The potential impacts of exploratory drilling and aquaculture wastes on cold-water cnidarians with a focus on cold-water corals

by © Kathryn Murray
A thesis submitted to the School of Graduate Studies
in partial fulfillment of the requirements for the degree of

Master of Science (Marine Biology)
Department of Ocean Sciences
Memorial University

September 2024
St. John's, Newfoundland and Labrador

Abstract

The study of anthropogenic impacts on benthic fauna, including cold-water corals (CWC), has increased in recent decades, but the state of knowledge fails to keep up with the rapid expansion of industry in the marine environment. Here, I aimed to experimentally probe the effects of two prominent marine industries, aquaculture and oil and gas exploration, on three representative benthic species. The first study explored the effects of finfish waste (feed and feces) on the soft coral *Gersemia rubiformis* and the sea anemone *Aulactinia stella* over 28 days. Results showed a potential difference in expansion behaviour in waste-exposed individuals compared to controls (no or inorganic sediment) for both species, as well as higher instances of pharynx eversion in *A. stella*. No change in lipid composition was detected as a result of waste sedimentation, although an increase in tracer fatty acids associated with salmon waste showed positive trends across treatments, especially in *A. stella*. The second study evaluated the effects of drilling muds from oil and gas exploration on the cup coral *Flabellum* (*Ulocyathus*) *alabastrum.* After 10 days of exposure to barite, bentonite, or a combination of the two, marked changes in behaviour (excessive polyp expansion) and mucus secretion were observed, along with a potentially higher density of stinging cells (nematocytes) in the combination treatment. An additional 10-day recovery period revealed a high recovery potential to the tested sedimentation rate (<6.3 mm). These findings contribute to the knowledge of potential impacts of marine industries on ecologically significant benthic animals like CWC and hopefully provide useful data towards their management and conservation.

General Summary

Ocean-based human activities, such as aquaculture and oil/gas exploration, may pose a threat to benthic (bottom-dwelling) animals. This project explored the impacts of wastes produced by these industries on two species of cold-water corals and a sea anemone through two experimental studies. The first study exposed the coral *Gersemia rubiformis* and the sea anemone *Aulactinia stella* to fish waste (feed and feces) over 28 days. Results indicated that fish waste may influence behaviour and fatty acid composition of their tissue. The second study investigated the effects of drilling mud products (barite and bentonite), supplied separately and together over 10 days, on the deep-sea coral *Flabellum* (*Ulocyathus*) *alabastrum*. The results showed strong responses in the polyp behaviour and mucus secretion due to exposure, especially when barite and bentonite were combined. These studies were the first of their kind for species ubiquitous in the Newfoundland region and can provide the basis for future research questions.

Acknowledgements

Firstly, I would like to thank my supervisors Dr. Bárbara de Moura Neves and Dr. Annie Mercier. Without your immense support, enthusiasm and guidance, I would not have become so immersed in the world of cold-water corals. I would also like to thank Jean-Francois Hamel and my committee member Dr. Johanne Vad for readily and enthusiastically sharing their expertise with me through this process.

A special thank you to Craig Kelly (Fisheries and Oceans Canada, DFO) whose help was instrumental in the experimental set-up and maintenance within the facility. Brandon Piercy (DFO) for his technical work in experimental preparations and assistance sampling. As well, the commissioners of the Northwest Atlantic Fisheries Centre for checking on the water flows and chillers of my tanks overnight while I had live animals.

I would also like to thank the members of the Gamperl Lab (Memorial University), specifically Rebeccah Sandrelli and Dr. Eric Ignaz, as well as Danny Boyce who were essential for the collection of fish waste used in this thesis. Dr. Joanna Potter (CREAIT, Memorial University) for the carbon and nitrogen analysis of the fish waste. Philip Sargent, Ana Storch and Dwight Drover (DFO) for the dive collections of *Gersemia* and *Aulactinia*. Jeanette Wells (CREAIT) for the training of lipid and fatty acid protocols, and analyses of raw lipid and fatty acid data. As well, Jeanette Wells and Efe Obade (CREAIT) for their work on the lipid and fatty acid extractions and processing of the *Gersemia* samples. Thank you to Dr. Dounia Hamoutene for the suggestion of including an inorganic treatment, and Dr. Chris Parrish for providing insights on lipid and fatty acid analyses.

I am appreciative for the Canadian Coast Guard and DFO team present on the multispecies trawl survey (CCGS *John Cabot*, Leg 4, May 2022) who made the installation, water supply, and

dismount of the set-up for live *Flabellum* possible. Vonda Hayes for her assistance with the field tank design for keeping live *Flabellum* at sea and Rachelle Dove (DFO) for assistance and support at sea. Dylan Goudie (CREAIT) for his guidance and expertise with the EDX and MLA analyses. Justin So and Lara Miles for their effort in helping me source materials for my second experiment. As well, a special thanks to Hedley Forward who supplied the barite and bentonite needed for this thesis. Shannah Rastin (DFO) for the water nutrient analyses and Brooklin Caines (DFO) for assistance during the preservation and sampling of *Flabellum*. As well as Danielle Gardiner (Medical Laboratories, Memorial University) for processing the histology samples and her histological expertise.

This research was funded by a Competitive Science Research Fund (CSRF) grant from DFO awarded to Dr. Bárbara de Moura Neves, and a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) awarded to Dr. Annie Mercier. I am extremely grateful for the opportunities and learning experiences this afforded me.

I am very grateful to the members of the Benthic Ecology Team (DFO-NL) including Vonda Hayes, Rachelle Dove, Brooklin Caines, Cynthia Mercer, Rylan Command, Marion Boulard, as well as Dr. Emilie Geissinger, Coady Fitzpatrick and Courtney King for making me feel welcome and their constant encouragement. Likewise, the members of the Mercier Lab, especially Sara Jobson and Sophie Wolvin. Finally, I would like to thank my family, friends, and my best friend Vilde Bentzen, whose unrelenting support facilitated the completion of this thesis.

Table of Contents

Lists of Figures

List of Tables

List of Abbreviations

ARC – Aquatic Research Centre

BA – Barite

BA+BE – Barite and bentonite combined

BE – Bentonite

CCGS – Canadian Coast Guard Ship

CSRF – Competitive Science Research Fund

CREAIT – Core Research Equipment & Instrument Training Network

CWC – Cold-water corals

DFO – Fisheries and Oceans Canada

FA – Fatty acid

MUFA – Monounsaturated fatty acids

MUN – Memorial University

MLA – Mineral Liberation Analysis

NAFC – Northwest Atlantic Fisheries Centre

NL – Newfoundland and Labrador

PUFA – Polyunsaturated fatty acids

SEM – Scanning Electron Microscopy

Statement of Co-Authorship

The work in this thesis was conducted by Kathryn Murray under the supervision and guidance of Dr. Bárbara de Moura Neves and Dr. Annie Mercier. Experimental design, data collection, data analysis and manuscript creation were executed by Kathryn Murray with consultation and input from the listed co-authors:

- Dr. Bárbara de Moura Neves and Dr. Annie Mercier co-authored all chapters, provided funding and guidance on all components (experimental design, data collection, analysis, writing).
- Jean-François Hamel provided input on experimental design, data collection and writing on Chapters 2 and 3.
- Dr. Daria Gallardi provided input on study design and editorial input for Chapter 2.
- Dr. Johanne Vad provided insights into the experimental design and statistical analyses, as well as editorial input for Chapter 3.
- Vonda Hayes provided logistical conceptualization and coordination for the collection of *Flabellum alabastrum* and editorial input for Chapter 3.

Chapter 1 - General Introduction

Termed as the 'blue acceleration', extraction and use of ocean resources has been intensifying over the past decades to sustain global development (Figure 1-1, Jouffray et al. 2020). Unprecedented rates of industrial expansion in the ocean are accelerated by technological advances, and coupled with increased demands for food, space and material (Jouffray et al. 2020). This may pose risk to marine organisms through physical, chemical and biological disturbances (Ragnarsson et al. 2016).

Benthic organisms (those living on or closely associated with the seafloor), especially those with limited or no mobility, are particularly susceptible to anthropogenic disturbance (Aguilar et al. 2017). Many of these animals, such as sponges and corals, live attached to hard substratum or anchored in the sediment. By feeding on particulates in the water column, they play a vital role in benthic-pelagic coupling (nutrient cycling, Rossi et al. 2017). However, as suspension feeders are often non-selective (within their particle size range), they are susceptible to exposure and potential ingestion of particles of anthropogenic origin (Hamann and Blanke 2022). This could include microplastics (Hamann and Blanke 2022), as well as waste produced through ocean-based industries (e.g. aquaculture, oil and gas exploration). This may increase the vulnerability of many sessile benthic taxa (e.g. corals) to disturbance from industry activities, leading to broader ecosystem effects.

1.1 Cold-water corals (CWC)

1.1.1 Importance and life-history traits

CWC are important contributors to species richness and abundance in cold-water and deep-sea environments (Buhl-Mortensen and Mortensen 2005, Edinger et al. 2007, Baillon et al. 2014, De Clippele et al. 2015) as they can function as ecosystem engineers. Previous studies have

shown that CWC can represent important habitats for benthic organisms (Neves et al. 2020, Caulier et al. 2021, Boulard et al. 2022), including nurseries for larvae of commercially harvested fish (Baillon et al. 2012). Unlike their tropical counterparts, they lack photosynthesizing zooxanthellae in their tissues, meaning they do not depend on sunlight for survival. Though this allows them to inhabit depths beyond that of the photic zone, as 65% of known coral species exist below 50 m (Roberts et al. 2009), they can be faced with challenges of deep, cold environments (e.g. food availability) which when combined with certain life-history traits (i.e. slow growth, later maturation) may further hamper recovery from disturbance.

Data on the life history of CWC is sparse or non-existent for most species. Slow growth rates and long life-spans seen in many CWC species suggest that recovery from disturbances can take decades, if not centuries (Risk et al. 2002, Sherwood and Edinger 2009, Neves et al. 2015, Neves 2016, Prouty et al. 2016). As a result of slow growth, a late age (several years) at sexual maturity is presumed (Waller et al. 2023). Fecundity can vary greatly with species (<10 to >1000 oocytes per polyp) and while data is limited (Waller et al. 2023), indications of low recruitment have been reported *in situ* (Huvenne et al. 2016, Beazley et al. 2021). Thus, dependence on regrowth or recolonization are not effective means of conservation (Huvenne et al. 2016).

1.1.2 Research gaps and impacts of ocean-based industries on CWC

Without an extensive fundamental understanding of their biology and life history, predicting the effects of anthropogenic disturbances on CWC is difficult and warrants investigation. The lack of information on the influence of expanding industries (e.g. fishing, deep-sea mining, submarine cabling, wind farms, aquaculture, oil and gas) on CWC is concerning for management of these organisms and, by association, other marine species that depend on them.

CWCs are widely known to be damaged by fishing activities including bottom trawling, bottom-set longlines, bottom-set gill nets, pots and traps (Ragnarsson et al. 2016). With these methods, gear is set or dragged across the seabed, which can result in physical damage to CWC. The detrimental impacts of fishing on CWC are well documented (Althaus et al. 2009, Rooper et al. 2011, Sampaio et al. 2012, Pierdomenico et al. 2018) and in some cases measures (e.g. establishing Marine Protected Areas (MPAs)) have been taken to conserve CWC in areas of fishing pressure (Ragnarsson et al. 2016, Huvenne et al. 2016).

Research efforts on the effects of deep-sea mining have recently begun. To my knowledge, the work by Ramirez-Llodra et al. (2015) was the first to investigate the effects of deep-sea mining on CWC. Since then, research on this topic has accelerated and shown that exposure to metals and sediment associated with mining (copper, sulphide, polymetallic sulphide particles) can lead to tissue loss and mortality (Martins et al. 2018, 2022, Carreiro-Silva et al. 2022). Bioaccumulation and physiological impacts (increased respiration and ammonia excretion) have also been recorded, highlighting the importance of considering combined effects of mechanical and toxicological properties of different particle types (Carreiro-Silva et al. 2022).

The impacts of submarine cables on CWC are also not well understood. Nearly 1 million kilometers of submarine cables were installed in marine environments as of 2016 (Ragnarsson et al. 2016). They are often buried under the seabed, or otherwise laid on top with a form of protection (e.g. concrete mattresses, Taormina et al. 2018). Though specific impacts on CWC are not prevalent in the literature, general impacts on the benthic habitat from submarine cable operations may include physical destruction, sediment resuspension during burial (may also release chemicals, e.g. hydrocarbons), heat emission, and noise pollution (Taormina et al. 2018).

An increased interest in the operation of offshore wind farms for energy production has risen over the past few years with a recognized need for renewable energy sources. Effects of installation and operation of offshore wind farms on CWC still needs assessment, with negative effects on the abundance and biomass of benthic species reported around wind farms (Watson et al. 2024). Though it is noted that offshore structures may provide settlement habitat for macro-invertebrates, potential proliferation of invasive or opportunistic species on these structures has been raised as a concern, which may impact local benthic communities (Watson et al. 2024). As well, offshore wind farms are predicted to increase total carbon flux to the benthos (Ivanov et al. 2021), which could have implications for suspension-feeding organisms such as CWC.

It is clear much is yet to be learned regarding the impacts of industry activities on CWC, especially those which have had major advancements in the last decade. Of focus in this thesis are two industries that are experiencing growth in many regions worldwide yet the effects on CWC are largely unknown: finfish aquaculture and oil and gas exploration.

1.1.2.1 The effects of finfish aquaculture waste on CWC

Deposition of wastes (e.g. excess feed/feces) underneath finfish aquaculture pens is known to alter benthic habitats via organic enrichment, potentially leading to hypoxic/anoxic conditions, as well as declines in sediment habitat quality (Karakassis et al. 1999, Hargrave 2010, Valdemarsen et al. 2015, Hamoutene et al. 2016, Salvo et al. 2017a). Waste deposition has also been shown to induce shifts in benthic community assemblages towards those of opportunistic species (e.g. *Capitella capitata*, Keeley et al. 2014, 2015, Valdemarsen et al. 2015, Hamoutene et al. 2016, Salvo et al. 2017a, 2017b). However, literature available on the effects of wastes produced through aquaculture activities on CWC is minimal and only published in recent years (Table 1-1). To date, only a few studies (Kutti et al. 2015, 2022, Laroche et al. 2022, Taormina et

al. 2024a, 2024b) and one field survey (Dunlop et al. 2021) have probed the potential effects of aquaculture-produced wastes on CWC. All were conducted in Norway, and studies were conducted solely on the reef-building scleractinian *D. pertusum,* sea pens (*Pennatula phosphorea* and *Virgularia mirabilis*) or soft coral *D. florida*. Though published literature on the topic is expanding, there are major gaps in knowledge, both taxonomically and geographically, regarding the effects of aquaculture waste on CWC.

In a short-term laboratory study, Kutti et al. (2015) showed that within 2 months, fish feed particles increased the respiration rate and mucus production in *D. pertusum* fragments. A complementary field study conveyed that within 5 months of transplantation there was high survival of *D. pertusum* and skeletal growth was reduced only in colonies placed <250 m from an aquaculture site (Kutti et al. 2015).

In a later experiment, Kutti et al. (2022) transplanted fragments of *D. pertusum* on a gradient at variable distances (250 – 2000 m) within and outside the predicted depositional footprint of an aquaculture site for 13 months. Results showed that fragments in closer proximity to aquaculture sites had lower oxygen consumption, lower growth rates, lower lipid content, and higher lysosomal membrane destabilization rates. The results of this study indicate that CWC located within the depositional footprint of aquaculture sites seemingly experience negative physiological and biochemical effects, which may imply a state of metabolic depression (Kutti et al. 2022).

Another transplantation experiment evaluated the effects on the soft coral *Duva florida* after 7 months under aquaculture sites. *Duva florida* showed higher respiration correlated closeness to the site, as well as changes in both the microbial profile and fatty acid profile of transplanted specimens (Laroche et al. 2022). Additionally, Dunlop et al. (2021) surveyed

benthic epifauna in northern Norway using towed videos. This study described the decline of colony density of *D. florida* in the areas of highest depositional flux of particulate material (within 200 m from aquaculture sites).

Research on the effects of emamectin benzoate (chemotherapeutant used for sea-lice treatment in some aquaculture settings) on the sea pen *Pennatula phosphorea* over 8 days of high exposure (0.8 mg L^{-1}) was recently published by Taormina et al. (2024a). No mortality of *P. phosphorea* was observed, but the emamectin benzoate was detected within their tissues, suggesting a potential for bioaccumulation.

Finally, another recent study by Taormina et al. (2024b) explored the effects of aquaculture waste on sea pen *V. mirabilis in situ*. Under an operating salmon farm, video transects revealed that mortality, tissue necrosis, mucus production, and polyp contraction were associated with closeness to the farm. As well, reductions in associated fauna were also observed in close proximity (<500 m). Complementary to Taormina et al. (2024a), emamectin benzoate was detected in the tissues of sea pens collected directly under the farm 56 days after exposure to the chemotherapeutant (Taormina et al. 2024b). Overall, only a handful of research studies have been published on the topic of the effects of aquaculture on CWC and more research in this field is needed as expansion of industry in CWC habitat continues.

1.1.2.2 The impacts of oil and gas exploration on CWC

Though oil and gas exploration is commonly listed as a threat to CWC (Roberts et al. 2009, Roberts and Cairns 2014, Ragnarsson et al. 2016, Cordes et al. 2016), research in this field remains limited. Published experimental studies on the impacts of wastes produced by oil and gas exploration (drill cuttings, drill muds with barite and/or bentonite) on CWC have almost entirely focused on *D. pertusum* (e.g. Larsson and Purser 2011, Larsson et al. 2013, Allers et al.

2013, Buhl-Mortensen et al. 2015, Järnegren et al. 2017, 2020, Baussant et al. 2018, 2022, Vianna da Rocha et al. 2021). This species of coral has shown a high degree of resilience against sedimentation of drilling waste under the examined conditions, with polyp mortality occurring only at higher concentrations (Larsson and Purser 2011, Larsson et al. 2013, Allers et al. 2013, Baussant et al. 2022).

To my knowledge, only one experimental study has been conducted on a species of coral outside of *D. pertusum*. This study by Raimondi et al. (1997) experimentally explored the effects of suspended drilling muds on the brown cup coral, *Paracyathus stearnsii*. Results showed increased mortality and tissue loss with increased concentrations of drilling muds.

The known recovery potential of CWC communities to effects of drilling wastes has also been of interest. In Gates and Jones (2012), video transects conducted 1 day pre-drilling, as well as 27 days, 76 days and 3 years post-drilling revealed noticeable reductions in densities of sea pens (*Kophobelemnon stelliferum, Funiculina* sp*., Pennatula phosphorea*, unidentified pennatulid sp.) in areas visibly disturbed by drilling, which did not show recovery 3 years post-drilling. Recolonization of disturbed areas may be further impeded by potential impacts of drilling muds and cuttings on the functioning (Järnegren et al. 2017, 2020) and survival of CWC larvae (Larsson et al. 2013, Järnegren et al. 2017, 2020). Settlement success and survival of CWC on benthos disturbed by drilling wastes remain unknown, especially for those that grow within or on soft substrate where they would be in closer contact with remaining waste.

Additionally, with oil and gas exploration/exploitation comes the increased risk of oil spills. CWC research on this topic was advanced as a result of the 2010 Deepwater Horizon oil spill in the Gulf of Mexico, which provided the opportunity to monitor *in situ* responses, with impacted colonies observed as far as 11 km away from the spill site (White et al. 2012). Slow

recovery of colonies and impacts persisting 7 years later were recorded (Girard and Fisher 2018) with recovery estimated to take up to 30 years (Girard et al. 2018). Chemical dispersants used in clean-up responses following oil spills were observed to induce reductions in CWC health, which were sometimes greater than those elicited by oil exposure alone (DeLeo et al. 2016), and were traceable in flocculent matter in contact with colonies 6 months after use (White et al. 2014).

1.1.2.3 Multi-stressor effects

Of note is the fact that stress caused by industrial activity may be exacerbated within environments that are subject to multiple pressures (e.g. habitats affected by climate change). For example, increasing temperatures have the potential to reduce growth and increase respiration in CWC (Reynaud et al. 2021). As well, effects of ocean acidification under predicted future conditions have shown potential changes in gene expression (Glazier et al. 2020, Servetto et al. 2021), calcification (Gómez et al. 2018, Steiner et al. 2018, Maier et al. 2021), respiration (Gammon et al. 2018), reproduction (Rossin et al. 2019), as well as polyp and tissue loss (Gammon et al. 2018). Energetic requirements needed for CWC survival under future climate scenarios, such as those associated with maintaining calcification under lower pH (Wall et al. 2015), may be more demanding when combined with additional stressors from industry activity. For example, the gorgonian *Primnoa resedaeformis* had physiological changes (in respiration, nitrogen excretion, lysosome stability, O:N ratios) when exposed to simulated mine tailings (crushed granite) and elevated temperature conditions (13 °C), which were sometimes more pronounced under a combination of the two treatments (Scanes et al. 2018).

1.1.3 Challenges in CWC experimental research

CWC research has long been limited by the constraints associated with the collection and surveying of organisms, especially for deep-water taxa. Though there have been advances (e.g.

habitat mapping technology, remotely operated vehicles) in the past decades (Roberts et al. 2009), challenges remain. Among them is physical access to CWC, especially deep-sea species. For experimental or *in situ* studies, collecting statistically powerful sample sizes can prove difficult. These difficulties often lead to small sample sizes which can create high variation in results. Collection constraints can also introduce a depth bias to favour shallower, more accessible samples (Waller et al. 2023). As such, colony fragmentation (breaking a colony into pieces) to increase sample size is commonplace in experimental studies, particularly for colonial scleractinians or large gorgonian species (e.g. Larsson and Purser 2011, Allers et al. 2013, Gammon et al. 2018, Baussant et al. 2018, Rossin et al. 2019, Weinnig et al. 2020, DeLeo et al. 2021) which can present genetic entanglements among other caveats. For example, in both Glazier et al. (2020) and DeLeo et al. (2021) variation in gene expression among fragments were explained more clearly by original colony source than experimental treatment.

Maintaining live corals long enough for proper recovery from collection, acclimation to laboratory conditions and experiments themselves can be difficult. Effects of treatment can be obscured when mortality also occurs in corals used as controls (unmanipulated) during experiments, indicating underlying stress either from collection or the laboratory environment. It can also present a source of variation, for example Büscher et al. (2022) found that orange colour morphs of *Desmophylum pertusum* (published as *Lophelia pertusa*) were more likely to have polyp mortality under both control and treatment conditions compared to polyps of the white colour morph. Collecting and maintaining a sufficient sample size of live corals remains difficult for the study of many species, especially those from bathyal and abyssal depths.

1.2 Region and species of interest

Situated in the Northwest Atlantic, the province of Newfoundland and Labrador has been experiencing growth in both aquaculture and oil/gas sectors over the past decades, making the need for research in these areas pressing. However, although reef-building CWC species do occur in Atlantic Canada (Beazley et al. 2021), they are not known to inhabit Newfoundland and Labrador waters (Wareham and Edinger 2007, Murillo et al. 2011, Baker et al. 2012). This makes management of CWC using existing published data (largely focused on the reef-building species *D. pertusum*) difficult and leaves the impacts on species present in Newfoundland and Labrador unknown.

1.2.1 Aquaculture in Newfoundland and Labrador

The main aquaculture production in Newfoundland and Labrador revolves around salmonids and shellfish. Atlantic salmon is the main finfish harvested, with 106 commercial licenses held for salmonid aquaculture in 2021 (FFA 2022). Finfish aquaculture cages are concentrated in the southern coast of the province (Figure 1-2), covering 6,744 ha of water with intentions to expand (FFA 2022). Aquaculture sites are clustered in coastal areas such as bays and fjords to provide adequate shelter from harsh conditions such as wave action and ice cover (AMEC 2002). Sites in these areas generally have low bottom currents and sills (Donnet et al. 2018), minimizing waste dispersal and resuspension. Therefore, the probability of waste accumulation on sessile benthic species, including CWC, beneath aquaculture pens is high (Verhoeven et al. 2018, Armstrong et al. 2020, Knight et al. 2021).

1.2.2 Oil and gas exploration off Newfoundland and Labrador

Oil and gas drilling in offshore regions of Newfoundland and Labrador has been occurring for over 50 years, with 167 exploratory wells drilled between 1966 and 2016 (NSB 2016). Many

of these wells were concentrated in the Jeanne d'Arc region at an average depth ~100 m, but deep-water drilling activity (e.g. in the Flemish Pass basin) has increased in recent years (NSB 2016). Exploratory drilling is expected to accelerate in the region with the hopes of 100 new wells drilled by 2030 (Oil and Gas Industry Development Council 2018). As well, with $2.6 billion in exploratory commitments, industry growth over the next decades is projected to be generated by new projects rather than production from existing ones (Oil and Gas Industry Development Council 2018).

Areas of important benthic habitat have been identified offshore of Newfoundland and Labrador (Figure 1-3). These have been identified as VMEs (19 areas, outside of Canada's exclusive economic zone), significant benthic areas (SiBAs, 4 areas), ecologically and biologically significant areas (EBSA, 16 areas), marine refuges (MR, 5 areas) or marine protected areas (MPA, 1 area). Currently, all designated areas except MPAs do not prohibit exploratory drilling; only one MPA exists in offshore Newfoundland and Labrador (Gullage et al. 2022).

1.2.3 Focal species

Focal species for the present studies were chosen based on their presence in the region of interest combined with logistical constraints of collecting and maintaining CWC in aquaria. *Gersemia rubiformis* (shown in Figure 1-4) is a soft coral found ubiquitously across the Northwest Atlantic (Wareham and Edinger 2007), including shallow, coastal areas in close vicinity to aquaculture sites (Salvo et al. 2018). Its broad distribution, small size (<5 cm contracted height, Wareham and Edinger 2007), and previous maintenance success in laboratory environments (Henry et al. 2003, Caulier et al. 2021) make it an ideal target species for an experimental study on the effects of aquaculture waste.

In addition to *G. rubiformis*, the sea anemone *Aulactinia stella* (Figure 1-5) was included opportunistically in the study on aquaculture waste. As sea anemones can be abundant in temperate and cold-water habitats (Hargrave et al. 2004, Metaxas and Davis 2005, Nelson and Craig 2011), this study provided the possibility to concurrently collect data on this group which have also been recorded commonly near aquaculture sites (Salvo et al. 2018, Hamoutene et al. 2015). Additionally, as sea anemones share a similar morphology to a coral polyp, often share the same feeding strategy (i.e. suspension-feeding), and are understudied in the same respect, their inclusion was intended to be complementary to that of *G. rubiformis*. *Aulactinia stella* is common in shallow, coastal environments (0-178 m, Ivanova and Grebelnyi 2021) of the north-eastern coast of North America (Brunel et al. 1998) and was present at the same site as *G. rubiformis*, which was convenient for collection purposes. *Aulactinia stella* has also previously been maintained in laboratory conditions for long-term experiments (Mercier et al. 2016), making it a suitable addition to this study.

Flabellum (*Ulocyathus*) *alabastrum* is a solitary cup-coral that inhabits the deep sea (218 to 1433 m, Wareham and Edinger 2007). Its distribution range includes the Flemish Pass (Murillo et al. 2011), which is an active area of interest for oil and gas exploration (Figure 1-6). This species has a singular, large polyp sheltered within an external skeleton (Figure 1-7). *Flabellum alabastrum* lives unattached on soft sediment and is often found in high densities, making collection of many individuals with limited physical damage feasible compared to species that live attached to hard substratum. In addition, *F. alabastrum* has had long-term success surviving in laboratory settings (Buhl-Mortensen et al. 2007, Hamel et al. 2010). Thus, *F. alabastrum* was chosen as a species of interest to explore the effects of wastes from oil/gas exploration.

1.3 Objectives and chapter structure

This thesis aims to explore the potential effects of waste from two prominent marine industries, i.e. aquaculture and oil/gas exploration, on representative epibenthic species from cold-temperate benthic environments that occur in the Newfoundland and Labrador region. Two experimental studies were conducted towards the completion of the thesis and are structured as the two following data chapters.

The objective of Chapter 2 was to begin to understand the effects of aquaculture wastes (fish feed and feces) on *Gersemia rubiformis* and *Aulactinia stella*. This was done through the combination of behavioural monitoring and fatty acid/lipid analyses to investigate whether waste was being avoided or potentially assimilated.

The objective of Chapter 3 was to investigate the impacts of wastes produced by offshore drilling (barite and bentonite) on the deep-sea cup coral *Flabellum alabastrum*. This was accomplished by analyzing behaviour through daily photography to observe physical reactions to sedimentation, as well as conducting histological work to explore potential changes in cellular defense mechanisms (cnidocytes, or stinging cells).

The final chapter summarizes the key findings and furthers the discussion of the two data chapters, as well as identifies directions for future research.

It is anticipated the findings will provide valuable insights with respect to the conservation of benthic environments, including the conservation of CWC habitats, where knowledge is currently limited or non-existent. As well, this project hopes to further our fundamental understanding of CWC and sea anemones, which are dominant in many coastal and deep-water benthic communities (Hargrave et al. 2004, Metaxas and Davis 2005, Wareham and Edinger 2007).

1.4 Literature cited

Aguilar, R., A. L. Perry, and J. López. 2017. Conservation and management of vulnerable marine benthic ecosystems. Pages 1165–1207 Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots. Springer International Publishing.

Allers, E., R. M. M. Abed, L. M. Wehrmann, T. Wang, A. I. Larsson, A. Purser, and D. de Beer. 2013. Resistance of *Lophelia pertusa* to coverage by sediment and petroleum drill cuttings. Marine Pollution Bulletin 74:132–140.

Althaus, F., A. Williams, T. A. Schlacher, R. J. Kloser, M. A. Green, B. A. Barker, N. J. Bax, P. Brodie, and M. A. Schlacher-Hoenlinger. 2009. Impacts of bottom trawling on deep-coral ecosystems of seamounts are long-lasting. Marine Ecology Progress Series 397:279–294.

Armstrong, E. G., J. Mersereau, F. Salvo, D. Hamoutene, and S. C. Dufour. 2020. Temporal change in the spatial distribution of visual organic enrichment indicators at aquaculture sites in Newfoundland, Canada. Aquaculture International 28:569–586.

AMEC. 2002. Aquaculture information review - an evaluation of known effects and mitigations on fish and fish habitat in Newfoundland and Labrador. Can Tech. Rep. Fish. Aquat. Sci. 2434: vii + 47 p.

Baillon, S., J.-F. Hamel, and A. Mercier. 2014. Diversity, distribution and nature of faunal associations with deep-sea pennatulacean corals in the northwest Atlantic. PLoS ONE 9:e111519.

Baillon, S., J.-F. Hamel, V. E. Wareham, and A. Mercier. 2012. Deep cold-water corals as nurseries for fish larvae. Frontiers in Ecology and the Environment 10:351–356.

Baker, K. D., V. E. Wareham, P. V. R. Snelgrove, R. L. Haedrich, D. A. Fifield, E. N. Edinger, and K. D. Gilkinson. 2012. Distributional patterns of deep-sea coral assemblages in three

submarine canyons off Newfoundland, Canada. Marine Ecology Progress Series 445:235–249.

Baussant, T., M. Arnberg, E. Lyng, S. Ramanand, S. Bamber, M. Berry, I. Myrnes Hansen, D. Van Oevelen, and P. Van Breugel. 2022. Identification of tolerance levels on the cold-water coral *Desmophyllum pertusum* (*Lophelia pertusa*) from realistic exposure conditions to suspended bentonite, barite and drill cutting particles. PLOS ONE 17:e0263061.

Baussant, T., M. Nilsen, E. Ravagnan, S. Westerlund, and S. Ramanand. 2018. Effects of suspended drill cuttings on the coral *Lophelia pertusa* using pulsed and continuous exposure scenarios. Journal of Toxicology and Environmental Health - Part A: Current Issues 81:361–382.

Beazley, L., E. Kenchington, M. Korabik, D. Fenton, and M. King. 2021. Other effective area-based conservation measure promotes recovery in a cold-water coral reef. Global Ecology and Conservation 26:e01485.

Boulard, M., P. Lawton, K. Baker, and E. Edinger. 2022. The effect of small-scale habitat features on groundfish density in deep-sea soft-bottom ecosystems. Deep-Sea Research Part I: Oceanographic Research Papers 193:e103891.

Brunel, P., Bossé, L., and G. Lamarche. 1998. Catalogue of the marine invertebrates of the Estuary and Gulf of Saint Lawrence. Can. Spec. Publ. Fish. Aquat. Sci. 126. 405 p.

Buhl-Mortensen, L., P. Mortensen, S. Armsworthy, and D. Jackson. 2007. Field observations of *Flabellum* spp. and laboratory study of the behavior and respiration of *Flabellum alabastrum*. Bulletin of Marine Science 81:543–522.

Buhl-Mortensen, L., and P. B. Mortensen. 2005. Distribution and diversity of species associated with deep-sea gorgonian corals off Atlantic Canada. Pages 849–879 *in* A. Freiwald and J.

Murray Roberts, editors. Cold-water corals and ecosystems. Springer-Verlag Berlin Heidelberg.

Buhl-Mortensen, P., E. Tenningen, and A. B. S. Tysseland. 2015. Effects of water flow and drilling waste exposure on polyp behaviour in *Lophelia pertusa*. Marine Biology Research 11:725–737.

Büscher, J. V., A. U. Form, M. Wisshak, R. Kiko, and U. Riebesell. 2022. Cold-water coral ecosystems under future ocean change: live coral performance vs. framework dissolution and bioerosion. Limnology and Oceanography 67:2497–2515.

Carreiro-Silva, M., I. Martins, V. Riou, J. Raimundo, M. Caetano, R. Bettencourt, M. Rakka, T. Cerqueira, A. Godinho, T. Morato, and A. Colaço. 2022. Mechanical and toxicological effects of deep-sea mining sediment plumes on a habitat-forming cold-water octocoral. Frontiers in Marine Science 9:e915650.

Caulier, G., J.-F. Hamel, E. A. Hendrycks, K. E. Conlan, and A. Mercier. 2021. Mutualistic relationship between the amphipod *Stenula nordmanni* (Stephensen, 1931) and the nephtheid coral *Gersemia rubiformis* (Ehrenberg, 1834). Symbiosis 85:93–104.

De Clippele, L. H., P. Buhl-Mortensen, and L. Buhl-Mortensen. 2015. Fauna associated with cold water gorgonians and sea pens. Continental Shelf Research 105:67–78.

Cordes, E. E., D. O. B. Jones, T. A. Schlacher, D. J. Amon, A. F. Bernardino, S. Brooke, R. Carney, D. M. DeLeo, K. M. Dunlop, E. G. Escobar-Briones, A. R. Gates, L. Génio, J. Gobin, L. A. Henry, S. Herrera, S. Hoyt, M. Joye, S. Kark, N. C. Mestre, A. Metaxas, S. Pfeifer, K. Sink, A. K. Sweetman, and U. Witte. 2016. Environmental impacts of the deep-water oil and gas industry: a review to guide management strategies. Frontiers in Environmental Science 4:58.

DeLeo, D. M., A. Glazier, S. Herrera, A. Barkman, and E. E. Cordes. 2021. Transcriptomic responses of deep-sea corals experimentally exposed to crude oil and dispersant. Frontiers in Marine Science 8:e649909.

DeLeo, D. M., D. V. Ruiz-Ramos, I. B. Baums, and E. E. Cordes. 2016. Response of deep-water corals to oil and chemical dispersant exposure. Deep-Sea Research Part II: Topical Studies in Oceanography 129:137–147.

Donnet, S., Ratsimandresy, A.W., Goulet, P., Doody, C., Burke, S., and S. Cross. 2018. Coast of Bays metrics: geography, hydrology and physical oceanography of an aquaculture area of the South Coast of Newfoundland. DFO Can. Sci. Advis. Sec. Res. Doc. 2017/076. x + 109 p.

Dunlop, K., A. Harendza, R. Bannister, and N. Keeley. 2021. Spatial response of hard- and mixed-bottom benthic epifauna to organic enrichment from salmon aquaculture in northern Norway. Aquaculture Environment Interactions 13:455–475.

Edinger, E. N., V. E. Wareham, and R. L. Haedrich. 2007. Patterns of groundfish diversity and abundance in relation to deep-sea coral distributions in Newfoundland and Labrador waters. Pages 101–122 *in* R. Y. George and S. D. Cairns, editors. Conservation and adaptive management of seamount and deep-sea coral ecosystems. Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami.

Gammon, M. J., D. M. Tracey, P. M. Marriott, V. J. Cummings, and S. K. Davy. 2018. The physiological response of the deep- sea coral *Solenosmilia variabilis* to ocean acidification. PeerJ 6:e5236.

Gates, A. R., and D. O. B. Jones. 2012. Recovery of benthic megafauna from anthropogenic disturbance at a hydrocarbon drilling well (380 m depth in the Norwegian Sea). PLoS ONE 7:e44114.

Girard, F., and C. R. Fisher. 2018. Long-term impact of the Deepwater Horizon oil spill on deep-sea corals detected after seven years of monitoring. Biological Conservation 225:117–127.

Girard, F., K. Shea, and C. R. Fisher. 2018. Projecting the recovery of a long-lived deep-sea coral species after the Deepwater Horizon oil spill using state-structured models. Journal of Applied Ecology 55:1812–1822.

Glazier, A., S. Herrera, A. Weinnig, M. Kurman, C. E. Gómez, and E. Cordes. 2020. Regulation of ion transport and energy metabolism enables certain coral genotypes to maintain calcification under experimental ocean acidification. Molecular Ecology 29:1657–1673.

Gómez, C. E., L. Wickes, D. Deegan, P. J. Etnoyer, and E. E. Cordes. 2018. Growth and feeding of deep-sea coral *Lophelia pertusa* from the California margin under simulated ocean acidification conditions. PeerJ 6:e5671.

Gullage, L., V. Hayes, B. M. Neves, N. Wells, F. Cyr, and F. J. Murillo. 2022. Avoidance and mitigation of coral and sponge species during exploratory drilling activities offshore Newfoundland and Labrador. DFO Can. Sci. Advis. Sec. Res. Doc. 2022/059. vii + 141 p.

Hamann, L., and A. Blanke. 2022. Suspension feeders: diversity, principles of particle separation and biomimetic potential. Journal of the Royal Society Interface 19:e20210741.

Hamel, J.-F., Z. Sun, and A. Mercier. 2010. Influence of size and seasonal factors on the growth of the deep-sea coral *Flabellum alabastrum* in mesocosm. Coral Reefs 29:521–525.

Hamoutene, D., F. Salvo, T. Bungay, G. Mabrouk, C. Couturier, A. Ratsimandresy, and S. C. Dufour. 2015. Assessment of finfish aquaculture effect on Newfoundland epibenthic communities through video monitoring. North American Journal of Aquaculture 77:117–127.

Hamoutene D., F. Salvo, S. Donnet, and S. Dufour. 2016. The usage of visual indicators in regulatory monitoring at hard-bottom finfish aquaculture sites in Newfoundland (Canada). Marine Pollution Bulletin 108:232-241.

Hargrave, B. T. 2010. Empirical relationships describing benthic impacts of salmon aquaculture. Aquaculture Environment Interactions 1:33–46.

Hargrave, B. T., V. E. Kostylev, and C. M. Hawkins. 2004. Benthic epifauna assemblages, biomass and respiration in The Gully region on the Scotian Shelf, NW Atlantic Ocean. Marine Ecology Progress Series 270:55–70.

Henry, L.-A., E. L. Kenchington, and A. Silvaggio. 2003. Effects of mechanical experimental disturbance on aspects of colony responses, reproduction, and regeneration in the cold-water octocoral *Gersemia rubiformis*. Canadian Journal of Zoology 81:1691–1701.

Huvenne, V. A. I., B. J. Bett, D. G. Masson, T. P. Le Bas, and A. J. Wheeler. 2016. Effectiveness of a deep-sea cold-water coral Marine Protected Area, following eight years of fisheries closure. Biological Conservation 200:60–69.

Ivanov, E., A. Capet, E. De Borger, S. Degraer, E. J. M. Delhez, K. Soetaert, J. Vanaverbeke, and M. Grégoire. 2021. Offshore wind farm footprint on organic and mineral particle flux to the bottom. Frontiers in Marine Science 8:e631799.

Ivanova, N. Y., and S. D. Grebelnyi. 2021. The history of study, the taxonomic composition and the origin of the sea anemone fauna of the Kara Sea (Actiniaria, Anthozoa, Cnidaria). Proceedings of the Zoological Institute of the Russian Academy of Sciences 325:156–182.

Järnegren, J., S. Brooke, and H. Jensen. 2017. Effects of drill cuttings on larvae of the cold-water coral *Lophelia pertusa*. Deep Sea Research Part II: Topical Studies in Oceanography 137:454–462.

Järnegren, J., S. Brooke, and H. Jensen. 2020. Effects and recovery of larvae of the cold-water coral *Lophelia pertusa* (*Desmophyllum pertusum*) exposed to suspended bentonite, barite and drill cuttings. Marine Environmental Research 158:e104996.

Jouffray, J. B., R. Blasiak, A. V. Norström, H. Österblom, and M. Nyström. 2020. The blue acceleration: the trajectory of human expansion into the ocean. One Earth 2 2:43–54.

Karakassis, I., E. Hatziyanni, M. Tsapakis, and W. Plaiti. 1999. Marine ecology progress series: Benthic recovery following cessation of fish farming: a series of successes and catastrophes. Mar Ecol Prog Ser 184:205–218.

Keeley, N. B., B. M. Forrest, and C. K. Macleod. 2015. Benthic recovery and re-impact responses from salmon farm enrichment: Implications for farm management. Aquaculture 435:412–423.

Keeley, N. B., C. K. Macleod, G. A. Hopkins, and B. M. Forrest. 2014. Spatial and temporal dynamics in macrobenthos during recovery from salmon farm induced organic enrichment: When is recovery complete? Marine Pollution Bulletin 80:250–262.

Knight, R., J. T. P. Verhoeven, F. Salvo, D. Hamoutene, S. C. Dufour. 2021. Validation of visual bacterial mat assessment at aquaculture sites through abiotic and biotic indicators. Ecological Indicators 122:e107283.

Kutti, T., E. Legrand, V. Husa, S. Olsen, Ø. Gjelsvik, M. Carvajalino-Fernandez, and I. Johnsen. 2022. Fish-farm effluents cause metabolic depression, reducing energy stores and growth in the reef forming coral *Lophelia pertusa*. Aquaculture Environment Interactions 14:279–293.

Kutti, T., K. Nordbø, R. Bannister, and V. Husa. 2015. Oppdrettsanlegg kan true korallrev i fjordene. Havforskningsrapporten: fisken og havet, særnummer. 220 pp. Havforskningsinstituttet.

Laroche, O., S. Meier, S. A. Mjøs, and N. Keeley. 2022. Suspension-feeding benthic species' physiological and microbiome response to salmon farming and associated environmental changes. Frontiers in Marine Science 9:e841806.

Larsson, A. I., D. van Oevelen, A. Purser, and L. Thomsen. 2013. Tolerance to long-term exposure of suspended benthic sediments and drill cuttings in the cold-water coral *Lophelia pertusa*. Marine Pollution Bulletin 70:176–188.

Larsson, A. I., and A. Purser. 2011. Sedimentation on the cold-water coral *Lophelia pertusa*: cleaning efficiency from natural sediments and drill cuttings. Marine Pollution Bulletin 62:1159–1168.

Oil and Gas Industry Development Council. 2018. Advance 2030: a plan for growth in the Newfoundland and Labrador oil and gas industry. Retrieved from: https://www.gov.nl.ca/iet/files/advance30-pdf-oil-gas-sector-final-online.pdf (accessed June 12, 2024).

Maier, S. R., C. Jantzen, J. Laudien, V. Häussermann, G. Försterra, A. Cornils, J. Niggemann, T. Dittmar, and C. Richter. 2021. The carbon and nitrogen budget of *Desmophyllum dianthus*-a voracious cold-water coral thriving in an acidified Patagonian fjord. PeerJ 9:e12609.

Martins, I., A. Godinho, J. Goulart, and M. Carreiro-Silva. 2018. Assessment of Cu sub-lethal toxicity (LC50) in the cold-water gorgonian *Dentomuricea meteor* under a deep-sea mining activity scenario. Environmental Pollution 240:903–907.

Martins, I., A. Godinho, M. Rakka, and M. Carreiro-Silva. 2022. Beyond deep-sea mining sublethal effects: delayed mortality from acute Cu exposure of the cold-water octocoral *Viminella flagellum*. Marine Pollution Bulletin 183:e114051.

Mercier, A., Z. Sun, C. C. Parrish, and J.-F. Hamel. 2016. Remarkable shifts in offspring provisioning during gestation in a live-bearing cnidarian. PLoS ONE 11:e0154051.

Metaxas, A., and J. Davis. 2005. Megafauna associated with assemblages of deep-water gorgonian corals in Northeast Channel, off Nova Scotia, Canada. Journal of the Marine Biological Association of the United Kingdom 85:1381–1390.

Murillo, F. J., P. Durán Muñoz, A. Altuna, and A. Serrano. 2011. Distribution of deep-water corals of the Flemish Cap, Flemish Pass, and the Grand Banks of Newfoundland (Northwest Atlantic Ocean): Interaction with fishing activities. ICES Journal of Marine Science 68:319–332.

Nelson, M. L., and S. F. Craig. 2011. Role of the sea anemone *Metridium senile* in structuring a developing subtidal fouling community. Marine Ecology Progress Series 421:139–149.

Neves, B. M. 2016. Growth in cold-water octocorals: rates, morphology and environmental controls. Memorial University, St. John's.

Neves, B. M., E. Edinger, G. D. Layne, and V. E. Wareham. 2015. Decadal longevity and slow growth rates in the deep-water sea pen *Halipteris finmarchica* (Sars, 1851) (Octocorallia: Pennatulacea): implications for vulnerability and recovery from anthropogenic disturbance. Hydrobiologia 759:147–170.

Neves, B. M., V. Wareham Hayes, E. Herder, K. Hedges, C. Grant, and P. Archambault. 2020. Cold-water soft corals (Cnidaria: Nephtheidae) as habitat for juvenile basket stars (Echinodermata: Gorgonocephalidae). Frontiers in Marine Science 7:e547896.

[NSB] NSB Energy Consulting. 2016. Drilling performance review offshore Newfoundland and Labrador. Retrieved from: https://oilconl.com/wp-content/uploads/2016/09/Drilling-Performance-Review-Final.pdf (accessed June 12, 2024).

Pierdomenico, M., T. Russo, S. Ambroso, A. Gori, E. Martorelli, L. D'Andrea, J. M. Gili, and F. L. Chiocci. 2018. Effects of trawling activity on the bamboo-coral *Isidella elongata* and the sea pen *Funiculina quadrangularis* along the Gioia Canyon (Western Mediterranean, southern Tyrrhenian Sea). Progress in Oceanography 169:214–226.

Prouty, N. G., C. R. Fisher, A. W. J. Demopoulos, and E. R. M. Druffel. 2016. Growth rates and ages of deep-sea corals impacted by the Deepwater Horizon oil spill. Deep-Sea Research Part II: Topical Studies in Oceanography 129:196–212.

Ragnarsson, S. Á., J. M. Burgos, T. Kutti, I. van den Beld, H. Egilsdóttir, S. Arnaud-Haond, and A. Grehan. 2016. The impact of anthropogenic activity on cold-water corals. Pages 6–35 Marine Animal Forests. Springer International Publishing.

Raimondi, P. T., A. M. Barnett, and P. R. Krause. 1997. The effects of drilling muds on marine invertebrate larvae and adults. Environmental Toxicology and Chemistry 16:1218–1228.

Ramirez-Llodra, E., H. C. Trannum, A. Evenset, L. A. Levin, M. Andersson, T. E. Finne, A. Hilario, B. Flem, G. Christensen, M. Schaanning, and A. Vanreusel. 2015. Submarine and deep-sea mine tailing placements: A review of current practices, environmental issues, natural analogs and knowledge gaps in Norway and internationally. Marine Pollution Bulletin 97:13-35.

Reynaud, S., C. Orejas, A. Campagno, C. Rottier, C. Jimenez, and C. Ferrier-Pagès. 2021. Dendrophylliidae cold-water corals in a warm ocean: the effect of exposure duration on their physiological response. Deep-Sea Research Part II: Topical Studies in Oceanography 193:e104962.

Risk, M. J., J. M. Heikoop, M. G. Snow, and & R. Beukens. 2002. Lifespans and growth patterns of two deep-sea corals: *Primnoa resedaeformis* and *Desmophyllum cristagalli*. Hydrobiologia 471:125–131.

Roberts, J. M., and S. D. Cairns. 2014. Cold-water corals in a changing ocean. Current Opinion in Environmental Sustainability 7:118–126.

Roberts, J. M., A. Wheeler, A. Freiwald, and S. Cairns. 2009. Cold-Water Corals. Cambridge University Press.

Rooper, C. N., M. E. Wilkins, C. S. Rose, and C. Coon. 2011. Modeling the impacts of bottom trawling and the subsequent recovery rates of sponges and corals in the Aleutian Islands, Alaska. Continental Shelf Research 31:1827–1834.

Rossi, S., M. Coppari, and N. Viladrich. 2017. Benthic-pelagic coupling: New perspectives in the animal forests. Pages 855–885 Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots. Springer International Publishing.

Rossin, A. M., R. G. Waller, and R. P. Stone. 2019. The effects of in-vitro pH decrease on the gametogenesis of the red tree coral, *Primnoa pacifica*. PLoS ONE 14:e0203976.

Salvo F., J. Mersereau, D. Hamoutene, R. Belley, and S. Dufour. 2017a. Spatial and temporal changes in epibenthic communities at deep, hard bottom aquaculture sites in Newfoundland. Ecological Indicators 76:207-218.

Salvo F., S. Dufour, and D. Hamoutene. 2017b. Temperature thresholds of opportunistic annelids used as benthic indicators of aquaculture impact in Newfoundland (Canada). Ecological Indicators 79:103-105.

Salvo, F., Oldford, V., Bungay, T., Boone, C., and D. Hamoutene. 2018. Guide for video monitoring of hard bottom benthic communities of the south coast of Newfoundland for

aquaculture impact assessments. Can. Data Rep. Fish. Aquat. Sci. Fs 97-13/1284E-PDF: ix + 41 p.

Sampaio, I., A. Braga-Henriques, C. Pham, O. Ocaña, V. De Matos, T. Morato, and F. M. Porteiro. 2012. Cold-water corals landed by bottom longline fisheries in the Azores (north-eastern Atlantic). Journal of the Marine Biological Association of the United Kingdom 92:1547–1555.

Scanes, E., T. Kutti, J. K. H. Fang, E. L. Johnson, P. M. Ross, and R. J. Bannister. 2018. Mine waste and acute warming induce energetic stress in the deep-sea sponge *Geodia atlantica* and coral *Primnoa resedeaformis*; results from a mesocosm study. Frontiers in Marine Science 5:e129.

Servetto, N., M. C. de Aranzamendi, R. Bettencourt, C. Held, D. Abele, J. Movilla, G. González, D. M. Bustos, and R. Sahade. 2021. Molecular mechanisms underlying responses of the Antarctic coral *Malacobelemnon daytoni* to ocean acidification. Marine Environmental Research 170:e105430.

Sherwood, O. A., and E. N. Edinger. 2009. Ages and growth rates of some deep-sea gorgonian and antipatharian corals of Newfoundland and Labrador. Canadian Journal of Fisheries and Aquatic Sciences 66:142–152.

Steiner, Z., A. V. Turchyn, E. Harpaz, and J. Silverman. 2018. Water chemistry reveals a significant decline in coral calcification rates in the southern Red Sea. Nature Communications 9:e3615.

Taormina, B., J. Bald, A. Want, G. Thouzeau, M. Lejart, N. Desroy, and A. Carlier. 2018. A review of potential impacts of submarine power cables on the marine environment:

knowledge gaps, recommendations and future directions. Renewable and Sustainable Energy Reviews 96:380–391.

Taormina, B., R. H. Escobar-Lux, E. Legrand, A. E. Parsons, T. Kutti, V. Husa, R. Hannisdal, O. B. Samuelsen, and A. L. Agnalt. 2024a. Effects of the sea lice chemotherapeutant, emamectin benzoate, on metabolism and behaviour of the sea-pen *Pennatula phosphorea*. Marine Pollution Bulletin 198:e115903.

Taormina, B., T. Kutti, S. A. Olsen, P. N. Sævik, R. Hannisdal, V. Husa, and E. Legrand. 2024b. Effects of aquaculture effluents on the slender sea pen *Virgularia mirabilis*. Scientific Reports 14:e9385.

Valdemarsen, T., P. K. Hansen, A. Ervik, and R. J. Bannister. 2015. Impact of deep-water fish farms on benthic macrofauna communities under different hydrodynamic conditions. Marine Pollution Bulletin 101:776–783.

Verhoeven, J. T. P., F. Salvo, R. Knight, D. Hamoutene, and S. C. Dufour. 2018. Temporal bacterial surveillance of salmon aquaculture sites indicates a long lasting benthic impact with minimal recovery. Frontiers in Microbiology 9:e3054.

Vianna da Rocha, I., E. Cardinot Reis, P. Reis da Silva, G. de Hollanda Cavalcanti, R. Coutinho, and M. Vieira Reynier. 2021. Deep-sea coral *Lophelia pertusa* laboratory maintenance and exposure to barite using water recirculation systems. Journal of Toxicology and Environmental Health Sciences 13:1–17.

Wall, M., F. Ragazzola, L. C. Foster, A. Form, and D. N. Schmidt. 2015. pH up-regulation as a potential mechanism for the cold-water coral *Lophelia pertusa* to sustain growth in aragonite undersaturated conditions. Biogeosciences 12:6869–6880.

Waller, R. G., S. Goode, D. Tracey, J. Johnstone, and A. Mercier. 2023. A review of current knowledge on reproductive and larval processes of deep-sea corals. Marine Biology 170:e58.

Wareham, V. E., and E. N. Edinger. 2007. Distribution of deep-sea corals in the Newfoundland and Labrador region, Northwest Atlantic Ocean. Pages 289–313 Conservation and adaptive management of seamount and deep-sea coral ecosystems. Rosenstiel School of Marine and Atmospheric Science, University of Miami. Watling and Auster.

Watson, S. C. L., P. J. Somerfield, A. J. Lemasson, A. M. Knights, A. Edwards-Jones, J. Nunes, C. Pascoe, C. L. McNeill, M. Schratzberger, M. S. A. Thompson, E. Couce, C. L. Szostek, H. Baxter, and N. J. Beaumont. 2024. The global impact of offshore wind farms on ecosystem services. Ocean and Coastal Management 249:e107023.

Weinnig, A. M., C. E. Gómez, A. Hallaj, and E. E. Cordes. 2020. Cold-water coral (*Lophelia pertusa*) response to multiple stressors: High temperature affects recovery from short-term pollution exposure. Scientific Reports 10:e1768.

White, H. K., P. Y. Hsing, W. Cho, T. M. Shank, E. E. Cordes, A. M. Quattrini, R. K. Nelson, R. Camilli, A. W. J. Demopoulos, C. R. German, J. M. Brooks, H. H. Roberts, W. Shedd, C. M. Reddy, and C. R. Fisher. 2012. Impact of the Deepwater Horizon oil spill on a deep-water coral community in the Gulf of Mexico. Proceedings of the National Academy of Sciences of the United States of America 109:20303–20308.

White, H. K., S. L. Lyons, S. J. Harrison, D. M. Findley, Y. Liu, and E. B. Kujawinski. 2014. Long-term persistence of dispersants following the Deepwater Horizon oil spill. Environmental Science and Technology Letters 1:295–299.

1.5 Tables

Table 1-1: Potential effects of finfish aquaculture waste on cold-water corals described in published literature.

Measured parameter	Effect (elevated ↑ or depressed ↓)	Study duration (months)	Species and reference
Ammonia excretion	Variable	13	*D. pertusum*; Kutti et al. (2022)
Associated fauna	↓	N/A	*V. mirabilis*; Taormina et al. (2024b)
Bioaccumulation potential of chemotherapeutants	↑	8 d, 2	*P. phosphorea, V. mirabilis*; Taormina et al. (2024a, 2024b)
Cellular stress (lysosomal membrane destabilization rates)	↑	13	*D. pertusum*; Kutti et al. (2022)
Colony density	↓	N/A	*D. florida*; Dunlop et al. (2021)
Energy reserves (lipid content)	↓	13	*D. pertusum*; Kutti et al. (2022)
Erosion of dead coral skeleton	↑	5	*D. pertusum*; Kutti et al. (2015)
Growth	↓	5, 13	*D. pertusum*; Kutti et al. (2015, 2022)
Mucus production	↑	2	*D. pertusum, V. mirabilis*; Kutti et al. (2015) and Taormina et al. (2024b)
Polyp extension	↓	N/A	*V. mirabilis*; Taormina et al. (2024b)
Respiration	↑ / ↓	2 / 7, 13	*D. pertusum, D. florida*; Kutti et al. (2015, 2022), Laroche et al. (2022)
Tissue necrosis	↑	N/A	*V. mirabilis*; Taormina et al. (2024b)

D. florida = Duva florida
D. pertusum = Desmophyllum pertusum
P. phosphorea = Pennatula phosphorea
V. mirabilis = Virgularia mirabilis

1.6 Figures

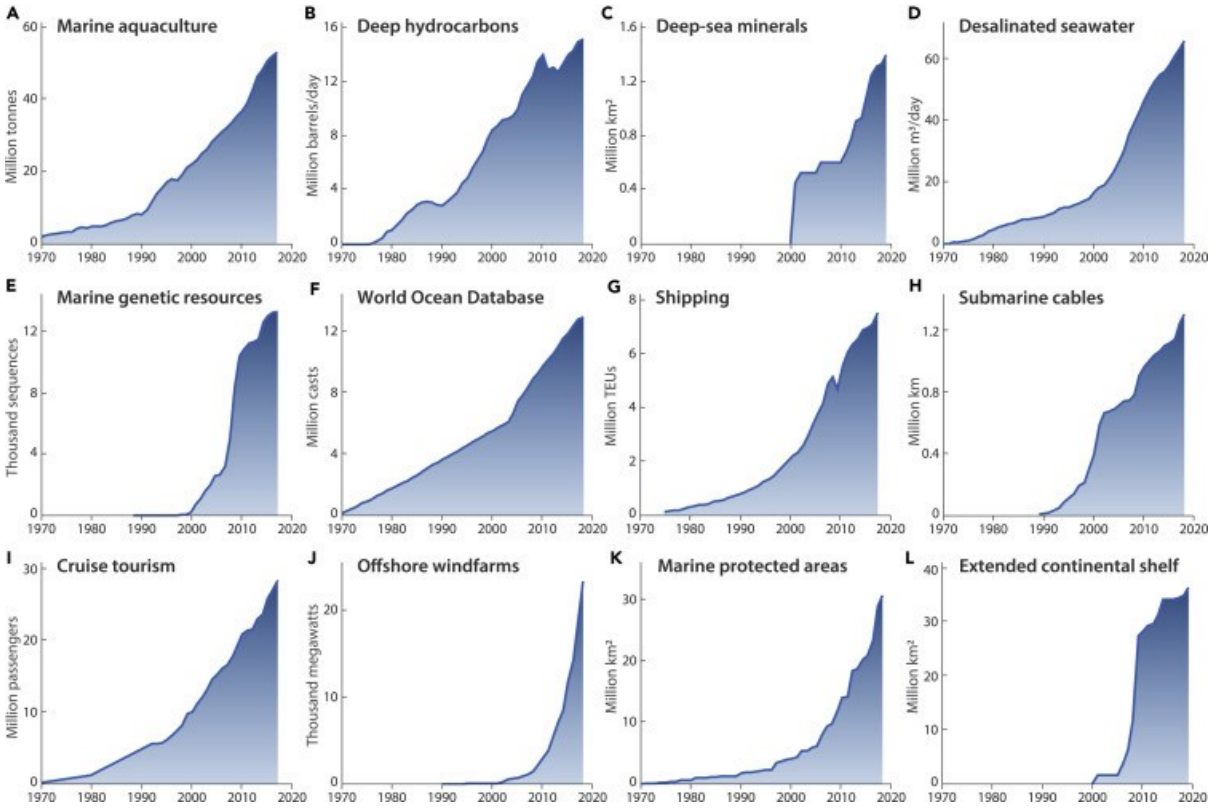


Figure 1-1: Global trends in ocean activities between 1970 and 2020, unmodified from Jouffray et al. (2020; open access under CC by 4.0).

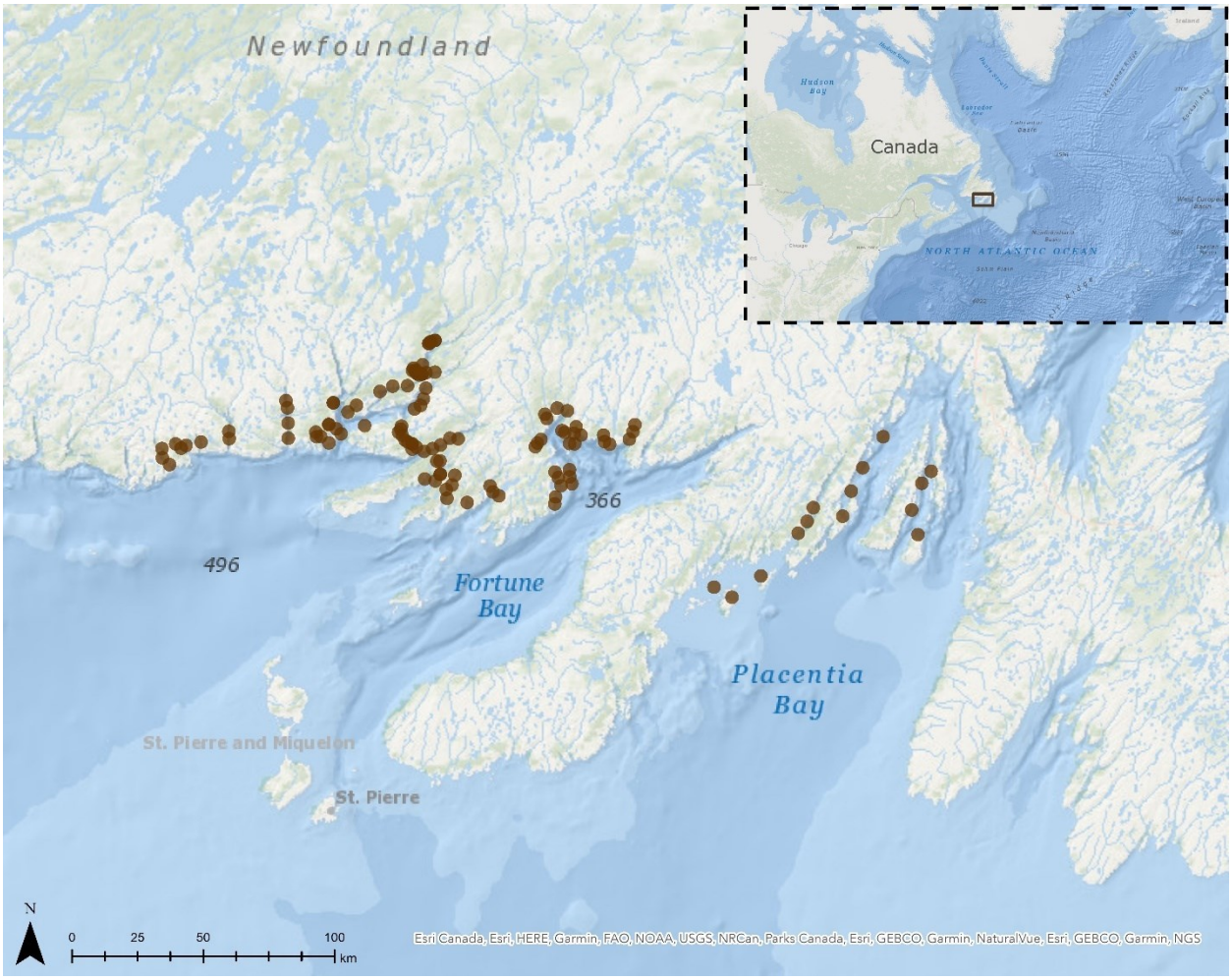


Figure 1-2: Sites of cage aquaculture (indicated by brown dots) along the south coast of Newfoundland and Labrador (NL), Canada. Inset situates NL cage aquaculture sites in a broader context. Created using the Canadian Marine Planning Atlas – Atlantic (Fisheries and Oceans Canada, Open Government Liscense).

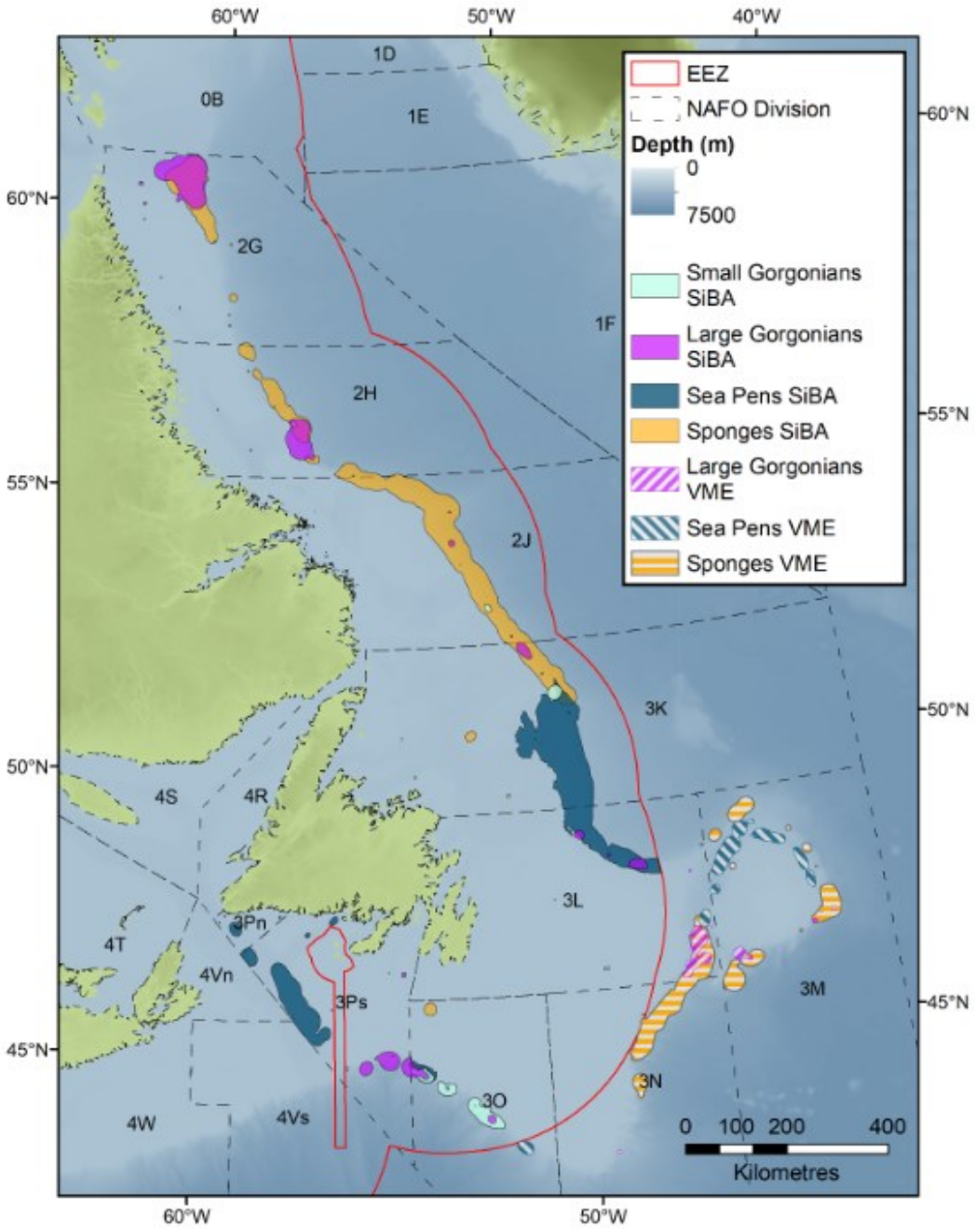


Figure 1-3: Identified vulnerable marine ecosystem (VME) habitats and significant benthic areas (SiBAs) in the Newfoundland and Labrador region. Figure retrieved from Gullage et al. (2022, © His Majesty the King in Right of Canada, as represented by the Minister of the Department of Fisheries and Oceans).

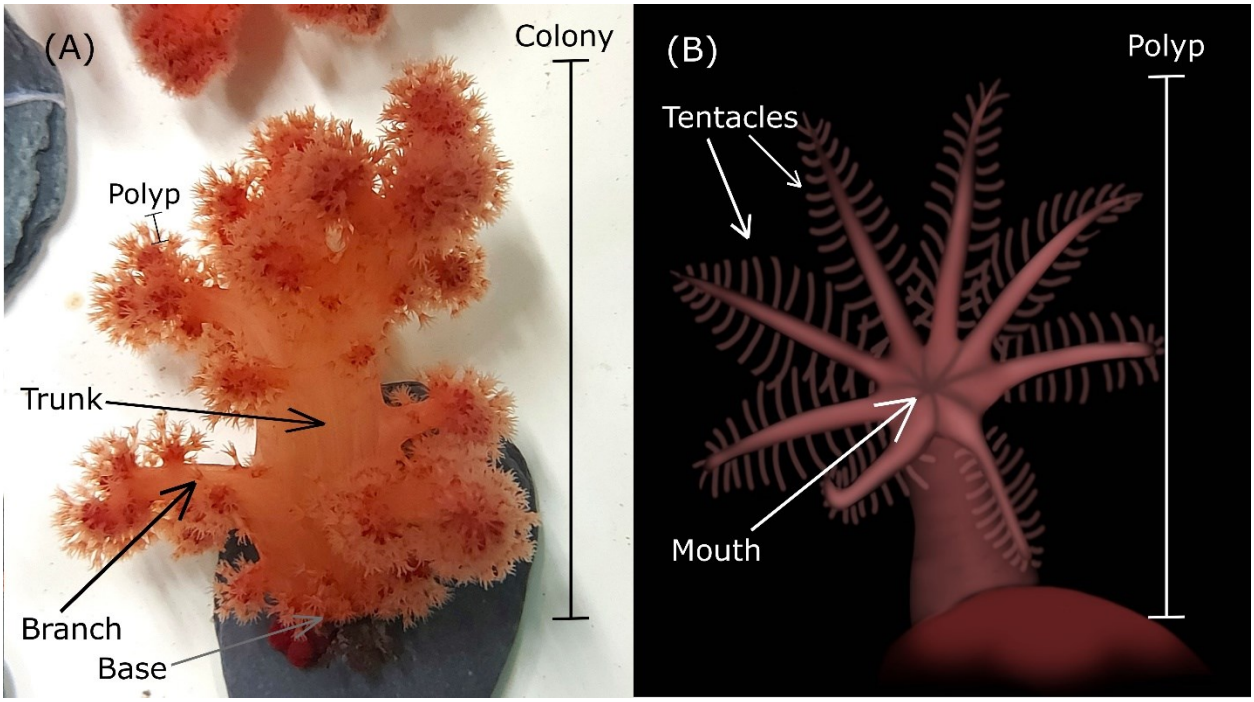


Figure 1-4: Illustration of *Gersemia rubiformis*, including (A) a live whole colony (height in photo ~8 cm) and (B) illustration of a single polyp.

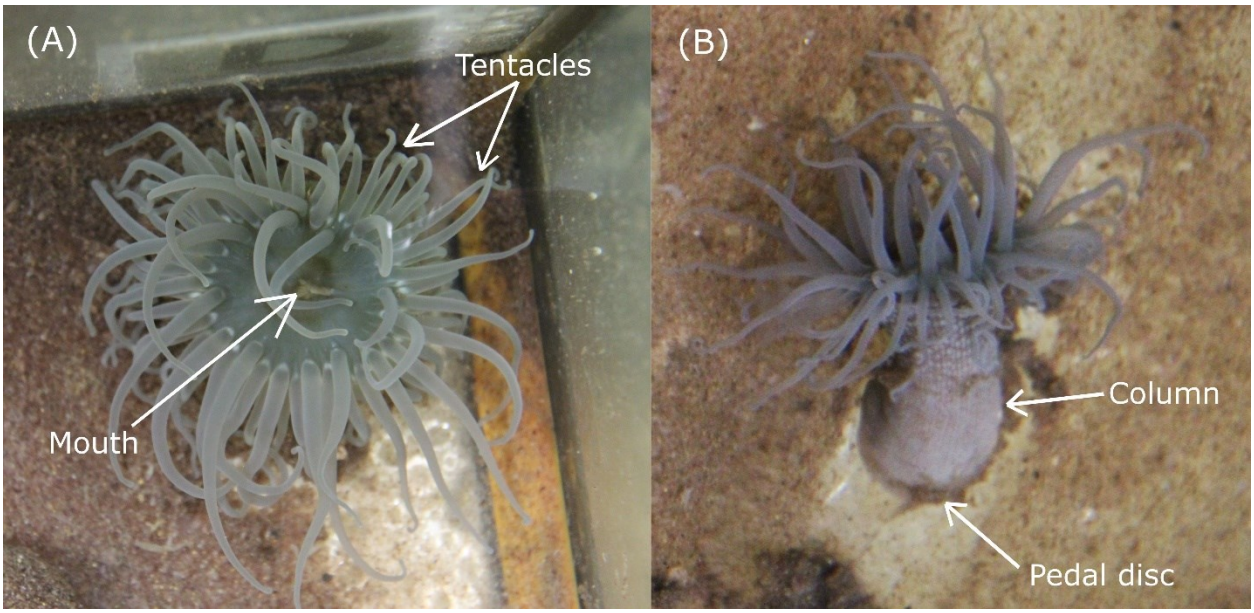


Figure 1-5: Illustration of *Aulactinia stella* from (A) a top (diameter in photo ~4 cm) and (B) side view.

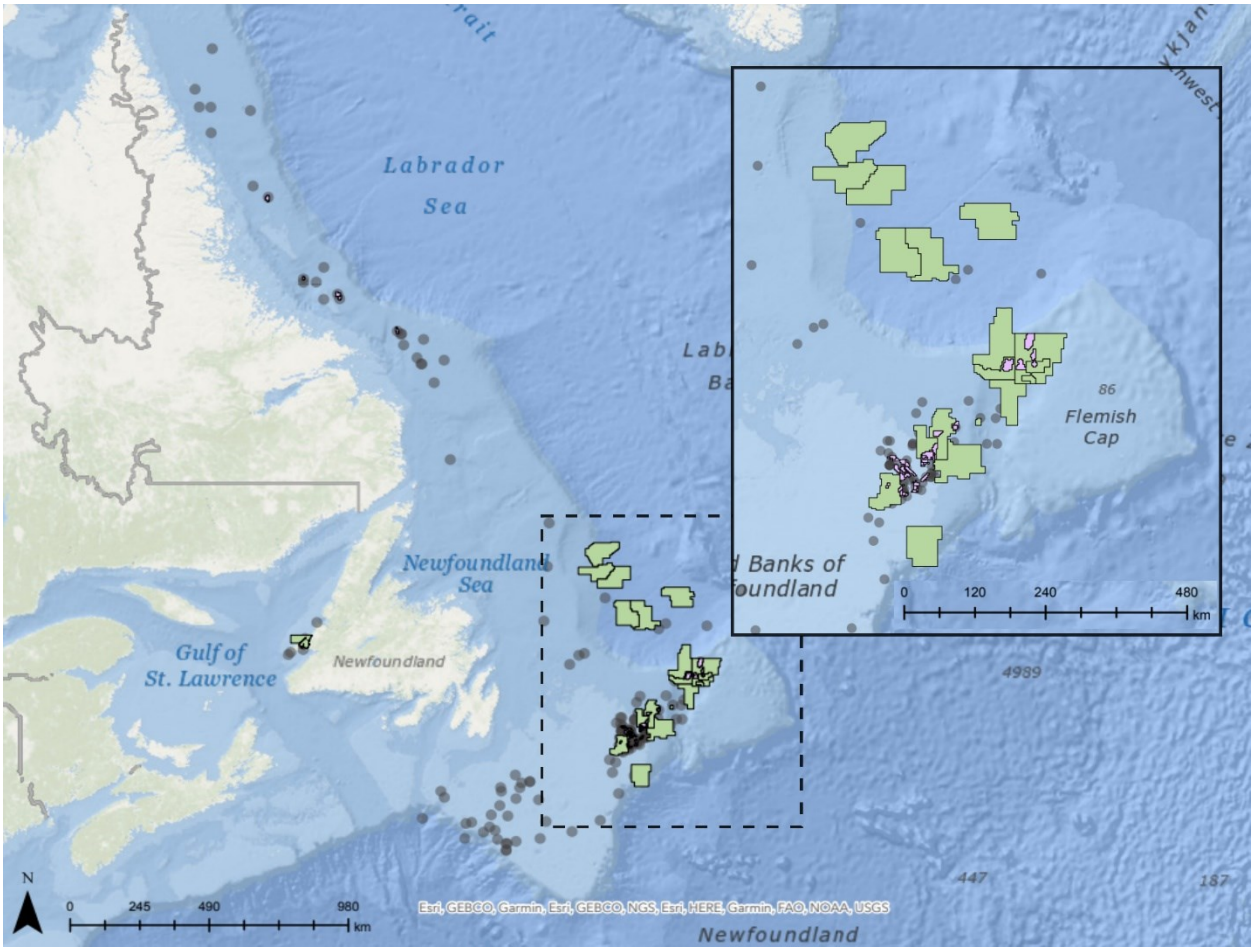


Figure 1-6: Active oil and gas exploration licenses (green polygons; as of April 2023), significant discovery licences (pink polygons) and previous exploratory wells (grey dots) in the Newfoundland and Labrador region, Canada. Inset shows closer view of region with active exploratory licenses. Created using the Canadian Marine Planning Atlas – Atlantic (Fisheries and Oceans Canada, Open Government Liscense).

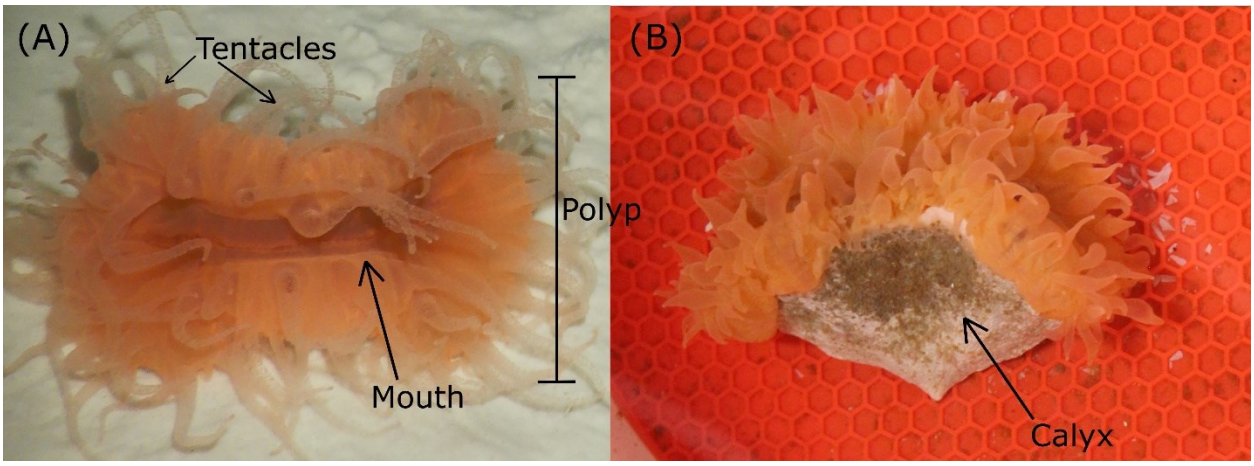


Figure 1-7: Illustration of *Flabellum alabastrum* from (A) front and (B) top view. Polyp diameter ~7 cm in photos.

Chapter 2 - Effects of finfish aquaculture waste on the soft coral *Gersemia rubiformis* and sea anemone *Aulactinia stella*

Abstract

Offshore finfish aquaculture production can alter benthic communities through the sedimentation of fish feces and excess feed. The impact of aquaculture wastes on habitat-forming species such as cold-water corals, and other sessile taxa, is not well understood. Here, the soft coral *Gersemia rubiformis* and the sea anemone *Aulactinia stella* were exposed twice daily to low (5 g C m^{-2} d^{-1}) or high (10 g C m^{-2} d^{-1}) concentrations of salmon feed/feces, inorganic sediment (0 g C m^{-2} d^{-1}), or no sedimentation over 28 d. When exposed to wastes, *Gersemia rubiformis* spent more time with its polyps deployed and fully contracted its main trunk less frequently, which may assist with sediment removal. Individuals of *A. stella* exposed to wastes spent more time with their tentacles extended and increased eversions of their pharynx, potentially as feeding mechanisms. Microbial mats began to develop on the salmon waste in the bottom of tanks after 21 d of high-waste and 25 d of low-waste treatment. Lipid composition of the tissues changed over time independent of treatment in *A. stella*, with no detectable change in *G. rubiformis.* Fatty acids associated with salmon waste (18:2ω6 and 18:1ω9) were elevated in the biochemical profiles of both species, potentially suggesting selective assimilation of salmon feed. Results from this study indicate that behavioural and biochemical effects can be observed over a short period of exposure to aquaculture wastes in both *G. rubiformis* and *A. stella,* with trends raising important questions regarding the effects of long-term sedimentation.

2.1 Introduction

By 2030, aquaculture is projected to account for ~60% of the aquatic food produced for human consumption (FAO 2022). Marine and coastal aquaculture alone generated 8.3 million tonnes of finfish in 2020 and growth is expected as food demands increase (FAO 2022). As aquaculture continues to expand across many coastal habitats worldwide, it is important to consider the ecological consequences of waste byproducts generated by this industry.

The two main wastes produced by finfish aquaculture are fish feces and excess (unconsumed) feed. Discharge of waste varies in particle size, density and settling velocity (Hills et al. 2005), while dispersal is influenced by factors including water currents and bathymetry (Hills et al. 2005, Valdemarsen et al. 2015). Aquaculture sites are often located in bays and fjords where protection from harsh environmental conditions (e.g. wave action, low temperatures, ice cover) is provided (Levings et al. 1995, Bay et al. 2018). Hence, oceanographic conditions often characteristic of these environments (e.g. low currents, DFO 2016) could increase the probability of waste accumulation on the benthos beneath aquaculture pens (Hamoutene et al. 2016).

The impact of aquaculture wastes on benthic habitats can be visually identified through the presence of bacterial mats, flocculent matter, and high densities of opportunistic polychaetes (Hamoutene et al. 2015). Organic enrichment can affect near-bottom water quality leading to anoxic conditions (Hills et al. 2005, Keeley et al. 2015), and result in changes to the benthic community structure (Hills et al. 2005, Keeley et al. 2015, Sanchis et al. 2021). Declines in benthic taxon abundance and richness have also been associated with proximity to aquaculture sites (Hamoutene et al. 2015, González-Gaya et al. 2022). Recovery after the cessation of aquaculture activities is difficult to predict and environmental remediation could take years

(Brooks et al. 2004, Keeley et al. 2014, 2015, Hamoutene et al. 2018b, Verhoeven et al. 2018). Our current understanding of how aquaculture wastes impact benthic communities is largely derived from broad assessments of biodiversity or species richness (Neofitou et al. 2012, Valdemarsen et al. 2015, Keeley et al. 2015, Salvo et al. 2017, Hamoutene et al. 2018a). Direct impacts, especially those on sessile epibenthic taxa such as cold-water corals and sea anemones who have no/limited mobility, are not well understood.

Cold-water soft corals, common in temperate coastal environments, provide shelter and habitat for benthic organisms (Poulos et al. 2013, Neves et al. 2020, Caulier et al. 2021). *In situ*, the soft coral *Duva florida* has shown reduced densities within the depositional footprint (<200 m) of salmonid aquaculture farms at depths of 67-110 m (Dunlop et al. 2021). It has also shown potential feeding difficulty suggested by reductions in fatty acids and increased respiration after 7 months of transplantation close to farms (<350 m, Laroche et al. 2022). The known effects of waste deposition from aquaculture on soft corals is minimal, however a limited number of studies exist regarding other cold-water coral species. For instance, the scleractinian coral *Desmophyllum pertusum* (formerly called *Lophelia pertusa*) showed increased respiration and mucus production after exposure to fish feed over a period of two months in the laboratory (Kutti et al. 2015). Complementary field studies conducted by Kutti et al. (2015) in Norway revealed that within 250 m of salmonid aquaculture sites, transplanted fragments of *D. pertusum* had high survival but lower growth rates after 5 months. The respiration, lipid content, growth and budding rates of *D. pertusum* have also shown to be lower inside than outside of the predicted area of aquaculture impact after 13 months of transplantation (Kutti et al. 2022). As well, a recent study by Taormina et al. (2024) explored the effects of aquaculture waste on the sea pen *Virgularia mirabilis in situ*. Under an operating salmon farm, video transects revealed that higher

instances of mortality, tissue necrosis, mucus production, and polyp contraction were associated with proximity to the farm. Reductions in associated fauna were also observed in close distances (<500 m, Taormina et al. 2024).

Other sessile suspension-feeding cnidarians such as sea anemones may also be exposed to chronic sedimentation of aquaculture waste. While they can be abundant and often dominant in temperate and cold-water habitats (Hargrave et al. 2004, Nelson and Craig 2011), their vulnerability to anthropogenic disturbances remains understudied (Steinberg et al. 2020). A 7-month transplantation experiment of the sea anemone *Hormathia digitata* showed mortality (7% of transplanted individuals) correlated with the proximity to salmon aquaculture sites in Norway (Laroche et al. 2022). Potential increases in energy stores (mono-unsaturated fatty acids), as well as changes in the microbiome profile of their tissue were also reported (Laroche et al. 2022). Additionally, higher abundances of plumose anemones (*Metridium* spp.) have been recorded at sites within the depositional footprint of salmon aquaculture in comparison to reference sites off the west coast of Canada (Sutherland et al. 2018), which may indicate a resilience to waste sedimentation in some species. Similarly, previous mesocosm experimentation has indicated the potential ability of the tropical sea anemone *Mesactinia genesis* to dominate competitors, including the tropical scleractinian *Acropora muricata*, under increased nutrient-rich sedimentation (Liu et al. 2015). Experimental studies on the impacts of aquaculture waste on cold-water soft corals and sea anemones are scarce and have yet to be conducted in the laboratory where parameters such as behaviour can be directly observed.

Here, we present an experimental study focused on the soft coral *Gersemia rubiformis* (Ehrenberg, 1834) and sea anemone *Aulactinia stella* (Verrill, 1864). *Gersemia rubiformis* inhabits depths between 1 m (Henry et al. 2003) and 1249 m (Wareham and Edinger 2007) but is

commonly found <250 m (Wareham and Edinger 2007, Sswat et al. 2015, Nadtochy et al. 2017, Imbs and Dang 2017). *Aulactinia stella* is common in shallow water environments (0-178 m, Ivanova and Grebelnyi 2021) of the north-eastern coast of North America (Brunel et al. 1998). Both *Gersemia* sp. and sea anemones have been commonly recorded under current (Salvo et al. 2018a) and proposed (DFO 2022a, 2022b) aquaculture sites in the Newfoundland region (Canada).

The purpose of this study was to investigate the potential impacts of waste (fish feed/feces) sedimentation from finfish aquaculture on the behaviour and biochemistry of *G. rubiformis* and *A. stella*. The specific objectives were to (1) assess behavioural responses to two concentrations of aquaculture waste; (2) explore whether concurrent changes in lipid and fatty acid profiles could indicate assimilation or tracing of aquaculture wastes; (3) compare behaviour and biochemical profiles between individuals exposed to waste vs. inorganic sedimentation to determine whether observed effects are caused by sedimentation alone.

2.2 Methodology

2.2.1 Species collection and acclimation

Colonies of *G. rubiformis* (n = 60) and individuals of *A. stella* (n = 60) were collected by divers from Island Cove (Newfoundland, eastern Canada, 47.1000, -52.8980) at depths of approximately 11-12 m and 3-5 m, respectively, on October 14, 2021. Both species were transferred temporarily to cylindrical fibreglass mesocosms (0.94 x 0.47 m) with continuous flow of unfiltered seawater (~360 L h^{-1}) at the Northwest Atlantic Fisheries Centre (NAFC), Fisheries and Oceans Canada (DFO) in St. John's (Newfoundland).

Each colony of *G. rubiformis* was attached to a single beach cobble (54-91 mm length; 10-25 mm height; collected from the sampling site) using cyanoacrylate adhesive to maintain

their natural upright positioning, a commonly used method for coral fragment attachment (e.g. Forsman et al. 2015, Duckworth et al. 2017, Renegar and Turner 2021). Individuals of *A. stella* attached to the tank bottom unassisted. *Gersemia rubiformis* and *A. stella* were acclimated to laboratory conditions for six weeks prior to being transferred to experimental tanks, where they were acclimated for an additional twelve days before the start of the experiment.

2.2.2 Waste collection and preparation

A mix of salmon feces and remnant feed (hereinafter referred to as waste) was collected from tanks holding Atlantic Salmon (*Salmo salar*) located in the Dr. Joe Brown Aquatic Research Building (JBARB) at the Ocean Sciences Centre (Memorial University). Salmon held in the tanks were fed a commercial diet of Signature Salmon Ration (Northeast Nutrition Inc.; 4 mm pellets). Waste appeared as flocculent brown fecal matter with visible food pellets and occasional presence of fish scales. It was collected from the outflow of the standpipe of each holding tank once daily over 17 d using a strainer with a mesh size of ~600 µm. Waste was immediately frozen at -80 °C and transported to NAFC. Two weeks prior to the initiation of the experiment, waste was thawed, homogenized, and stored at 4 °C until use upon the determination of carbon content. Dried samples of the homogenized waste (~1 mg dry wt, n = 3) were analyzed for carbon and nitrogen content using a Carlos Erba Elemental Analyser coupled to a Thermo Delta V IRMS at the Stable Isotope Laboratory, Core Research Equipment & Instrument Training Network (CREAIT) of Memorial University. Percent carbon and nitrogen of the samples were acquired using a calibration curve made with an acetanilide standard.

2.2.3 Experimental treatments and procedure

Five colonies of *G. rubiformis* and five individuals of *A. stella* were placed within each of twelve tanks (0.61 x 0.31 x 0.31 m) (Supplementary Figure S2-1). Two temperature loggers

(HOBO TidbiT v2) were placed inside of each tank to continuously monitor temperature. Flow in each tank was maintained at a constant rate of ~180 L h^{-1} for the duration of the experiment, leading to an estimated complete turnover of ~3.2 times h^{-1}. The photoperiod in the room mimicked that of seasonal conditions in the south-eastern Newfoundland region and ranged between a maximum daytime value of 800 lux and a low nighttime value of 1 lux during dark hours. Cameras (Brinno TLC 200 Pro) placed in a water-resistant housing and set to low-light mode were used for time-lapse photography. They were mounted above each tank such that the lens was positioned in the water, and set to take a picture every 20 s. An LED infrared light was mounted above each tank to capture night activity.

Each of the twelve experimental tanks were randomly assigned to one of four conditions: low-waste (5 g C m^{-2} d^{-1}, n = 3 tanks), high-waste (10 g C m^{-2} d^{-1}, n = 3 tanks), inorganic sedimentation (n = 3 tanks), or control (n = 3 tanks). Waste concentrations were based on estimated carbon sedimentation ranges under aquaculture pens (DFO 2004, Hargrave 2010) as well as standard values used in finfish aquaculture carbon deposition modelling (DFO 2018). Quantities of waste needed to achieve each concentration were calculated based on the area of the tank bottom and average carbon content of the waste samples (44 ± 2%). Quikrete Premium Play Sand ® was used for inorganic sedimentation. Prior to trials, sand was sieved through a 500 µm mesh, sterilized by heating at 550 °C for 6 h, then stored at -20 °C until use, as per Legrand et al. (2021). A particle size of <500 µm is comparable to sedimentation studies of other cold-water corals (Larsson and Purser 2011). The volume of sand applied daily in inorganic treatments (53 g m^{-2} day^{-1}) was estimated to be equivalent in volume to salmon waste particles added in the high-waste treatment. Targeted concentrations of the three sedimentation treatments were achieved by adding sand or waste to the tanks twice daily (09:00 and 14:00, referred to as

AM and PM additions). Materials were mixed with seawater prior to addition to facilitate a homogenous distribution before being gently poured into the tanks. Control tanks received only seawater. Slight loss of waste particles through outflow was assumed to be negligible due to their high settling velocity (~3 and 11 cm^{-1} s^{-1} for feces and feed respectively, DFO 2013).

Experimental trials were conducted over 28 days from December 7, 2021 to January 3, 2022. Water parameters (pH, salinity, dissolved oxygen, temperature) were measured twice daily (approximately 2.5 cm from the bottom as to not disturb the sediment) using a multi-parameter probe (YSI ProDSS). The pH was measured only from the third day onward. Ammonia concentrations were monitored twice daily using a colorimetric test kit (API). Two water samples (50 mL) were collected from each tank at the beginning and end of experimental trials for analysis of additional water parameters (nitrate, phosphate, silicate). Samples were stored at -20 °C until analyzed at NAFC following Seal Analytical AutoAnalyzer methods (Method No. G-172-96 Rev. 18, G-297-03 Rev. 6, G-177-96 Rev. 12).

A depuration period (3-4 days) was implemented at the end of the experiment to minimize the amount of waste present within and on the surface of the individuals at the time of sampling to prevent contamination of lipid and fatty acid analyses. During this time, tank bottoms were siphoned to remove sediment, and flow was switched to filtered seawater to minimize additional food uptake. A period of 3 days was previously determined to be sufficient time for sea anemone *Aiptasia pallida* to empty its gastrovascular cavity prior to biochemical sampling (Leal et al. 2013).

Directly prior to the preservation of *G. rubiformis* and *A. stella,* the total wet weight, base/disk diameter and height of contracted colonies/individuals were measured. Organisms

were then frozen at -80 °C in preparation for lipid and fatty acid sampling (see below). A visual timeline of the experimental period can be found in Figure 2-1.

2.2.4 Behaviour

Behavioural scores were assigned to *G. rubiformis* and *A. stella* during daily visual inspections of the tanks, immediately prior to each treatment addition, and then after 20 min, 40 min, 1 h, and 2 h after each addition (Figure 2-1B) to observe immediate (≤ 2 h) responses to waste sedimentation. Scores of *G. rubiformis* were assigned on a scale from 1–6 as detailed in Table 2-1 and Figure 2-2, where values range from the most contracted (1) to most expanded (6) state. Likewise, behavioural scores for *A. stella* were determined on a scale from 1–5 as detailed in Table 2-2 and Figure 2-3, where values again range from the most contracted (1) to the most expanded (5) state. Additionally, instances of pharynx eversion (white tissue which precedes the gastrovascular cavity is visible, Figure 2-3F) were noted at these time points for *A. stella*.

As well, videos created through time-lapse photography were used to detect trends in behaviour that might occur too slowly to be captured during daily visual inspections. They were analyzed at timepoints beginning with a pre-trial baseline (referred to as “Day 0”) and then 4, 8, 12, 16, 20, 24 and 28 d after the onset of the trial. To explore whether individuals were potentially avoiding sedimentation of their respective treatments, the time spent per day with polyps in a fully retracted state for *G. rubiformis* (behavioural scores 1-3) and *A. stella* (scores 1-2), with no polyp/tentacles visible, was measured. The frequency of full contractions was also recorded, defined as the number of times per day a colony/individual reached the lowest level(s) of hydrostatic expansion. This was done by counting each instance in which a colony/individual transitioned from any partially or fully deployed state (scores 2-6 for *G. rubiformis* and 3-5 for *A. stella*) to a fully contracted state (score 1 for *G. rubiformis* and scores 1-2 for *A. stella*). Instances

where individuals were out of view, or where *G. rubiformis* and *A. stella* were interacting (i.e. contracting as a result of contact) were omitted.

2.2.5 Lipids and fatty acids

Sample preparation for lipids and fatty acids (FA) followed the protocols detailed by Parrish and Wells (2021). Tissue samples (0.1–1 g) were collected from *G. rubiformis* and *A. stella* while frozen, then preserved in 2 mL of chloroform, flushed with nitrogen and stored at -20 °C until lipid extraction. Tissue samples of *G. rubiformis* were collected by removing the branches from each colony and preserving only the trunk in an effort to avoid potential contamination of physical waste particles within or between polyps. Epidermal tissue samples were taken from *A. stella* to avoid contamination from waste potentially present in the gastrovascular cavity. All tissue samples were visually inspected under a stereomicroscope prior to preservation to ensure that no waste particulates were present; doubtful samples were excluded. Samples had also been obtained in a similar manner from *G. rubiformis* (n = 3) and *A. stella* (n = 3) at the onset of the trials (referred to as "pre-trial baseline", beginning their depuration period on Day 1) for the purpose of a time-zero reference. Triplicate samples of the salmon waste were also frozen at -80 °C, both prior to and after the experiment, and subsequently as above to determine if lipid and FA signatures in the waste could be detected in the experimental tissue samples.

Lipid and FA extractions, lipid class and FAME (fatty acid methyl esters) characterization were performed at the Aquatic Research Cluster (ARC), CREAIT of Memorial University per Parrish and Wells (2021). Lipid classes were determined using an Iatroscan MK-6. FAME derivatization was conducted with H_2SO_4 in MeOH and analyzed using an Agilent 7890 GC-FID.

2.2.6 Bacteria

To further characterize experimental conditions, samples of the bulk salmon waste added to the waste treatment tanks (n = 6), and samples of suspected bacteria mats (n = 12) that formed towards the end of the experimental period were analyzed for bacterial species assemblages. Salmon waste samples were frozen at -80 °C at the beginning (n = 3) and end (n = 3) of experimental waste additions. Samples of waste matter with visible microbial mats from the low-waste (n = 2 per tank, 6 samples total) and high-waste (n = 2 per tank, 6 samples total) were collected using individual transfer pipettes at the end of the 28-d exposure period and stored in 1.5 mL vials at -80 °C. Samples were transported on dry ice to the Integrated Microbiome Resource (IMR), Dalhousie University, Nova Scotia, Canada. There, samples were extracted using a QIAGEN PowerFecal DNA Kit and prepared for PacBio Sequel2 sequencing (Comeau and Filloramo 2023) of 16S rRNA using bacteria-specific primers (B969F, BA1406R) targeting the V6-V8 region (Comeau et al. 2011). Bioinformatic analyses were conducted by IMR (https://github.com/LangilleLab/microbiome_helper/wiki, Comeau et al. 2017). All three bulk salmon waste samples from the first experimental day as well as one preserved after the experimental period had a weak (<1000) number of reads. One of the bacterial mat samples from the high-waste treatment also had a weak number of reads. Unless specified, the weak samples were excluded from analyses.

2.2.7 Data processing and statistical analyses

Data values reported in the text and figures were processed by calculating a tank mean (for n = 5 individuals per tank per species), to subsequently calculate an overall treatment mean (n = 3 tanks per treatment) for a specified period, or a daily treatment mean. Values in the text are presented as mean ± se (one standard error), except data for pre-trial baseline samples of *G.*

rubiformis and *A. stella* sampled for lipids/FA, which are reported as mean ± sd (one standard deviation), as are data pertaining to salmon waste samples (carbon content, lipid/FA, bacteria) and water quality.

Statistical analyses were conducted in R (version 4.2.2, R Core Team 2022) and Primer 7 with PERMANOVA+ (for PERMANOVAs only, Anderson et al. 2008, Clarke and Gorley 2015) using $p < 0.05$ for significance. A full description of statistical analyses can be found in Supplementary Text 2.10.3.1.

2.2.7.1 Behaviour

Mean daily scores were compared at 3-day intervals over the experimental period. Behavioural scores were not statistically analyzed out of caution due to a lack of available model validation support for ordinal mixed effect models, and model misfit to other explored distributions.

Daily treatment means of the time spent retracted (h) and frequency of full contractions (contr d^{-1}) were compared across experimental days and treatments. Mixed effect models (using lme4 package, Bates et al. 2015) were used to account for the non-independence of observations (individuals within tanks, repeated measures over time). Significant values reported in the text reflect the influence of fixed factors (treatment) and covariates (experimental day) on the response variable (behaviour) where appropriate, as well as pair-wise differences of the response between treatments. Time spent with polyps/tentacles retracted (h) was evaluated using linear mixed effects models (model 1, Supplementary Table S2-1). Retraction time was square-root transformed to help with assumptions. Experimental day was included as a covariate. Tank and subject ID were included as random factors. Generalized linear mixed effect models with

Poisson distributions were used to determine the effect of treatment on full contraction frequency (model 2, Supplementary Table S2-1).

For *A. stella*, the proportion of individuals within each tank showing visible pharynx was reported as a daily treatment mean (% ind d^{-1}). Presence/absence of this behaviour was analyzed using a generalized linear mixed effect model with a binomial distribution (model 3, Supplementary Table S2-1). Experimental day and time of day (AM or PM) were included as fixed factors (time of day nested in day). Tank was included as a random factor.

2.2.7.2 Lipid composition

Percent lipid composition was compared among treatments, as well as between pre-trial baseline and experimental animals (with sample date as a fixed effect) using PERMANOVAS. Data was square-root transformed, Bray-Curtis distance measures were used and permutations set to 9999. Tank was included as a random factor nested within treatment.

2.2.7.3 Fatty acid composition

FA representing 1% of at least one sample were analyzed (Mercier et al. 2016, Salvo et al. 2018b). One coral in the inorganic treatment was omitted as an influential outlier due to high concentrations (>12%) in FA rare in all other individuals. Square-root transformed FA data were examined using multivariate analyses with Bray-Curtis distance measures. They were compared among treatments, as well as between pre-trial baseline and experimental animals using PERMANOVAs (permutations set to 9999). Tank was included as a random factor nested within treatment. Dissimilarities of FA compositions were also explored using multivariate analyses of similarity (ANOSIM) of Bray-Curtis distance measures, as well as principal coordinate analyses (PCoA), guided by Couturier et al. (2020) and Bakker (2024). An ANOSIM, where the R value represents the extent of dissimilarity between groups, was conducted both at an individual-level

and at a tank-level to determine if the FA profiles varied more between treatment (R = 1) than within group (R = 0).

2.2.7.4 Bacteria

Mean percent compositions (%) of identified bacteria was compared among bulk salmon waste preserved at Day 1 and Day 28, as well as between bulk waste and waste/microbial mats in the tanks. Alpha diversity was compared using Kurskal-Wallis one-way analyses of variance in QIIME 2 (Bolyen et al. 2019).

2.3 Results

2.3.1 Waste composition and water quality

Carbon and nitrogen contents of waste used for these experiments were 44.2 ± 2.2 and 5.8 ± 0.7%, respectively. Temperature, pH, salinity were similar among treatments over the experimental duration (Supplementary Figure S2-2). Temperature decreased over time due to seasonal changes in the ambient seawater, ranging from 7 °C at the start of the experiment to 3 °C at the end. Salinity increased slightly, ranging from 31.2 to 32.5 psu and pH remained between 7.7-7.9. Dissolved oxygen varied between 99.8-110%. Ammonia in all tanks remained <0.25 mg L^{-1}.

Water nutrients (nitrate, silicate, phosphate) increased between pre-exposure and post-exposure samples but remained consistent between treatments at each sample point (Supplementary Figure S2-3). Nitrate increased from 1.27 ± 0.09 to 2.80 ± 0.20 (SD) µmol L^{-1} between pre-exposure and post-exposure sampling, phosphate from 0.44 ± 0.04 to 0.53 ± 0.01 µmol L^{-1} and silicate increased from 2.20 ± 0.07 pre-exposure to 3.13 ± 0.17 µmol L^{-1} post-exposure sampling.

2.3.2 Behaviour

2.3.2.1 Gersemia rubiformis

Daily scores were lowest on Day 1 across all groups, with 3.2 ± 0.4 in controls, 3.1 ± 0.1 in colonies under inorganic sedimentation, 3.8 ± 0.7 under low-waste and 3.1 ± 0.2 under high-waste treatment. Scores of controls and colonies exposed to inorganic sedimentation generally increased with time until a peak on Day 19 (5.1 ± 0.3) and Day 22 (5.2 ± 0.4), respectively, followed by a decline until the end of the trial. Colonies exposed to low and high-waste treatments generally increased in scores over the exposure period (Supplementary Figure S2-4). On Day 28, scores had returned to 4.1 ± 0.4 in controls and 3.6 ± 0.2 in colonies under inorganic sedimentation but remained higher under low-waste (4.8 ± 0.3) and high-waste treatments (4.8 ± 0.4) (Supplementary Figure S2-5). Daily scores were higher before the addition of waste than 20 min after it across all treatments; scores for colonies in the control and inorganic treatments then further declined after 2 h. Forty minutes after waste addition, colonies in the low-waste treatment had begun to return to higher behavioural scores; those in the high-waste treatment did the same but to a lesser degree.

Both treatment (χ^2 =24.8, df = 3, $p < 0.001$) and experimental day (χ^2 =17.7, df = 1, $p < 0.001$) influenced polyp retraction over the exposure period. The daily hours spent with polyps retracted declined in all treatments over the trial, with the greatest reductions observed in the waste treatments throughout (Figure 2-5). Control colonies spent more time with polyps retracted compared to colonies in the low-waste ($z = -2.9$, $p = 0.02$), as well as the high-waste treatment ($z = -3.4$, $p = 0.004$). Colonies in the inorganic treatment also spent more time retracted than both low-waste ($z = -3.6$, $p = 0.002$) and high-waste ($z = -4.0$, $p < 0.001$) treatments. No difference in

retraction time was determined between colonies in the low-waste and high-waste treatments ($z = -0.6$, $p = 0.9$), nor between those in the control and inorganic treatment ($z = 0.7$, $p = 0.9$).

On Day 0 (prior to waste additions), frequencies of full contractions between treatments were similar with a mean of 3.5 ± 0.1 contr d^{-1}. Over the exposure period, treatment ($\chi^2 = 13.2$, df = 3, $p = 0.004$) and experimental day ($\chi^2 = 30.0$, df = 1, $p < 0.001$) both had a statistically significant influence, as the number of contractions was reduced in all treatments over the exposure period with the least contractions in the waste treatments (Figure 2-5). On Day 28, contractions had declined to 1.8 ± 0.3 contr d^{-1} in the controls, 2.1 ± 0.1 contr d^{-1} under inorganic sedimentation, while further reduced under the high-waste (1.1 ± 0.2 contr d^{-1}) and low-waste (1.2 ± 0.3 contr d^{-1}) treatments. Contractions observed under inorganic sedimentation over the exposure period were significantly more frequent than in the low-waste treatment ($z = -3.0$, $p = 0.01$). No statistical difference was detected between the other treatments/controls over the exposure period ($p > 0.05$).

On Day 18, planula (coral larvae) began appearing in the tanks, see Supplementary Text 2.10.3.2 for details.

2.3.2.2 *Aulactinia stella*

Individuals of *A. stella* were usually either fully contracted (score of 1) or fully expanded (score of 5, Figure 2-4). On Day 1, mean behavioural scores were 2.5 ± 0.4 in controls, while higher in those under inorganic (3.5 ± 0.4), low-waste (3.3 ± 0.5) and high-waste (3.7 ± 0.0) treatments. Scores fluctuated across the experimental period in all treatment/control groups (Supplementary Figure S2-4), but generally remained higher in individuals under the high-waste treatment throughout. At the end of the experiment (Day 25 and Day 28), a divergence between scores of individuals in the waste treatments compared to the inorganic/control was observed. On

Day 28, scores were lower but similar between individuals in the control (2.2 ± 0.4) and inorganic treatment (2.0 ± 0.2). However, scores remained elevated in individuals under high-waste (3.6 ± 0.2) and low-waste (3.3 ± 0.2) treatments on Day 28 (Supplementary Figure S2-5). In general, scores slightly increased in low and high-waste treatment individuals between timepoints scored prior to each waste addition and those scored in the 2 h afterwards, while scores for inorganic and control remained constant or decreased, respectively.

Both treatment ($\chi^2 = 14.8$, df = 3, p = 0.002) and experimental day ($\chi^2 = 7.5$, df = 1, p = 0.006) had a statistically significant effect on the time spent with tentacles retracted. There was a decrease in retraction time after the onset of the experiment (between Day 0 and 4) by ~3 h in controls, 15 h in high-waste and 9 h in low-waste treatment individuals (Figure 2-5). In contrast, an increase by 1 h of retraction was observed for inorganic treatment individuals. Over the exposure period, the individuals in the high-waste treatment retracted their tentacles for a significantly shorter amount of time than both the control (z = -3.0, p = 0.01) and the inorganic treatment (z = -3.1, p = 0.01). There was no clear difference in time spent with tentacles retracted between individuals of the low-waste treatment and those in the inorganic treatment (z = -2.3, p = 0.09) or control (z = -2.3, p=0.1). There was no difference detected between individuals in the high-waste and low-waste treatments (z = -0.7, p = 0.9), or between those in the inorganic treatment and control (z = 0.1, p = 1.0).

Neither treatment ($\chi^2 = 5.7$, df = 3, p = 0.13) nor experimental day ($\chi^2 = 0.9$, df = 1, p = 0.4) had a statistically significant influence on full contraction frequency. Mean daily contractions for *A. stella* fluctuated with time between 0 and 1 contr d^{-1} in all treatments (Figure 2-5).

Pharynx eversion was significantly influenced by treatment ($\chi 2 = 89.3$, df = 3, $p < 0.001$) and experimental day ($\chi 2 = 15.9$, df = 1, $p < 0.001$), but not by time of waste addition ($\chi 2 = 3.6$, df = 1, $p = 0.06$). Instances in all treatments were highest at the beginning of the experimental period (Day 1, Day 4, Day 7). Occurrence of partial or complete pharynx eversion was recorded most often in the high-waste treatment (48 ± 6 % ind d^{-1}), followed by the low-waste (28 ± 4 % ind d^{-1}), inorganic treatment (5 ± 3% ind d^{-1}), then control individuals (2 ± 0% ind d^{-1}) (Figure 2-6A). Individuals within the high-waste treatment showed pharynx visibility more often compared to low-waste ($z = 2.7$, $p = 0.03$), inorganic treatments ($z = 7.2$, $p < 0.001$), and controls ($z = 7.6$, $p < 0.001$). Individuals in the low-waste treatment had significantly higher instances of visible pharynx compared to inorganic treatment ($z = 5.0$, $p < 0.001$) and controls ($z = 5.9$, $p < 0.001$). Individuals in the control and inorganic treatment did not statistically differ in terms of the presence of visible pharynx ($z = 2.0$, $p = 0.19$). The highest percentage of individuals with visible pharynx in the waste treatments occurred following waste addition and up until 1 h post addition (Figure 2-6B).

Details of expulsion of juvenile sea anemones from the adults during the experiment are noted in Supplementary Text 2.10.3.3. Unanticipated contacts where *A. stella* partially or wholly engulphed *G. rubiformis* are described in Supplementary Text 2.10.3.4.

2.3.3 Lipid composition

2.3.3.1 Waste

The total lipid concentration in the salmon waste added to the tanks was 54.4 ± 2.7 mg g^{-1} on Day 1 and 62.0 ± 7.0 mg g^{-1} on Day 28. Lipid composition differed between the time points, but the dominant lipid classes (free FA and triacylglycerols) remained the same. A summary of lipid composition of the waste can be found in Supplementary Table S2-2.

2.3.3.2 Gersemia rubiformis

The total lipid content of colonies at the end of the exposure was 4.3 ± 0.4 mg g^{-1} in controls, 3.6 ± 0.2 mg g^{-1} under inorganic sedimentation, 3.9 ± 0.4 mg g^{-1} under the high-waste, and 4.6 ± 0.7 mg g^{-1} under low-waste. All these concentrations appeared higher than those measured in the pre-trial baseline colonies (3.2 ± 0.1 mg g^{-1}).

The lipid composition of *G. rubiformis* was mainly comprised of phospholipids, followed by sterols, and acetone mobile polar lipids (see Table 2-3 for details). The lipid composition was not significantly influenced by treatment (PERMANOVA, p = 0.3), nor was a difference detected between pre-trial baseline and experimental samples of *G. rubiformis* (PERMANOVA, p = 0.2).

2.3.3.3 Aulactinia stella

At the end of the trial, lipid content was highest in control individuals (10.9 ± 0.1 mg g^{-1}), followed by individuals in the low-waste (10.6 ± 0.7 mg g^{-1}), inorganic (10.2 ± 0.7 mg g^{-1}) and high-waste treatments (8.7 ± 1.4 mg g^{-1}). All appeared higher than the pre-trial samples (6.7 ± 1.2 mg g^{-1}).

The lipid composition of *A. stella* was mainly comprised of phospholipids and sterols (see Table 2-3 for details). Lipid composition was not significantly influenced by treatment (PERMANOVA, p = 0.3), however there was a significant change in lipid composition between pre-trial baseline and experimental samples of *A. stella* (PERMANOVA, p = 0.02).

2.3.4 Fatty acid composition and tracers

2.3.4.1 Waste

The three dominant fatty acids (FA) in wastes added to tanks on Day 1 and Day 28 were 18:1ω9 (33.5 ± 0.5%), 16:0 (16.5 ± 0.3%), and 18:2ω6 (14.0 ± 0.3%). A summary of FA compositions of the waste material can be found in Supplementary Table S2-3.

2.3.4.2 Gersemia rubiformis

The most abundant FA in *G. rubiformis* were 20:4ω6, 20:1ω7, 20:5ω3 and 16:0 (Table 2-4). The FA composition of *G. rubiformis* was significantly influenced by treatment (PERMANOVA, $p = 0.04$), though a significant difference with pair-wise comparisons between treatments could not be determined. FA composition of colonies (ANOSIM, $R = 0.1$, $p = 0.01$) showed a significant dissimilarity between treatments or controls compared to within-group differences. When tank averages were used, dissimilarity between treatments and controls was more pronounced (ANOSIM, $R = 0.4$, $p = 0.03$, visualized with a PCoA in Figure 2-7A). The FA composition did not significantly change between pre-trial baseline samples and experimental samples (PERMANOVA, $p = 0.3$).

The three dominant FA identified within the waste samples (18:1ω9, 16:0 and 18:2ω6) were used as tracers to determine whether waste was being assimilated by experimental colonies/individuals. The 18:1ω9 contribution was 2.1 ± 0.4% in control colonies, which was elevated in colonies of high-waste (2.6 ± 0.3%) and low-waste treatments (2.6 ± 0.1%), while similar under inorganic sedimentation (2.2 ± 0.3%, Figure 2-8). Inversely, 16:0 was highest in control colonies (7.4 ± 0.9%), while 18:2ω6 showed an increasing trend with waste concentration where its contribution was lower in colonies of the control (0.7 ± 0.1%) and

inorganic sedimentation (0.8 ± 0.1%), while higher in those of the low-waste (1.4 ± 0.1%) and high-waste treatments (1.9 ± 0.1%).

In control colonies, Σω3 made up 17.5 ± 0.8%, which was higher than in the inorganic treatment (17.0 ± 0.4%) and both low-waste (15.8 ± 1.0%) and high-waste treatments (15.0 ± 0.6%). The inverse was observed for Σω6, which was lowest in colonies in the control (43.5 ± 2.3%), followed by those in the inorganic (44.2 ± 1.1%), then low-waste treatments (45.2 ± 1.9%) and highest in the high-waste treatment (46.3 ± 0.7%).

2.3.4.3 Aulactinia stella

FA composition of *A. stella* was dominated by 20:5ω3, followed by 22:4ω6, 22:1ω9, 22:5ω3, 18:1ω5, 18:0 and 16:4ω3 (Table 2-4). FA compositions were not significantly influenced by treatment (PERMANOVA, p = 0.08) but differed from pre-trial baseline samples (PERMANOVA, p = 0.01). The dissimilarity of FA composition between treatments and controls was neither significant when individual values (ANOSIM, R = 0.01, p = 0.3) or tank averages were used (ANOSIM, R = 0.1, p = 0.2). However, a PCoA showed potential clustering of high-waste tank averages (Figure 2-7B).

All three FA tracers identified in salmon waste showed an increasing trend with waste treatment concentration in *A. stella* (Figure 2-8). The proportion of 18:1ω9 was lower under both the control (0.9 ± 0.04 %) and inorganic conditions (0.8 ± 0.0%) while elevated in low-waste (1.3 ± 0.2%) and high-waste treatments (1.5 ± 0.1%). The 16:0 was largely consistent across individuals of the control (2.3 ± 0.1%), inorganic (2.3 ± 0.0%), low-waste (2.4 ± 0.2%) and high-waste treatment (2.5 ± 0.1%). The proportion of 18:2ω6 was again lowest in individuals from the control (0.4 ± 0.1%) and inorganic treatments (0.3 ± 0.0%), and higher in the low-waste (0.8 ± 0.04%) and high-waste treatments (1.0 ± 0.1%).

Aulactinia stella had differing trends in Σω3 across treatments. The contributions of Σω3 were 38.6 ± 0.5% in individuals of the control, lower in individuals of the high-waste (37.1 ± 0.1%) and low-waste treatments (37.9 ± 1.6%), and higher in individuals of inorganic treatment (39.4 ± 0.9). Values of Σω6 were similar across controls (20.1 ± 0.6%) and treatments, with concentrations barely higher in individuals of the high-waste treatment (21.1 ± 0.6%), than those exposed to low-waste (20.3 ± 0.5%), inorganic sedimentation (20.2 ± 0.4%).

2.3.5 Bacteria

Waste added to the tanks on Day 1 contained mainly *Mycoplasma* sp. (79 ± 5%) and *Photobacterium phosphoreum* (8 ± 2%). Both *Lactobacillus* and *Fusobacterium* spp. were low (<0.3%) in these samples. Waste added to the tanks on Day 28 was dominated by *Mycoplasma* sp. (38 ± 14%) and *Lactobacillus* spp. (36 ± 8%), as well as *Fusobacterium* sp. (13 ± 5%). Day 28 samples had a low number of reads (<1000) and should be interpreted with caution.

White microbial mats began to develop on accumulated waste matter after 21 d in the high-waste, and 25 d in the low-waste treatment tanks. These mats continued to develop until the end of the trials. No microbial activity was visible in the control or inorganic tanks. Samples from mats in the high-waste tanks contained a high percentage of Arcobacteraceae bacteria (mainly *Arcobacter* sp., 37 ± 1 %), followed by *Fusibacter* spp. (10 ± 7%), and *Marinifilum* spp. (10 ± 4%). Samples from low-waste tanks were dominated by *Tenacibaculum* (16 ± 11%), *Colwellia* spp. (14 ± 4%) and *Fusibacter* spp. (14 ± 9%).

The samples collected from the waste treatment tanks (mix of waste and microbial mats) had a higher Shannon index (alpha diversity) of bacterial taxa compared to those from the bulk waste that was added to the tanks (Kruskal-Wallis, $H = 8.3$, $p = 0.004$). The Shannon index did not significantly differ between samples collected from the low or high-waste treatments (Kruskal-

Wallis, H = 2.3, p > 0.05), nor between salmon waste added to the tanks on Day 1 or Day 28 of the trial (Kruskal-Wallis, H = 2.3, p > 0.05). Full bacterial assemblages are detailed in Supplementary Table S2-4.

2.4 Discussion

2.4.1 Behavioural responses

Colonies of *G. rubiformis* exposed to aquaculture wastes (mix of salmon feed and feces) spent increasingly more time in an expanded state as the experimental trials progressed than those under inorganic sedimentation or control conditions. This is in line with another study showing that colonies of *G. rubiformis* exposed to mechanical disturbance (crushing) did not differ in expansion relative to control colonies 4- or 7-d post disturbance, with most of them staying fully expanded (Henry et al. 2003). Long-term sediment build-up on branches and polyps was not observed in the present study, supporting that the morphology of soft corals (in an expanded state) may have a natural resilience to sedimentation by assisting sediment movement off the sloping colony edges (Reigl 1995). Remaining in an expanded state may therefore in turn be effective (and less energetically taxing) for preventing sediment accumulation in soft corals, although this interpretation does not explain the differing reactions to organic wastes and inorganic sediment.

The overall temporal trends in expansion observed in *G. rubiformis* may be explained by the seasonal decrease in seawater temperature over the experimental period. In colder temperatures, metabolic rates of cold-water corals are slower (Dorey et al. 2020), likely leading to reduced activity, which could include a lower full contraction frequency. The most reduced contraction frequency in colonies under the waste treatments may indicate waste sedimentation had an additive impact on metabolic activity. This aligns with Kutti et al. (2022) who reported

that *D. pertusum* transplanted under aquaculture sites for 13 months showed signs of metabolic depression (e.g. reduced respiration). The reduction in full contraction frequency here in waste treatments was not large compared to controls and it was obscured by temperature trends, so interpretations of metabolism over the short experimental period must be made cautiously. *Aulactinia stella* did not show the same distinct trends with time/season suggesting their behaviours may be less sensitive to seasonal changes in comparison to *G. rubiformis*.

Expansion behaviour of *A. stella* increased with waste concentration (5 and 10 g C m^{-2} d^{-1}) during the trial and individuals were generally more deployed under waste-treatments than under inorganic treatment and control. Avoidant behaviour, such as tentacle retraction, has been observed in the cold-water sea anemone *Metridium senile* (Shumway 1978) during environmental stress (i.e. low salinity) and in the tropical sea anemone *Aiptasia pallida* during chemical exposure via contaminated water (tributyltin, Mercier et al. 1997). Interestingly, *A. pallida* remained in an expanded state when the contaminant was introduced through food (Mercier et al. 1997), meaning the mechanism of delivery may have been influential on the behavioural response. As well, the waste being organic in nature (feed and feces) may not present as an environmental stressor that would initially induce avoidant behaviour, it could appear as food to opportunistic suspension feeders (i.e. sea anemones and corals) and induce expansion. For example, Rossi et al. (2019) reported that the red coral *Corallium rubrum* expanded its polyps following the addition of food, i.e. live/dead zooplankton filtrate or particles, presumably representing an evolutionary adaptation to food pulses common to passive suspension feeders (Rossi et al. 2019).

Pharynx eversion was observed in *A. stella*, increasing with waste concentration, while rarely observed in the inorganic treatment and control individuals. Though pharynx eversion has

been previously noted in the sea anemone *Phyllactis concinnata* as a temporary response to mechanical disturbance (i.e. handling, Pickens 1988), it has also been described as part of the feeding behaviour of sea anemones (*Calliactis parasitica, Cereus pedunculatus*) in response to food stimuli (McFarlane 1975, Riedel et al. 2008). As the highest instance of pharynx eversion was recorded in the first hour after waste addition (when the concentration of waste particles in the water would have been highest), it may be a tool to increase the surface area for feeding on the particulate waste matter. This is complemented by an observation of noticeably higher tentacle activity in *A. stella* directly after waste addition, as well by the fact sedimentation from inorganic treatments did not elicit this behaviour as often (48% of the time under high-waste versus 5% under inorganic treatment).

Although the metrics used in the present study to evaluate the effects of aquaculture waste on the behaviour of *G. rubiformis* and *A. stella* were the same for both species, observed changes do not necessarily hold the same implications for each and effects should be considered species-specific. Both *G. rubiformis* and *A. stella* are often found in shallow waters (<200 m, Wareham and Edinger 2007, Ivanova and Grebelnyi 2021) up to the intertidal zone, which could be reflective of their ability to cope with increased sedimentation loads. With indications of potential feeding, and less pronounced trends in behaviour over time in *A. stella*, sea anemones may be more tolerant to aquaculture waste sedimentation than soft corals. A 7-month caged transplantation of suspension feeders, including the soft coral *D. florida* and the sea anemone *H. digitata*, revealed an almost 3-fold difference in mortality between the two species, with *D. florida* having the higher mortality. As well, a higher respiration rate was measured in *D. florida* near the cage, which was not detected in *H. digitata* (Laroche et al. 2022). Though similar in morphology to sea anemones, the polyps of soft corals are much smaller and

smothering/clogging of the polyp with waste particles is likely of greater concern. Clogging of tentacles/feeding structures with waste particulates could inhibit typical food capture and was previously hypothesized to contribute to the reduced lipid content of *D. florida* transplanted under salmon farms (Laroche et al. 2022).

Relative to colonies in waste treatments, *G. rubiformis* in inorganic treatments tended to display behaviour similar to that of the controls or sometimes more avoidant, highlighting that, contrary to initial assumptions, behavioural responses to waste treatments go beyond those elicited by sedimentation itself. Coral responses have been shown to differ with particle type in other studies because of their chemical nature or shape. For example, Carreiro-Silva et al. (2022) found that polymetallic sulphide particles (related to deep-sea mining) affected the octocoral *Dentomuricea* aff. *meteor* differently than quartz in terms of survival, tissue condition, respiration and ammonia excretion. As well, when exposed to abrasive mine tailings, *D. florida* showed contraction behaviour that was not observed when exposed to smooth glass beads (Liefmann et al. 2018). This highlights the need for source material to be as realistic as possible for experiments evaluating the effects of industry waste, as well as calls for caution when using studies of natural sedimentation as indicators of the impacts that industrial waste may have.

Hypoxic conditions could be expected under 5-10 g C m^{-2} d^{-1} and anoxic conditions at >10 g C m^{-2} d^{-1} (DFO 2013). The study length may have limited behavioural observations related to expected changes in dissolved oxygen and bacterial activity with waste deposition. For example, sediment anoxia under Chinook salmon (*Oncorhynchus tshawytscha*) farms have been seen to take up to 24 months to develop (Keeley et al. 2015). Though dissolved oxygen measured in the tanks remained saturated during trials, the presence of anaerobic bacteria (e.g. *Fusibacter*) in samples of waste matter taken from treatment tanks indicates a potential decline of dissolved

oxygen at the sediment level. Visible bacterial mats began developing on top of the collected flocculent matter after 21 d of sedimentation within the high-waste treatment, and 25 d in the low-waste treatment. This is consistent with field observations made at two salmonid aquaculture sites located in Newfoundland, which noted the presence of bacterial mats after 1 month of production (Armstrong et al. 2020). However it should be noted that the bacterial community identified in this study was not expected to be reflective of *in situ* conditions as the collection site, freezing/thawing, as well as the storage of collected waste at 4 °C is likely to have influenced the bacterial composition (Sawicka et al. 2009, Johnson et al. 2023). Verhoeven et al. (2016) identified bacterial communities within flocculent matter collected underneath salmonid aquaculture pens from the south coast of Newfoundland. In comparison to Verhoeven et al. (2016), *Marinifilum* was the only genus dominant in microbial mats within both studies. With a study length more reflective of long-term sedimentation experienced *in situ*, the development of hypoxia/anoxia may further drive changes in behaviour and/or survival that could not be evaluated here.

2.4.2 Lipid composition and reserves

No effect of treatment on the lipid composition of *G. rubiformis* or *A. stella* was detected, however it was influenced by experimental duration in *A. stella*. Lipid reserves may change seasonally in both CWC (*D. pertusum,* Maier et al. 2020) and cold-water sea anemones (*M. senile,* Hill-Manning and Blanquet 1979), so changes seen between pre-trial baseline and day 28 samples may not be solely nutrition-based.

In both *G. rubiformis* and *A. stella*, total lipids concentration increased from the onset of the experiment (between pre-trial baseline and day 28) in all groups. Though waste treatment did not have a clear effect on the concentration of total lipids in *G. rubiformis,* the lowest value was

observed in colonies exposed to higher sedimentation concentrations (high-waste and inorganic treatments). Lipid reserves are often depleted during unfavourable conditions and a drop in lipid content has been observed in colonies of both *D. florida* and *D. pertusum* transplanted within the depositional footprint of salmon farms after 7 months and a year, respectively (Laroche et al. 2022, Kutti et al. 2022). This finding suggested that transplanted colonies were not feeding on the waste (Laroche et al. 2022, Kutti et al. 2022). Therefore, we may expect lipid reserves to deplete in response to long-term sedimentation of aquaculture waste, which was not clearly detected here in the short timeframe of the experiment. Total lipids in the individuals of *A. stella* exposed to high-waste treatment were noticeably lower than in the other experimental and control individuals, though a decline in storage classes (i.e. triacylglyerols, wax esters) was not observed. The difference was largely attributed to a lower phospholipid content in two individuals and high variation between samples. Sea anemones have previously shown strong individual variation in terms of behavioural responses (Hensley et al. 2012, Osborn and Briffa 2017, Maskrey et al. 2020, 2021), which will likely also appear in feeding behaviour. This can create high intra-species variability and make conclusions from smaller sample sizes difficult. Here, the biochemical analyses were restricted to the trunks of *G. rubiformis* and the epidermal tissue of *A. stella* to prevent contamination from remnant waste particles. Further exploration of the whole animals (especially where lipid stores may be more prevalent) as well as a longer experimental period, may yield stronger effects on the lipid composition.

2.4.3 Fatty acids as tracers

The most prevalent fatty acids in the salmon waste (18:1ω9, 16:0, 18:2ω6) were explored as potential indicators for waste assimilation. If waste was incorporated into the tissue profile of the cnidarians, we would expect an increase in tracers coinciding with waste treatment

concentration (i.e. similar values between control and inorganic treatment individuals, elevated concentrations in low-waste treatment individuals and highest concentrations in high-waste treatment individuals). Both *G. rubiformis* and *A. stella* showed this trend in their fatty acid composition after 28 d of exposure in terms of 18:2ω6 (linoleic acid). *Gersemia rubiformis* showed elevated concentrations of 18:1ω9 (oleic acid) in waste treatments compared to the control and inorganic treatments, though the low-waste showed higher concentrations and average differences were slim. In contrast, *A. stella* again showed a strong pattern with the lowest concentrations in individuals of the control and inorganic treatment, followed by increased concentrations in low-waste and finally high-waste treatments. Trends for 16:0 (palmitic acid) were not detected with treatment in *G. rubiformis*; they were weak but present in *A. stella*. Fatty acid analyses in the gonads and eggs of the sea urchin *Echinus acutus* showed assimilation of aquaculture waste after 10 weeks of exposure to either fish feed, natural feed or a combination (White et al. 2017). White et al. (2017) similarly indicated assimilation by *E. acutus* through the increase of 18:1ω9 and 18:2ω6, while 16:0 was not a distinct link as seen here. However, it should be noted fatty acids may hold different assimilation potentials or be biosynthesized/catabolized (Kelly and Scheibling 2012), meaning the lower 16:0 concentrations may not necessarily be a direct reflection of uptake through feeding. As our study was only 28 d, assimilation may become clearer with a longer experimental period.

The two primary fatty acids reported in salmon feed are 18:1ω9 and 18:2ω6, while those for salmon feces are 18:1ω9 and 16:0 (Bergvik et al. 2019). The experimental waste had a carbon content of ~44% which was between the reported carbon content for salmon feces (~30%) and feed (~50%, Handå et al. 2012, Callier et al. 2013, Bergvik et al. 2019), suggesting a fairly even mix of the two (though likely slightly higher in feed). While 18:1ω9 (33% total FA)

and 16:0 (16% total FA) were most prevalent in the waste, the strongest trend was seen in 18:2ω6 (14% total FA). This may indicate that although trends in *G. rubiformis* and *A. stella* suggest incorporation of fatty acids from waste into their tissues, feeding may be more selective towards particles from the salmon feed rather than feces. As feces are expected to be the main depositional component of aquaculture waste due to reductions in feed wastage over the past decades (Cromey and Black 2005), the assimilation potential and effects of feed and feces both separately and combined may be considered in future research.

An effect of treatment on the overall fatty acid composition was observed in *G. rubiformis* but not *A. stella*. A similar finding by Laroche et al. (2022) was reported where the fatty acid profile of *D. florida* appeared more sensitive to salmon aquaculture waste compared to *H. digitata*. Reductions in ω3 PUFA (polyunsaturated fatty acids) and increases in ω6 PUFA contributions with waste concentration observed here in *G. rubiformis*, are also consistent with observations made of *D. florida* transplanted under salmon farms. A strong reduction in MUFA with waste concentration in *G. rubiformis* was not seen here as it was in Laroche et al. (2022), though this could be due to the shorter length of our study (1 month versus 7 months). An effect of treatment on fatty acid composition does not necessarily indicate waste assimilation, as changes in Laroche et al. (2022) were suggested to be a result of catabolism of MUFA and ω3 PUFA due to feeding-related issues (i.e. polyp clogging).

2.5 Conclusions

Exposure to salmon waste sedimentation (5 and 10 g C m^{-2} d^{-1}) elicited shifts in the behaviour of both *G. rubiformis* and *A. stella*. Individuals exposed to waste behaved more similarly to each other than to those in inorganic or control treatments, suggesting the organic nature of the waste influenced their response to sedimentation. Elevated expansion behaviour

observed in *G. rubiformis* may help prevent sediment build-up, though expansion in this species also appeared to be confounded by time (potentially via seasonal changes in temperature). *Aulactinia stella* displayed increased levels of expansion behaviour, as well as higher instances of gastrovascular eversion in the waste treatments. These may suggest a feeding response, which was supported by stronger indications of waste assimilation compared to *G. rubiformis*. *Gersemia rubiformis* and *A. stella* showed an increasing trend for two of the three salmon waste tracer fatty acids (18:1ω9, 18:2ω6) with waste concentration. Only *A. stella* showed a weak trend with concentration for 16:0. Lipid reserves did not appear to be influenced over the trial in either species. Exposure to salmon waste altered the fatty acid composition of *G. rubiformis*, potentially supporting assimilation or alluding to changes in biochemical pathways. Results indicate that species-specific responses to aquaculture waste should be considered and explored with different taxa. Future studies should consider longer experimental periods, which may be more reflective of long-term effects experienced *in situ*.

2.6 Acknowledgements

We would like to thank the members of the Gamperl Lab (Memorial University), specifically Rebeccah Sandrelli and Eric Ignaz, as well as Danny Boyce (Memorial University) who were instrumental in the collection of fish waste used in this experiment. Joanna Potter (Stable Isotope Laboratory, CREAIT, Memorial University) for the carbon and nitrogen analysis of the fish waste. Philip Sargent, Ana Storch and Dwight Drover (DFO) for the dive collections of soft corals and sea anemones. Brandon Piercy (DFO) for his technical work in experimental preparations and assistance sampling. Jeanette Wells (Aquatic Research Cluster, CREAIT, Memorial University) for her training of lipid and fatty acid protocols, and analyses of raw lipid and fatty acid data, as well as Jeanette Wells and Efe Obade (Aquatic Research Cluster,

CREAIT) for their work on the lipid and fatty acid extractions of the *Gersemia*. Thank you to Dounia Hamoutene (DFO) for the suggestion of including an inorganic treatment, and Chris Parrish (Memorial University) for lipid and fatty acid resources that influenced the experimental design. This research was funded by a Competitive Science Research Fund (CSRF) grant awarded to Bárbara de Moura Neves, and by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) awarded to Annie Mercier.

2.7 Literature cited

Anderson, M. J., R. N. Gorley, and K. R. Clarke. 2008. PEMANOVA+ for PRIMER. Guide to software and statistical methods. Primer-e Ltd., Plymouth, UK.

Armstrong, E. G., J. Mersereau, F. Salvo, D. Hamoutene, and S. C. Dufour. 2020. Temporal change in the spatial distribution of visual organic enrichment indicators at aquaculture sites in Newfoundland, Canada. Aquaculture International 28:569–586.

Bakker, J. D. 2024. Applied Multivariate Statistics in R. University of Washington.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Bay, K., K. Ahrendt, H. Sterr, P. Krost, W. Windhorst, and M. Schultz. 2018. Potential, constrains and solutions for marine aquaculture in Kiel Bay & Fjord. Journal of Coastal Conservation 22:115–130.

Bergvik, M., L. Stensås, A. Handå, K. I. Reitan, Ø. Strand, and Y. Olsen. 2019. Incorporation of feed and fecal waste from salmon aquaculture in great scallops (*Pecten maximus*) co-fed by different algal concentrations. Frontiers in Marine Science 5:e524.

Bolyen, E., et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37:852–857.

Brooks, K. M., A. R. Stierns, and C. Backman. 2004. Seven year remediation study at the Carrie Bay Atlantic salmon (*Salmo salar*) farm in the Broughton Archipelago, British Columbia, Canada. Aquaculture 239:81–123.

Brunel, P., L. Bossé, and G. Lamarche. 1998. Catalogue of the marine invertebrates of the Estuary and Gulf of Saint Lawrence. Can. Spec. Publ. Fish. Aquat. Sci. 126. 405 p.

Buhl-Mortensen, L., T. H. Thangstad, G. Søvik, and H. Wehde. 2023. Sea pens and bamboo corals in Skagerrak and the Norwegian trench. Marine Biology Research 19:191–206.

Callier, M. D., S. Lefebvre, M. K. Dunagan, M. P. Bataille, J. Coughlan, and T. P. Crowe. 2013. Shift in benthic assemblages and organisms' diet at salmon farms: Community structure and stable isotope analyses. Marine Ecology Progress Series 483:153–167.

Carreiro-Silva, M., I. Martins, V. Riou, J. Raimundo, M. Caetano, R. Bettencourt, M. Rakka, T. Cerqueira, A. Godinho, T. Morato, and A. Colaço. 2022. Mechanical and toxicological effects of deep-sea mining sediment plumes on a habitat-forming cold-water octocoral. Frontiers in Marine Science 9:e915650.

Caulier, G., J.-F. Hamel, E. A. Hendrycks, K. E. Conlan, and A. Mercier. 2021. Mutualistic relationship between the amphipod *Stenula nordmanni* (Stephensen, 1931) and the nephtheid coral *Gersemia rubiformis* (Ehrenberg, 1834). Symbiosis 85:93–104.

Clarke, K. R., and R. N. Gorley. 2015. Primer. Primer-e Ltd., Plymouth, UK.

Comeau, A. M., G. M. Douglas, and M. G. I. Langille. 2017. Microbiome helper: a custom and streamlined workflow for microbiome research. mSystems 2:e00127-16.

Comeau, A. M., and G. V Filloramo. 2023. Preparing multiplexed 16S/18S/ITS amplicon SMRTbell libraries with the Express TPK2.0 for the PacBio Sequel2 V.1. protocols.io.

Comeau, A. M., W. K. W. Li, J. É. Tremblay, E. C. Carmack, and C. Lovejoy. 2011. Arctic ocean microbial community structure before and after the 2007 record sea ice minimum. PLoS ONE 6:e27492.

Cornwall, C. E., and C. L. Hurd. 2016. Experimental design in ocean acidification research: problems and solutions. ICES Journal of Marine Science 73:572–581.

Couturier, L. I. E., L. N. Michel, T. Amaro, S. M. Budge, E. Da Costa, M. De Troch, V. Di Dato, P. Fink, C. Giraldo, F. Le Grand, I. Loaiza, M. Mathieu-Resuge, P. D. Nichols, C. C. Parrish, F. Sardenne, M. Vagner, F. Pernet, and P. Soudant. 2020, December 1. State of art and best practices for fatty acid analysis in aquatic sciences. Oxford University Press.

Cromey, C. J., and K. D. Black. 2005. Modelling the impacts of finfish aquaculture. Pages 129–155 Environmental Effects of Marine Finfish Aquaculture. Springer-Verlag.

DFO. 2013. DEPOMOD Predictions for an Aquaculture Site at Cheney Head, New Brunswick. DFO Can. Sci. Advis. Sec. Sci. Resp. 2012/035.

DFO. 2016. State of knowledge of the oceanography and water exchange on the South Coast of Newfoundland to support the development of bay management areas for finfish aquaculture. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2016/039.

[DFO] Fisheries and Oceans Canada. 2018. AAR Monitoring Standard. Retrieved from: https://www.dfo-mpo.gc.ca/aquaculture/management-gestion/doc/AAR-Monitoring-Standard-2018-eng.pdf (accessed June 12, 2024).

[DFO] Fisheries and Oceans Canada. 2004. A scientific review of the potential environmental effects of aquaculture in aquatic ecosystems. Volume III. Near-field organic enrichment from marine finfish aquaculture (D.J. Wildish, M. Dowd, T.F. Sutherland and C.D. Levings). Can. Tech. Rep. Fish. Aquat. Sci. 2450: ix + 117 p.

DFO. 2022a. DFO Newfoundland and Labrador region science review of five proposed Grieg Aquaculture marine finfish aquaculture facilities in Placentia Bay, Newfoundland. DFO Can. Sci. Advis. Sec. Sci. Resp. 2022/019.

DFO. 2022b. DFO Newfoundland and Labrador region science review of three proposed marine harvest Atlantic Canada marine finfish aquaculture facilities in Chaleur Bay, Newfoundland. DFO Can. Sci. Advis. Sec. Sci. Resp. 2022/044.

Dorey, N., Ø. Gjelsvik, T. Kutti, and J. V. Büscher. 2020. Broad thermal tolerance in the cold-water coral *Lophelia pertusa* from arctic and boreal reefs. Frontiers in Physiology 10:e1636.

Duckworth, A., N. Giofre, and R. Jones. 2017. Coral morphology and sedimentation. Marine Pollution Bulletin 125:289–300.

Dunlop, K., A. Harendza, R. Bannister, and N. Keeley. 2021. Spatial response of hard- and mixed-bottom benthic epifauna to organic enrichment from salmon aquaculture in northern Norway. Aquaculture Environment Interactions 13:455–475.

FAO. 2022. The state of world fisheries and aquaculture 2022. Towards blue transformation. Rome, FAO.

Forsman, Z. H., C. A. Page, R. J. Toonen, and D. Vaughan. 2015. Growing coral larger and faster: micro-colony-fusion as a strategy for accelerating coral cover. PeerJ 20:e1313.

Fox, J., and S. Weisberg. 2019. An R companion to applied regression, third edition. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

González-Gaya, B., N. García-Bueno, E. Buelow, A. Marin, and A. Rico. 2022. Effects of aquaculture waste feeds and antibiotics on marine benthic ecosystems in the Mediterranean Sea. Science of the Total Environment 806:e151190.

Hamoutene, D., F. Salvo, T. Bungay, G. Mabrouk, C. Couturier, A. Ratsimandresy, and S. C. Dufour. 2015. Assessment of finfish aquaculture effect on Newfoundland epibenthic

communities through video monitoring. North American Journal of Aquaculture 77:117–127.

Hamoutene D., F. Salvo, S. Donnet, and S. Dufour. 2016. The usage of visual indicators in regulatory monitoring at hard-bottom finfish aquaculture sites in Newfoundland (Canada). Marine Pollution Bulletin 108:232-241.

Hamoutene, D., F. Salvo, S. Cross, S. C. Dufour, and S. Donnet. 2018a. Linking the presence of visual indicators of aquaculture deposition to changes in epibenthic richness at finfish sites installed over hard bottom substrates. Environmental Monitoring and Assessment 190:e750.

Hamoutene, D., F. Salvo, S. N. Egli, A. Modir-Rousta, R. Knight, G. Perry, C. S. Bottaro, and S. C. Dufour. 2018b. Measurement of aquaculture chemotherapeutants in flocculent matter collected at a hard-bottom dominated finfish site on the south coast of Newfoundland (Canada) after 2 years of fallow. Frontiers in Marine Science 5:e228.

Handå, A., A. Ranheim, A. J. Olsen, D. Altin, K. I. Reitan, Y. Olsen, and H. Reinertsen. 2012. Incorporation of salmon fish feed and feces components in mussels (*Mytilus edulis*): Implications for integrated multi-trophic aquaculture in cool-temperate North Atlantic waters. Aquaculture 370–371:40–53.

Hargrave, B. T. 2010. Empirical relationships describing benthic impacts of salmon aquaculture. Aquaculture Environment Interactions 1:33–46.

Hargrave, B. T., V. E. Kostylev, and C. M. Hawkins. 2004. Benthic epifauna assemblages, biomass and respiration in The Gully region on the Scotian Shelf, NW Atlantic Ocean. Marine Ecology Progress Series 270:55–70.

Hartig, F. 2022. DHARMa: residual diagnostics for hierarchical (multi-Level / mixed) regression models. R package version 0.4.6. http://florianhartig.github.io/DHARMa.

Henry, L.-A., E. L. Kenchington, and A. Silvaggio. 2003. Effects of mechanical experimental disturbance on aspects of colony responses, reproduction, and regeneration in the cold-water octocoral *Gersemia rubiformis*. Canadian Journal of Zoology 81:1691–1701.

Hill-Manning, D. N., and R. S. Blanquet. 1979. Seasonal changes in the lipids of the sea anemone *Metridium senile* (L.). J. exp. mar. Biol. Ecol. 36:249-257.

Hills, A., J. Spurway, S. Brown, and C. Cromey. 2005. Regulation and monitoring of marine cage fish farming in Scotland, Annex H: methods for modelling in-feed anti-parasites and benthic effects.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346–363.

Imbs, A. B., and L. T. P. Dang. 2017. The molecular species of phospholipids of the cold-water soft coral *Gersemia rubiformis* (Ehrenberg, 1834) (Alcyonacea, Nephtheidae). Russian Journal of Marine Biology 43:239–244.

Ivanova, N. Y., and S. D. Grebelnyi. 2021. The history of study, the taxonomic composition and the origin of the sea anemone fauna of the Kara Sea (Actiniaria, Anthozoa, Cnidaria). Proceedings of the Zoological Institute of the Russian Academy of Sciences 325:156–182.

Johnson, L. A., S. C. Dufour, D. D. N. Smith, A. J. Manning, B. Ahmed, S. Binette, D. Hamoutene. 2023. Descriptive analyses of bacterial communities in marine sediment microcosms spiked with fish wastes, emamectin benzoate, and oxytetracycline. Ecotoxicology and Environmental Safety 268:e115683.

Keeley, N. B., B. M. Forrest, and C. K. Macleod. 2015. Benthic recovery and re-impact responses from salmon farm enrichment: Implications for farm management. Aquaculture 435:412–423.

Keeley, N. B., C. K. Macleod, G. A. Hopkins, and B. M. Forrest. 2014. Spatial and temporal dynamics in macrobenthos during recovery from salmon farm induced organic enrichment: When is recovery complete? Marine Pollution Bulletin 80:250–262.

Kelly, J. R., and R. E. Scheibling. 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series 446:1-22.

Kutti, T., E. Legrand, V. Husa, S. Olsen, Ø. Gjelsvik, M. Carvajalino-Fernandez, and I. Johnsen. 2022. Fish-farm effluents cause metabolic depression, reducing energy stores and growth in the reef forming coral *Lophelia pertusa*. Aquaculture Environment Interactions 14:279–293.

Kutti, T., K. Nordbø, R. Bannister, and V. Husa. 2015. Oppdrettsanlegg kan true korallrev i fjordene. Havforskningsrapporten: fisken og havet, særnummer. 220 pp. Havforskningsinstituttet.

Laroche, O., S. Meier, S. A. Mjøs, and N. Keeley. 2022. Suspension-feeding benthic species' physiological and microbiome response to salmon farming and associated environmental changes. Frontiers in Marine Science 9:e841806.

Larsson, A. I., and A. Purser. 2011. Sedimentation on the cold-water coral *Lophelia pertusa*: cleaning efficiency from natural sediments and drill cuttings. Marine Pollution Bulletin 62:1159–1168.

Leal, M. C., C. Nunes, S. Kempf, A. Reis, T. L. da Silva, J. Serôdio, D. F. R. Cleary, and R. Calado. 2013. Effect of light, temperature and diet on the fatty acid profile of the tropical sea anemone *Aiptasia pallida*. Aquaculture Nutrition 19:818–826.

Legrand, E., T. Kutti, E. V. G. Casal, S. P. S. Rastrick, S. Andersen, and V. Husa. 2021. Reduced physiological performance in a free-living coralline alga induced by salmon faeces deposition. Aquaculture Environment Interactions 13:225–236.

Levings, C. D., A. Ervik, P. Johannessen, J. Aure, C. D. Levings', C. A. Ervik, and B. Norway. 1995. Ecological criteria used to help site fish farms in fjords. Estuaries 18:81–90.

Liefmann, S., J. Järnegren, G. Johnsen, and F. Murray. 2018. Eco-physiological responses of cold-water soft corals to anthropogenic sedimentation and particle shape. Journal of Experimental Marine Biology and Ecology 504:61–71.

Liu, P. J., M. C. Hsin, Y. H. Huang, T. Y. Fan, P. J. Meng, C. C. Lu, and H. J. Lin. 2015. Nutrient enrichment coupled with sedimentation favors sea anemones over corals. PLoS ONE 10:e0125175.

Maier, S., R. Bannister, D. van Oevelen, and T. Kutti. 2020. Seasonal controls on the diet, metabolic activity, tissue reserves and growth of the cold-water coral *Lophelia pertusa*. Coral Reefs 39:173-187.

McFarlane, I. D. 1975. Control of mouth opening and pharynx protrusion during feeding in the sea anemone *Calliactis Parasitica*. Journal of Experimental Biology 63:615–626.

Mercier, A., É. Pelletier, and J.-F. Hamel. 1997. Effects of butyltins on the symbiotic sea anemone *Aiptasia pallida* (Verrill). Journal of Experimental Marine Biology and Ecology 215:289–304.

Mercier, A., Z. Sun, C. C. Parrish, and J.-F. Hamel. 2016. Remarkable shifts in offspring provisioning during gestation in a live-bearing cnidarian. PLoS ONE 11:e0154051.

Mercier, A., Zhao Sun, S. Baillon, and J.-F. Hamel. 2011. Lunar rhythms in the deep sea: Evidence from the reproductive periodicity of several marine invertebrates. Journal of Biological Rhythms 26:82–86.

Nadtochy, V. A., N. V. Kolpakov, and I. A. Korneichuk. 2017. Distribution of macrozoobenthic taxa - potential indicators of vulnerable marine ecosystems in the western part of Bering Sea. 2. Chukotka and Koryak districts. Izvestiya TINRO 190:177–195.

Nelson, M. L., and S. F. Craig. 2011. Role of the sea anemone *Metridium senile* in structuring a developing subtidal fouling community. Marine Ecology Progress Series 421:139–149.

Neofitou, N., D. Vafidis, and S. Klaoudatos. 2012. Spatial and temporal effects of fish farming on benthic community structure in a semi-enclosed gulf of the Eastern Mediterranean. Aquaculture Environment Interactions 1:95–105.

Neves, B. M., V. Wareham Hayes, E. Herder, K. Hedges, C. Grant, and P. Archambault. 2020. Cold-water soft corals (Cnidaria: Nephtheidae) as habitat for juvenile basket stars (Echinodermata: Gorgonocephalidae). Frontiers in Marine Science 7:e547896.Parrish, C. C. 2013. Lipids in Marine Ecosystems. ISRN Oceanography 2013:e604045.

Parrish, C. C., and J. S. Wells. 2021. Determination of total lipid and lipid classes in marine samples. Journal of Visualized Experiments 178:e62315.

Pickens, P. E. 1988. Systems that control the burrowing behaviour of a sea anemone. Journal of Experimental Biology 135:133–164.

Poulos, D. E., D. Harasti, C. Gallen, and D. J. Booth. 2013. Biodiversity value of a geographically restricted soft coral species within a temperate estuary. Aquatic Conservation: Marine and Freshwater Ecosystems 23:838–849.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reigl, B. 1995. Effects of sand deposition on scleractinian and alcyonacean corals. Marine Biology 121:517–526.

Renegar, D. A., and N. R. Turner. 2021. Species sensitivity assessment of five Atlantic scleractinian coral species to 1-methylnaphthalene. Scientific Reports 11:e529.

Riedel, B., M. Stachowitsch, and M. Zuschin. 2008. Sea anemones and brittle stars: unexpected predatory interactions during induced *in situ* oxygen crises. Marine Biology 153:1075–1085.

Rossi, S., L. Rizzo, and J. C. Duchêne. 2019. Polyp expansion of passive suspension feeders: A red coral case study. PeerJ 7:e7076.

Salvo, F., Oldford, V., Bungay, T., Boone, C., and D. Hamoutene. 2018a. Guide for video monitoring of hard bottom benthic communities of the south coast of Newfoundland for aquaculture impact assessments. Can. Data Rep. Fish. Aquat. Sci. Fs 97-13/1284E-PDF: ix + 41 p.

Salvo, F., D. Hamoutene, V. E. W. Hayes, E. N. Edinger, and C. C. Parrish. 2018b. Investigation of trophic ecology in Newfoundland cold-water deep-sea corals using lipid class and fatty acid analyses. Coral Reefs 37:157–171.

Salvo, F., J. Mersereau, D. Hamoutene, R. Belley, and S. C. Dufour. 2017. Spatial and temporal changes in epibenthic communities at deep, hard bottom aquaculture sites in Newfoundland. Ecological Indicators 76:207–218.

Sanchis, C., E. H. Soto, and E. Quiroga. 2021. The importance of a functional approach on benthic communities for aquaculture environmental assessment: trophic groups – A polychaete view. Marine Pollution Bulletin 167:e112309.

Sawicka, J., A. Robador, C. Hubert, B. B. Jørgensen, and V. Brüchert. 2009. Effects of freeze–thaw cycles on anaerobic microbial processes in an Arctic intertidal mud flat. The ISME Journal 4:585-594.

Shumway, S. 1978. Activity and respiration in the anemone, *Metridium senile* (L.) exposed to salinity fluctuations. Journal of Experimental Marine Biology and Ecology 33:85–92.

Sswat, M., B. Gulliksen, I. Menn, A. K. Sweetman, and D. Piepenburg. 2015. Distribution and composition of the epibenthic megafauna north of Svalbard (Arctic). Polar Biology 38:861–877.

Steinberg, R. K., K. A. Dafforn, T. Ainsworth, and E. L. Johnston. 2020. Know thy Anemone: a review of threats to octocorals and anemones and opportunities for their restoration. Frontiers in Marine Science 7:e590.

Sutherland, T. F., A. M. Sterling, and M. Ou. 2018. Influence of salmonid aquaculture activities on a rock-cliff epifaunal community in Jervis Inlet, British Columbia. Marine Pollution Bulletin 127:297–309.

Taormina, B., T. Kutti, S. A. Olsen, P. N. Sævik, R. Hannisdal, V. Husa, and E. Legrand. 2024. Effects of aquaculture effluents on the slender sea pen *Virgularia mirabilis*. Scientific Reports 14:e9385.

Valdemarsen, T., P. K. Hansen, A. Ervik, and R. J. Bannister. 2015. Impact of deep-water fish farms on benthic macrofauna communities under different hydrodynamic conditions. Marine Pollution Bulletin 101:776–783.

Verhoeven, J. T. P., F. Salvo, D. Hamoutene, and S. C. Dufour. 2016. Bacterial community composition of flocculent matter under a salmonid aquaculture site in Newfoundland, Canada. Aquaculture Environment Interactions 8:637–646.

Verhoeven, J. T. P., F. Salvo, R. Knight, D. Hamoutene, and S. C. Dufour. 2018. Temporal bacterial surveillance of salmon aquaculture sites indicates a long lasting benthic impact with minimal recovery. Frontiers in Microbiology 9:e3054.

Wareham, V. E., and E. N. Edinger. 2007. Distribution of deep-sea corals in the Newfoundland and Labrador region, Northwest Atlantic Ocean. Pages 289–313 Conservation and adaptive management of seamount and deep-sea coral ecosystems. Rosenstiel School of Marine and Atmospheric Science, University of Miami. Watling and Auster.

White, C. A., R. J. Bannister, S. A. Dworjanyn, V. Husa, P. D. Nichols, T. Kutti, and T. Dempster. 2017. Consumption of aquaculture waste affects the fatty acid metabolism of a benthic invertebrate. Science of the Total Environment 586:1170–1181.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4.

2.8 Tables

Table 2-1: Scores used to access the behavioural state of *Gersemia rubiformis*.

Behavioural score	Description	Corresponding photo
1	Fully contracted state (raspberry appearance), branches tightly packed together, polyps retracted.	Figure 2-2A
2	Partially contracted colony, expansion of branches such that the trunk was partially visible, polyps retracted.	Figure 2-2B
3	Full expansion of the branches, trunk was fully visible, polyps retracted.	Figure 2-2C
4	Expansion of branches and trunk with either partially extended polyps (XD) or fully extended polyps observed on less than half the colony (XE).	Figure 2-2D, 2-2E
5	Expansion of branches and trunk with the majority/all polyps fully extended. Tentacles, if visible, were not fully deployed.	Figure 2-2F
6	Full expansion of branches and trunk, polyps fully extended, and deployment of tentacles (fuzzy appearance).	Figure 2-2G

Table 2-2: Scores used to access the behavioural state of *Aulactinia stella.*

Behavioural score	Description	Corresponding photo
1	Fully contracted state with no visible column or tentacles (flat appearance).	Figure 2-3A
2	Column partially expanded and appeared mound-like, tentacles retracted.	Figure 2-3B
3	Expanded column and partial visibility of the tentacles.	Figure 2-3C
4	Expanded column with most of the tentacles visible but not fully extended. Mouth not visible.	Figure 2-3D
5	Expanded column, all tentacles visible and fully extended, and the mouth visible.	Figure 2-3E

Table 2-3: Lipid composition (%) of corals (*Gersemia rubiformis*) and sea anemones (*Aulactinia stella*) sampled on the first experimental day ("Pre-trial") and sampled after a 28 d exposure period to either inorganic sediment ("inorganic") or fish waste ("waste"). "Low" and "high" waste refer to 5 and 10 g C m^{-2} d^{-1} target concentrations, respectively. Values are mean ± se for all except pre-trial values which are mean ± sd.

	Lipids	Pre-trial (%)	Control (%)	Inorganic (%)	Low-waste (%)	High-waste (%)
Gersemia rubiformis	Hydrocarbons	0.9 ± 0.0	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
	Steryl esters/wax esters	0.9 ± 0.1	1.9 ± 0.6	2.8 ± 0.2	1.1 ± 0.4	0.7 ± 0.3
	Ethyl esters/methyl esters	2.6 ± 0.1	5.8 ± 1.8	8.0 ± 0.6	3.9 ± 1.2	4.0 ± 0.7
	Ethyl ketones	1.3 ± 0.0	2.6 ± 1.3	3.2 ± 0.7	3.2 ± 0.5	4.1 ± 0.3
	Glyceryl ethers	1.0 ± 0.0	3.5 ± 0.9	2.4 ± 0.5	2.6 ± 0.6	2.0 ± 0.3
	Triacylglycerols	8.0 ± 0.2	4.9 ± 1.5	4.1 ± 1.0	4.7 ± 0.6	4.8 ± 0.2
	Free fatty acids	4.5 ± 0.0	4.9 ± 0.5	6.6 ± 1.5	5.6 ± 0.4	5.9 ± 0.5
	Alcohols	0.0 ± 0.0	0.2 ± 0.1	0.4 ± 0.3	0.1 ± 0.1	0.4 ± 0.2
	Sterols	18.4 ± 0.1	13.8 ± 1.0	15.4 ± 0.4	14.9 ± 1.1	15.8 ± 0.7
	Acetone mobile polar lipids	17.2 ± 0.2	10.1 ± 1.1	8.8 ± 0.7	12.0 ± 1.2	10.9 ± 0.3
	Phospholipids	45.2 ± 0.0	51.9 ± 2.2	48.0 ± 2.4	51.6 ± 1.2	51.4 ± 1.0
	Total lipids (mg g^{-1})	3.2 ± 0.1	4.3 ± 0.4	3.6 ± 0.2	4.6 ± 0.7	3.9 ± 0.4
Aulactinia stella	Hydrocarbons	0.1 ± 0.2	0.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
	Steryl esters/wax esters	1.8 ± 1.5	0.6 ± 0.3	0.8 ± 0.1	0.7 ± 0.3	1.2 ± 0.4
	Ethyl esters/methyl esters	8.7 ± 2.1	3.6 ± 1.3	4.1 ± 0.8	4.1 ± 1.5	5.0 ± 0.2
	Ethyl ketones	0.0 ± 0.0	1.3 ± 0.5	0.1 ± 0.0	0.4 ± 0.3	1.4 ± 0.7
	Glyceryl ethers	0.2 ± 0.3	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Triacylglycerols	0.1 ± 0.2	0.6 ± 0.1	0.6 ± 0.0	0.8 ± 0.2	0.7 ± 0.3
	Free fatty acids	2.7 ± 2.0	2.6 ± 0.2	2.0 ± 0.2	1.9 ± 0.0	1.8 ± 0.4
	Alcohols	0.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
	Sterols	30.2 ± 4.9	24.9 ± 1.0	25.2 ± 0.9	23.0 ± 1.0	26.3 ± 1.0
	Acetone mobile polar lipids	0.0 ± 0.0	2.7 ± 1.2	2.2 ± 0.3	2.2 ± 0.7	1.5 ± 0.4
	Phospholipids	56.0 ± 4.0	63.6 ± 1.6	64.9 ± 1.8	66.7 ± 1.9	62.0 ± 1.7
	Total lipids (mg g^{-1})	6.7 ± 1.2	10.9 ± 0.1	10.2 ± 0.7	10.6 ± 0.7	8.7 ± 1.4

Table 2-4: Fatty acid composition (%) of corals (*Gersemia rubiformis*) and sea anemones (*Aulactinia stella*) sampled on the first experimental day (Pre-trial") and after a 28 d exposure period to either inorganic sediment ("inorganic") or fish waste ("waste"). Values are mean ± se for all except pre-trial values which are mean ± sd. ΣBacterial = the sum of fatty acids associated with bacteria. ΣSAT = sum of saturated fatty acids. PUFA = polyunsaturated fatty acids. MUFA = Monounsaturated fatty acids. ΣEPA = eicosapentaenoic acid; (20:5ω3). ΣDHA = docosahexaenoic acid (22:6ω3).

	Fatty acid	Pre-trial (%)	Control (%)	Inorganic (%)	Low waste (%)	High waste (%)
Gersemia rubiformis	14:0	0.5 ± 0.3	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
	14:1	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.3	0.5 ± 0.1	0.4 ± 0.1
	ai16:0	0.2 ± 0.1	0.5 ± 0.0	0.4 ± 0.2	0.4 ± 0.1	0.6 ± 0.1
	16:0	9.7 ± 1.8	7.4 ± 0.9	6.7 ± 0.3	7.3 ± 0.5	6.9 ± 0.1
	16:1ω9	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
	16:1ω7	0.9 ± 0.3	0.7 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	1.1 ± 0.1
	16:1ω5	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
	i17:0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.4 ± 0.0
	16:2ω6	0.9 ± 0.4	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.0
	16:3ω4	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
	16:4ω3	4.3 ± 1.7	3.3 ± 0.4	3.3 ± 0.6	3.7 ± 0.4	3.7 ± 0.2
	18:0	2.9 ± 0.5	2.5 ± 0.1	2.5 ± 0.2	2.6 ± 0.2	2.6 ± 0.3
	18:1ω9	2.0 ± 0.6	2.1 ± 0.4	2.2 ± 0.3	2.6 ± 0.1	2.6 ± 0.3
	18:1ω7	1.5 ± 0.5	1.4 ± 0.2	1.3 ± 0.1	1.1 ± 0.1	1.2 ± 0.0
	18:1ω5	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
	18:2ω6	0.7 ± 0.3	0.7 ± 0.1	0.8 ± 0.1	1.4 ± 0.1	1.8 ± 0.1
	18:3ω3	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.7 ± 0.1
	20:1ω9	1.8 ± 0.7	1.9 ± 0.3	2.0 ± 0.2	1.5 ± 0.1	1.2 ± 0.2
	20:1ω7	13.8 ± 1.3	13.6 ± 0.6	14.2 ± 0.0	13.9 ± 0.2	14.2 ± 0.2
	20:4ω6	37.6 ± 9.0	39.3 ± 2.5	39.6 ± 1.1	40.2 ± 1.8	40.9 ± 0.9
	20:4ω3	1.2 ± 0.5	1.3 ± 0.2	1.4 ± 0.1	1.0 ± 0.1	0.7 ± 0.1
	20:5ω3	7.3 ± 3.6	8.9 ± 0.3	8.0 ± 0.3	7.6 ± 1.1	7.2 ± 0.4
	22:1ω9	1.3 ± 0.4	1.4 ± 0.1	1.3 ± 0.0	1.2 ± 0.1	1.1 ± 0.1
	22:2NMIDa	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

	Fatty acid	Pre-trial (%)	Control (%)	Inorganic (%)	Low waste (%)	High waste (%)
	22:4ω6	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.0	1.6 ± 0.1	1.5 ± 0.0
	22:5ω3	0.9 ± 0.6	1.2 ± 0.2	1.3 ± 0.0	0.9 ± 0.0	0.7 ± 0.1
	22:6ω3	2.1 ± 1.3	1.5 ± 0.2	1.4 ± 0.0	1.3 ± 0.1	1.2 ± 0.1
	ΣBacterial	1.7 ± 0.2	2.8 ± 0.2	2.5 ± 0.2	2.6 ± 0.3	3.2 ± 0.2
	ΣSat	14.2 ± 1.9	11.3 ± 1.0	10.4 ± 0.2	11.3 ± 0.7	10.9 ± 0.3
	ΣMUFA	24.4 ± 1.9	24.1 ± 0.8	25.2 ± 1.1	24.4 ± 0.7	23.9 ± 0.4
	ΣPUFA	60.2 ± 3.6	62.5 ± 1.6	62.6 ± 1.3	62.3 ± 1.3	62.6 ± 0.1
	P/S	4.3 ± 0.8	5.7 ± 0.6	6.2 ± 0.3	5.8 ± 0.3	5.9 ± 0.0
	Σ ω3	17.1 ± 5.1	17.5 ± 0.8	17.0 ± 0.4	15.8 ± 1.0	15.0 ± 0.6
	Σ ω6	41.7 ± 8.5	43.5 ± 2.3	44.2 ± 1.1	45.2 ± 1.9	46.3 ± 0.7
	ω6/ω3	2.7 ± 1.6	2.7 ± 0.2	2.9 ± 0.1	3.0 ± 0.3	3.4 ± 0.3
	DHA/EPA	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
Aulactinia stella	14:0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	14:1	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.0
	ai16:0	0.3 ± 0.1	1.0 ± 0.3	0.6 ± 0.2	0.8 ± 0.2	0.9 ± 0.1
	16:0	4.4 ± 1.5	2.3 ± 0.1	2.3 ± 0.0	2.4 ± 0.2	2.5 ± 0.1
	16:1ω9	0.8 ± 0.2	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.0
	16:1ω7	0.4 ± 0.2	0.6 ± 0.1	0.4 ± 0.1	0.6 ± 0.0	0.7 ± 0.0
	16:1ω5	0.4 ± 0.2	0.7 ± 0.2	0.4 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
	i17:0	1.7 ± 0.5	2.0 ± 0.0	1.8 ± 0.1	1.6 ± 0.1	1.9 ± 0.2
	16:2ω6	2.6 ± 1.5	4.0 ± 0.2	4.0 ± 0.3	3.8 ± 0.2	3.8 ± 0.2
	16:3ω4	0.1 ± 0.0	0.4 ± 0.1	0.7 ± 0.4	0.5 ± 0.2	0.6 ± 0.1
	16:4ω3	6.4 ± 0.5	5.6 ± 0.6	5.9 ± 0.4	5.6 ± 0.6	5.6 ± 0.3
	18:0	7.4 ± 1.0	4.7 ± 0.8	5.7 ± 0.3	6.2 ± 0.6	5.7 ± 0.4
	18:1ω9	1.3 ± 0.5	0.9 ± 0.0	0.8 ± 0.0	1.3 ± 0.2	1.5 ± 0.1
	18:1ω7	1.3 ± 0.4	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	18:1ω5	6.3 ± 0.8	6.8 ± 0.1	6.4 ± 0.1	6.4 ± 0.3	5.9 ± 0.1
	18:2ω6	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.8 ± 0.0	1.0 ± 0.1
	18:3ω3	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

Fatty acid	Pre-trial (%)	Control (%)	Inorganic (%)	Low waste (%)	High waste (%)
20:1ω9	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:1ω7	2.4 ± 0.8	1.7 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0
20:4ω6	3.0 ± 1.0	4.5 ± 0.2	4.7 ± 0.2	4.9 ± 0.2	5.5 ± 0.3
20:4ω3	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:5ω3	25.0 ± 0.6	24.7 ± 0.2	24.6 ± 0.5	24.1 ± 0.8	23.3 ± 0.1
22:1ω9	7.9 ± 0.9	9.0 ± 0.3	9.2 ± 0.4	8.9 ± 0.5	8.5 ± 0.3
22:2NMIDa	1.3 ± 0.5	1.8 ± 0.1	1.7 ± 0.0	1.8 ± 0.1	1.8 ± 0.0
22:4ω6	7.9 ± 1.8	10.3 ± 0.3	10.3 ± 0.1	9.8 ± 0.5	10.0 ± 0.3
22:5ω3	8.4 ± 1.7	6.4 ± 0.2	6.8 ± 0.2	6.2 ± 0.2	6.1 ± 0.1
22:6ω3	1.7 ± 0.6	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.4 ± 0.1
ΣBacterial	4.0 ± 0.5	5.0 ± 0.4	4.2 ± 0.4	4.3 ± 0.4	4.8 ± 0.2
ΣSat	13.6 ± 2.3	8.6 ± 0.8	9.7 ± 0.3	10.2 ± 0.7	10.0 ± 0.4
ΣMUFA	22.8 ± 1.9	24.5 ± 0.5	23.0 ± 0.5	24.0 ± 0.8	23.7 ± 0.3
ΣPUFA	60.6 ± 3.5	63.0 ± 0.1	64.1 ± 0.4	62.5 ± 1.0	62.6 ± 0.5
P/S	4.6 ± 1.1	7.8 ± 0.9	7.0 ± 0.3	6.4 ± 0.5	6.7 ± 0.5
Σ ω3	42.1 ± 1.3	38.6 ± 0.5	39.4 ± 0.9	37.9 ± 1.6	37.1 ± 0.1
Σ ω6	14.8 ± 4.0	20.1 ± 0.6	20.2 ± 0.4	20.3 ± 0.5	21.1 ± 0.6
ω6/ω3	0.4 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
DHA/EPA	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0

2.9 Figures

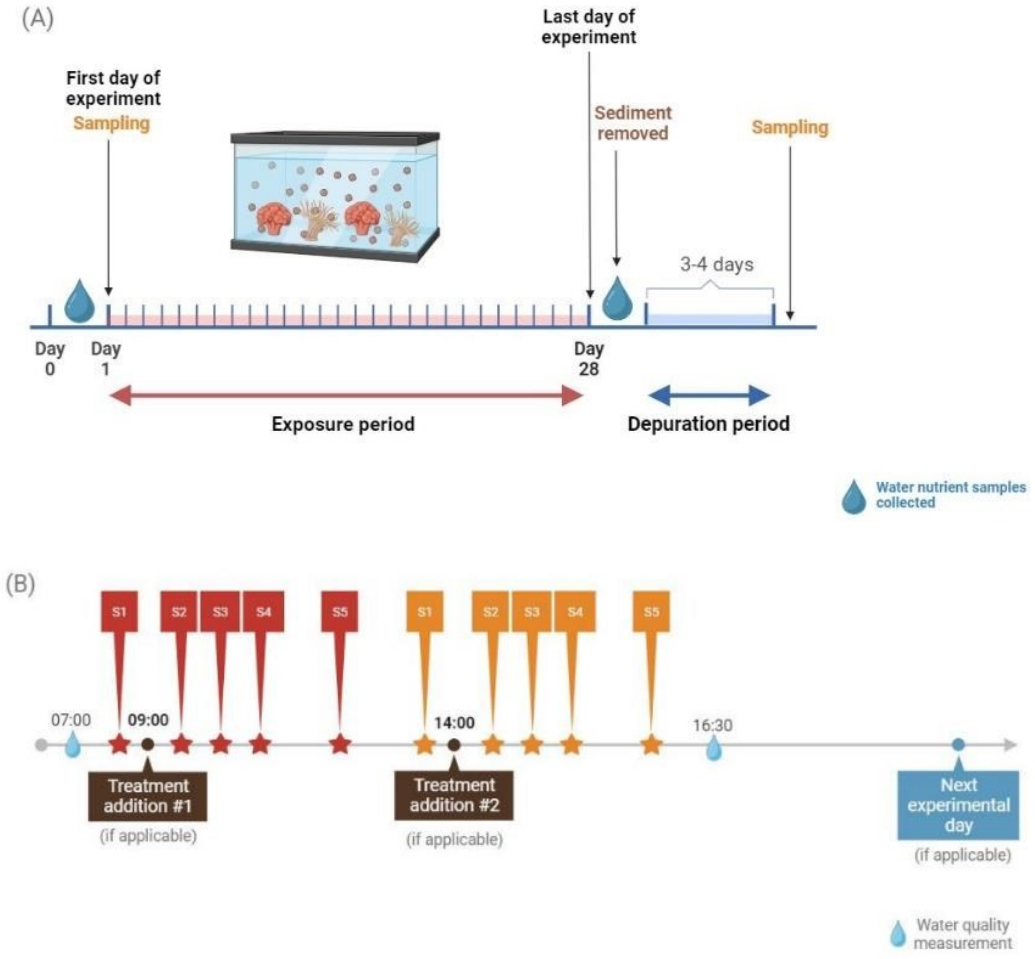


Figure 2-1: (A) Timeline of experimental period. "Day 0" denotes the day prior to experimental onset. "Sampling" reference corals and sea anemones were preserved for biochemical analyses. "Exposure period" corresponds to the 28 days over which treatments were applied to experimental tanks. "Depuration period" represents the 3-4 days after sediment was removed from tank bottoms and animals were measured and preserved for biochemical analyses. (B) Timeline of an experimental day. Treatments were added twice daily (09:00 and 14:00). Behavioural scores were assigned to each animal prior to each treatment addition (S1), then 20 min (S2), 40 min (S3), 1 h (S4) and 2 h (S5) after. Water drops denote the approximate onset of water quality measurements. Created in BioRender.com

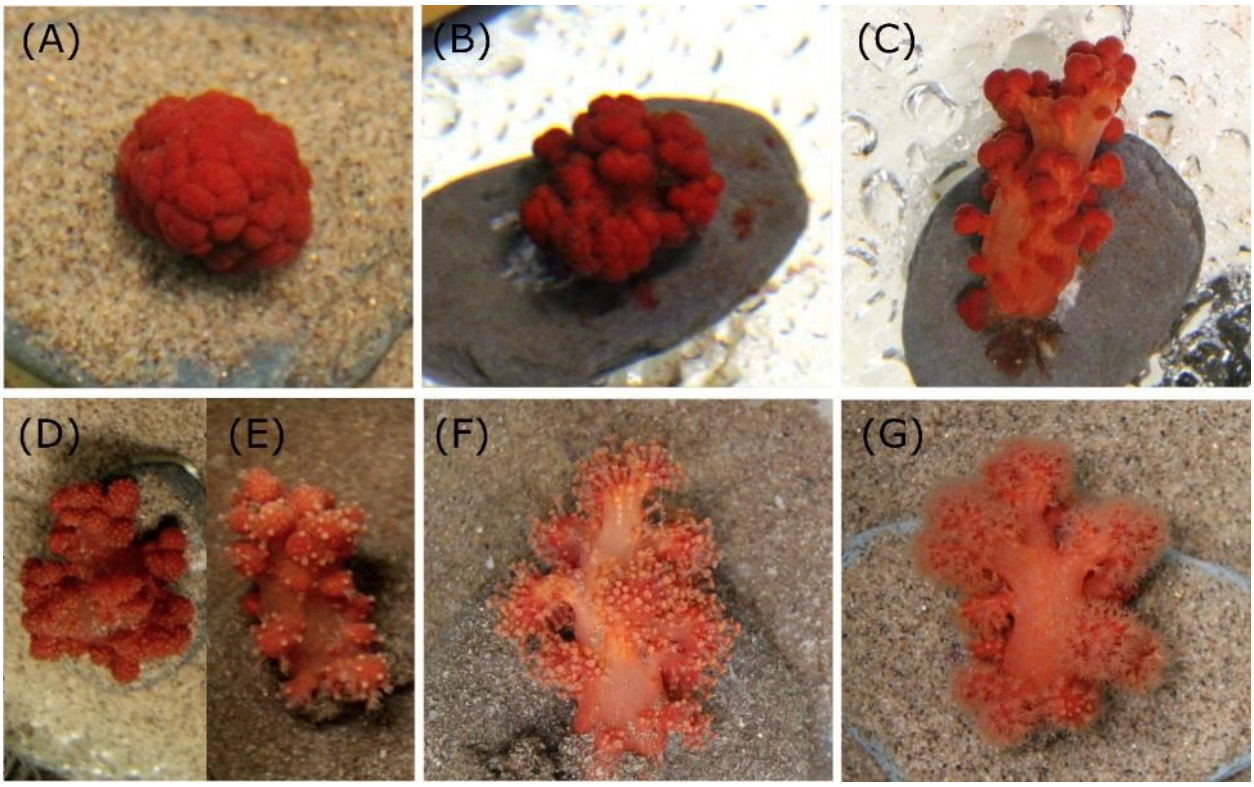


Figure 2-2: Behaviours of *Gersemia rubiformis* based on branch and polyp deployment, corresponding to behavioural scores 1-6 in Table 2-1. (A) Score 1; (B) score 2; (C) score 3; (D-E) score 4; (F) score 5; (G) score 6.

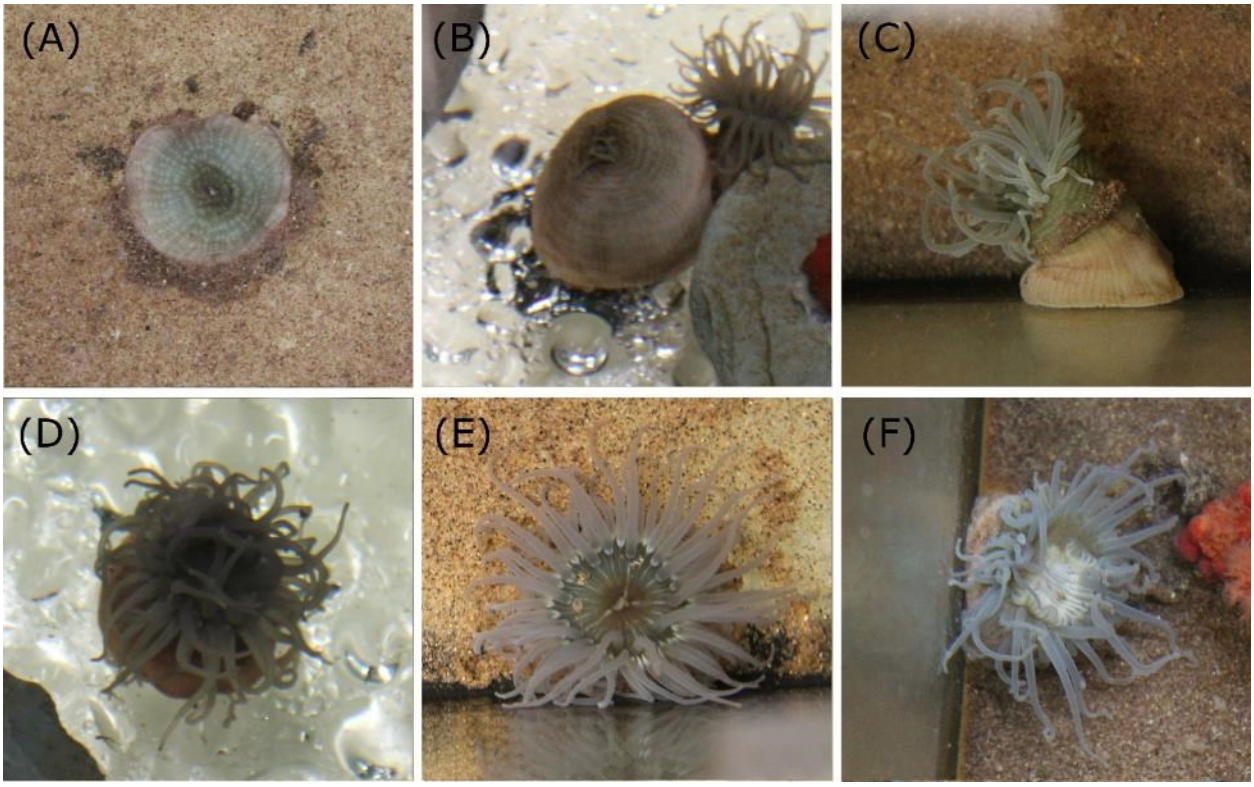


Figure 2-3: Behaviours of *Aulactinia stella* based on column extension and tentacle deployment, corresponding to behavioural scores 1-5 in Table 2-2. (A) Score 1; (B) score 2; (C) score 3; (D) score 4; (E) score 5; (F) an example of pharynx eversion.

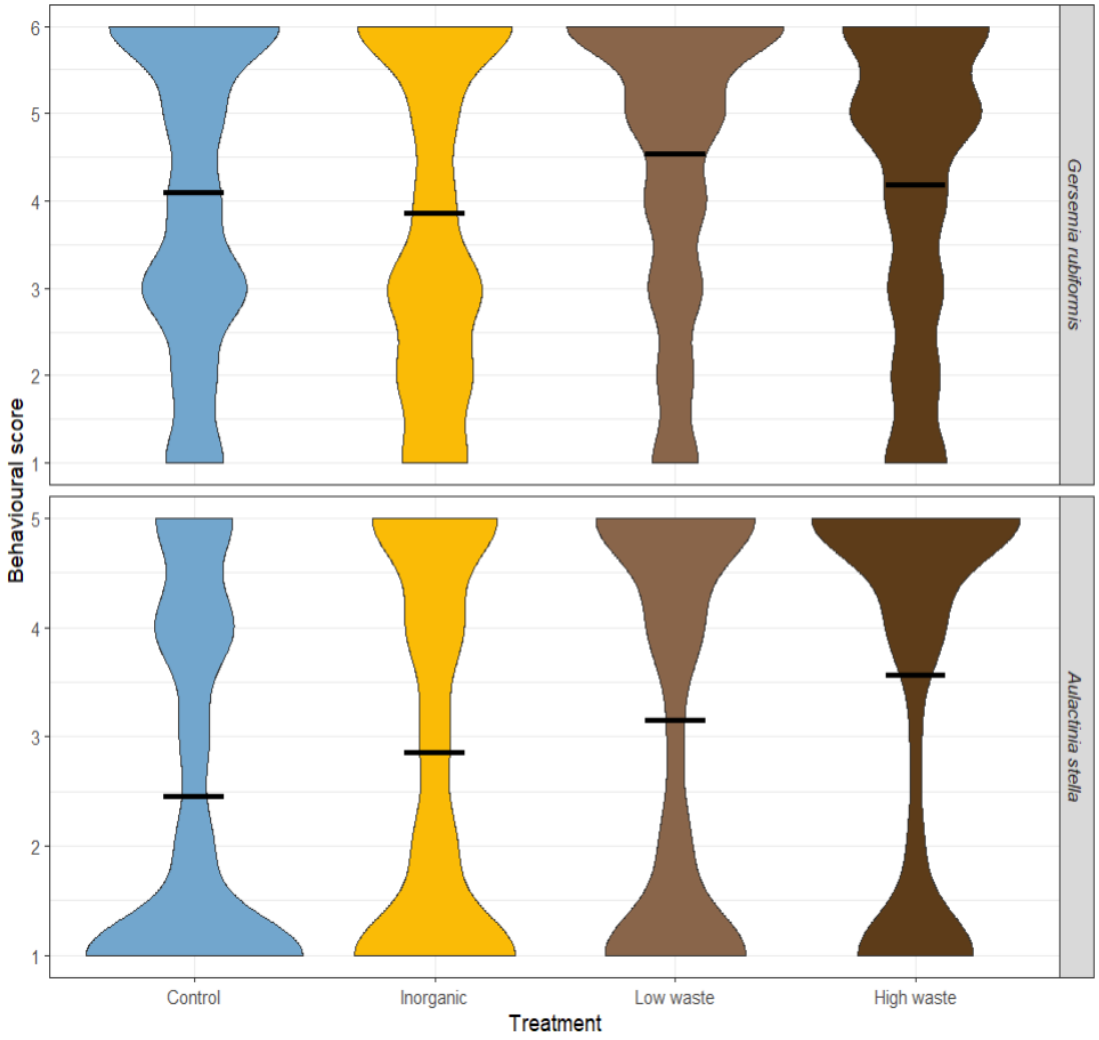


Figure 2-4: Behavioural scores of *Gersemia rubiformis* and *Aulactinia stella* measured over 28 d under low-waste (n = 15 colonies/individuals), high-waste (n = 15 colonies/individuals), inorganic sedimentation (n = 15 colonies/individuals) or control conditions (n = 15 colonies/individuals), with increasing level of column extension and/or polyp deployment (see scores in Table 2-1/Figure 2-2 for *G. rubiformis* and Table 2-2 and Figure 2-3 for *A. stella*). Behaviour was scored 20 min, 40 min, 1 h and 2 h after twice-daily with the treatment additions. Plots are distributions of unaveraged scores at every timepoint, treatment mean over the whole experimental period is indicated by black horizontal bars.

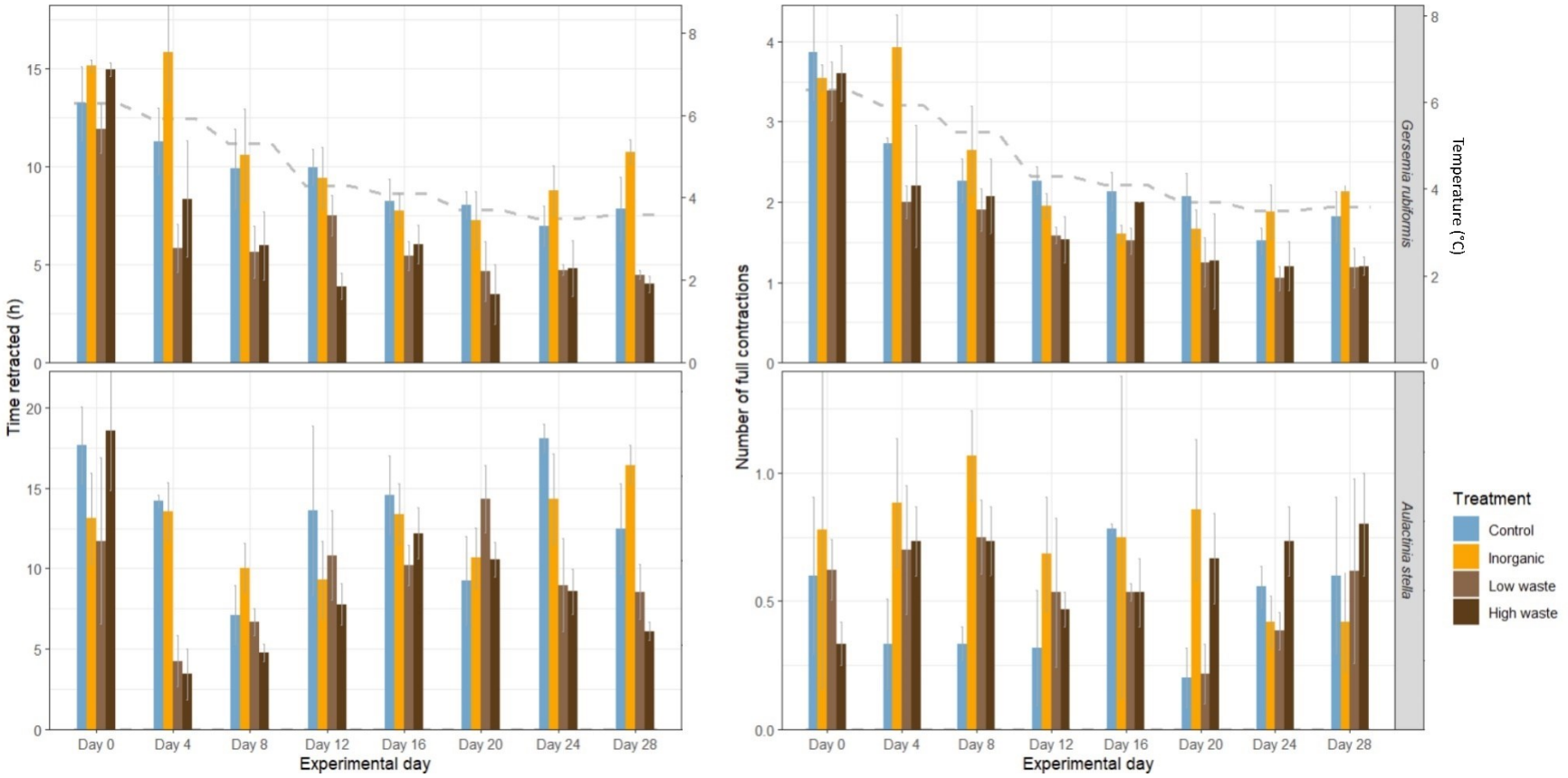


Figure 2-5: Time spent with all polyps/tentacles retracted and frequency of full contractions for *Gersemia rubiformis* (top) and *Aulactinia stella* (bottom) over 28 d of exposure to low-waste, high-waste, inorganic sedimentation or control conditions. Data shown as mean ± standard error (n = 3 tanks per treatment). Dashed line and secondary (right) y-axis represent average seawater temperature.

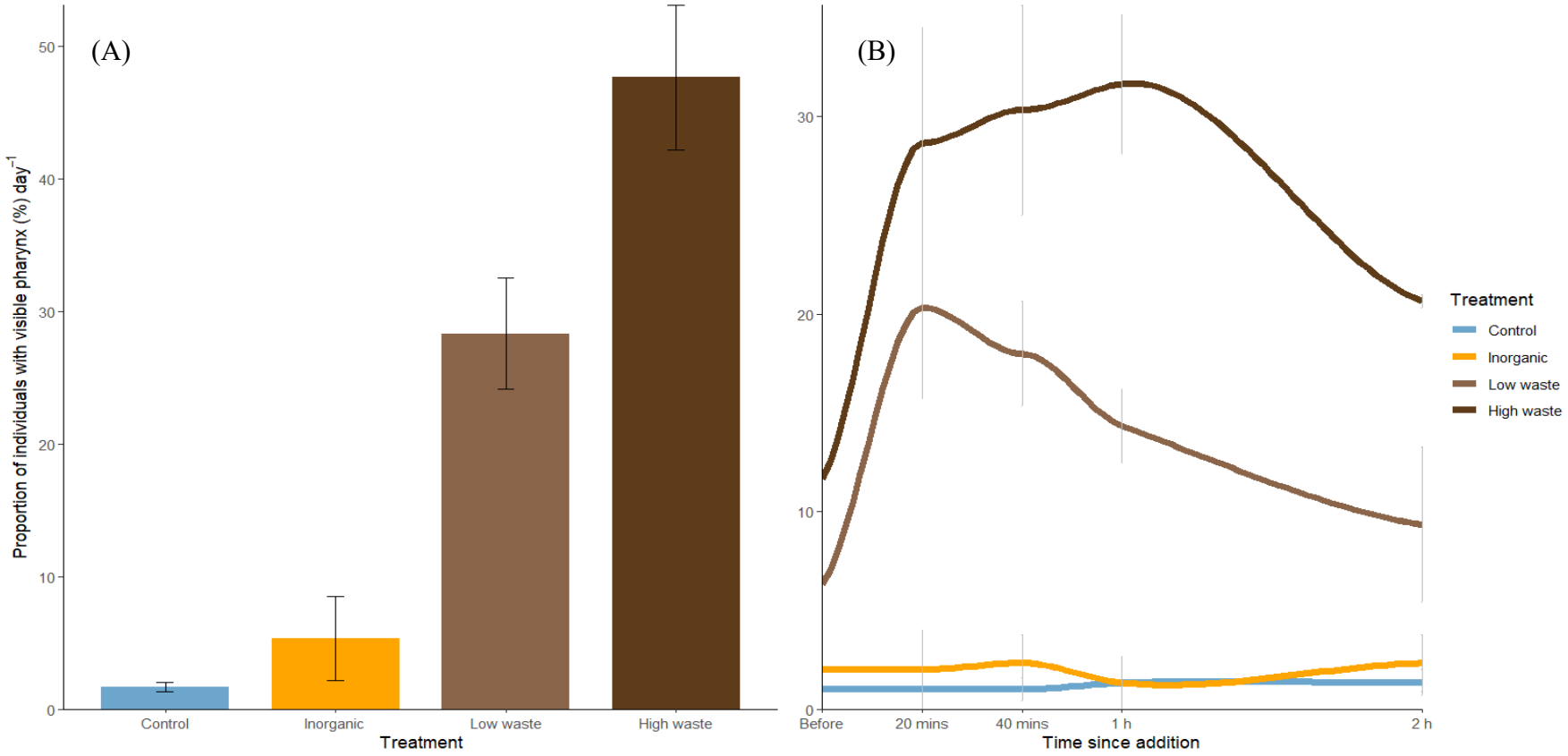


Figure 2-6: Proportion of individuals of *Aulactinia stella* showing visible pharynx (%) over 28 d of exposure under low-waste, high-waste, inorganic sedimentation or control conditions (A) per day and (B) relative to addition of treatment to tank (20 min, 40 min, 1 h or 2 h after). "Before" indicates time point scored prior to each treatment addition (twice a day). Data shown as mean ± standard error (n = 3 tanks per treatment).

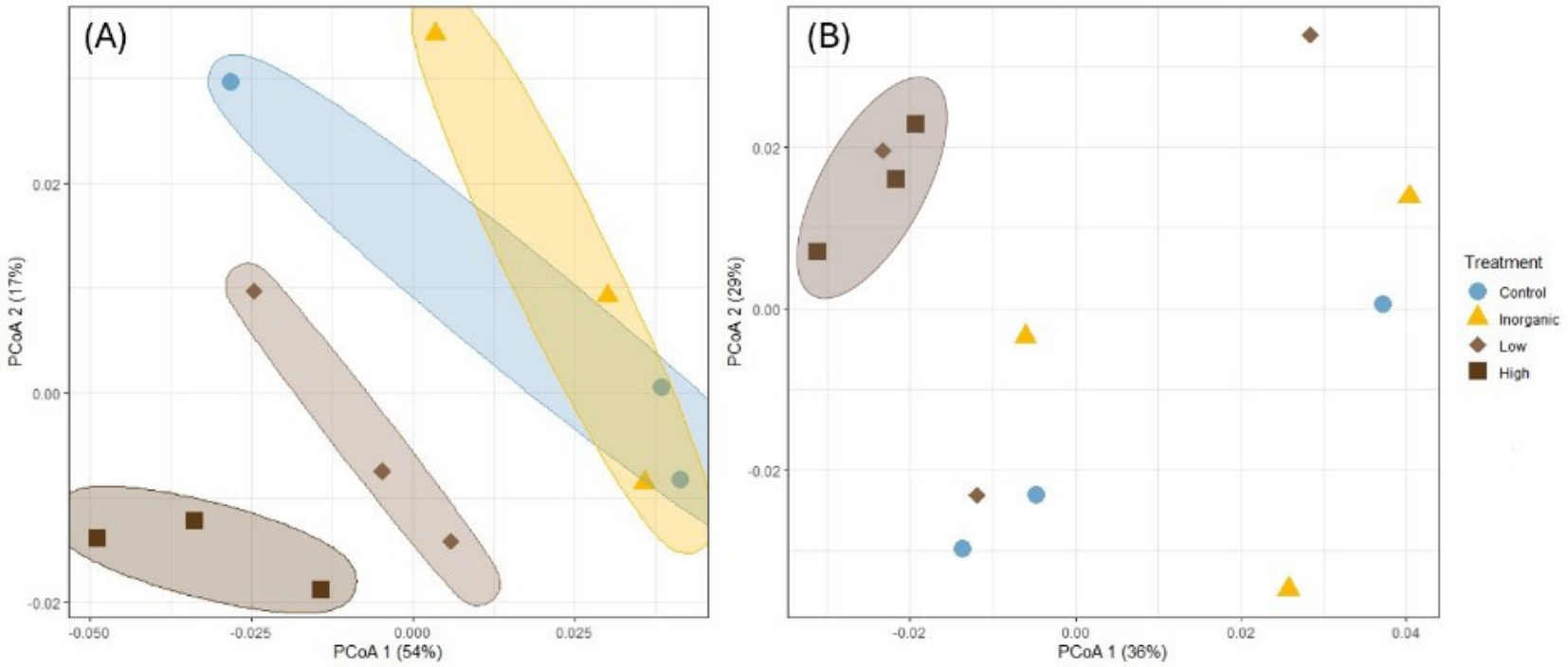


Figure 2-7: Principal coordinate analyses (PCoA) of the mean fatty acid profile of (A) *Gersemia rubiformis* and (B) *Aulactinia stella* after 28 d of exposure to low salmon waste (Low), high salmon waste (High), inorganic sedimentation or control conditions, (n = 3 tanks). Dots represent tanks (n = 3 per treatment), ellipses highlight clustering.

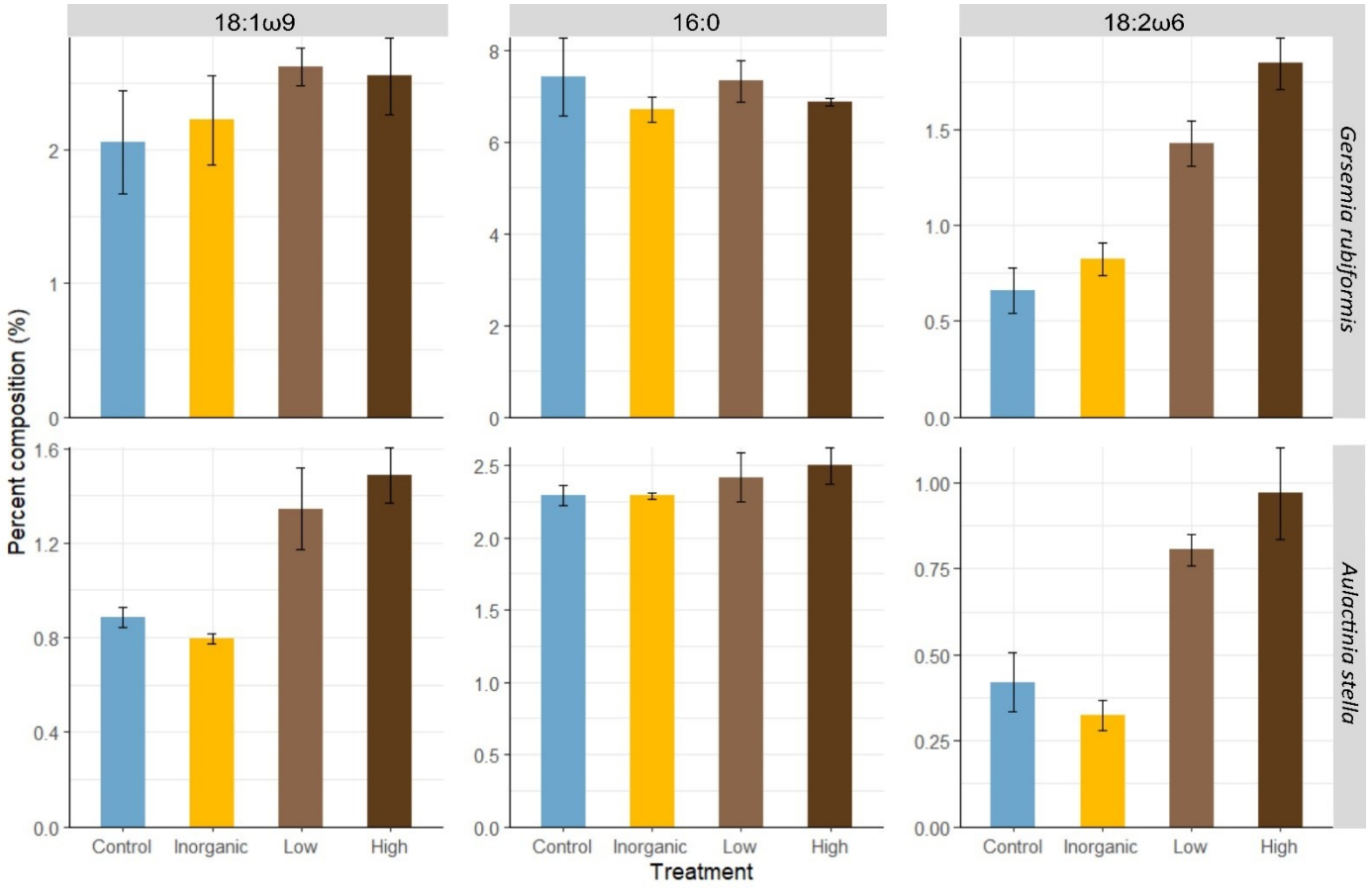


Figure 2-8: Percent (%) fatty acid composition (by weight) attributable to tracers of salmon waste (18:1ω9, 16:0 and 18:2ω6) in tissue samples of *Gersemia rubiformis* and *Aulactinia stella* after 28 d of exposure to low-waste (Low), high-waste (High), inorganic sedimentation or control conditions. Data shown as mean ± standard error (n = 3 tanks)

2.10 Supplementary materials

2.10.1 Supplementary Tables

Supplementary Table S2-1: Models selected for statistical analyses.

Model ID	Model
1	√ (Time spent retracted) ~ treatment + day + (1 | tank ID) + (1 | animal ID)
2	Full contraction frequency ~ treatment + day + (1 | tank ID) + (1 | animal ID)
3	Pharynx presence ~ treatment + day + time + (1 | day/time) + (1 | tank ID)

"day**"** = the number of days since experimental onset
"time" = identifying first or second treatment addition of the experimental day
"tank ID" = unique codes identifying each tank.
"animal ID" = unique codes identifying each coral or each sea anemone

Supplementary Table S2-2: Lipid composition (%) of fish waste used in treatments sampled on the first (Day 1) and last day (Day 28) of the experiment. Values are mean ± sd.

Lipids	Day 1 (%)	Day 28 (%)
Hydrocarbons	0.2 ± 0.3	0.3 ± 0.3
Steryl esters/wax esters	0 ± 0.0	0.5 ± 0.9
Ethyl esters/methyl esters	0.2 ± 0.4	0.3 ± 0.6
Ethyl ketones	1.4 ± 0.4	0.5 ± 0.4
Glyceryl ethers	0.0 ± 0.0	0.0 ± 0.0
Triacylglycerols	40.1 ± 4.5	20.3 ± 4.0
Free fatty acids	46.0 ± 2.5	56.7 ± 3.4
Alcohols	0.4 ± 0.7	0.0 ± 0.0
Sterols	4.6 ± 0.5	9.5 ± 2.7
Acetone mobile polar lipids	4.4 ± 4.1	5.3 ± 2.5
Phospholipids	2.7 ± 3.0	6.5 ± 4.0

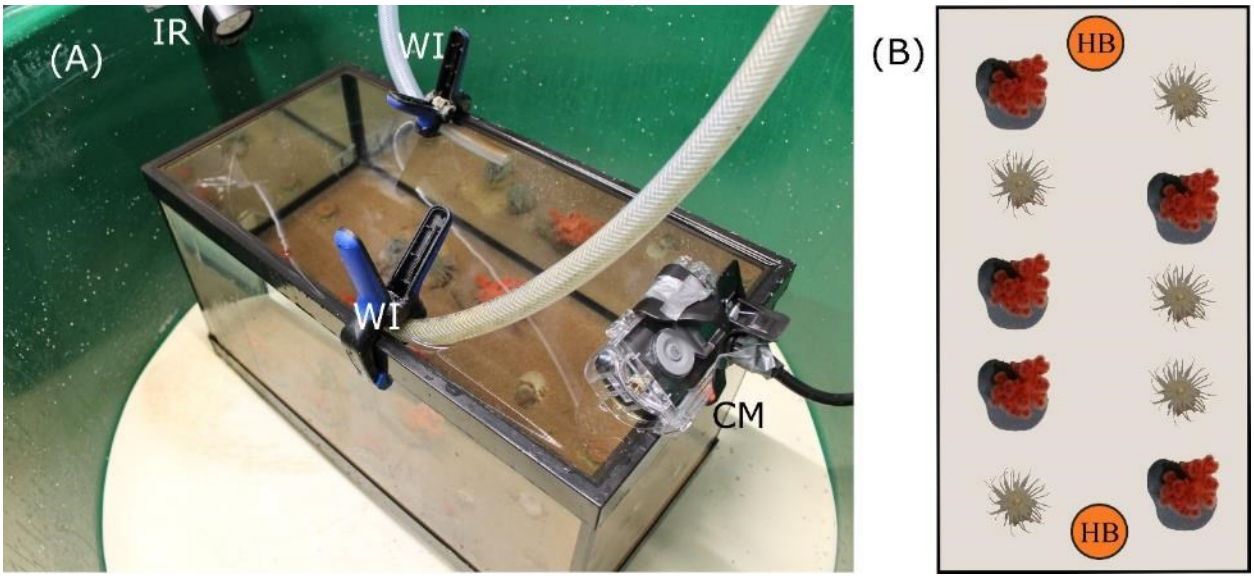
Supplementary Table S2-3: Fatty acid percent composition of the salmon waste added to tanks; sampled on the first ("Day 1") and last day ("Day 28") of the experiment. Values are mean ± sd. ΣBacterial = the sum of fatty acids associated with bacteria. ΣSAT = sum of saturated fatty acids. PUFA = polyunsaturated fatty acids. MUFA = Monounsaturated fatty acids. ΣEPA = eicosapentaenoic acid; (20:5ω3). ΣDHA = docosahexaenoic acid (22:6ω3).

Fatty acid	Day 1 (%)	Day 28 (%)
14:0	3.8 ± 0.1	3.7 ± 0.1
14:1	0.0 ± 0.0	0.0 ± 0.0
ai16:0	0.0 ± 0.0	0.0 ± 0.0
16:0	16.3 ± 0.2	16.7 ± 0.3
16:1ω9	0.2 ± 0.0	0.2 ± 0.0
16:1ω7	5.2 ± 0.0	5.2 ± 0.1
16:1ω5	0.1 ± 0.0	0.1 ± 0.0
i17:0	0.1 ± 0.0	0.1 ± 0.0
16:2ω6	0.7 ± 0.0	0.7 ± 0.0
16:3ω4	0.7 ± 0.0	0.6 ± 0.1
16:4ω3	0.0 ± 0.0	0.0 ± 0.0
18:0	4.5 ± 0.1	4.7 ± 0.1
18:1ω9	33.2 ± 0.3	33.7 ± 0.5
18:1ω7	2.9 ± 0.0	3.0 ± 0.0
18:1ω5	0.0 ± 0.0	0.0 ± 0.0
18:2ω6	14.2 ± 0.1	13.8 ± 0.2
18:3ω3	3.5 ± 0.1	3.3 ± 0.1
20:1ω9	1.1 ± 0.0	1.2 ± 0.0
20:1ω7	0.1 ± 0.0	0.1 ± 0.0
20:4ω6	0.5 ± 0.1	0.4 ± 0.1
20:4ω3	0.3 ± 0.0	0.3 ± 0.0
20:5ω3	4.9 ± 0.2	4.6 ± 0.1
22:1ω9	0.0 ± 0.0	0.0 ± 0.1
22:2NMIDa	0.0 ± 0.0	0.0 ± 0.0
22:4ω6	0.0 ± 0.0	0.0 ± 0.0
22:5ω3	0.8 ± 0.0	0.8 ± 0.0
22:6ω3	2.8 ± 0.1	2.7 ± 0.1
ΣBacterial	0.8 ± 0.0	0.8 ± 0.1
ΣSat	25.9 ± 0.4	26.5 ± 0.1
ΣMUFA	43.6 ± 0.4	44.5 ± 0.4
ΣPUFA	30.2 ± 0.5	28.7 ± 0.5
P/S	1.2 ± 0.0	1.1 ± 0.0
Σ ω3	13.3 ± 0.5	12.3 ± 0.3
Σ ω6	15.6 ± 0.1	15.2 ± 0.1
ω6/ω3	1.2 ± 0.0	1.2 ± 0.0
DHA/EPA	0.6 ± 0.0	0.6 ± 0.0

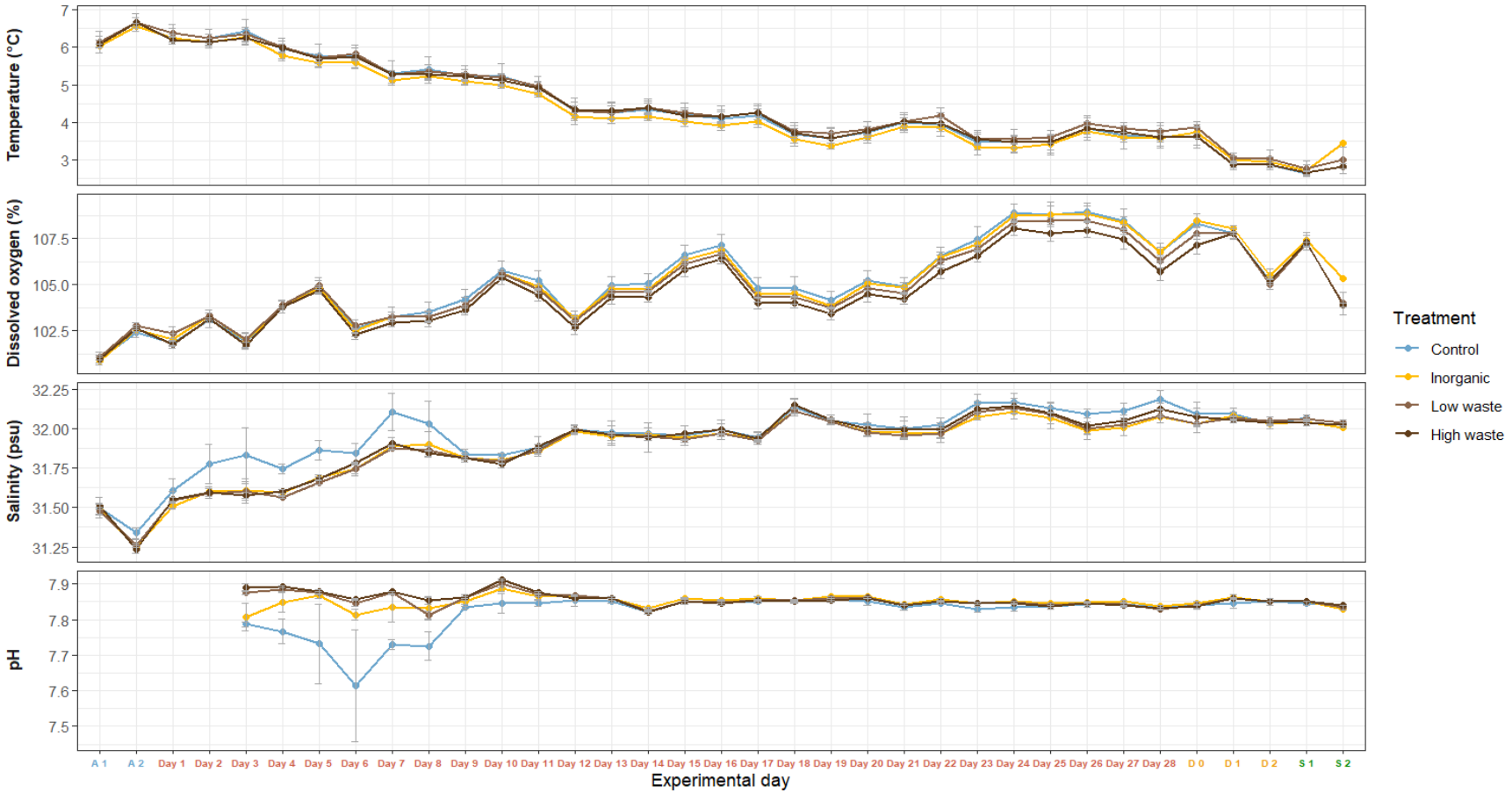
Supplementary Table S2-4: Percent (%) composition of major bacteria taxon (>2% in at least one sample) present in waste/mat samples form low-waste (LW) and high-waste (HW) tanks, as well as from Day 1 bulk waste (SWB) and Day 28 bulk waste (SWA). Data shown as mean ± standard deviation (n = 3 tanks).

Identification	LW (%)	SD (± %)	HW (%)	SD (± %)	SW B (%)	SD (± %)	SW A (%)	SD (± %)
d__Bacteria;p__Firmicutes;c__Bacilli;o__Mycoplasmatales;f__Mycoplasmataceae;g__Mycoplasma;s__uncultured_Mycoplasma	0.1	0.1	1.0	0.1	78.5	5.0	37.7	13.5
d__Bacteria;p__Campilobacterota;c__Campylobacteria;o__Campylobacterales;f__Arcobacteraceae;g__uncultured;s__Arcobacter_sp.	3.6	4.5	28.1	7.5	0.0	0.0	0.0	0.0
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Tenacibaculum;s__Tenacibaculum_ovolyticum	13.5	8.7	1.2	0.2	0.5	0.5	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Colwelliaceae;g__Colwellia;__	13.0	3.6	2.9	1.3	0.5	0.6	0.2	0.3
d__Bacteria;p__Campilobacterota;c__Campylobacteria;o__Campylobacterales;f__Arcobacteraceae;g__uncultured;__	2.9	2.9	9.0	6.5	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Photobacterium;s__Photobacterium_phosphoreum	0.0	0.0	0.0	0.0	7.7	2.2	0.0	0.0
d__Bacteria;p__Campilobacterota;c__Campylobacteria;o__Campylobacterales;f__Arcobacteraceae;g__Halarcobacter;__	3.3	2.9	7.5	3.8	0.0	0.0	0.0	0.0
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__Marinifilum;__	1.2	0.7	6.8	3.1	0.0	0.0	0.0	0.0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Fusibacteraceae;g__Fusibacter;s__bacterium_WH1-8	4.4	2.0	6.5	6.4	0.0	0.0	0.0	0.0
d__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_curvatus	0.0	0.0	0.0	0.0	0.1	0.2	18.8	6.3
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Fusibacteraceae;g__Fusibacter;s__uncultured_low	5.2	7.5	1.2	0.8	0.0	0.0	0.0	0.0
d__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Fusobacterium;s__Fusobacteriaceae_bacterium	0.1	0.1	0.5	0.5	0.1	0.0	13.3	4.7
d__Bacteria;p__Desulfobacterota;c__Desulfobulbia;o__Desulfobulbales;f__Desulfocapsaceae;g__Desulfotalea;s__uncultured_delta	1.1	0.9	4.4	0.6	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Psychromonadaceae;g__Psychromonas;__	0.7	1.1	4.0	1.2	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Sulfitobacter;__	0.5	0.5	0.0	0.0	3.3	4.1	0.3	0.5
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Fusibacteraceae;g__Fusibacter;__	3.0	3.3	2.0	0.1	0.0	0.0	0.0	0.0
d__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Psychrilyobacter;s__uncultured_bacterium	0.2	0.3	0.0	0.1	0.0	0.0	1.5	2.7
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Pseudofulvibacter;s__Flavobacteriaceae_bacterium	2.6	2.3	0.5	0.5	0.0	0.0	0.0	0.0
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Tenacibaculum;__	2.3	2.1	0.5	0.5	0.1	0.1	0.0	0.0
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Polaribacter;__	2.0	1.4	1.0	1.0	0.1	0.2	0.0	0.0
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Pseudofulvibacter;__	1.9	1.8	0.4	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Desulfobacterota;c__Desulfobulbia;o__Desulfobulbales;f__Desulfocapsaceae;g__Desulforhopalus;s__uncultured_delta	0.6	0.6	1.9	0.3	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Arenicellales;f__Arenicellaceae;g__Arenicella;s__uncultured_bacterium	1.2	1.9	0.1	0.1	1.7	2.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;s__Sphingomonas_echinoides	0.3	0.2	0.0	0.0	0.4	0.1	1.7	1.7
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__Marinifilum;s__uncultured_marine	0.3	0.5	1.7	0.1	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae;g__Paraglaciecola;__	1.6	1.8	0.2	0.1	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Cellvibrionaceae;g__uncultured;__	1.6	0.7	0.6	0.1	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oleiphilaceae;g__Oleiphilus;s__uncultured_gamma	1.6	2.3	0.0	0.0	0.0	0.0	0.1	0.2
d__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_sakei	0.0	0.1	0.0	0.0	0.0	0.0	17.4	1.5
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Lutibacter;s__uncultured_bacterium	0.5	0.8	1.4	0.5	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium;__	0.0	0.0	0.1	0.1	0.0	0.1	1.1	1.3
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Tenacibaculum;s__Tenacibaculum_haliotis	0.1	0.2	0.0	0.0	1.3	1.9	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Saccharospirillaceae;g__Oleispira;__	1.3	0.7	1.0	0.5	0.0	0.0	0.0	0.0
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__Marinifilum;s__uncultured_Bacteroidetes	0.3	0.4	1.3	0.6	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Psychromonadaceae;g__Psychromonas;s__Psychromonas_arctica	0.8	1.1	1.2	1.2	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Nitrincolaceae;g__Neptunomonas;__	1.1	1.0	0.4	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Myxococcota;c__Polyangia;o__Haliangiales;f__Haliangiaceae;g__Haliangium;s__uncultured_deep-sea	1.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Cellvibrionaceae;g__Marinagarivorans;s__gamma_proteobacterium	1.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;s__Sphingomonas_melonis	0.3	0.2	0.1	0.1	0.1	0.2	2.1	1.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Saccharospirillaceae;g__Oleispira;s__uncultured_bacterium	1.0	0.4	0.3	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Fusibacteraceae;g__Fusibacter;s__uncultured_bacterium	0.8	0.5	0.1	0.1	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Colwelliaceae;g__Colwellia;s__uncultured_Nitrospinaceae	0.8	0.9	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Colwelliaceae;g__Colwellia;s__Colwellia_psychrerythraea	0.5	0.8	0.7	0.9	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Gammaproteobacteria_Incertae_Sedis;f__Unknown_Family;g__Alkalimarinus;s__uncultured_bacterium	0.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Psychromonadaceae;g__Agarivorans;s__Agarivorans_litoreus	0.7	0.6	0.0	0.1	0.0	0.0	0.0	0.0
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Cryomorphaceae;g__uncultured;s__uncultured_bacterium	0.6	0.6	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Gammaproteobacteria_Incertae_Sedis;f__Unknown_Family;g__Unknown_Family;s__uncultured_bacterium	0.6	0.6	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Rubritaleaceae;g__Rubritalea;s__uncultured_bacterium	0.0	0.1	0.0	0.0	0.6	0.2	0.2	0.4
d__Bacteria;p__Firmicutes;c__Negativicutes;o__Acidaminococcales;f__Acidaminococcaceae;g__uncultured;s__uncultured_bacterium	0.0	0.0	0.0	0.0	0.6	0.1	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Cellvibrionaceae;g__uncultured;s__uncultured_bacterium	0.5	0.9	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__Methylobacterium-Methylorubrum;__	0.2	0.3	0.0	0.0	0.2	0.0	0.5	0.5
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Thiotrichaceae;g__Leucothrix;s__uncultured_bacterium	0.5	0.5	0.1	0.0	0.0	0.1	0.0	0.0
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Saprospiraceae;g__Lewinella;s__uncultured_bacterium	0.4	0.7	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Colwelliaceae;g__Thalassotalea;s__uncultured_bacterium	0.4	0.5	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Nitrincolaceae;g__Profundimonas;__	0.4	0.5	0.2	0.2	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Spongiibacteraceae;g__Dasania;s__uncultured_bacterium	0.4	0.6	0.0	0.0	0.1	0.1	0.2	0.4
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Polaribacter;s__Polaribacter_lacunae	0.4	0.5	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Bradyrhizobium;__	0.1	0.2	0.0	0.0	0.1	0.1	1.1	0.4
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Pseudofulvibacter;s__uncultured_bacterium	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Halocynthiibacter;s__Halocynthiibacter_namhaensis	0.1	0.2	0.0	0.0	0.1	0.2	0.0	0.0
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Yoonia-Loktanella;__	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
d__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Psychrilyobacter;__	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

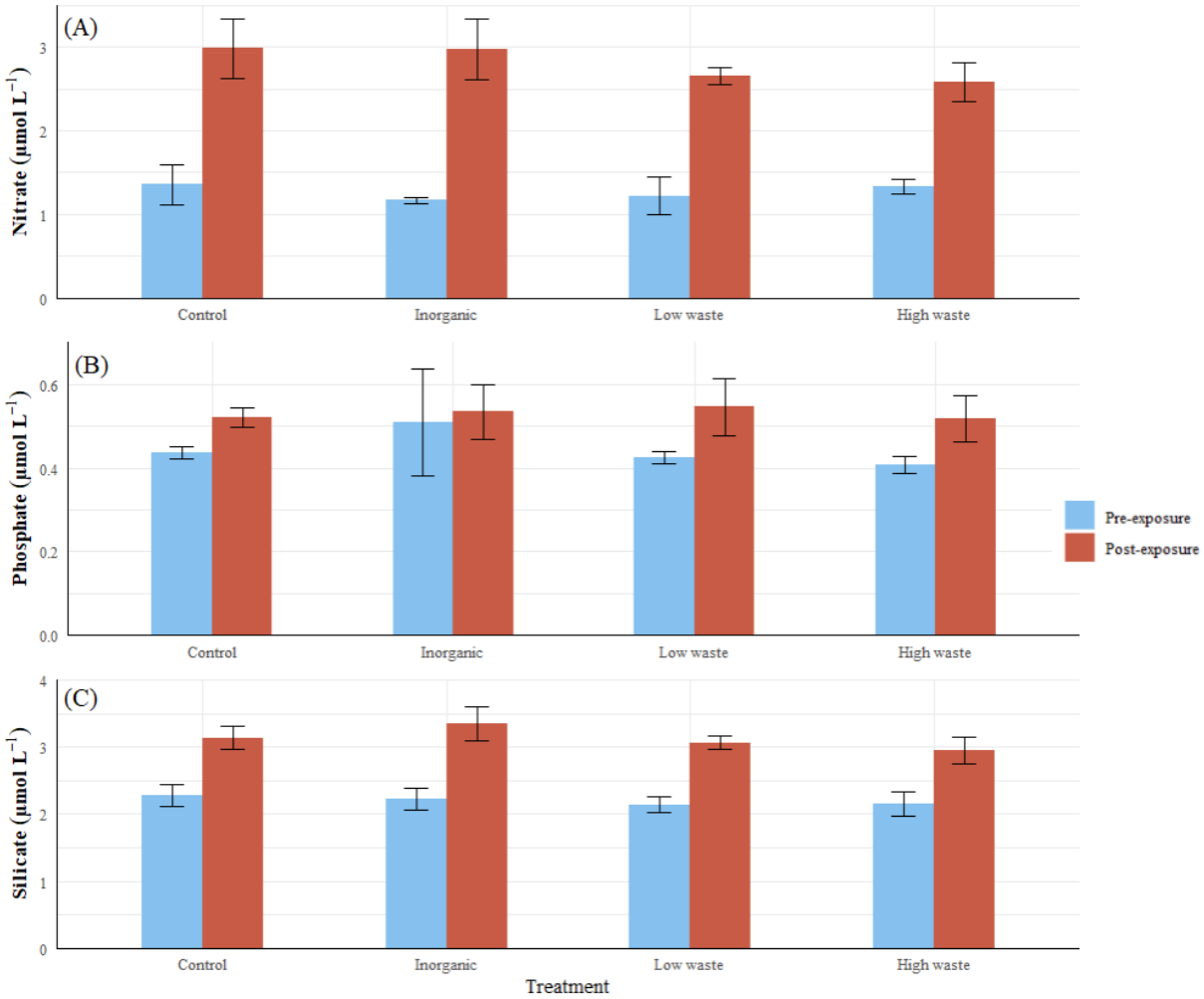
2.10.2 Supplementary Figures



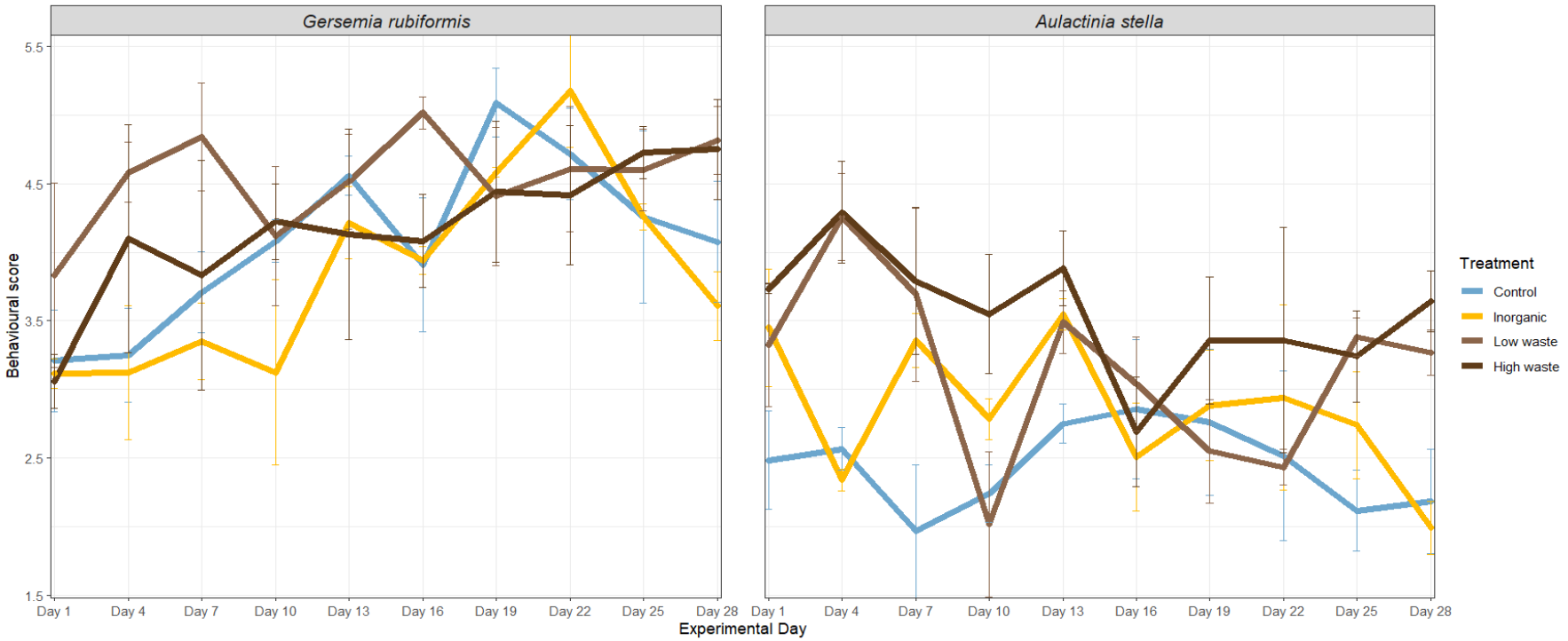
Supplementary Figure S2-1: Experimental tank set-up. (A) Exterior of tank (61 x 30.5 x 30.5 cm) depicting the camera mounted in water-resistant housing (CM), two water inflows (WI), and an infrared light mounted above the tank (IR). (B) Schematic example of randomized placement of five *Gersemia rubiformis* and five *Aulactinia stella* within tanks, along with two Hobo temperature loggers (HB).



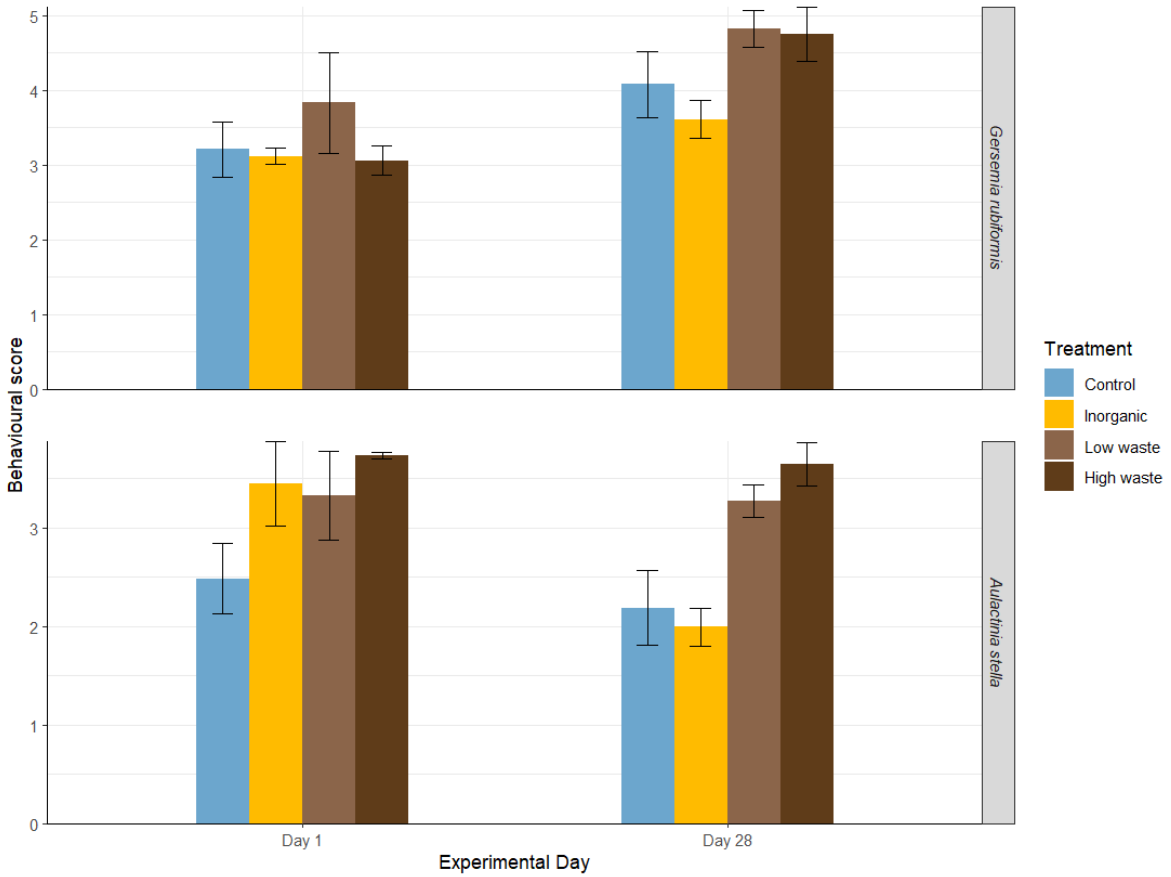
Supplementary Figure S2-2: Water quality parameters (temperature, dissolved oxygen, salinity, pH) measured across control, inorganic, low-waste and high-waste treatment tanks. Parameters were measured twice daily during two days of acclimatization (A1, A2), the exposure period (Day 1–Day 28), the depuration period (D0 - D2), and sampling days (S1, S2). Data shown as mean ± standard deviation (n = 3 tanks per treatment). Discrepancies between the control and treatment tanks between A1 and Day 9 were attributed to insufficient instrument warm-up time which was remediated on Day 10 onwards.



Supplementary Figure S2-3: Water nutrients in control, inorganic, low-waste and high-waste treatment tanks prior to any treatment additions (pre-exposure), after 28 d of additions (post-exposure). Data shown as mean ± standard deviation (n = 3 tanks per treatment)



Supplementary Figure S2-4: Behavioural scores of *Gersemia rubiformis* and *Aulactinia stella* on scales of 1 to 5 or 6, respectively (with increasing level of column extension and/or polyp deployment) measured during 28 d of exposure to low-waste, high-waste, inorganic sedimentation or control conditions. Behaviour scored 20 min, 40 min, 1 h and 2 h after bi-daily treatment additions was averaged. Data shown as overall treatment mean ± standard error (n = 3 tanks per treatment). Scores are described in Table 2-1/Figure 2-2 for *G. rubiformis* and Table 2-2/Figure 2-3 for *A. stella.*



Supplementary Figure S2-5: Behavioural scores of *Gersemia rubiformis* and *Aulactinia stella* on scales of 1 to 5 or 6, respectively (with increasing level of column extension and/or polyp deployment) measured on Day 1 and Day 28 of exposure to low-waste, high-waste, inorganic sedimentation or control conditions. Behaviour scored 20 min, 40 min, 1 h and 2 h after bi-daily treatment additions was averaged. Data shown as overall treatment mean ± standard error (n = 3 tanks per treatment). Scores are described in Table 2-1/Figure 2-2 for *G. rubiformis* and Table 2-2/Figure 2-3 for *A. stella.*

2.10.3 Supplementary Text

2.10.3.1 Data analyses details

Statistical analyses were conducted on raw, unaveraged data in R (version 4.2.2, R Core Team 2022) and Primer 7 with PERMANOVA+ (for PERMANOVAs only, Anderson et al. 2008, Clarke and Gorley 2015). Mixed effect models (using lme4 package, Bates et al. 2015) were used to account for the non-independence of observations. As individuals were nested within tanks, the non-independence of those kept within the same tank were considered by including tank as a random factor. As the time elapsed since experimental onset could have influenced the magnitude of treatment effects, the data was treated as repeated measures by including experimental day as a covariate. Tank was kept as a random factor, even if it did not explain a large amount of variance within the model, per recommendations on tank experiments from Cornwall and Hurd (2016). Model selection was aided by comparing AIC values using anova(). Model fit and assumption diagnostics (e.g. normality of residuals, homogeneity of variance, independence of residuals) were explored using the DHARMa package (Hartig 2022)as performed in studies with similar mixed model data (e.g. Bilan et al. 2023). The Anova() from the car package (Fox and Weisberg 2019) was used to determine the significance of the fixed effects, as the base anova() does not provide these. Pairwise comparisons between treatments were conducted using the multcomp package (Hothorn et al. 2008). Graphical representations were created using the ggplot2 package (Wickham 2016). A threshold of $p < 0.05$ was used to determine significant effects.

2.10.3.1.1 Behaviour

Behavioural scores are reported as means that were either averaged across timepoints, waste addition events, day and tank to give an overall daily treatment mean. Otherwise, scores

were averaged over timepoints, waste addition events and tank to visualize daily changes over the exposure period. Day 28 was specified for comparison as it was the best indicator of potential long-term effects. Mean daily scores were extracted from data measured after the addition of wastes; every 3 days of the experimental period. Behavioural scores were not statistically analyzed out of caution due to a lack of available model validation support for ordinal mixed effect models, and model misfit to other explored distributions.

Daily treatment means of the time spent retracted (h) and frequency of full contractions (contr d^{-1}) were compared across experimental days and treatments. Individuals of *G. rubiformis* were excluded from specific daily means if they were showing noticeable influence from interactions with *A. stella* (i.e. contracting as a result of a touching, colony damage). Individuals that moved outside the view of the camera were omitted whenever they could not be measured.

Time spent with polyps/tentacles retracted (h) was evaluated using linear mixed effects models (model 1, Supplementary Table S2-1). Retraction time was square-root transformed to meet normality assumptions. Experimental day was included as a covariate. Tank and coral ID (unique identifier for each coral) were included as random factors.

Generalized linear mixed effect models with Poisson distributions were used to determine the effect of treatment on full contraction frequency (model 2, Supplementary Table S2-1). The pre-trial baseline measurement was excluded from the models since treatments had not yet been applied at that timepoint.

For *A. stella*, the proportion of individuals within each tank showing visible pharynx was calculated per day, and reported as a daily treatment mean (% ind d^{-1}). Raw presence/absence data was analyzed using a generalized linear mixed effect model with a binomial distribution (model 3, Supplementary Table S2-1). Experimental day and time of day (AM or PM) were

included as fixed factors (time of day nested in day). Tank was included as a random factor. Sea anemone ID was not included as a random factor as observations made during the experiment did not confidently track the movement of each individual which often moved positions.

2.10.3.1.2 Lipid composition

Lipid content of coral and sea anemone tissue was standardized based on wet weight (mg g^{-1} WW). Lipid content of salmon waste preserved on Day 1 and Day 28 was reported as percent composition due to high water content. Within *G. rubiformis* and *A. stella*, lipid content was reported as a treatment mean to compare among treatments, as well as with pre-trial baseline individuals. Lipid compositions were compared among treatments, as well as between pre-trial baseline and experimental animals (with sample date as a fixed effect) using PERMANOVAS. Data was square-root transformed, Bray-Curtis distance measures were used and permutations set to 9999. Tank was included as a random factor nested within treatment.

2.10.3.1.3 Fatty acid composition

Fatty acids representing 1% of at least one sample were analyzed (Mercier et al. 2016, Salvo et al. 2018) to better detect slight changes in major fatty acids. One coral in the inorganic treatment was omitted as an influential outlier due to high concentrations (>12%) in fatty acids (18:1w9 and 22:4w6) that were less prevalent in all other colonies (<4%). Fatty acids for *G. rubiformis* and *A. stella* were reported as treatment means of percent compositions (%), which were compared among treatments. Fatty acid compositions were square-root transformed and statistically accessed using multivariate analyses with Bray-Curtis distance measures. Compositions were compared among treatments, as well as between pre-trial baseline and experimental animals (with sample date as a fixed effect) using PERMANOVAs (permutations set to 9999). Tank was included as a random factor nested within treatment. Fatty acids

compositions were statistically accessed using multivariate analyses of similarity (ANOSIM) of Bray-Curtis distance measures with permutations set to 9999, as well as graphically using a principal coordinate analysis (PCoA), guided by Couturier et al. (2020) and Bakker (2024). An ANOSIM (using the vegan package) was conducted both at an individual-level and at a tank-level (averaged across individuals within each tank) with treatment as a grouping factor to determine if the fatty acid profiles varied more within treatment (R = 1) than within group (R = 0).

2.10.3.1.4 Bacteria

Mean percent compositions (%) of identified bacteria was reported and compared among bulk salmon waste preserved at Day 1 and Day 28, as well as between bulk waste samples and samples of waste/microbial mats taken directly from the tanks. Kurskal-Wallis tests used to compare the alpha diversity between bulk waste and mat samples were conducted in QIIME 2 (Bolyen et al. 2019).

2.10.3.2 Planula release

On Day 18, planula (coral larvae) began appearing in the tanks. Planula release from *G. rubiformis* was recorded through opportunistic inspections of the tank. As larvae were hard to detect on the sand, counts were done after siphoning the material out from the tanks for the inorganic treatment. The opportunistic nature of the monitoring meant that not all planulae were likely recorded and reported values should be interpreted as minima.

Planulae were most often observed on the sediment/tank bottom; they were observed swimming in the tanks on a few occasions. At minimum, one planula was observed within each tank during the experimental period. Opportunistic visual observations of the tanks combined with post-experiment collection of larvae recorded a quantity of planula highest in one of the

low-waste tanks (50 planulae observed at once, range for all low-waste tanks: 2-50). Less planulae were recorded through visual observations (0-1) in inorganic tanks, but we believe that this was due to camouflage with the sand, since examination of sediment siphoned from these tanks yielded high amounts of planula (range of 3-30 in tanks). Control tanks ranged from 4-30 planulae and high-waste tanks had the lowest observed quantities during the experiment ranging from 2-17. Planulae release was highly variable within treatments, and methodology for counting was not consistent between treatments so ranges should be considered minimums. Due to the mobility of planulae it was not possible to discern which were the parent colonies.

To our knowledge, details of the planulation periodicity of *G. rubiformis* have not been published. A close relative, *G. fruticosa* (n=5), has been observed to release planulae in synchrony with the lunar cycle with the most released just after the full moon (observed over 3 lunar months, Mercier et al. 2011). The onset of planulae release during the present study occurred approximately 8 d after a full moon. Interestingly, planulae release in *G. rubiformis* has been observed by Henry et al. (2003) in response to mechanical disturbance (crushing). However, Henry et al. (2003) observed high mortality occurred in daughter colonies that propagated from prematurely-released planula. Observations of planulae did not appear to increase with waste concentration, but most planulae were observed in a low-waste treatment tank, and the inorganic treatment/control tanks. This may suggest that planulae release was stimulated under mild waste sedimentation, but less release occurred in colonies exposed to the high-waste concentration. Although observations could be biased as higher amounts of sedimentation in the high-waste treatment may have obscured visual counts of planulae in the tanks (i.e. if buried), further assessing the potential impact of waste concentration on larval release should be considered in a future study. As well, certain colonies may have been at a more

advanced larval production stage independent of the experiment, influencing which colonies had matured planulae to release. Both an indistinguishable trigger and exacerbation by mechanical disturbance (sedimentation) may have played a role in triggering the release of planulae.

2.10.3.3 Juvenile expulsion

In six instances, juvenile *A. stella* were expelled from the adults. This occurred three times during the acclimation period (with juveniles measuring 6, 8, 10 mm in pedal disc diameter) and three times during the exposure period (with juveniles measuring 3, 6, 7 mm in base diameter). Juvenile sea anemones were expelled once in a low-waste tank (6 mm pedal disk diameter) and twice in a high-waste tank (3 and 7 mm pedal disk diameter) during the exposure period. Only the two juveniles that were in the high-waste tank were expelled by the same parent individual, which was verified from the time-lapse videos.

2.10.3.4 Sea anemone and coral interactions

Unexpected contacts between *A. stella* and *G. rubiformis* were observed where *A. stella* would partially or completely engulf a coral colony with its mouth for variable periods of time. A total of 17 events were recorded over the acclimation (3 events), experimental period (13 events) and depuration period (1 event). Events ranged from 15 min – 28 h in length between contact initiation and the release/regurgitation of corals. This was observed in 10 of the 12 tanks, affecting 13 different coral colonies, with no discernable pattern across time or treatment. Of note, three events occurred within different tanks and treatments on Day 17 of the exposure period. In one instance, an individual of *A. stella* pulled a colony of *G. rubiformis* from the cobble it was glued to and engulfed it whole for 28 h before the coral was regurgitated. One event occurred during the depuration period and the coral was engulfed within the sea anemone at the time of sampling. No mortality of *G. rubiformis* was observed as a result of these

interactions. Longer events of engulfment resulted in darkened regions and mucus production on the affected area of the colony. Shedding of sclerite-containing matter on the tank bottom beneath the coral was occasionally noted.

During contacts, the pharynx of *A. stella* would be in direct contact with *G. rubiformis*, suggesting the interactions could be a feeding behaviour. This could highlight that the mobility and size of *A. stella* may provide an advantage in unfavourable conditions. Previous mesocosm experimentation has indicated the potential ability of sea anemones (*Mesactinia genesis*) to dominate competitors, including tropical scleractinians (*Acropora muricata)* under increased nutrient-rich sedimentation (Liu et al. 2015). No pattern of the interactions could be determined in the present study, consistent with Liu et al. (2015) who reported that mortality of *A. muricata* due to interactions with *M. ganesis* did not differ between control, inorganic and organic sedimentation treatments. However, these reported interactions involved modified tentacles and not attempted ingestion (engulfment), as seen here. Near-anoxic oxygen conditions seemingly induced predation of whole brittle stars by the sea anemones *Cereus pedunculatus* and *Calliactis parasitica* in a similar manner as the present study, in some instances ingesting the brittle star up to 12.5 h before regurgitation. This is suggested to indicate both a resilience to low-oxygen and a predatory advantage to the sea anemone (Riedel et al. 2008). Whether similar interactions between *A. stella* and *G. rubiformis* occur *in situ* is still unknown, though predatory interactions between the sea anemone *Ptychodactis patula* and the cold-water octocoral *Funiculina quadrangularis* have been reported (Buhl-Mortensen et al. 2023). Regardless, the opportunistic behaviour of *A. stella* witnessed here may provide an advantage over other benthic organisms in disturbed habitats (e.g. under aquaculture pens).

Chapter 3 - Impacts of barite and bentonite sedimentation on the behaviour, mucus production and cnidocytes of the deep-sea coral *Flabellum* (*Ulocyathus*) *alabastrum*[1]

Abstract

As oil and gas exploration expands in deep-sea environments, understanding the impacts of wastes produced by this industry is urgent. One of the main wastes of offshore drilling is the discharge of drill muds, commonly including barite and bentonite clay. Here, we investigated the effects of these two byproducts on the expansion behaviour, mucus secretion and cnidocyte density of the solitary cup-coral *Flabellum* (*Ulocyathus*) *alabastrum*. A total of 96 live corals were exposed over 10 d to one of three treatments (barite, bentonite, or barite and bentonite combined) or assigned as an untreated control. Daily waste additions aimed to reach a total sedimentation depth of 6.3 mm (common 'probable no-effects threshold'). Photography was used to monitor behavioural changes and mucus production, and histology was used to assess changes in tentacle cnidocytes. Overall, responses of *F. alabastrum* in the form of excessive polyp expansion (swelling) and mucus production were more severe under the barite and bentonite combined treatment, followed by barite alone, and bentonite alone. Moreover, elevated nematocyte densities were measured under exposure to the mix of barite and bentonite. However, *F. alabastrum* returned to baseline metrics during a 10-d recovery period. The results suggest that sedimentation of common drill mud ingredients to <6.3 mm depth can cause observable and non-lethal responses in *F. alabastrum*, but remain conservative relative to what may be experienced *in situ*. Long-term consequences, potential synergistic effects of barite and

[1] A version of this chapter is under review at Deep Sea Research Part II: Topical Studies in Oceanography.

bentonite, as well as impacts of more abrasive drilling waste (drill cuttings) on *F. alabastrum* require further study.

3.1 Introduction

The oil and gas sector represents one of the largest ocean-based industries, with over 9,000 offshore platforms operating worldwide (Jouffray et al. 2021) and activities now reaching deep-sea habitats as energy demands increase (Cordes et al. 2016). Since offshore deposits are responsible for over 70% of the recent (2000-2010) discoveries made in this sector (Sandrea and Sandrea 2010), the impacts of wastes produced through drilling activity on marine organisms need to be better understood.

The primary wastes produced by oil and gas exploration are drill cuttings consisting of fragments of rock dislodged during drilling, and drill muds, which are multipurpose fluids composed of additives, including barite and bentonite clay (IOGP 2016). Discharged drilling waste forms plumes where heavier particles (e.g. larger cuttings) settle rapidly close to the discharge site, whereas fine particles (e.g. drilling muds) are dispersed over a wider area (IOGP 2016) and can reach >1000 m downstream of the site (DNV 2013). The greatest accumulation of wastes is typically concentrated within 100 m of the drilling installation (DNV 2013), resulting in the smothering or burial of benthic organisms under particulate layers ranging from 1 to 50 cm in thickness (IOGP 2016). However, accumulation depends on factors such as currents, depth, volume of waste discharged as well as the quantity of wells drilled (IOGP 2016). Water-based muds (WBM) have become the most common type of drilling mud used in offshore operations (Neff 2008, Cordes et al. 2016) and are often permitted for discharge directly into the marine environment (Neff 2008, C-NLOPB et al. 2010, EPA 2011, IOGP 2016, Edge et al. 2016). Due

to their low toxicity and organic content, the potential effects of water-based drilling muds on benthic organisms are expected to primarily be a result of sedimentation (DNV 2013).

The deep sea off Newfoundland and Labrador (NL) is home to a rich benthic fauna, including over 70 species of cold-water coral (CWC). Cold-water corals are classified as indicators of vulnerable marine ecosystems (VME) due to their slow growth rates (Risk et al. 2002, Sherwood and Edinger 2009, Hamel et al. 2010, Carreiro-Silva et al. 2013, Neves et al. 2015, Neves 2016, Prouty et al. 2016, Piccirillo 2023) suggesting that their recovery from disturbances may take decades, if not centuries (Sherwood and Edinger 2009, Carreiro-Silva et al. 2013). Although NL is the largest offshore producer of oil in Canada (CER 2022), the effects of drilling on CWC present in this region are presently unstudied (Gullage et al. 2022). So far, the impacts of drilling wastes on CWC have heavily focused on the reef-building scleractinian *Desmophyllum pertusum* (previously known as *Lophelia pertusa*), which is common off the coast of Europe but rarer off Canada (Beazley et al. 2021) and not known from NL (Wareham and Edinger 2007, Murillo et al. 2011, Baker et al. 2012).

Previous studies have shown that *D. pertusum* has a resilience to sediment and drill cuttings alike, with polyp mortality and tissue reduction occurring only when completely smothered with deposited material (Larsson and Purser 2011, Allers et al. 2013). Under short-term (5 d) exposure to suspended drilling wastes in 4-h pulses, drill cuttings, barite or bentonite showed no effect on the growth or respiration rates of colony fragments 2 and 6 wk post-exposure. However, increased polyp mortality was seen at concentrations of drill cuttings ≥19 mg L^{-1} (Baussant et al. 2022). Increases in polyp activity have also been recorded during exposure to both suspended barite (Vianna da Rocha et al. 2021) and drill cuttings (Baussant et al. 2018), likely representing a behaviour that targets sediment removal (Vianna da Rocha et al.

2021). Additionally, larvae of *D. pertusum* subjected to suspended drill cuttings, barite or bentonite for 24 h displayed abnormal swimming behaviour, changes in shape, mucus secretion, particles attached to their body, and death at the highest treatment concentrations (>50 mg L^{-1}), with the most severe effects and lowest recovery potential observed in bentonite treatments (Järnegren et al. 2020). While studies on *D. pertusum* are informative, they may not necessarily be applicable to other CWC species. For instance, branching colonial corals may have a higher capability to reject sediment compared to non-branching morphologies (Duckworth et al. 2017) such as solitary corals. As such, high sedimentation loads are needed to maintain sediment coverage on *D. pertusum* (Allers et al. 2013), possibly overestimating effect thresholds appropriate for CWC species that are more susceptible to smothering.

Questions remain regarding the breadth of effects that drilling wastes may have on CWC. Much of the current literature has measured behaviour and physiological parameters, while little is known of cellular responses. Cnidocytes, the characteristic "stinging cells" of cnidarians, play functional roles such as food capture and defense (Kass-Simon and Scappaticci 2002). While mechanical and chemical stimuli can cause these cells to trigger (Kass-Simon and Scappaticci 2002), changes in cnidocyte densities can also be induced (e.g. by predation, Gochfeld 2004). Whether disturbance from human activity, such as the sedimentation of drilling waste, may influence the discharge or production of these cells is yet to be explored.

The solitary coral *Flabellum* (*Ulocyathus*) *alabastrum* is one of the most common scleractinian species off eastern Canada; it is known to inhabit depths ranging between 218 and 1433 m (Wareham and Edinger 2007) sometimes congruent to those where exploratory wells are drilled (NSB 2016). This species is expected to be vulnerable to anthropogenic activities due to their slow growth rates (~1 mm y^{-1}), potential high longevity (Hamel et al. 2010), and sedentary

nature. However, small CWC species (<30 cm) such as *F. alabastrum* are currently excluded from criteria established for use during regional pre-drilling surveys (Gullage et al. 2022).

The purpose of the present study was to investigate the potential effects of drilling wastes on *F. alabastrum*. The specific objectives were to (1) assess behavioural responses to sedimentation of barite and bentonite, (2) record observations of mucus presence, (3) explore concurrent changes in cnidocytes (stinging cells), and (4) evaluate whether the changes/damages recorded persist after a recovery period.

3.2 Materials and methods

3.2.1 Materials

Barite (BA) and bentonite (BE) products were obtained through an industry supplier. To verify their composition, mineral liberation analysis (MLA) of BA and BE samples were conducted in triplicate at the Micro Analysis Facility (MAF) of the Core Research and Equipment and Training Network (CREAIT) of Memorial University. Samples were embedded in epoxy, polished and then analyzed using a FEI MLA 650FEG scanning electron microscope equipped with MLA software. Each sample was automatically analyzed until a minimum particle count of 35,000 was reached, which is appropriate for a high degree of confidence in the results (Grant et al. 2018). The minerology of each particle was identified. The particle size distribution of each sample was also extracted from MLA.

3.2.2 Coral collection and holding

About 400 individuals of *F. alabastrum* were collected opportunistically as by-catch during multi-species trawl surveys conducted by Fisheries and Oceans Canada in May 2022. Individuals included in this study were collected at two sites within the Laurentian Channel (45.033 N, -56.460 W and 45.035 N, -54.833 W, trawl start positions) at mean depths of 385 and

638 m (see Supplementary Table S3-1 for details). They were maintained alive onboard for 9–10 d in dark conditions and flow-through seawater between 3-8 °C until their transfer to tank facilities at the Northwest Atlantic Fisheries Centre (NAFC, Fisheries and Oceans Canada), St. John's, NL. Once at NAFC, they were acclimated to laboratory conditions for a period of 10 wk before being transferred to experimental tanks, where they were acclimated for an additional 2 d prior to the trial (see Supplementary Table S3-2 for all holding and acclimation conditions). Individuals were kept in dark (<15 lux) conditions and supplied with flow-through, natural seawater continuously pumped through an intake pipe 3 km North of St. John's Harbour (47.593, -52.663) from a depth of 30 m. In-line chillers (Advantage M1D-5W-MG-SP) were used to maintain appropriate seawater temperature (<8 °C), based on Buhl-Mortensen et al. (2007). The largest 96 individuals in healthy condition were selected (i.e. individuals with intact calices, no visible tissue damage, and with typical expansion behaviour). Individual sizes ranged from 25-75 mm calyx width, and 23-56 mm calyx length (measured from the base of the calyx to the edge of the central septal margin).

3.2.3 Experimental treatments

Eight individuals of *F. alabastrum* were placed in each of 12 glass tanks (57 L; 61 cm x 31 cm x 31 cm), positioned on their side to mimic their natural posture (Hamel et al. 2010). Seawater was supplied to each tank (see above for details) through two inflow tubes (1.9 cm diameter), each set at a flow rate of ~108 L h^{-1} (30 mL s^{-1}, see Supplementary Figure S3-3 for set-up). A HOBO Pendant® MX Temperature/Light Data Logger was added to the bottom of each tank to record temperature and light intensity at 5 min intervals for the duration of the trial. Water quality parameters (pH, salinity, dissolved oxygen, temperature) were recorded twice daily using a multi-parameter probe (YSI Pro DSS). From each tank, duplicate water samples of

50 mL were collected prior to the experiment, after the 10-d exposure period and again after the 10-d recovery period for nutrient analyses (nitrate, phosphate, silicate). Samples were stored at -20 °C until analyzed at NAFC following Seal Analytical AutoAnalyzer methods (Method No. G-172-96 Rev. 18, G-297-03 Rev. 6, G-177-96 Rev. 12).

Each of the 12 tanks was randomly assigned as either a control (n = 3 tanks) or one of three treatments: barite alone "BA" (n = 3 tanks), bentonite alone "BE" (n = 3 tanks), or a combination of barite and bentonite together "BA+BE" (n = 3 tanks). BA+BE had a dry weight ratio of 14:6, respectively, based on typical water-based drilling mud compositions (IOGP 2016). Control tanks received no sediment. Each daily treatment addition was portioned to reach a total depositional depth of 6.3 mm over 10 d. The 6.3 mm value (based on Smit et al. 2008) has been widely recommended to industry as a 'probable no-effect threshold' (PNET) for smothering (Larsson and Purser 2011, Gullage et al. 2022). The duration of 10 d was chosen to replicate the time over which drilling (NSB 2016) and the discharge of water-based muds are expected to be deposited onto the seabed during drilling activities (BP 2017).

Daily waste addition amounts to achieve the 6.3 mm target were based on preliminary testing that estimated the swelling and settling behaviour of BA alone, BE alone, and BA+BE in seawater. Triplicate testing the settlement of 1 cm^3 of each waste in seawater within 10 mL falcon tubes showed that deposited BA expanded to ~2 times its dry volume, BE to ~6.5 times its dry volume, and BA+BE to ~4 times its initial volume. Based on these results, 133 g of BA, 17 g of BE, and 47 g of mixed BA+BE (33 g BA + 14 g BE) were added once daily to the respective treatment tanks to aim for the target depositional depth of 6.3 mm.

Dry materials were mixed with seawater prior to addition to the tanks to achieve a homogenized mixture, as per Allers et al. (2013). Similar to previous work (Larsson and Purser

2011, Allers et al. 2013), the water flow was interrupted for 8 h following addition to allow particles to settle. During flow cessation, a chilled water bath surrounding the outside of the tank was used to maintain temperatures. Final depositional depths at the end of the exposure period were measured in petri dishes (5 cm diameter, Weber et al. 2006) positioned at each end of the experimental tanks.

After 10 d of exposure, half of the *F. alabastrum* (n = 4 per tank) were preserved in 5% formalin (Lopez et al. 2006). The remaining individuals (n = 4 per tank) were monitored for an additional 10 d recovery period with no waste additions, after which they were preserved in 5% formalin.

3.2.4 Behaviour

Time-lapse videos were recorded throughout the exposure and recovery periods using a Brinno TLC200 Pro placed in a water-resistant housing and affixed to one end of each tank (see Supplementary Figure S3-3 for set-up). Two LED infrared lights were secured above each tank to help capture activity under dark conditions. To circumvent anticipated video quality issues, underwater photos were taken twice daily from the third day onward, using a Nikon Coolpix W300. The first daily timepoint for photos was set after 8 h of waste exposure plus an additional hour for water clarity to return after the reinstatement of flows (referred to as "T1"). The second daily time point for photos (referred to as "T2") was taken prior to the second waste addition (~14 h after T1). See Supplementary Figure S3-4 for visual timeline. Based on the photos taken at T1 and T2, behavioural scores were assigned to each individual (n = 8 per tank) twice daily throughout the exposure period (beginning on the third experimental day) and during the recovery period (n = 4 per tank). Scores on a scale of 1-8 (from most contracted to most

expanded) were based on the criteria outlined in Table 3-1 with visual examples shown in Figure 3-1.

3.2.5 Mucus production

Corals (n = 8 per tank) were monitored for the absence or presence of visible mucus strands twice daily from photos taken at T1 and T2 timepoints, aided by direct observations made during the experiment. The location of the mucus was recorded as either in contact with the tentacles, originating from the mouth, or both.

3.2.6 Cnidocytes

Histology was performed to explore the cellular effects of barite and bentonite on *F. alabastrum.* Whole tentacle samples were collected from individuals preserved after the 10-d exposure period (see Supplementary Figure S3-5 for sample location). The same was done for individuals preserved after the additional 10 d of recovery. Samples were transferred to 70% ethanol prior to processing at the Histology Medical Laboratories of Memorial University. Samples were dehydrated, mounted in paraffin, sectioned longitudinally to 5 µm at the estimated tentacle midpoint, and stained with hematoxylin and eosin (H&E). Histological slides were analyzed using a stereoscope (Nikon Eclipse 80i) equipped with a digital camera (Olympus DP73), and cellSens imaging software. Cnidocyte densities were quantified by identifying and counting the cnidocysts (prominent organelle within cnidocytes) visible in each tentacle along a 100 µm length of epithelium (n = 3 replicates per tentacle). Cnidocyte and mucocyte identification was based on Cordie and Budd (2016) and Strömberg and Östman (2017). Two types of cnidocytes were identified as either spirocyte or nematocyte. Spirocytes were identified by the presence of a coiled spring-like tubule which stained pink (spirocyst, Figure 3-2B). Nematocytes (heteroneme type) were identified based on the presence of a prominent, linear

tubule that tapered towards the end (nematocyst, Figure 3-2C). The tubule stained pink, while the capsule housing the tubule stained purple. The presence of a notch in the proximal end of the tubule could not be determined from the slides, so identification of nematocysts could not distinguish between p- and b-mastigophores types (Cordie and Budd 2016). Mucocytes did not stain but were identified by their ovular shape and a visible purple web-like texture (Figure 3-2D).

3.2.7 Data processing and analyses

3.2.7.1 Data reporting

Reported values for the measured parameters were obtained by calculating tank mean (for n = 8 individuals per tank), to get a treatment mean (n = 3 tanks per treatment) for each observational period (twice daily), and per experimental day. Means were compared among treatment/control groups, across exposure days, between T1 and T2, as well as at Day 10 of the exposure and recovery periods where appropriate. Unless otherwise specified, values in the text are presented as mean ± se (standard error).

Reported values for behavioural scores were calculated as a treatment mean and averaged across experimental days (dubbed “daily score”). Treatment and control means were compared between T1 and T2, as well as between exposure and recovery periods.

Reported values for mucus presence were calculated as the proportion of individuals showing visible mucus strands (%) per day in each tank. Treatment means were compared between T1 and T2 measurements for the exposure period.

Cnidocyte densities within each treatment were pooled as a low number of samples (n = 2) were analyzed per tank per sample point. Mean densities were compared both between treatments and separately for the exposure and the recovery periods. Cnidocyte % composition

was calculated as the proportion of nematocytes and spirocytes to the total cnidocyte population in each subsample and then followed the same averaging procedure as cnidocyte density.

3.2.7.2 Statistical analyses

Statistical analyses were conducted in R (version 4.2.2). Mixed effect models using the *lme4* package (Bates et al. 2015) were used to account for the nested (individuals held in the same tanks, timepoints of same day) and repeated measures nature of the data (the same individuals scored every observation). Behavioural scores were not statistically analyzed out of caution due to a lack of available model validation support for ordinal mixed effect models, and model misfit to other explored distributions.

Presence/absence counts of mucus were evaluated over the exposure period through generalized mixed effect models with binomial distributions. As no mucus was observed within either the control group or recovery period, statistics were used to determine differences between treatments during the exposure period. Model 1a (Supplementary Table S3-3) was used to evaluate the effect of time after exposure (T1 or T2) on mucus presence. Tank and coral ID (unique codes identifying each coral) were included as random factors, and time after exposure was nested within experimental day. Experimental day was included as a covariate, and time after exposure and treatment as fixed factors. As a significant effect of the time after exposure and treatment on mucus presence were determined, comparisons of treatment were conducted separately for T1 and T2 measurements using Model 1b (Supplementary Table S3-3).

Cnidocyte density counts were analysed through linear mixed effect models. Separate models were run to determine if there was an effect of treatment on total cnidocyte (Model 2a, Supplementary Table S3-3), nematocyte (Model 3a, Supplementary Table S3-3) and spirocyte densities (Model 4a, Supplementary Table S3-3). Due to a low sample size from each tank (n = 2

corals per tank per timepoint), only coral ID was included as a random effect. To determine if there was a change within treatments between the exposure and recovery period, a model with sampling time included as a fixed factor was run for cnidocyte (Model 2b, Supplementary Table S3-3), nematocyte (Model 3b, Supplementary Table S3-3), and spirocyte densities (Model 4b, Supplementary Table S3-3).

Model assumption diagnostics (i.e. normality of residuals, homogeneity of variance, independence of residuals) were explored using the *DHARMa* package (Hartig 2022) as in studies with similar mixed model data (e.g. Bilan et al. 2023).

3.3 Results

3.3.1 Mineral liberation analysis (MLA) of wastes

The weight-based composition of BA consisted of 85 ± 3% (sd) barite, 5 ± 2% quartz with minor impurities, and 5 ± 1% BE. The BE samples were confirmed 89 ± 1% bentonite, with 6 ± 1% plagioclase feldspar, and 4 ± 1% quartz with minor impurities (Supplementary Table S3-5). About 90% of the weight of barite was attributed to particles <53 µm and ~90% of the weight of bentonite was attributed to particles <90 µm.

3.3.2 Deposition depths and water quality

Final depositional depths achieved within each treatment were 5.3 ± 0.1 (sd) mm for BA, 4.3 ± 0.2 mm for BE, and 4.4 ± 0.2 mm for BA+BE.

Temperature, DO, salinity, and pH were consistent among tanks over the experimental period (Supplementary Figure S3-6). Temperature ranged from 3-6 °C and light intensities varied between 0-12 lux during the trials. DO remained between 75–90% saturation and fluctuations inversely mirrored those of temperature. Salinity decreased slightly over the

experimental period ranging between 32.3 and 31.1 psu. The pH remained stable between 8.0–8.2.

Nitrate, phosphate, and silicate were similar among control and treatment tanks within the exposure and recovery periods (Supplementary Figure S3-7). Nitrate increased over time across all treatments from 1.44 ± 0.02 to 2.03 ± 0.10 (sd) µmol L^{-1}. Phosphate ranged from 0.34 ± 0.01 µmol L^{-1} to 0.40 ± 0.02 µmol L^{-1}. Silicate increased across treatments only after the recovery period, from 1.24 ± 0.02 pre-exposure to 1.48 ± 0.09 µmol L^{-1} post-recovery.

3.3.3 Behaviour

Daily scores for each treatment were consistent over the exposure period with only slight variations (Figure 3-3). At T1 in treatments, the mix of BA+BE elicited a daily score over the exposure period of 5.6 ± 0.1, followed by BA alone at 5.2 ± 0.1, BE alone at 4.9 ± 0.1, and the control individuals at 4.0 ± 0.1 (Figure 3-3). At T2, individuals in BA+BE showed the highest daily score (most expanded) at 5.4 ± 0.1, followed by those exposed to BA at 5.0 ± 0.1, BE individuals at 4.7 ± 0.1, and 4.0 ± 0.1 in control individuals.

Within each treatment, scores varied among individuals with some reaching higher scores (levels of expansion) than others (Supplementary Figure S3-8). For example, 63% of individuals within BA+BE reached the maximum behavioural score (level 8, fully swollen) at least once, followed by 29% of individuals within BE, 20% of individuals within BA and 4% of individuals within the control. Notably, an individual exposed to BE swelled to a score of 8 on day 3 and was recorded at a score of 8 for every subsequent observation until day 8, reaching a maximum polyp size of ~21 cm across (~10 cm baseline polyp size). During the recovery period, daily scores were 4.3 ± 0.1 for BA, 4.2 ± 0.4 for BA+BE, 4.1 ± 0.1 for control, and 4.0 ± 0.1 for BE individuals overall.

No mortality occurred over the exposure or recovery period.

3.3.4 Mucus production

Visible strands of sediment-coated mucus were observed at least once on all individuals exposed to waste treatments, but never among control individuals. Individuals with visible mucus were observed an average of 6 times in the BA+BE treatment, 5 times in BA, and 3 times in BE, between days 3 and 10 of the exposure period. Most mucus strands were seen encompassing the tentacles (97% observations, n = 422, Figure 3-4A). Mucus originating from the mouth was also observed, either as a single strand or as a larger mass (11% observations, Figure 3-4B). On 33 occasions, mucus was observed both wrapped around the tentacles and originating from the mouth (28 of these instances were in the BA+BE treatment, 4 in BA and 1 in BE).

At T1, BA+BE yielded the highest daily proportion of individuals displaying visible mucus (79 ± 4%), followed by BA (66 ± 3%) and BE (43 ± 4%) over the exposure period (Figure 3-5). Pairwise comparisons highlighted a significant difference between BA+BE and BE ($z = 5.0$, $p = <0.001$), as well as BA and BE treatments ($z = -3.0$, $p = 0.007$). However, the proportion of individuals with visible mucus was not statistically different between BA+BE and BA at T1 ($z = 2.1$, $p = 0.08$). There was an interaction between treatment and experimental day during the T1 measurements over the exposure period ($\chi^2 = 8.8$, $df = 2$, $p = 0.01$) likely as the proportion of individuals with visible mucus in the BE treatment gradually increased over time, from 21 ± 4 % on day 3 to 63 ± 7 % on day 9 at T1 (Figure 3-5), while those in the BA and BA+BE treatments remained relatively constant across experimental days.

At T2 (~14 h later), the daily proportion of individuals with visible mucus was reduced to 18 ± 3% ind in BA+BE, 10 ± 2% ind in BA, and 3 ± 2% ind in BE over the exposure period

(Figure 3-5; $\chi^2 = 257.7$, df = 1, $p < 0.001$). A difference between the treatments at T2 was detected between BE and BA ($z = -2.4$, $p = 0.04$), as well as BE and BA+BE individuals ($z = 4.0$, $p < 0.001$), though there was no significant difference between BA and BA+BE individuals ($z = 2.0$, $p = 0.1$). Mucus strands were also observed on the sediment surrounding the calyx (Figure 3-4D). Two individuals (one treated with BA, and one treated with BA+BE) showed residual mucus on the first day of recovery, but no mucus was present on any individual thereafter.

3.3.5 Cnidocytes

3.3.5.1 Cnidocyte densities after exposure

After 10 d of exposure, total cnidocyte densities were similar across treatments ($\chi^2 = 2.6$, df = 3, $p = 0.5$; Supplementary Table S3-4). In terms of nematocytes, controls had a density of 17 ± 11 (sd) mm^{-1} (Figure 3-6A). Individuals exposed to BA+BE had 28 ± 14 mm^{-1}, BA had 17 ± 7 mm^{-1} and BE had 14 ± 9 mm^{-1}, although differences were not significant ($\chi^2 = 5.9$, df = 3, $p = 0.1$). Spirocyte densities were 169 ± 19 mm^{-1} in controls. Individuals exposed to BA+BE had 172 ± 34 mm^{-1}, BA had 153 ± 29 mm^{-1} and BE had 178 ± 25 mm^{-1}, without any statistical differences ($\chi^2 = 2.6$, df = 3, $p = 0.5$).

3.3.5.2 Cnidocyte densities after recovery

After 10 d of recovery, total cnidocyte densities were consistent among treatments ($\chi^2 = 7.5$, df = 3, $p = 0.6$, Supplementary Table S3-4) and between sample times (post-exposure or post-recovery, $\chi^2 = 1.5$, df = 1, $p = 0.2$). Nematocyte densities were 21 ± 9 mm^{-1} in controls. Individuals exposed to BA had 23 ± 18 mm^{-1}, BA+BE had 22 ± 14 mm^{-1} and BE had 17 ± 10 mm^{-1} with no statistical differences between treatments ($\chi^2 = 0.7$, df = 3, $p = 0.9$, Figure 3-6A) or sample times ($\chi^2 = 0.2$, df = 1, $p = 0.6$). The highest density of nematocytes was recorded in an individual exposed to BA, which had an average of 57 ± 29 mm^{-1} after recovery. Spirocyte

densities were 144 ± 32 mm^{-1} in controls, those exposed to BA had 134 ± 39 mm^{-1}, BA+BE had 178 ± 17 mm^{-1} and BE had 166 ± 43 mm^{-1} (Figure 3-6B). No effect of treatment (χ^2= 6.2, df = 3, p = 0.1) or sampling time (χ^2= 2.1, df = 1, p = 0.2) was determined.

3.4 Discussion

Excessive polyp expansion (swelling) and mucus production were observed in individuals of *F. alabastrum* exposed to barite and bentonite, most markedly when the two were combined, compared to control conditions. The combination of stressors was also associated with trends showing elevated densities of nematocytes in the tentacles.

Swelling in *F. alabastrum* has been previously documented *in situ* (Supplementary Figure S3-10) and individuals expanding >10 times their original size have also been reported in laboratory studies (Buhl-Mortensen et al. 2007). However, the frequency of swelling detected in individuals exposed to treatments in the present study was atypical when compared to those in the controls. It was previously hypothesized that swelling might be related to inter-individual interactions in *F. angulare* (Mercier et al. 2011a) and to functions including metabolite transfer, or to increase buoyancy for movement in *F. alabastrum* (Buhl-Mortensen et al. 2007). Active buoyancy adjustments (ABA) have been recorded in sedentary benthic organisms to expand their dispersal capabilities when exposed to suboptimal environmental conditions (Hamel et al. 2019). Although no movement directly due to polyp swelling occurred in the experimental tanks, *in situ* currents might have increased the likelihood of movement through ABA, thus providing an evolutionary advantage to escape unfavourable conditions. Excessive tissue expansion has been observed as a common mechanism through which some solitary tropical scleractinian species react to various stressors (*Heliofungia actiniformis*; Lewis et al. 2016, *Herpolitha limax*; Hoeksema and Bongaerts 2016) including sediment burial (*Lobactis scutaria* and *Herpolitha*

limax; Bongaerts et al. 2012). In an investigation by Stafford-Smith and Ormond (1992) on colonial and solitary species of scleractinian corals exposed to 50 mg cm^{-2} of sand (calcium carbonate and quartz), all species displayed tissue expansion (coenosarc or polyp), mucus production and ciliary movement as active mechanisms of sediment rejection. The utilization of the same mechanisms (hydrostatic expansion, mucus secretion) to remove sediment by tropical scleractinians (*Favia favus, Favites pentagona, Platygyra daedalea, Gyrosmilia interrupta*) was described by Riegl (1995) in response to 6 wk of biogenic sand sedimentation. From this combined evidence, swelling in *F. alabastrum* as a means of sediment removal is likely.

Mucus was visible on the corals after exposure to waste, but not in the controls. Our results were consistent with previous observations in scleractinian corals, where the formation of mucus strands plays an important role in particle removal, particularly for finer particles (<250 μm) (Stafford-Smith and Ormond 1992). The small particle size (mostly <100 μm) of barite and bentonite may have contributed to the high levels of mucus made visible here in response to sedimentation, through successful particle-trapping. Mucus discharge was similarly noted in response to barite or bentonite exposure in *D. pertusum* (Järnegren et al. 2020, Vianna da Rocha et al. 2021). Here, the majority of mucus secreted in response to the first waste addition was likely shed before the second daily waste addition, given the reduction of visible mucus between the two daily time points. This suggests that *F. alabastrum* produced new mucus throughout the 10-d exposure, even though individuals did not produce mucus in response to every addition of waste. Possible energy constraints or time requirements may explain this variation, as mucus production in response to sedimentation in tropical scleractinians (*F. favus, F. pentagona, P. daedalea, G. interrupta*) has been shown to be energetically costly (Riegl and Branch 1995). Mucocyte presence appeared to vary among tentacle cut orientation, with the highest densities

observed in non-target cut orientations (Supplementary Figure S3-9). The variable presence and location of mucocytes within the samples, combined with their inability to uptake hematoxylin and eosin stains, made them difficult to quantify.

The most instances of polyp swelling and excess mucus secretion were associated with corals exposed to a combination of barite and bentonite, followed by barite alone and bentonite alone. No visible mucus strands and rare instances of polyp swelling occurred in control individuals. Experimental work on cold-water *D. pertusum* previously evaluated barite and bentonite separately (Järnegren et al. 2020, Vianna da Rocha et al. 2021, Baussant et al. 2022) and to our knowledge this is the first CWC study to test them both independently and in combination. As barite reached the highest sedimentation depth of the treatments (5.3 mm), the combination treatment having more severe effects at a lower depth (4.3 mm) emphasizes the importance of testing the materials together. Because barite is non-reactive with bentonite (Ibrahim et al. 2017), the higher instances of swelling and mucus in individuals submitted to the combination treatment were not likely induced by new or unique waterborne compounds. Overall, the fact that the treatment representing the most realistic outcome of drilling activities showed the highest responses indicates that it will be important to further study the potential additive effects of these wastes.

No mortality in *F. alabastrum* occurred during the experiment. In addition, behavioural and mucosal responses of waste-exposed individuals had returned to baseline control levels within two days of post-experiment recovery. These observations suggest that *F. alabastrum* has a potential for recovery after exposure to barite and/or bentonite under the sedimentation depths and experimental conditions tested here. This is consistent with observed robustness to drilling wastes in the scleractinian *D. pertusum* (Larsson and Purser 2011, Larsson et al. 2013, Allers et

al. 2013, Buhl-Mortensen et al. 2015, Weinnig et al. 2020, Baussant et al. 2022) where polyp mortality mainly occurred at higher concentrations (19 and 40 mg L^{-1}, Baussant et al. 2022) or when they were completely covered (Larsson and Purser 2011, Allers et al. 2013). In contrast, exposure to suspended drill muds (0.02, 2, and 200 mg L^{-1}) increased instances of polyp mortality and tissue damage in the brown cup coral *Paracyathus stearnsii* (Raimondi et al. 1997).

Cnidocyte types found in the tentacles of *F. alabastrum* were predominantly spirocytes with occasional heteroneme nematocytes. Interestingly, a higher density of nematocytes was observed in individuals after 10 d of exposure to combined barite and bentonite, though no statistical difference was detected likely due to high variability between individuals. Gochfeld (2004) showed densities of microbasic p-mastigophores (nematocytes) had increased as a result of predation grazing intensity in the tropical scleractinian *Porites compressa*. There was not a significant difference between control and grazed colonies directly after predation exposure, but an increase was recorded after an 11-d recovery period, suggesting it takes days for the production or translocation of nematocytes at a particular location (Gochfeld 2004). It may thus be possible that the present 10-d exposure period was just long enough to allow for early changes in nematocyte densities to be detected, such as the ones observed in the individuals exposed to combined barite and bentonite. It is also possible that higher densities of nematocytes developed as a result of increased contacts between conspecifics due to polyp swelling, which was more frequent in this treatment. In tropical colonial scleractinians, nematocyte-rich tentacles termed "sweeper tentacles" are known to develop as a response to threats of competition (Chornesky 1983, Hidaka and Yamazato 1984). Whether stimulated directly by chemical exposure or indirectly as a consequence of swelling, investment into cnidocyte production may have trade-

offs with energetically costly processes such as growth (Rivera and Goodbody-Gringley 2014). Although we would have expected changes in nematocyte density to persist beyond exposure based on Gochfeld (2004), differences between treatments were not detected post-recovery. This suggests the potentially higher nematocyte density observed in individuals exposed to a combination of barite and bentonite may only be a short-term occurrence, though high variation between individuals was present.

It is important to note that the results of the present study were conservative and likely reflective of far-field sedimentation depths (90–250 m from drilling activity; DNV 2013), given that much greater sedimentation rates can occur closer to the discharge site or in areas with multiple wells. The selection of larger individuals of *F. alabastrum* for this experiment may have also played a role in their ability to successfully remove sediment through polyp swelling since sediment rejection success in scleractinians can be influenced by calyx size (Stafford-Smith and Ormond 1992). Importantly, drill cuttings that make up most deposited drilling wastes were not tested in the present study. Sharp particles, such as those found in drill cuttings, are expected to have more harmful impacts on tissue condition relative to smoother particles (Liefmann 2016) such as clay (e.g. barite, bentonite), further emphasizing the conservative nature of our results. Similar to Larsson and Purser (2011), the present study shows that sedimentation below the 6.3 mm target has observable and potentially costly effects on CWC species. This suggests that a lower PNET, such as 1.5 mm as suggested by Kjeilen-Eilertsen et al. (2004), may be more appropriate for sessile benthic taxa.

3.5 Conclusions

The present study revealed that sedimentation of barite and bentonite clay below the commonly predicted no-effect threshold (PNET) of 6.3 mm over 10 d induced observable

changes in both the behaviour and mucus production of the deep-sea cup coral *F. alabastrum*. Cnidocyte densities were variable between individuals within treatments, but trends alluded to a higher density of nematocytes in individuals exposed to a combination of barite and bentonite, which could be confirmed by further study. The corals showed recovery potential, having returned to baseline control conditions within 10 d post-exposure. However, energetic trade-offs and long-term effects (e.g. impacts on fitness, growth, reproduction) of these exposures remain unknown, and could be explored using physiological endpoints. Because the greatest impacts were observed in the treatment that combined barite and bentonite, research should strive to assess the possible synergistic effects of these materials, as would be experienced *in situ*. Effects might also be evaluated on corals of different size classes (ages). Overall, the present findings underscore the need for further research to help predict near-field, *in situ*, as well as long-term impacts of exploratory drilling activities on nearby deep-sea corals. They also support the adoption of a more conservative PNET, such as 1.5 mm (Kjeilen-Eilertsen et al. 2004, Gullage et al. 2022).

3.6 Acknowledgements

We would like to the Canadian Coast Guard and Fisheries and Oceans Canada team present on the multispecies trawl survey (CCGS *John Cabot*, Leg 4, May 2022) who greatly helped with the installation, water supply and dismount of the set-up for live cup corals. Brandon Piercy (DFO) is thanked for his assistance with collection and experimental preparations. Rachelle Dove (DFO) for her assistance and support collecting cup corals at-sea. Dylan Goudie (Micro Analysis Facility, CREAIT, Memorial University) for his guidance and expertise with the mineral liberation analyses. Justin So and Lara Miles for their assistance obtaining barite and bentonite. Headley Forward for supplying the barite and bentonite needed for this experiment.

Craig Kelly (DFO) for his help with the laboratory water supply and chiller set-up. Shannah Rastin (DFO) for the water nutrient analyses. Brooklin Caines (DFO) for her assistance during the preservation and sampling of cup corals. Danielle Gardiner (Medical Laboratories, Memorial University) for processing the histology samples. This work was supported by a Competitive Science Research Fund (CSRF) grant awarded to BMN; and a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) awarded to AM.

3.7 Literature cited

Allers, E., R. M. M. Abed, L. M. Wehrmann, T. Wang, A. I. Larsson, A. Purser, and D. de Beer. 2013. Resistance of *Lophelia pertusa* to coverage by sediment and petroleum drill cuttings. Marine Pollution Bulletin 74:132–140.

Baker, K. D., V. E. Wareham, P. V. R. Snelgrove, R. L. Haedrich, D. A. Fifield, E. N. Edinger, and K. D. Gilkinson. 2012. Distributional patterns of deep-sea coral assemblages in three submarine canyons off Newfoundland, Canada. Marine Ecology Progress Series 445:235–249.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Baussant, T., M. Arnberg, E. Lyng, S. Ramanand, S. Bamber, M. Berry, I. Myrnes Hansen, D. Van Oevelen, and P. Van Breugel. 2022. Identification of tolerance levels on the cold-water coral *Desmophyllum pertusum* (*Lophelia pertusa*) from realistic exposure conditions to suspended bentonite, barite and drill cutting particles. PLOS ONE 17:e0263061.

Baussant, T., M. Nilsen, E. Ravagnan, S. Westerlund, and S. Ramanand. 2018. Effects of suspended drill cuttings on the coral *Lophelia pertusa* using pulsed and continuous exposure scenarios. Journal of Toxicology and Environmental Health - Part A: Current Issues 81:361–382.

Beazley, L., E. Kenchington, M. Korabik, D. Fenton, and M. King. 2021. Other effective area-based conservation measure promotes recovery in a cold-water coral reef. Global Ecology and Conservation 26:e01485.

Bilan, M., A. Gori, J. Grinyó, M. Biel-Cabanelas, X. Puigcerver-Segarra, A. Santín, S. Piraino, S. Rossi, and P. Puig. 2023. Vulnerability of six cold-water corals to sediment resuspension from bottom trawling fishing. Marine Pollution Bulletin 196:e115423.

Bongaerts, P., B. W. Hoeksema, K. B. Hay, and O. Hoegh-Guldberg. 2012. Mushroom corals overcome live burial through pulsed inflation. Coral Reefs 31:399.

BP. 2017. Newfoundland Orphan Basin exploration drilling program: drilling mud and cuttings dispersion modelling. Appendix B. Retrieved from: https://iaac-aeic.gc.ca/050/documents/p80147/125913E.pdf (accessed June 12, 2024).

Buhl-Mortensen, L., P. Mortensen, S. Armsworthy, and D. Jackson. 2007. Field observations of *Flabellum* spp. and laboratory study of the behavior and respiration of *Flabellum alabastrum*. Bulletin of Marine Science 81:543–522.

Buhl-Mortensen, P., E. Tenningen, and A. B. S. Tysseland. 2015. Effects of water flow and drilling waste exposure on polyp behaviour in *Lophelia pertusa*. Marine Biology Research 11:725–737.

Carreiro-Silva, M., A. H. Andrews, A. Braga-Henriques, V. De Matos, F. M. Porteiro, and R. S. Santos. 2013. Variability in growth rates of long-lived black coral *Leiopathes* sp. from the Azores. Marine Ecology Progress Series 473:189–199.

[CER] Canadian Energy Regulator. 2022. Provincial and territorial energy profiles: Newfoundland and Labrador. https://www.cer-rec.gc.ca/en/data-analysis/energy-markets/provincial-territorial-energy-profiles/provincial-territorial-energy-profiles-newfoundland-labrador.html (accessed June 12, 2024).

Chornesky, E. A. 1983. Induced development of sweeper tentacles on the reef coral *Agaricia agaricites*: a response to direct competition. Bulletin 165:569–581.

[C-NLOPB et al.] Canada-Newfoundland and Labrador Offshore Petroleum Board, National Energy Board, and Canada-Nova Scotia Offshore Petroleum Board. 2010. Offshore waste treatment guidelines. National Energy Board.

Cordes, E. E., D. O. B. Jones, T. A. Schlacher, D. J. Amon, A. F. Bernardino, S. Brooke, R. Carney, D. M. DeLeo, K. M. Dunlop, E. G. Escobar-Briones, A. R. Gates, L. Génio, J. Gobin, L. A. Henry, S. Herrera, S. Hoyt, M. Joye, S. Kark, N. C. Mestre, A. Metaxas, S. Pfeifer, K. Sink, A. K. Sweetman, and U. Witte. 2016. Environmental impacts of the deep-water oil and gas industry: a review to guide management strategies. Frontiers in Environmental Science 4:e58.

Cordie, D. R., and A. F. Budd. 2016. Histological data in a combined phylogenetic analysis of scleractinian reef corals. Journal of Morphology 277:494–511.

[DNV] Det Norske Veritas. 2013. Monitoring of drilling activities in areas with presence of cold water corals.

Duckworth, A., N. Giofre, and R. Jones. 2017. Coral morphology and sedimentation. Marine Pollution Bulletin 125:289–300.

Edge, K. J., E. L. Johnston, K. A. Dafforn, S. L. Simpson, T. Kutti, and R. J. Bannister. 2016. Sub-lethal effects of water-based drilling muds on the deep-water sponge *Geodia barretti*. Environmental Pollution 212:525–534.

[EPA] United States Environmental Protection Agency. 2011. Information sheet: regulating petroleum industry wastewater discharges in the United States and Norway. Retrieved from: https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100B9AW.txt. (accessed June 12, 2024).

Gochfeld, D. J. 2004. Predation-induced morphological and behavioral defenses in a hard coral: implications for foraging behavior of coral-feeding butterflyfishes. Marine Ecology Progress Series 267:145–158.

Grant, D. C., D. J. Goudie, and E. Baird. 2018. Analysis of 98 individual −200 mesh iron ore samples in a single scanning electron microscope-automated mineralogy session. Applied Earth Science: Transactions of the Institute of Mining and Metallurgy 127:38–43.

Gullage, L., V. Hayes, B. M. Neves, N. Wells, F. Cyr, and F. J. Murillo. 2022. Avoidance and mitigation of coral and sponge species during exploratory drilling activities offshore Newfoundland and Labrador. DFO Can. Sci. Advis. Sec. Res. Doc. 2022/059. vii + 141 p.

Hamel, J.-F., J. Sun, B. L. Gianasi, E. M. Montgomery, E. L. Kenchington, B. Burel, S. Rowe, P. D. Winger, and A. Mercier. 2019. Active buoyancy adjustment increases dispersal potential in benthic marine animals. Journal of Animal Ecology 88:820–832.

Hamel, J.-F., Z. Sun, and A. Mercier. 2010. Influence of size and seasonal factors on the growth of the deep-sea coral *Flabellum alabastrum* in mesocosm. Coral Reefs 29:521–525.

Hartig, F. 2022. DHARMa: residual diagnostics for hierarchical (multi-Level / mixed) regression models. R package version 0.4.6. http://florianhartig.github.io/DHARMa.

Hidaka, M., and K. Yamazato. 1984. Coral reefs *Galaxea fascicularis*: induced formation of sweeper tentacles. Coral Reefs 3:77–85.

Hoeksema, B. W., and P. Bongaerts. 2016. Mobility and self-righting by a free-living mushroom coral through pulsed inflation. Marine Biodiversity 46:521–524.

Ibrahim, D. S., N. A. Sami, and N. Balasubramanian. 2017. Effect of barite and gas oil drilling fluid additives on the reservoir rock characteristics. Journal of Petroleum Exploration and Production Technology 7:281–292.

[IOGP] International Association of Oil & Gas Producers. 2016. Environmental fates and effects of ocean discharge of drill cuttings and associated drilling fluids from offshore oil and gas operations. Report 543.

Järnegren, J., S. Brooke, and H. Jensen. 2020. Effects and recovery of larvae of the cold-water coral *Lophelia pertusa* (*Desmophyllum pertusum*) exposed to suspended bentonite, barite and drill cuttings. Marine Environmental Research 158:e104996.

Jouffray, J.-B., R. Blasiak, M. Nyström, H. Österblom, K. Tokunaga, C. C. C. Wabnitz, and A. V Norström. 2021. Blue acceleration: an ocean of risks and opportunities. Ocean risk and resilience action alliance (ORRAA) report.

Kass-Simon, G., and A. A. Scappaticci. 2002. The behavioral and developmental physiology of nematocysts. Canadian Journal of Zoology 80:1772–1794.

Kjeilen-Eilertsen, G., H. Trannum, R. Jak, M. Smit, J. Neff, and G. Durell. 2004. Literature report on burial: derivation of PNEC as component in the MEMW model tool. Report AM 2004/024.

Larsson, A. I., D. van Oevelen, A. Purser, and L. Thomsen. 2013. Tolerance to long-term exposure of suspended benthic sediments and drill cuttings in the cold-water coral *Lophelia pertusa*. Marine Pollution Bulletin 70:176–188.

Larsson, A. I., and A. Purser. 2011. Sedimentation on the cold-water coral *Lophelia pertusa*: cleaning efficiency from natural sediments and drill cuttings. Marine Pollution Bulletin 62:1159–1168.

Lewis, B. M., L. D. Nothdurft, and L. N. Nothdurft. 2016. Expulsion of *Symbiodinium* by pulsed inflation under hyperthermal stress in *Heliofungia actiniformis*. Coral Reefs 35:1405.

Liefmann, S. 2016. Eco-physiological responses of cold-water corals to anthropogenic sedimentation and particle shape. Norwegian University of Technology, Trondheim.

Lopez, J. V, P. Etnoyer, S. D. Cairns, J. A. Sanchez, J. K. Reed, J. V Lopez, W. W. Schroeder, S. D. Brooke, L. Watling, A. Baco-Taylor, G. C. Williams, A. Lindner, S. C. France, and A. W. Bruckner. 2006. Deep-sea coral collection protocols. NOAA Technical Memorandum NMFS-OPR-28, Silver Spring, MD. 53 pp.

Mercier, A., Z. Sun, and J. F. Hamel. 2011. Reproductive periodicity, spawning and development of the deep-sea scleractinian coral *Flabellum angulare*. Marine Biology 158:371–380.

Murillo, F. J., P. Durán Muñoz, A. Altuna, and A. Serrano. 2011. Distribution of deep-water corals of the Flemish Cap, Flemish Pass, and the Grand Banks of Newfoundland (Northwest Atlantic Ocean): Interaction with fishing activities. ICES Journal of Marine Science 68:319–332.

Neff, J. M. 2008. Estimation of bioavailability of metals from drilling mud barite. Integrated Environmental Assessment and Management 4:184–193.

Neves, B. M. 2016. Growth in cold-water octocorals: rates, morphology and environmental controls. Memorial University.

Neves, B. M., E. Edinger, G. D. Layne, and V. E. Wareham. 2015. Decadal longevity and slow growth rates in the deep-water sea pen *Halipteris finmarchica* (Sars, 1851) (Octocorallia: Pennatulacea): implications for vulnerability and recovery from anthropogenic disturbance. Hydrobiologia 759:147–170.

[NSB] NSB Energy Consulting. 2016. Drilling performance review offshore Newfoundland and Labrador. Retrieved from: https://oilconl.com/wp-content/uploads/2016/09/Drilling-Performance-Review-Final.pdf (accessed June 12, 2024).

Piccirillo, L. F. 2023. Sclerochronology of deep-sea bamboo corals from the Northwest Atlantic: *Acanella arbuscula* and *Keratoisis* spp. Memorial University, St. John's.

Prouty, N. G., C. R. Fisher, A. W. J. Demopoulos, and E. R. M. Druffel. 2016. Growth rates and ages of deep-sea corals impacted by the Deepwater Horizon oil spill. Deep-Sea Research Part II: Topical Studies in Oceanography 129:196–212.

Raimondi, P. T., A. M. Barnett, and P. R. Krause. 1997. The effects of drilling muds on marine invertebrate larvae and adults. Environmental Toxicology and Chemistry 16:1218–1228.

Riegl, B. 1995. Effects of sand deposition on scleractinian and alcyonacean corals. Marine Biology 121:517–526.

Riegl, B., and G. M. Branch. 1995. Effects of sediment on the energy budgets scleractinian (Bourne 1900) and five alcyonacean (Lamouroux 1816) corals. Journal of Experimental Marine Biology and Ecology 186:259–275.

Risk, M. J., J. M. Heikoop, M. G. Snow, and & R. Beukens. 2002. Lifespans and growth patterns of two deep-sea corals: *Primnoa resedaeformis* and *Desmophyllum cristagalli*. Hydrobiologia 471:125–131.

Rivera, H. E., and G. Goodbody-Gringley. 2014. Aggregation and cnidae development as early defensive strategies in *Favia fragum* and *Porites astreoides*. Coral Reefs 33:1079–1084.

Sandrea, R., and I. Sandrea. 2010. Deepwater crude oil output: how large with the uptick be? Oil & Gas Journal 108:48–53.

Sherwood, O. A., and E. N. Edinger. 2009. Ages and growth rates of some deep-sea gorgonian and antipatharian corals of Newfoundland and Labrador. Canadian Journal of Fisheries and Aquatic Sciences 66:142–152.

Smit, M. G. D., K. I. E. Holthaus, H. C. Trannum, J. M. Neff, G. Kjeilen-Eilertsen, R. G. Jak, I. Singsaas, M. A. J. Huijbregts, and A. J. Hendriks. 2008. Species sensitivity distributions for suspended clays, sediment burial, and grain size change in the marine environment. Environmental Toxicology and Chemistry 27:1006–1012.

Stafford-Smith, M. G., and R. F. Ormond. 1992. Sediment-rejection mechanisms of 42 species of Australian scleractinian corals. Aust. J. Mar. Freshwater Res. 43:683–705.

Strömberg, S. M., and C. Östman. 2017. The cnidome and internal morphology of *Lophelia pertusa* (Linnaeus, 1758) (Cnidaria, Anthozoa). Acta Zoologica 98:191–213.

Vianna da Rocha, I., E. Cardinot Reis, P. Reis da Silva, G. de Hollanda Cavalcanti, R. Coutinho, and M. Vieira Reynier. 2021. Deep-sea coral *Lophelia pertusa* laboratory maintenance and exposure to barite using water recirculation systems. Journal of Toxicology and Environmental Health Sciences 13:1–17.

Wareham, V. E., and E. N. Edinger. 2007. Distribution of deep-sea corals in the Newfoundland and Labrador region, Northwest Atlantic Ocean. Pages 289–313 Conservation and adaptive management of seamount and deep-sea coral ecosystems. Rosenstiel School of Marine and Atmospheric Science, University of Miami. Watling and Auster.

Weber, M., C. Lott, and K. E. Fabricius. 2006. Sedimentation stress in a scleractinian coral exposed to terrestrial and marine sediments with contrasting physical, organic and geochemical properties. Journal of Experimental Marine Biology and Ecology 336:18–32.

Weinnig, A. M., C. E. Gómez, A. Hallaj, and E. E. Cordes. 2020. Cold-water coral (*Lophelia pertusa*) response to multiple stressors: High temperature affects recovery from short-term pollution exposure. Scientific Reports 10:e1768.

3.8 Tables

Table 3-1: Descriptions of scores used to assess behavioural responses of *Flabellum* (*Ulocyathus*) *alabastrum.*

Behavioural score	Description	Corresponding photo
1	Polyp fully retracted, visible septal ridges of the skeleton and no visible tentacles	Figure 3-1A
2	Polyp retracted with short tentacles visible	Figure 3-1B
3	Polyp showing normal expansion with short tentacles visible	Figure 3-1C
4	Polyp showing normal expansion with long tentacles visible	Figure 3-1D
5	Polyp showing excessive expansion, i.e. swelled, tentacles long	Figure 3-1E
6	Polyp swelled, tentacles short and wide, mouth extended out/away from calyx, often ovular	Figure 3-1F
7	Polyp swelled, tentacles short and wide, mouth extended out/away from calyx, geometric shape	Figure 3-1G
8	Polyp swelled, tentacles short and wide, mouth fully extended away from calyx, 'ballooning' appearance	Figure 3-1H

3.9 Figures

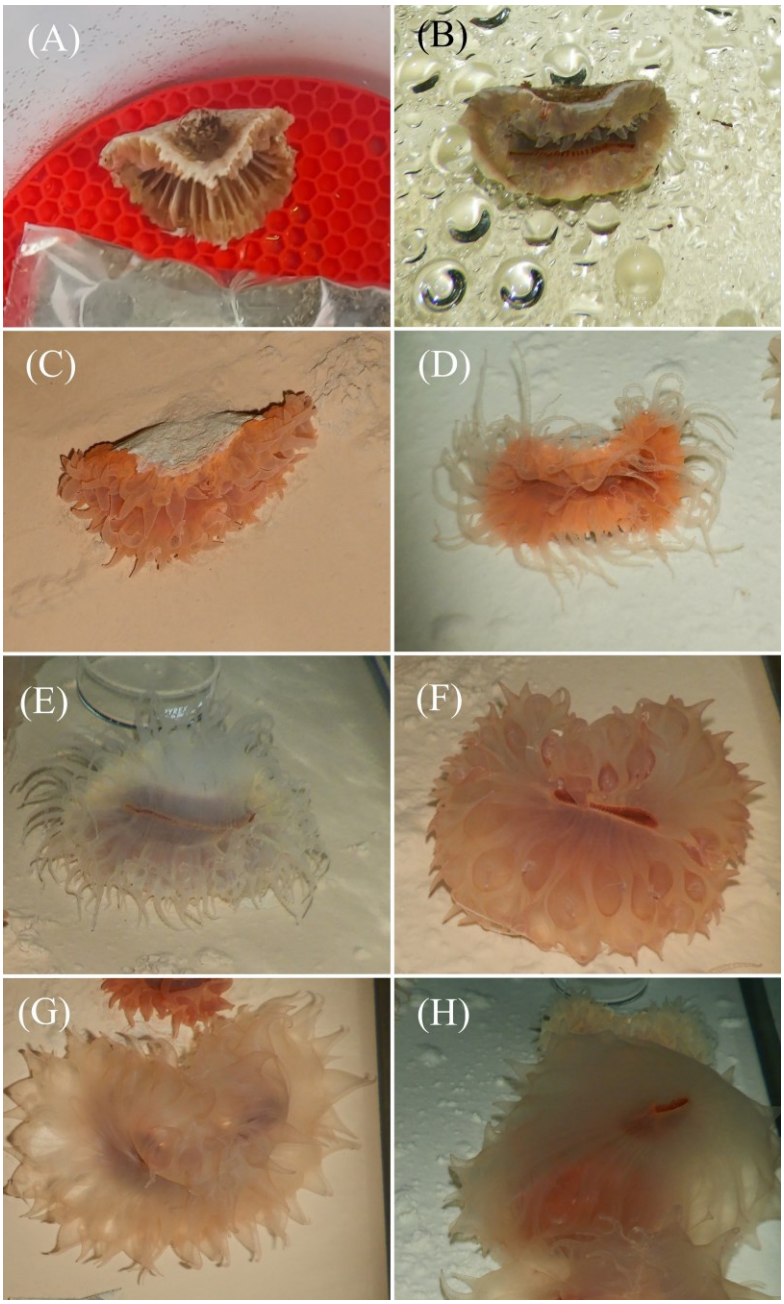


Figure 3-1: Photos representative of each behaviour score used to assess *Flabellum* (*Ulocyathus*) *alabastrum.* (A) Polyp fully retracted, visible septal ridges of the skeleton and no visible tentacles. (B) Polyp retracted with short tentacles visible. (C) Polyp showing normal expansion with short tentacles visible. (D) Polyp showing normal expansion with long tentacles visible. (E)

Polyp showing excessive expansion, i.e. swelled, tentacles long. (F) Polyp swelled, tentacles short and wide, mouth extended out/away from calyx, often ovular in shape. (G) Polyp swelled, tentacles short and wide, mouth extended out/away from calyx, often geometric in shape. (H) Polyp swelled, tentacles short and wide, mouth fully extended away from calyx, 'ballooning' appearance.

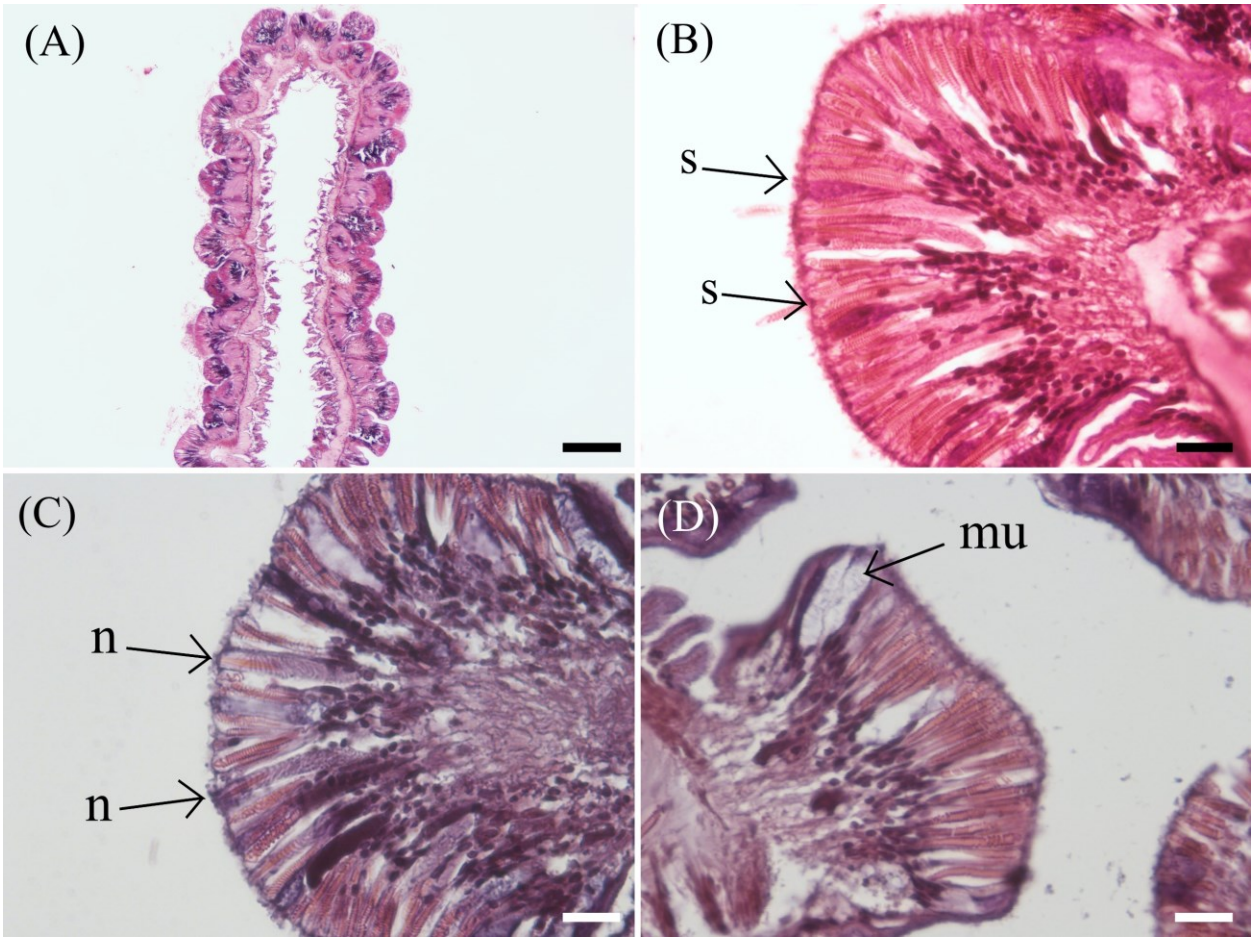


Figure 3-2: Micrographs of longitudinal sections of tentacles sampled from individuals of *Flabellum* (*Ulocyathus*) *alabastrum* exposed to barite (BA), bentonite (BE), barite and bentonite combined (BA+BE) or control conditions. (A) Overview of shape of tentacle. (B-D) Close-up of cnidocyte batteries. Labels: s, spirocytes; n, nematocyte; mu, mucocyte. Scale bar represents 200 µm in A and 20 µm in B-D.

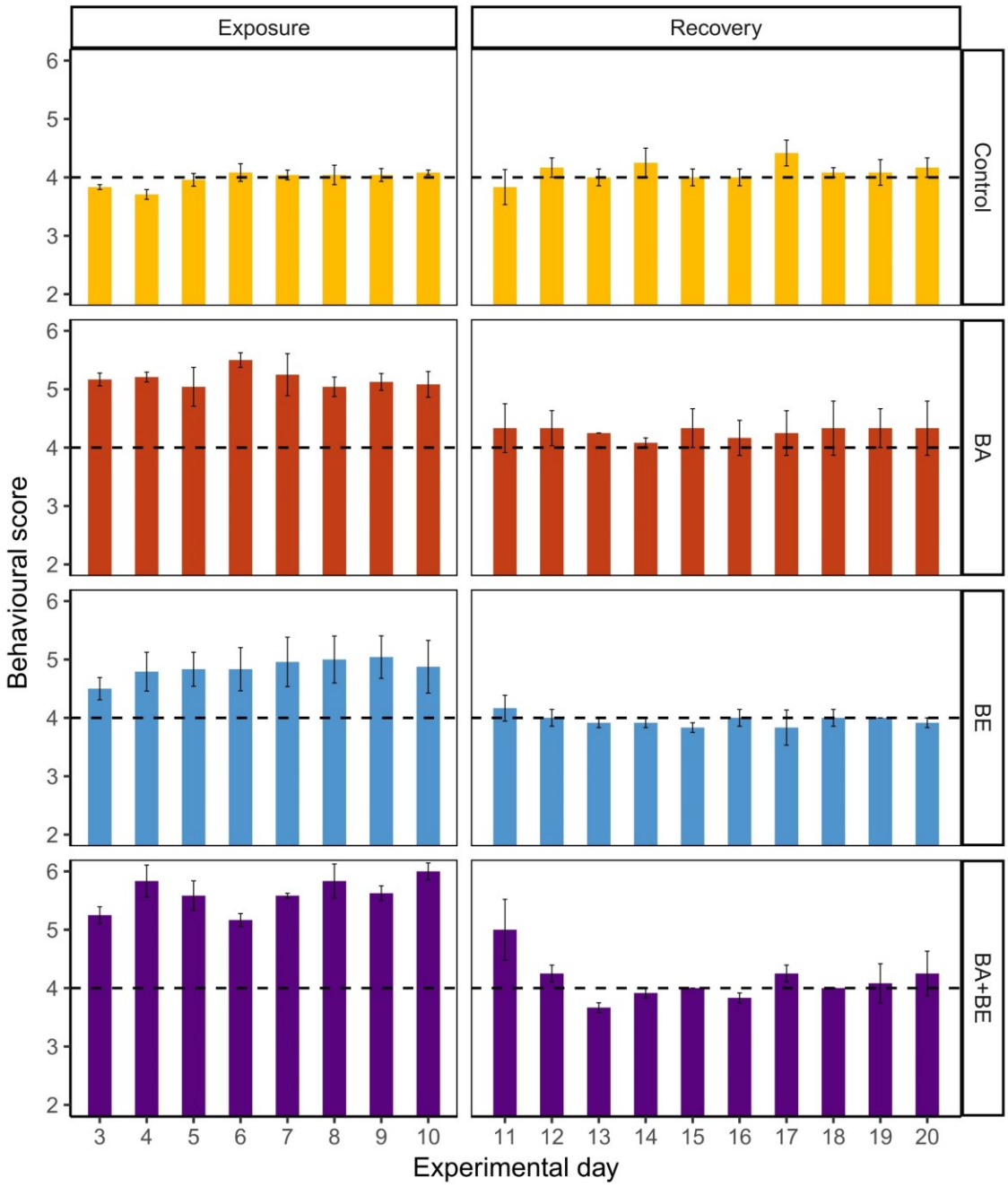


Figure 3-3: Behavioural scores of *Flabellum* (*Ulocyathus*) *alabastrum* at T1 (after 8 h of waste exposure) during 10 d of exposure to barite (BA), bentonite (BE), or barite and bentonite combined (BA+BE), and after a 10-d recovery period. Dashed line was added at baseline behaviour score (score 4) for easier comparison between treatments. Data shown as mean ± standard error (n = 3 tanks per treatment).

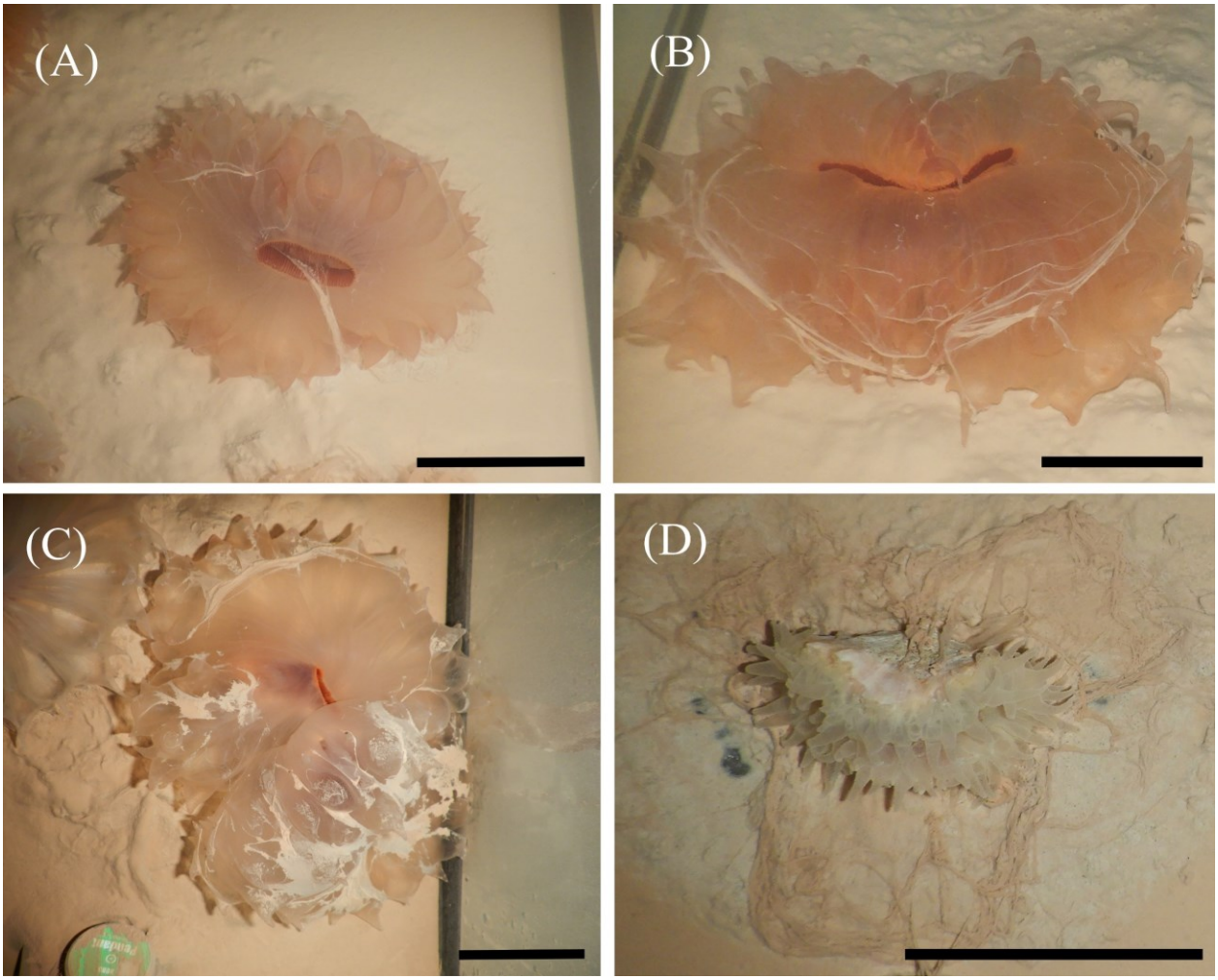


Figure 3-4: Examples of visible mucus secretion in *Flabellum* (*Ulocyathus*) *alabastrum* exposed to barite, bentonite, or barite and bentonite combined for 10 d. (A) Mucus expulsion from mouth, (B) mucus strands encompassing tentacles, (C) swollen individual coated in mucus-sediment mix, (D) shed mucus strands surrounding an individual. Scale bars represent ~5 cm.

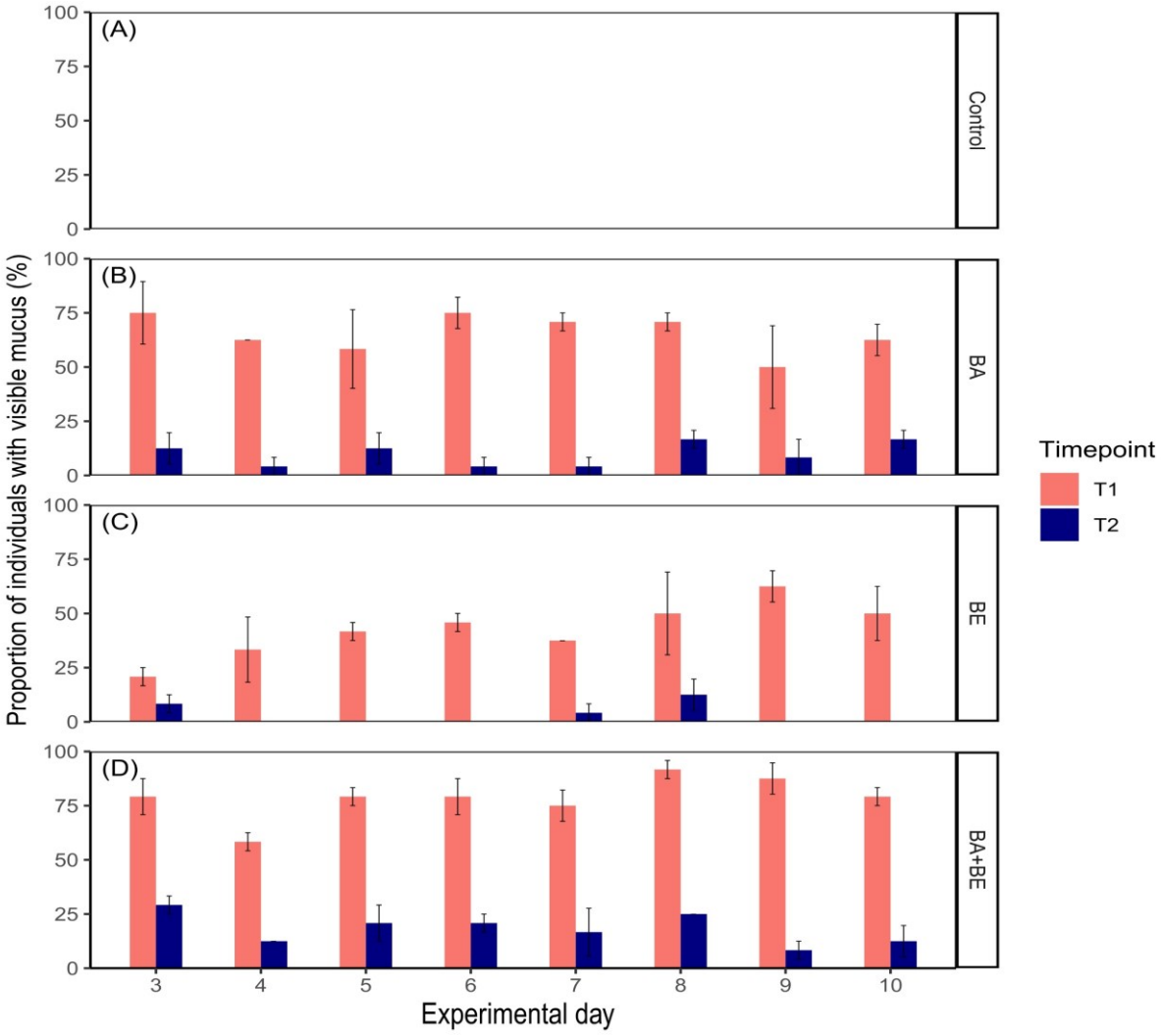


Figure 3-5: Proportion of individuals observed with visible mucus across days 3–10 of the exposure period for (A) controls, (B) barite treatment, (C) bentonite treatment, and (D) barite and bentonite treatment, showing results at T1 (after 8 h of waste exposure) and T2 (after 14 without waste exposure). Data shown as mean ± standard error (n = 3 tanks per treatment).

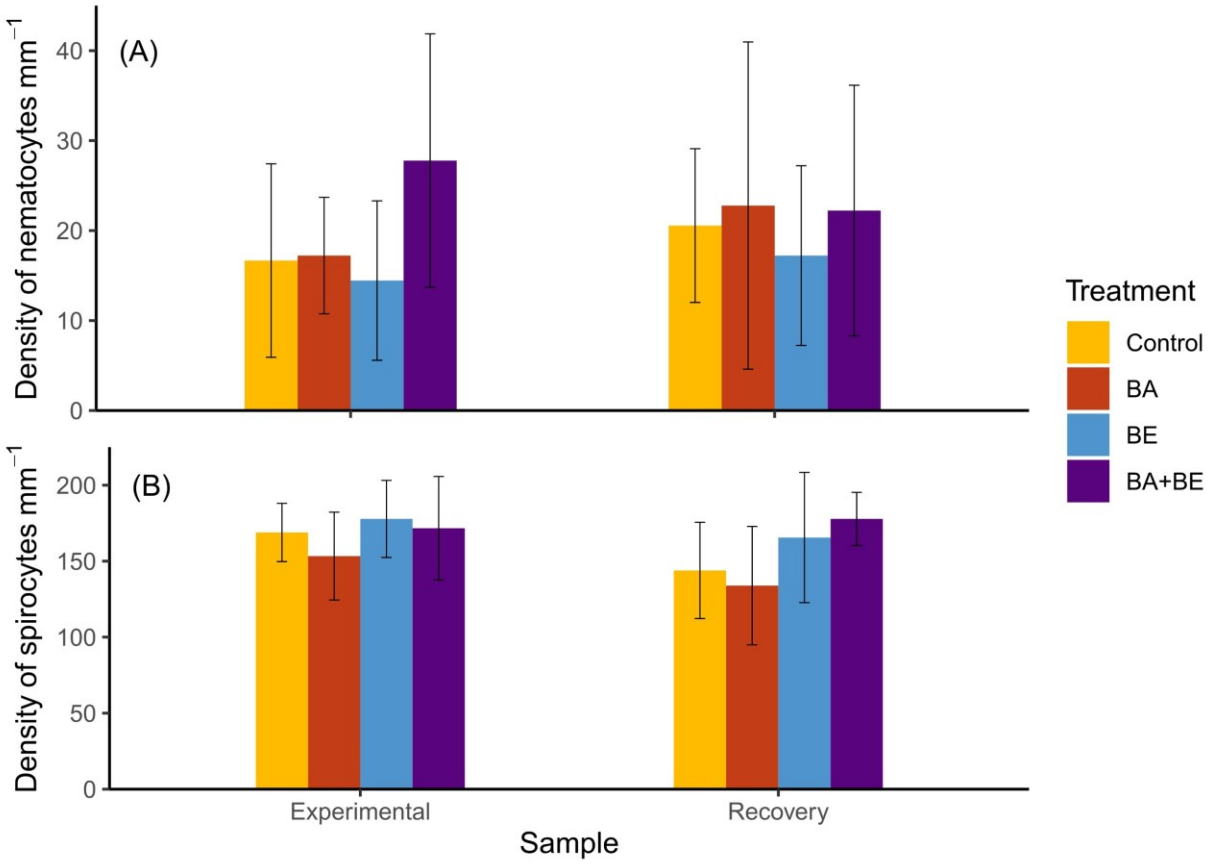


Figure 3-6: Density of (A) nematocytes and (B) spirocytes measured from the tentacles of *Flabellum* (*Ulocyathus*) *alabastrum* after a 10-d period of exposure ("Experimental") to barite (BA), bentonite (BE), a combination of barite and bentonite (BA+BE), no treatment (Control), or after an additional 10-d with no exposure ("Recovery"). Data shown as mean ± standard deviation (n = 6 corals per treatment per sample point).

3.10 Supplementary materials

3.10.1 Supplementary tables

Supplementary Table S3-1: Collection details of live individuals of *Flabellum* (*Ulocyathus*) *alabastrum* with the vessel CCGS *John Cabot* during multispecies trawl surveys conducted by DFO in May 2022.

	Site 1 (Set 17)	**Site 2 (Set 21)**
Date of trawl	May 7, 2022	May 8, 2022
Trawl coordinates		
Start position	45.033 N, -56.460 W	45.035 N, -54.833 W
End position	45.023 N, -56.448 W	45.028 N, -54.845 W
NAFO division	3P	3P
Mean depth (m)	385	638
Number of *F. alabastrum* included in experiment	20	76

Supplementary Table S3-2: Holding conditions onboard the CCGS *John Cabot*, laboratory holding conditions and experimental conditions.

	Onboard Holding	**Holding Tanks**	**Experimental Tanks**
Duration	9-10 d (May 7/8–May 17, 2022)	10 wk (May 17–July 27, 2022)	2 d acclimation (July 28–July 29) 20 d experiment (July 30–August 19, 2022)
Flow supply	Unfiltered seawater Flow rate: not measured	Unfiltered seawater Flow rate: 45-60 mL s^{-1}	Unfiltered seawater Flow rate: 20-40 mL s^{-1}
Temperature	3-8 °C Method: digital thermometer	1–8 °C* Method: HOBO logger, YSI	3-6 °C Method: HOBO logger, YSI
Light	Dark Not measured	Dark (0 lux) Method: HOBO logger	Dark (<10 lux) Method: HOBO logger
Density	95–238 ind m^{-2} 4–10 individuals per bucket (based on size)	8–49 ind m^{-2} 13–75 individuals per tank (depended on how many had been removed based on condition)	43 ind m^{-2} 8 individual per tank
Tank size and material	Fish tote (1000 L, 1.2 x 1.1 x 1.2 m) with suspended plastic buckets (9 L, 23 cm diameter, 23 cm height).	Fiberglass tank (192 L, 0.7 m diameter, 0.5 m height)	Glass aquarium (57 L, 61 x 30.5 x 30.5 cm)
Photos of set-up	Supplementary Figure S3-1	Supplementary Figure S3-2	Supplementary Figure S3-3
Description	*F. alabastrum* was held onboard the vessel. Individuals were placed in buckets which were suspended within a large fish tote. Buckets were separated by collection location	After transport from the vessel, *F. alabastrum* was allowed to recover in fiberglass tanks to acclimate to laboratory conditions, and to remove individuals	Two days prior to each experiment, eight individuals were randomly positioned into each experimental tank. Individuals were staggered in two rows

	Onboard Holding	Holding Tanks	Experimental Tanks
	(trawl sets). Density within buckets was based on size (e.g., buckets with larger individuals had lower densities, and vice-versa).	who showed visible damage by trawl. Tanks were separated by trawl set.	of four, facing the camera lens.

* = at one instance one of the chillers has stopped, and temperatures rose to 10.7 °C over a period of 1 h before being returned to <9 °C which took an additional 30 min (this affected one of the three holding tanks).

Supplementary Table S3-3: Models selected for statistical analyses.

Model ID	Model
1a	Mucus presence ~ treatment * day + time + (1 | tank ID) + (1 | coral ID) + (1 | day / time)
1b	Mucus presence ~ treatment * day + (1 | tank ID) + (1 | coral ID)
2a	Cnidocyte density ~ treatment + (1 | coral ID)
2b	Cnidocyte density ~ treatment + sample time + (1 | coral ID)
3a	Nematocyte density ~ treatment + (1 | coral ID)
3b	Nematocyte density ~ treatment + sampling time + (1 | coral ID)
4a	Spirocyte density ~ treatment + (1 | coral ID)
4b	Spirocyte density ~ treatment + sample time + (1 | coral ID)

"day**"** = number of days since experimental onset.
"time" = T1 or T2 measurement.
"tank ID" = unique codes identifying each tank.
"coral ID" = unique codes identifying each coral.
"sample time" = sampled either post-exposure or post-recovery period

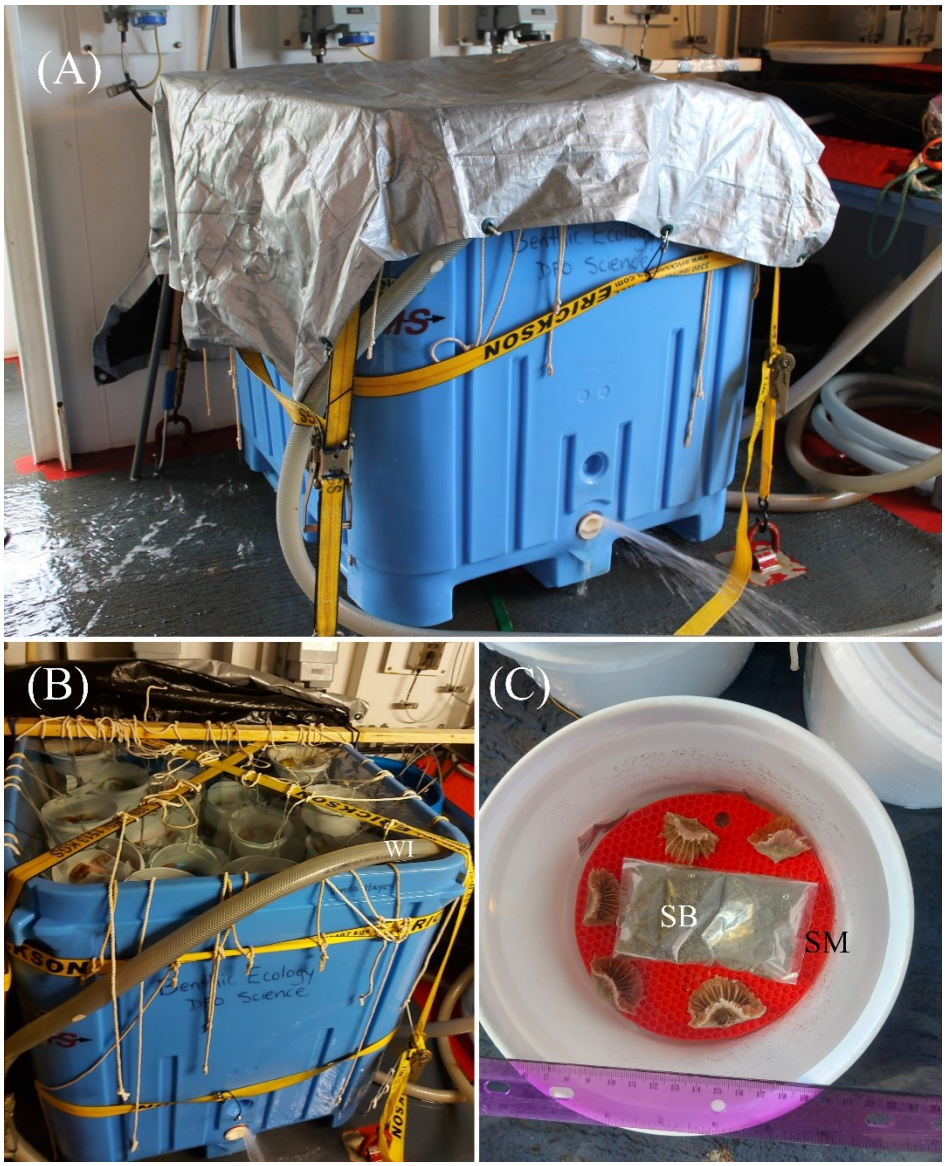
Supplementary Table S3-4: Cnidocyte densities counted along three 100-µm subsamples of tentacle cross sections in *Flabellum* (*Ulocyathus*) *alabastrum*. Individuals were sampled either after 10 d of exposure ("Exposure") to barite (BA), bentonite (BE) or a combination of barite and bentonite (BA+BE), or after 10 d of a recovery period ("Recovery"). Values are mean ± sd.

Treatment	Sample period	Nematocytes (mm^{-1})	Spirocytes (mm^{-1})	Total cnidocytes (mm^{-1})	Cnidocyte assemblage	
					Nematocyte (%)	Spirocyte (%)
Control	Exposure	17 ± 11	169 ± 19	186 ± 20	9 ± 6	91 ± 6
	Recovery	21 ± 9	144 ± 32	164 ± 24	13 ± 7	87 ± 7
BA	Exposure	17 ± 7	153 ± 29	171 ± 28	11 ± 5	89 ± 5
	Recovery	23 ± 18	134 ± 39	157 ± 31	17 ± 13	83 ± 13
BE	Exposure	14 ± 9	178 ± 25	192 ± 31	7 ± 4	93 ± 4
	Recovery	17 ± 10	166 ± 43	183 ± 45	9 ± 7	91 ± 7
BA+BE	Exposure	28 ± 14	172 ± 34	199 ± 46	14 ± 4	87 ± 4
	Recovery	22 ± 14	178 ± 18	201 ± 10	11 ± 7	89 ± 7

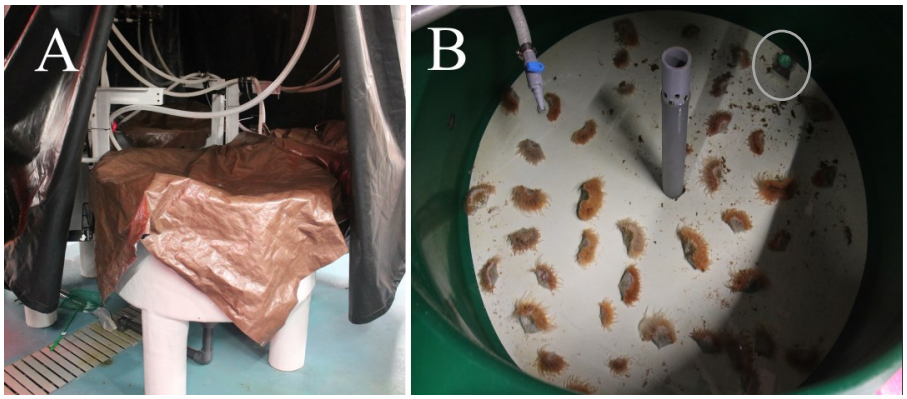
Supplementary Table S3-5: Total estimated weight and scanned area (as proportions) of barite (n = 3) and bentonite samples (n = 3) quantified by mineral liberation analysis.

	Mineral	Wt%	Area%	Particle Count
Barite	Barite	84.87	77.93	105257
	Bentonite	5.35	9.11	4237
	Quartz	5.38	8.40	3070
	Iron Oxide	2.64	2.05	2025
	Orthoclase Feldspar	0.53	0.85	681
	Calcite	0.50	0.76	477
	Plagioclase Feldspar	0.36	0.54	344
	Fluorite	0.12	0.16	70
	Mn Fe Oxide	0.17	0.14	66
	Pyrite	0.04	0.03	40
	Galena	0.04	0.02	60
	Fe Ti Oxide	0.00	0.01	24
	Total	100.00	100.00	109591
Bentonite	Bentonite	88.51	89.54	67600
	Plagioclase Feldspar	5.70	5.15	16854
	Quartz	4.12	3.81	13412
	Orthoclase Feldspar	1.23	1.17	2070
	Calcite	0.18	0.16	337
	Fluorite	0.09	0.07	171
	Pyrite	0.08	0.04	147
	Barite	0.04	0.02	149
	Fe Ti Oxide	0.01	0.02	9
	Iron Oxide	0.02	0.01	45
	Galena	0.02	0.01	10
	Total	100.00	100.00	89014

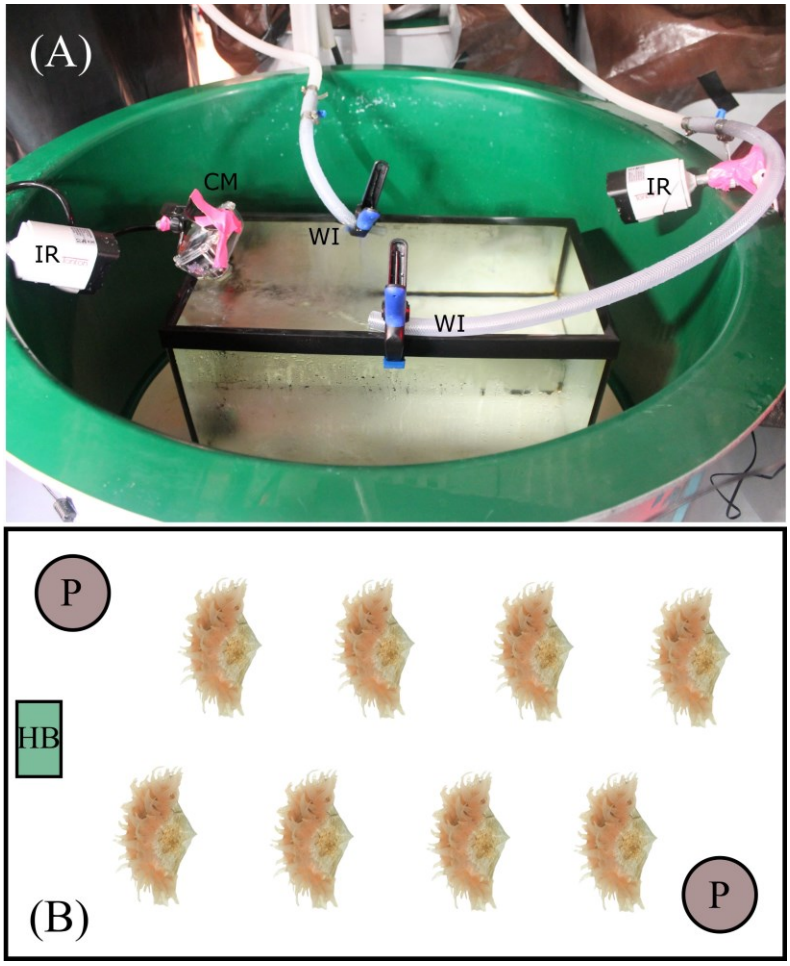
3.10.2 Supplementary figures



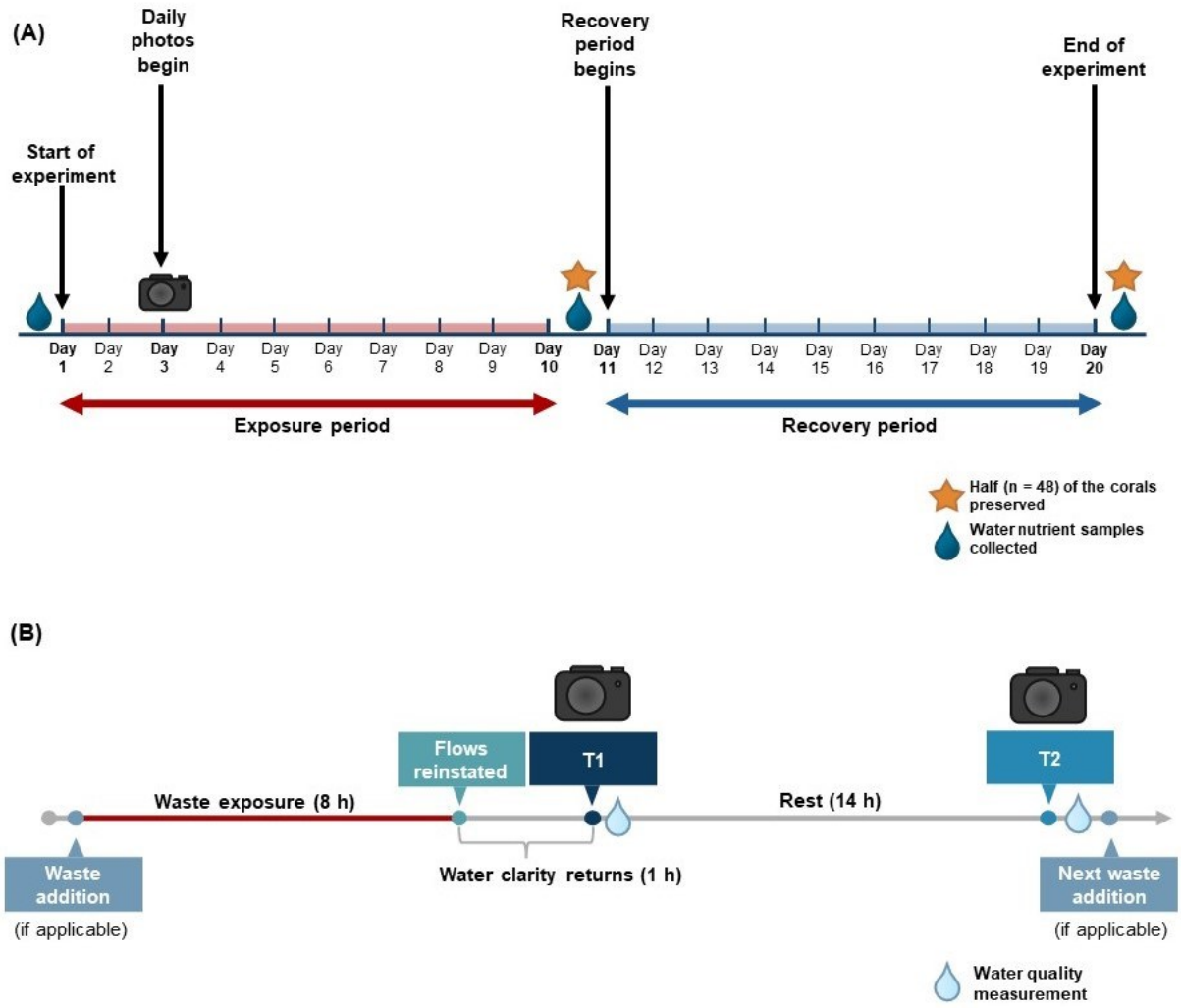
Supplementary Figure S3-1: Holding conditions onboard the CCGS *John Cabot*. (A) A large, insulated container (1000 L, 1.2 x 1.1 x 1.2 m) covered with a tarp and secured to the ship deck. (B) Individuals of *F. alabastrum* suspended in buckets by cotton rope inside the container. Water inflow was supplied by a large hose (WI). (C) Close up on bucket holding individuals of *F. alabastrum*, a sand bag (SB) for weight, and a silicon mat (SM) to prevent sliding.



Supplementary Figure S3-2: Laboratory holding conditions. (A) One of the fiberglass tanks (0.7 m diameter, 0.5 m height) covered with tarp and isolated by curtains to maintain darkness. (B) Tank interior with individuals of *F. alabastrum* and HOBO logger (circled).

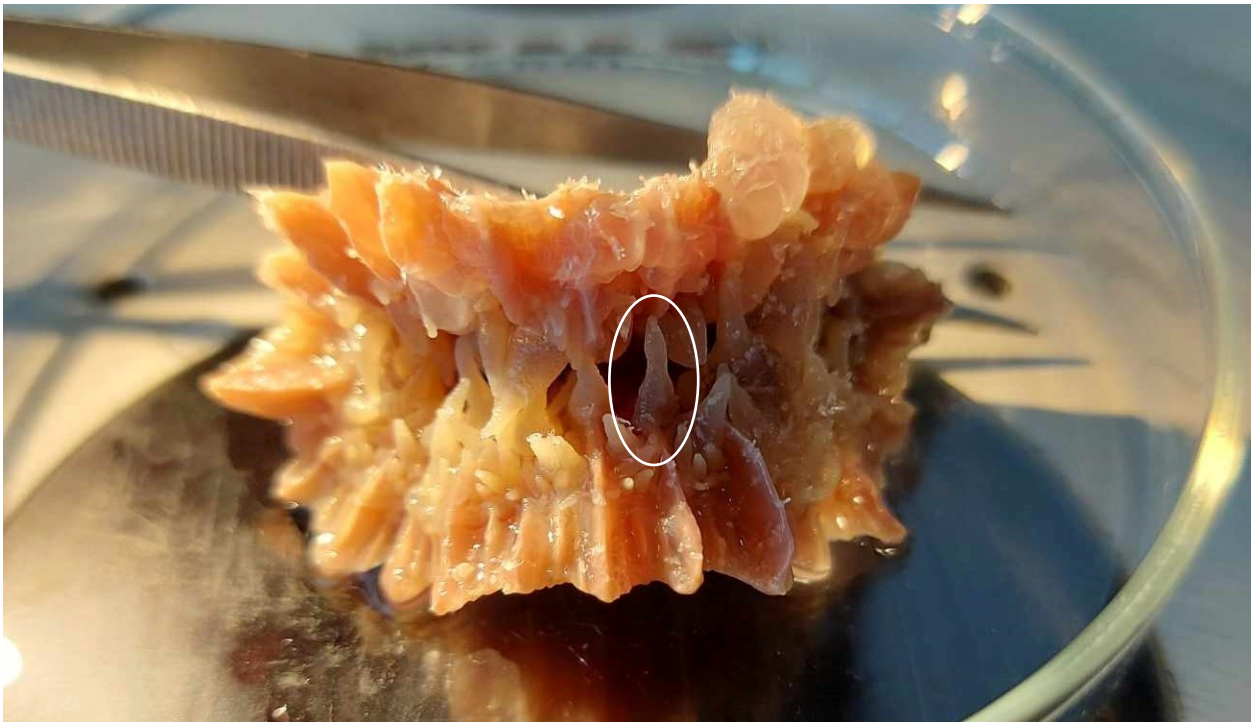


Supplementary Figure S3-3: Experimental tank set-up. (A) Experimental tank exterior (61 x 30.5 x 30.5 cm). Camera (CM) mounted with lens immersed in water. Water inflow provided by tubing clipped to the tank sides (WI). Infrared lights (IR) to allow time-lapse in dark conditions. (B) Schematic of tank interior with eight individuals of *F. alabastrum* in two rows, positioned to face the camera lens. A HOBO light and temperature logger (HB) at one end of the tank and glass petri dishes ("P", 5 cm diameter) at opposite corners of the tank.

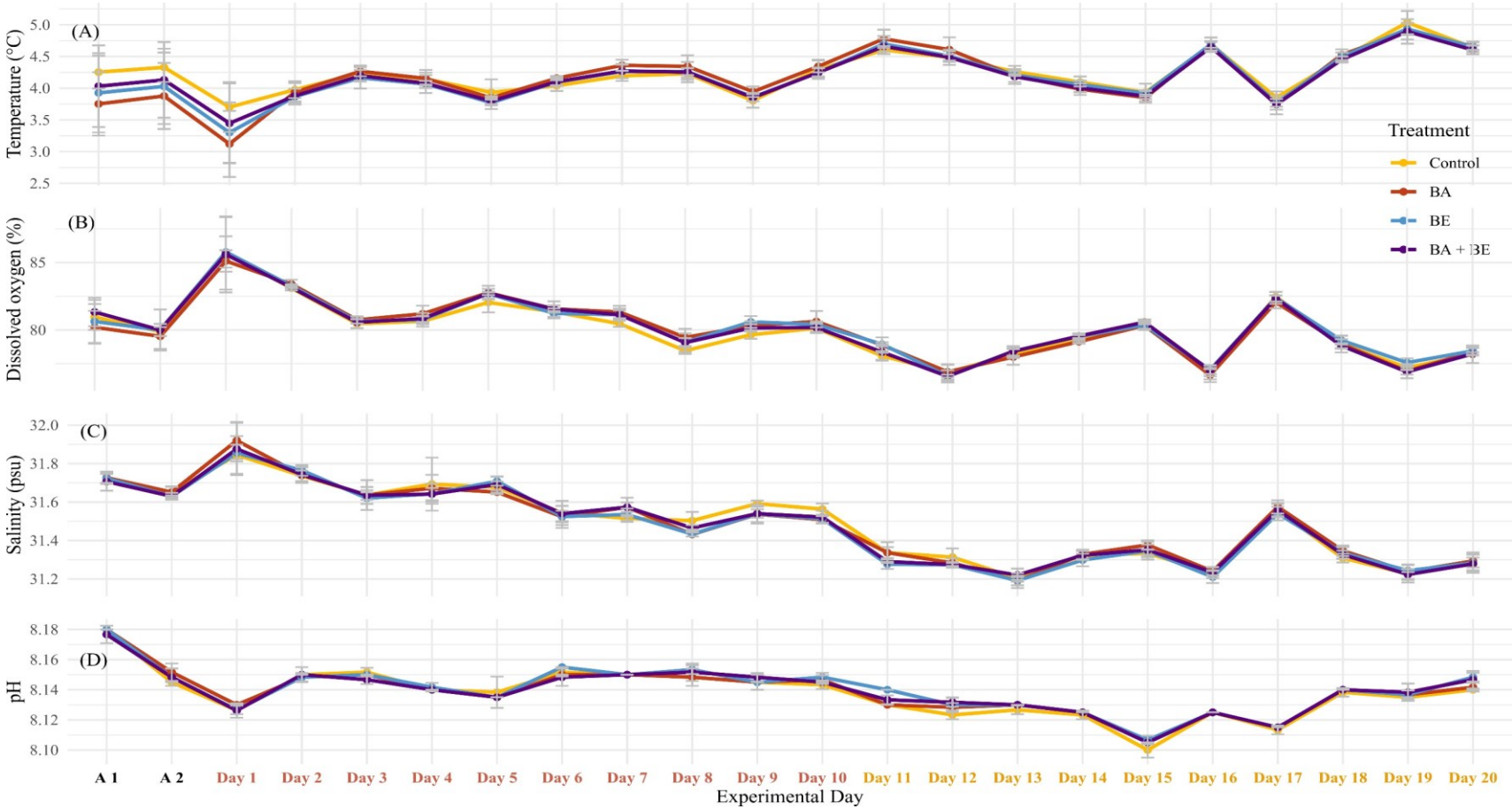


Supplementary Figure S3-4: Timeline of experimental period (A) and experimental days (B). (A) Corals were exposed to assigned treatments (BA, BE or BA+BE) or control conditions for 10 d ("Exposure Period"), followed by 10 d of no treatments ("Recovery Period"). Sampling (indicated by a star) occurred after each period, during which half the corals (n = 48) were preserved for histological work. Water samples were preserved for nutrient analysis prior to the onset of the experiment, after the exposure period, and again after the recovery period. Underwater photos used for behavioural and mucus observations began on the third experimental day. (B) Each experimental day began with the addition of waste to treatment tanks (excluding controls, and all individuals during the recovery period). At this time, the flows to the tanks were

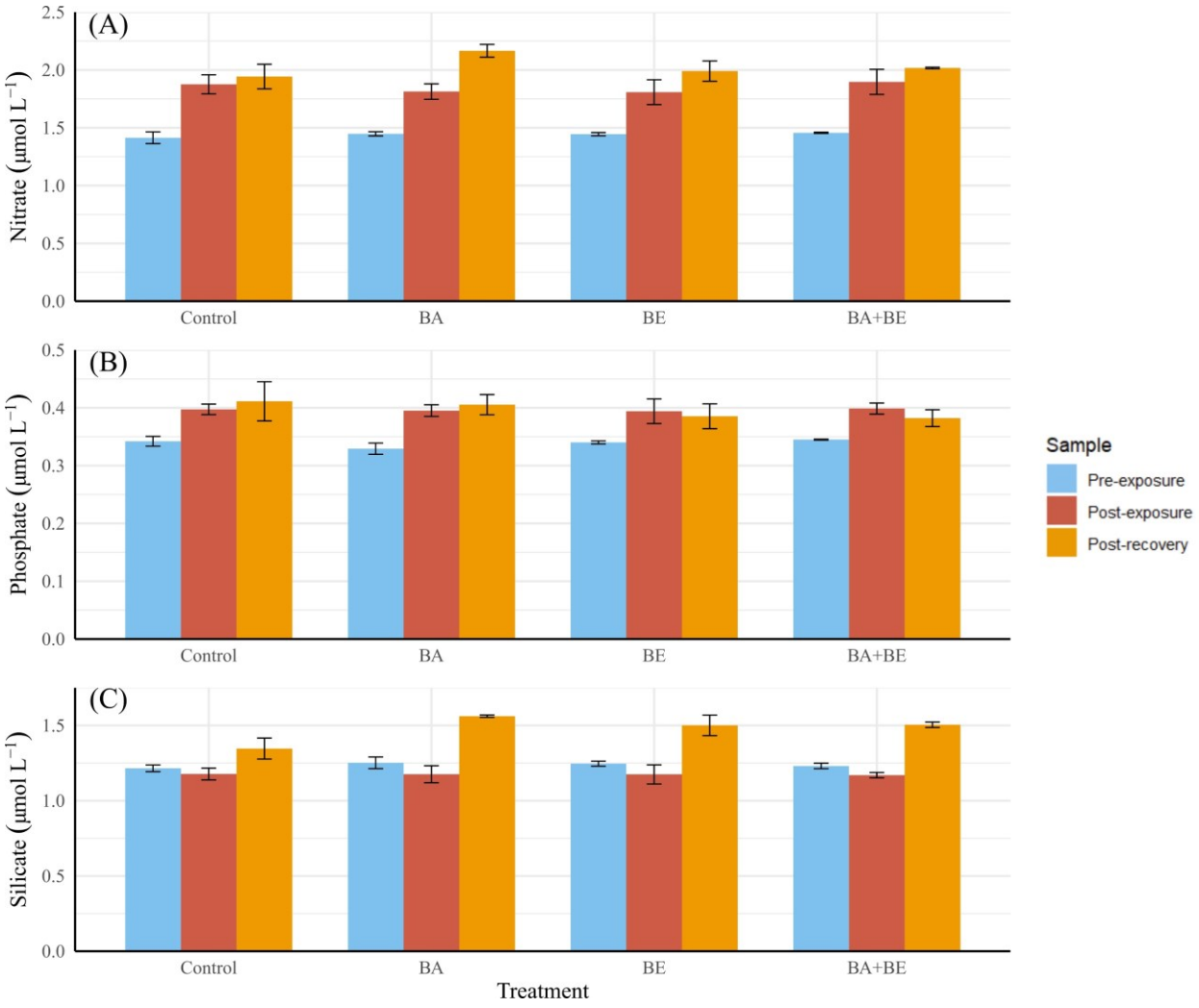
stopped. After 8 h, the flows were reinstated. After another ~1 h when water clarity returned, underwater photos were taken of each tank (referred to as “T1” timepoint). A second photo was taken ~14 hours later before the onset of the next experimental day (referred to as “T2” timepoint). Water quality was measured after T1 and T2 timepoints daily.



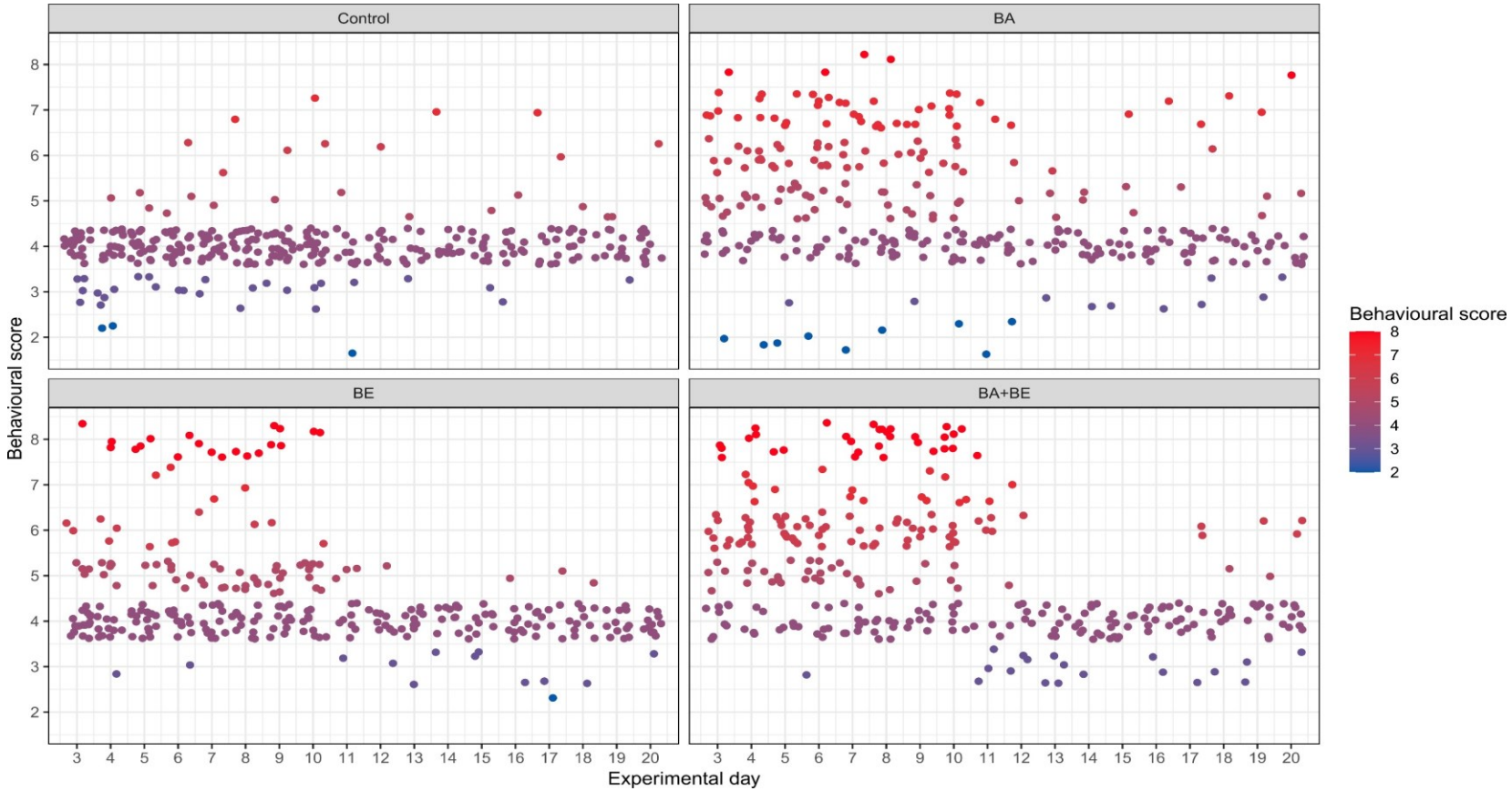
Supplementary Figure S3-5: Location of tentacle sampling from preserved *Flabellum* (*Ulocyathus*) *alabastrum* for histological work (indicated by white circle). Target tentacle was situated in the row of tentacles closest to the mouth, directly adjacent to the central tentacle of that row.



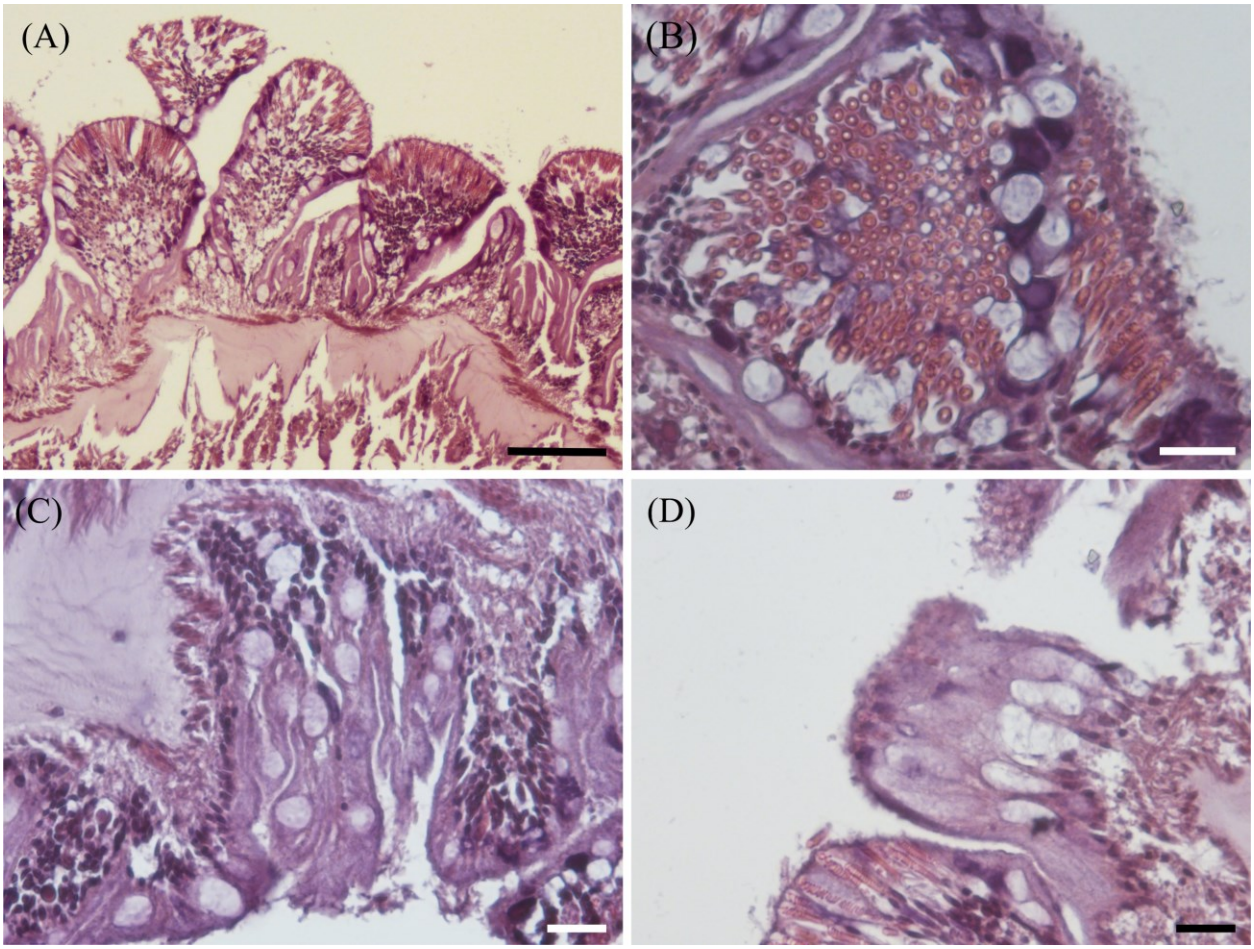
Supplementary Figure S3-6: Water quality parameters (temperature, dissolved oxygen, salinity, pH) measured across control, barite (BA), bentonite (BE), and barite + bentonite (BA+BE) treatment tanks (A-D, respectively). Parameters were measured twice daily during two days of acclimation (A1, A2), the exposure period (days 1–10), and the recovery period (days 11–20). Data shown as mean ± standard deviation (n = 3 tanks per treatment).



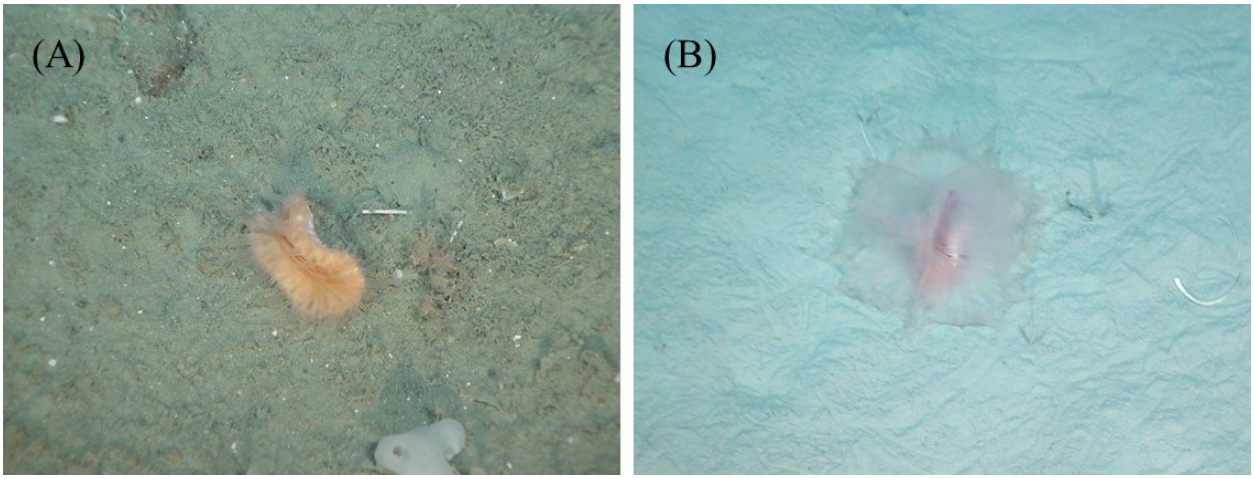
Supplementary Figure S3-7: (A) Nitrate, (B) phosphate and (C) silicate concentrations in control, barite (BA), bentonite (BE), or barite and bentonite combined (BA+BE) treatment tanks prior to any treatment additions (Pre-exposure), after 10 d of additions (Post-exposure), and after 10 d of recovery (Post-recovery). Data shown as mean ± standard deviation (n = 3 tanks per treatment).



Supplementary Figure S3-8: Jitter plot of behavioural scores of *Flabellum* (*Ulocyathus*) *alabastrum* at T1 (after 8 h of waste exposure) throughout the exposure (days 3 to 10) and recovery periods (days 11 to 20). During the exposure period, *F. alabastrum* experienced sedimentation of either barite (BA), bentonite (BE), or barite and bentonite combined (BA+BE). No sedimentation was experienced by control individuals or during the recovery period. Each dot represents one behavioural score recorded during each experimental day at T1.



Supplementary Figure S3-9: High densities of mucocytes observed (A) along epithelial tissue, (B) arranges around outside of cnidocyte battery (viewing cnidocytes from top), (C) in non-target tissue, (D) on edge of cnidocyte battery. Scale bar represents 100 µm in A and 20 µm in B-D.



Supplementary Figure S3-10: *Flabellum* (*Ulocyathus*) *alabastrum in situ* on the Grand Banks (south) of Newfoundland (A) at a baseline expansion behaviour and (B) in an excessive expansion or swelling. Photos courtesy of ROPOS, and Fisheries and Oceans Canada.

Chapter 4 - General discussion

4.1 Chapter summaries

The objective of this thesis was to expand the current knowledge of the effects of wastes produced by aquaculture and exploratory drilling activity on cnidarians common in the Newfoundland and Labrador region, with a focus on cold-water corals (CWC). More generally, this thesis provided the first results on the impacts of aquaculture waste on *Gersemia rubiformis* and *Aulactinia stella* and of exploratory drilling waste on *Flabellum* (*Ulocyathus*) *alabastrum*.

4.1.1 Chapter 2: aquaculture waste sedimentation on *Gersemia rubiformis* and *Aulactinia stella*

The potential effects of aquaculture waste (salmon feed and feces) on the soft coral *G. rubiformis* and the sea anemone *A. stella* were explored through a 28-d experiment. Experimental groups included control, inorganic sediment (0 g C m^2 d^{-1}), low waste (5 g C m^2 d^{-1}) and high waste (10 g C m^2 d^{-1}) treatments. No mortality was observed over the experimental period. Trends in behaviour over sedimentation time observed for *G. rubiformis* and *A. stella* were more similar between waste-exposed individuals, compared to inorganic sedimentation or control individuals. Higher levels of expansion of the polyps/tentacles were observed. As well, reductions in contraction frequencies of the colonies/individuals were noted over time, potentially indicating metabolic interference as this behaviour in *G. rubiformis* was also seemingly confounded by seasonal temperature declines. *Aulactinia stella* showed increased eversion of the pharynx under waste treatments, potentially as a feeding mechanism. To determine whether waste materials were being assimilated, lipid and fatty acid content was analyzed. Lipid content did not appear to be altered by treatment, and indications of declines in lipid reserves were not detected in this time-period for either species. *Aulactinia stella* had higher

indications of waste assimilation compared to *G. rubiformis*, showing an increasing trend with waste concentration for the three tracer fatty acids: strongly in 18:1ω9 and 18:2ω6, and weakly in 16:0. Consequently, both species indicated a potential for selectively assimilating feed over fecal particles. The fatty acid profile of *G. rubiformis* was influenced by treatment, possibly due to a higher sensitivity compared to *A. stella.* Emerging trends observed in behaviour and fatty acids show potential short-term effects of aquaculture waste exposure and these may become more pronounced over a longer period. This was highlighted by the appearance of microbial mats in the tanks on top of the waste towards the end of the experiment, suggesting effects on behaviour and biochemistry observed here may become more pronounced with chronic exposure, as expected *in situ.*

4.1.2 Chapter 3: barite and bentonite sedimentation on *Flabellum* (*Ulocyathus*) *alabastrum*

To evaluate potential effects of exploratory drilling on CWC, the cup coral *F. alabastrum* was exposed to sedimentation of barite (BA) and bentonite (BE), i.e. the main materials in water-based drilling muds, for 10 d. The results of this study showed that sedimentation <6.3 mm (often used as a probable no-effect threshold, PNET) did not result in mortality but had strong impacts on polyp expansion and mucus secretion. The greatest magnitude of effects was observed in a combined BA + BE treatment, followed by BA alone, then BE alone. These results are novel and could imply interactive effects, so should be considered in future experimental designs as to not underestimate the effects of these materials when combined. High levels of polyp swelling and mucus production as a response to BA and BE sedimentation may have energetic costs or other unknown long-term consequences. During a subsequent 10-d recovery period, behaviour returned to baseline expansion and visible mucus was not observed, suggesting

a high recovery potential after the cessation of BA and/or BE sedimentation. Histology was also used to explore potential effects of BA and BE sedimentation on cnidocytes (stinging cells) including nematocyte and spirocyte types. Results exhibited high variation within treatments but trends indicated a potential elevation in nematocyte density after 10 d of exposure to BA+BE, requiring further exploration. As well, this study provided the first insights of the cnidocyte types found within *F. alabastrum*. Results from this chapter show that low quantities (<6.3 mm) of common wastes produced by exploratory drilling have observable effects on *F. alabastrum*.

4.2 Management considerations

4.2.1 The conservative nature of this thesis

As this was the first study investigating the effects of wastes produced by aquaculture and the oil and gas industry on the targeted species, a conservative approach to the experimental designs was taken and should be acknowledged.

In Chapter 2, the concentrations of waste material (5 g C m^2 d^{-1} and 10 g C m^2 d^{-1}) were chosen based on regional aquaculture modeling requirements of organic material by industry (AAR 2015). Although the experiment only lasted 28 d, which is short in the context of aquaculture operations, effects in the behaviour and potential trends within the fatty acid compositions could nevertheless be observed. However, chronic sedimentation over multiple seasons, especially with a build-up of organic matter on the benthos and changes in microbial activity (as seen towards the end of the experiment), may yield stronger effects with time. Therefore, effects experienced *in situ* are expected to be more severe than those only beginning to show in the 28-d period tested here.

In Chapter 3, a target sedimentation depth of 6.3 mm was chosen to be comparable to existing studies (Larsson and Purser 2011, Allers et al. 2013) as well as a PNET included in

depositional modeling of drill wastes by industry (e.g. Amec Foster Wheeler 2017, BP 2017, Wood 2021). The target concentration used here was much lower than those which can be expected closer to a well head (>50 mm, DNV 2013) and does not account for multiple wells being drilled in the same area. Importantly, the present study considered the wet volume (volume after hydration of material) of BA and BE, which to my knowledge has not been mentioned in previous CWC experimental designs. A dry sedimentation volume of 6.3 mm of the materials was not transferable to a wet depth, so the wet volume of BA and BE was accounted for when determining quantities of material to be added to the tanks. This means material added to the tanks was 50% less for BA, 85% less for BE and 75% less for BA+BE relative to what would have been added to reach a 6.3 mm dry volume. This aspect should be considered when comparing the results to other studies or depositional models.

Finally, drill cuttings and other drilling mud types (synthetic and oil-based drilling muds) were not evaluated for this project. These are presumed to have more severe effects (physical, toxicological) on CWC relative to BA and BE. Therefore, the conclusions from this study of the effects of exploratory drilling on CWC remain preliminary and it must be acknowledged that those of clay-like drill mud materials and effects *in situ* will likely be more severe when combined with drill cuttings.

4.2.2 The issues of 6.3 mm as a probable no-effect threshold (PNET) for CWC

The widespread use of the 6.3 mm PNET (sometimes mentioned as 6.5 mm), within Canada and internationally, reflects the need for more relevant data and standards regarding CWC management. This value first mentioned in Kjeilen-Eilertsen et al. (2004) and most commonly attributed to Smit et al. (2008) has been cited as a threshold below which adverse

effects on benthic organisms are not expected to occur, and is included in the modeling of predicted depositional depths of drilling waste (e.g. Amec Foster Wheeler 2017, BP 2017, Wood 2021). Not only was the 6.3 mm value calculated from *burial* depths (depth of sediment accumulation after total burial of an organism) rather than depositional depth (depth of sediment deposited on an unburied organism) as assumed for CWC, but this value was also determined from a species sensitivity distribution derived from 32 species; 75% of which were molluscs, 16% crustaceans and 9% polychaetes (Smit et al. 2008). Many of these taxa are endobenthic and others have a range of mobility that sessile forms of CWC (e.g. those attached to substratum), and other benthic organisms lack.

To date, studies evaluating this 6.3 mm value have not demonstrated the applicability of this threshold to CWC and have instead found potential negative effects at this value (Larsson and Purser 2011), including the results of the present thesis. At the time of determination of this value, there were limited available data and so it was recommended "for the time being" (Smit et al. 2006). Almost twenty years later, this value persists.

A more conservative PNET of 1.5 mm has also been suggested (Kjeilen-Eilertsen et al. 2004), though less widely applied, and has been incorporated into some industry reports along side the 6.3 mm value (e.g. Amec Foster Wheeler 2017, Wood 2021). To my knowledge, the 1.5 mm PNET has not been experimentally tested. Given the effects seen here with the 6.3 mm target depth, and the seeming lack of practical applicability to CWC, a more precautionary approach of a lower PNET may be considered.

4.2.3 Particle Type

The results of this study highlight the importance of including experimental treatments true to realistic waste material. Though studies exist testing the effects of natural sedimentation

on CWC, these should not be used as indicative of potential effects of industry material. The present thesis provides an example in which different particle types were seen to invoke different levels of response. This was observed in Chapter 2, with differing behavioural effects between treatment subjects exposed to aquaculture waste versus inorganic sediment, as well as in Chapter 3 with individuals exposed to BA and/or BE treatment reacting to different extents. This is consistent with the results of previous CWC studies. For example, Liefmann et al. (2018) reported that when exposed to abrasive mine tailings, *Primnoa resedaeformis* showed increases in food intake, as well as polyp mortality and had particles embedded in their tissue, which were not observed after exposure to smooth glass beads. Likewise, *Duva florida* additionally displayed prolonged contraction behaviour, reductions in food intake and had particles embedded in their tissue only under the mine tailing treatment (Liefmann et al. 2018). Finally, Carreiro-Silva et al. (2022) found that polymetallic sulphide particles (related to deep-sea mining) and quartz particles affected the octocoral *Dentomuricea* aff. *meteor* differently in terms of survival, tissue condition, respiration and ammonia excretion. These findings emphasize the importance of using experimental material realistic to or derived from industry waste expected *in situ*.

4.2.4 *Flabellum* expansion and imagery surveys

The insights into the behaviour of *F. alabastrum* gained in this thesis hold value for *in situ* benthic imagery surveys. The high levels of extreme polyp expansion exhibited by *F. alabastrum* in the study of Chapter 3 and previously observed by Buhl-Mortensen et al. (2007), may disguise *F. alabastrum* from its typical (expected) appearance. This may have implications for the proper identification of expanded individuals of *F. alabastrum* recorded during image-based surveys, especially in areas close to drilling sites where higher levels of expansion may be expected to occur. Care should therefore be taken to include over-expanded individuals in visual guides in

order to facilitate identification in support of more accurate estimates of the species presence, distribution and densities.

4.3 Future directions

To gain a clearer understanding on how the expansion or marine industries will impact CWC and other epibenthic, sessile/sedentary taxa, more research needs to be conducted within this field. Below are directions and knowledge gaps that have been identified throughout this thesis project that warrant future research.

- *The impacts of drill cuttings on* F. alabastrum. Despite substantial efforts, drill cuttings could not be successfully obtained for the experiments conducted in Chapter 3. Based on the differences in particle shape between drill cuttings (sharp) and drill muds (clay-like, less abrasive), drill cuttings are expected to have an abrasive physical impact on tissue (similar to those of mine tailings tested in Liefmann 2018). Polyp swelling, as seen in this thesis, combined with potential tissue damage from sharp particles could be detrimental to *F. alabastrum*. Additionally, as individuals *in situ* would be exposed to both drilling muds and drill cuttings together, more accurate reflections of impacts occurring in the field will be obtained by the inclusion of drill cuttings along with drilling muds in future work.
- *The impacts of drilling and aquaculture on different CWC life stages and size classes.* The largest (adult) individuals were collected and chosen for the experiments within this thesis for the purposes of visibility in time-lapse videos. However, smaller and more vulnerable life stages and size classes will presumably have different mechanisms and energy reserves to cope with sedimentation stress. So far, studies on the effects of oil/gas wastes have been conducted on larvae of *D. pertusum* (Järnegren et al. 2017, 2020) and

larval settlement in the tropical coral *Pocillopora acuta* has been shown to be impeded by aquaculture waste (Quimpo et al. 2020). However, the non-mobile early life stages of CWC (i.e. primary polyps) may be even more vulnerable.

- *Long-term effects of aquaculture and drilling wastes remain understudied*. Benthic video surveys often employed to monitor impacts at aquaculture and drilling sites will likely not reflect the potential hidden impacts of waste exposure (e.g. reproductive costs, physiological consequences). Longer-term impacts of aquaculture were recently explored by Kutti et al. (2022) through transplantation experiments, which indicated lipid reserves may assist corals in coping with waste exposure. However, previous studies have indicated that mechanisms such as oocyte reabsorption (Rossin et al. 2019) can be employed by CWC under stress to increase energy reserves, which would negatively impact fecundity. As more research is conducted in this field, long-term impacts of industrial activities on CWC will become clearer.
- *In situ sampling and experiments.* As seen in Kutti et al. (2022), valuable data can be extracted by *in situ* transplantation (in their case under aquaculture sites). A transplantation study of CWC placed at different distances from a planned drilling site could be beneficial for tracking the magnitude of impacts on them at different distances from the well. Likewise, comparing samples of CWC collected at different distances from aquaculture or drilling sites could provide complementary insights into laboratory or field observations.
- *More species need to be evaluated regarding impacts of marine industry expansion*. Data is limited to only a few species outside *D. pertusum* for both aquaculture and oil/gas exploration, so information regarding the sensitivity of taxonomic groups with different

morphologies remains largely unknown. For example, sea pens (superfamily Pennatuloidea) were shown to hold ecological importance (Baillon et al. 2012, 2014, Boulard et al. 2022), however to my knowledge nothing is known yet about the effects of drilling exploration on this group.

- *Cumulative effects of anthropogenic activities.* With increasing habitat alteration from sources such as climate change (e.g. ocean warming, acidification) and industry activity, the cumulative and interactive effects of multiple stressors may pose additional threats to CWC (as in the study by Scanes et al. 2018). While many basic questions with regards to the effects of aquaculture and oil/gas exploration on CWC still need to be investigated, more advanced topics such as potential cumulative effects should be considered as data is collected.

4.4 Literature cited

[AAR] Aquaculture Activities Regulations, SOR/2015-177.

Allers, E., R. M. M. Abed, L. M. Wehrmann, T. Wang, A. I. Larsson, A. Purser, and D. de Beer. 2013. Resistance of *Lophelia pertusa* to coverage by sediment and petroleum drill cuttings. Marine Pollution Bulletin 74:132–140.

Amec Foster Wheeler. 2017. Flemish Pass exploration drilling program, drill cuttings dispersion modelling. Appendix D. Retrieved from: https://iaac-aeic.gc.ca/050/documents/p80117/122070E.pdf (accessed June 12, 2024).

Baillon, S., J. F. Hamel, and A. Mercier. 2014. Diversity, distribution and nature of faunal associations with deep-sea pennatulacean corals in the northwest Atlantic. PLoS ONE 9:e111519.

Baillon, S., J.-F. Hamel, V. E. Wareham, and A. Mercier. 2012. Deep cold-water corals as nurseries for fish larvae. Frontiers in Ecology and the Environment 10:351–356.

Boulard, M., P. Lawton, K. Baker, and E. Edinger. 2022. The effect of small-scale habitat features on groundfish density in deep-sea soft-bottom ecosystems. Deep-Sea Research Part I: Oceanographic Research Papers 193:e103891.

BP. 2017. Newfoundland Orphan Basin exploration drilling program: drilling mud and cuttings dispersion modelling. Appendix B. Retrieved from: https://iaac-aeic.gc.ca/050/documents/p80147/125913E.pdf (accessed June 12, 2024).

Buhl-Mortensen, L., P. Mortensen, S. Armsworthy, and D. Jackson. 2007. Field observations of *Flabellum* spp. and laboratory study of the behavior and respiration of *Flabellum alabastrum*. Bulletin of Marine Science 81:543–522.

Carreiro-Silva, M., I. Martins, V. Riou, J. Raimundo, M. Caetano, R. Bettencourt, M. Rakka, T. Cerqueira, A. Godinho, T. Morato, and A. Colaço. 2022. Mechanical and toxicological effects of deep-sea mining sediment plumes on a habitat-forming cold-water octocoral. Frontiers in Marine Science 9:e915650.

[DNV] Det Norske Veritas. 2013. Monitoring of drilling activities in areas with presence of cold water corals.

Järnegren, J., S. Brooke, and H. Jensen. 2017. Effects of drill cuttings on larvae of the cold-water coral *Lophelia pertusa*. Deep Sea Research Part II: Topical Studies in Oceanography 137:454–462.

Järnegren, J., S. Brooke, and H. Jensen. 2020. Effects and recovery of larvae of the cold-water coral *Lophelia pertusa* (*Desmophyllum pertusum*) exposed to suspended bentonite, barite and drill cuttings. Marine Environmental Research 158:e104996.

Kjeilen-Eilertsen, G., H. Trannum, R. Jak, M. Smit, J. Neff, and G. Durell. 2004. Literature report on burial: derivation of PNEC as component in the MEMW model tool. Report AM 2004/024.

Kutti, T., E. Legrand, V. Husa, S. Olsen, Ø. Gjelsvik, M. Carvajalino-Fernandez, and I. Johnsen. 2022. Fish-farm effluents cause metabolic depression, reducing energy stores and growth in the reef forming coral *Lophelia pertusa*. Aquaculture Environment Interactions 14:279–293.

Larsson, A. I., and A. Purser. 2011. Sedimentation on the cold-water coral *Lophelia pertusa*: cleaning efficiency from natural sediments and drill cuttings. Marine Pollution Bulletin 62:1159–1168.

Liefmann, S. 2016. Eco-physiological responses of cold-water corals to anthropogenic sedimentation and particle shape. Norweigian University of Technology, Trondheim.

Quimpo, T. J. R., C. A. Ligson, D. P. Manogan, J. N. C. Requilme, R. L. Albelda, C. Conaco, and P. C. Cabaitan. 2020. Fish farm effluents alter reef benthic assemblages and reduce coral settlement. Marine Pollution Bulletin 153:e111025.

Rossin, A. M., R. G. Waller, and R. P. Stone. 2019. The effects of in-vitro pH decrease on the gametogenesis of the red tree coral, *Primnoa pacifica*. PLoS ONE 14:e0203976.

Scanes, E., T. Kutti, J. K. H. Fang, E. L. Johnson, P. M. Ross, and R. J. Bannister. 2018. Mine waste and acute warming induce energetic stress in the deep-sea sponge *Geodia atlantica* and coral *Primnoa resedeaformis*; results from a mesocosm study. Frontiers in Marine Science 5:e129.

Smit, M. G. D., K. I. E. Holthaus, H. C. Trannum, J. M. Neff, G. Kjeilen-Eilertsen, R. G. Jak, I. Singsaas, M. A. J. Huijbregts, and A. J. Hendriks. 2008. Species sensitivity distributions for suspended clays, sediment burial, and grain size change in the marine environment. Environmental Toxicology and Chemistry 27:1006–1012.

Smit, M. G. D., J. E. Tamis, R. G. Jak, C. C. Karman, G. Kjeilen-Eilertsen, H. Trannum, and J. Neff. 2006. Threshold levels and risk functions for non-toxic sediment stressors: burial, grain size changes and hypoxia. Summary report. TNO report 2006-DH-0046/A.

Taormina, B., R. H. Escobar-Lux, E. Legrand, A. E. Parsons, T. Kutti, V. Husa, R. Hannisdal, O. B. Samuelsen, and A. L. Agnalt. 2024. Effects of the sea lice chemotherapeutant, emamectin benzoate, on metabolism and behaviour of the sea-pen *Pennatula phosphorea*. Marine Pollution Bulletin 198:e115903.

Wood. 2021. Flemish Pass exploration drilling project 2021 drilling discharges follow-up monitoring program.