and physiologically challenge pelagic fauna.

The exact responses, including metabolic costs and ultimately survival rates, likely depend on sediment concentrations and the species. Therefore, the experiments executed here should be repeated for other taxa, to gather information on species-specific responses. Given the fundamental role of gelatinous zooplankton in ecosystem services, more data are needed to properly evaluate the impact of warming and mining on midwater ecosystems. Society is getting close to starting to exploit deep seafloor resources, yet scientific research is relatively slow-paced. We should therefore act rapidly to be able to make well-informed decisions and protect the deep sea for future generations.

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# 6. Document Information

| EU Project N° | 818123 | Acronym | iAtlantic |
|---|---|---|---|
| Full Title | Integrated Assessment of Atlantic Marine Ecosystems in Space and Time | | |
| Project website | www.iatlantic.eu | | |

| Deliverable | N° | D4.1 | Title | Stressor impacts on planktonic organisms |
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