Nadia Frontier- 02.11.2020

Diving project plans- Marine Biologist 2021-2022

Index- In order of priority

i) **Aim:** Obtain seasonal, field growth rates of abundant benthic species kept in mesocosms

Overview: Six invertebrate species will be maintained in *in situ* 'mesocosms' for a 12- month period. Some mesocosms will be filled with filtered sediment (devoid of life) others will be installed with existing sediment containing natural prey densities. Repeated sampling (4 x events across ~12 months) of the mesocosms will be achieved using a suction sampler to empty the mesocosm contents. Contents will be bought back to the laboratory to obtain growth measurements and conduct physiological tests. Contents should be returned as soon as possible to the mesocosm.

Method & Timeline:

Site requirements: Shallow plateau, relatively fine sediment of at least ~10 cm depth, and subject to as little ice scour disturbance as possible. Install protective concrete blocks in front of mesocosms.

Gold standard (x2 sites, 30 mesocosms):

Hangar cove, 15 replicate mesocosms

Runway boulder support/ Backbay Lagoon, install 15 mesocosms

The main purpose of replicating this experiment twice is to account for the destruction of the experimental system. Installing 15 replicate mesocosms is accounting for the loss/failure of 5. Having a 'safety' site would be ideal but I appreciate this might not be possible given logistical and time constraints (esp. because Backbay Lagoon is relatively far).

Bronze standard: Enough data can be generated from one site with 10 mesocosms.

1) **Sediment collection**, two proposed methods:
   a) Collect sufficient sediment to fill the base of 18 mesocosms (**15 cm** high x **41 cm** wide). This will be utilised in the cleared mesocosms containing the bivalves *Aequiyolida eightsi* and *Laternula elliptica* and the polychaetes *Leitoscoloplos kerguelensis, Aglaophamus trissophyllus.*

During 1 dive at Hanger Cove, the diver will use a shovelling tool to add sediment into heavy duty **30 L** buckets that will be deployed from the RIB. Line signals will be given to topside to haul the buckets when appropriately full. This process will be repeated ~6 times.

b) Collect sediment adjacent to the mesocosm installation site and pass it though a sieve above the mesocosm. Visibility will be the limiting factor here.

2) Species collection: Sufficient species will be collected to replicate natural densities. Species will be collected over as large size range as possible. Table 1 demonstrates the **Gold standard.**

Table 1: Anticipated collection densities of the study invertebrates based on natural densities.

Species	No. of individuals per mesocosm	No.of mesocosms	Total no. of species to collect	Collection site
Aequiyolida eightsi- Mollusc	200	6	1,200	Hanger
Laternula elliptica- Mollusc	4	6	24	Hanger
Aglaophamus trissophyllus *	18	3*	54	Hanger
Leitoscoloplos kerguelensis *	158	3*	474	Hanger or South Cove
Ophionotus victoriae	4	6	24	South Cove
Sterechinus neumayeri	5	6	30	RMAsst collection site

Aglaophamus trissophyllus and *Leitoscoloplos kerguelensis* will share a mesocosm.
I will conduct some initial growth trials using these polychaetes given that they are mobile species. Alternatively, I could work with *Edwardsia* anenomes and repeatedly sample their biomass

Bronze standard: Halving the project to collect three species: *Aequiyolida eightsi, Ophionotus victoriae* and *Sterechinus neumayeri*. This will result in 3 replicate mesocosms for each species, at one site, with one spare mesocosm.

3) Mesocosm installation: X 30 100 L catering bins made of 250 mm HDPE have been ordered and will arrive on station with the JCR (internal diameter **41 cm** x height **66.5 cm**). The bins will be sawed into three units (~33 L, internal diameter **41 cm** x height **22 cm**). The possible methods are outline below for transforming the bins into mesocosms and installing them *in situ*:

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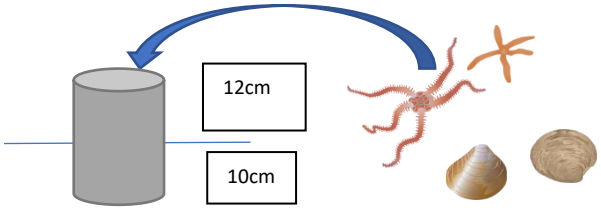


12 cm should allow sufficient water movement and sediment oxygenation

a) *Pre instalment*: saw the bottom off the bin. Place the bin on the sediment surface and suction sample the inner contents to install the bin. Use the flexible lip of the bin to enable divers to hold the bin and enact a 'cookie cutter' movement to wedge the bins into the sediment. Line the base of the bin with a 1mm mesh to prevent non-experimental species burrowing up/ experimental species burying down. Concrete weights (made from milk tins) will keep the mesocosm in place during the four sampling intervals when the internal mesocosm contents is removed.
b) Use a trowel or a soft sediment suction sampler to excavate a **~15 cm** pit in the sediment. Place the mesocosm in the hole and add large rocks to the bin's base to weight it down.
c) . A tight seal should be created between the mesocosm and the adjacent sediment to limit movement as much as possible and stabilise the system.

Species will the be added into the corresponding mesocosms. *Sterechinus* and *Ophionouts* will be put into mesocosms with unfiltered sediment in order for natural food sources to be present (n = 12). *Aequiyolida eightsi, Laternula elliptica, Leitoscoloplos kerguelensis* and *Aglaophamus trissophyllus* will be distributed between sieved sediment mesocosms (n = 18).

Gold standard: 3 repeated sampling events

Bronze standard: 1 repeated sampling event

4) **Repeated sampling:** Three repeated sampling events conducted in Autumn, Winter and Summer.

   Description of a single sampling period:
   Using the suction sampler (**10 cm** diameter, **1 mm²** sampling bag) to excavate the contents of the mesocosms containing *Aequiyolida eightsi, Laternula elliptica, Leitoscoloplos kerguelensis* and *Aglaophamus trissophyllus.* Hopefully, this task could be completed during one dive at each site (n = 9 per site).
   Change the sampling bag for each mesocosms and immediately transfer them into a pre-prepared buckets of water.

   During the second dive, the diver will hand-collect *Ophionotus victoriae* and *Sterechinus neumayeri* at each site. These species will have been added in low densities into the mesocosm system and therefore collecting them should be feasible during one dive.

5) **Re-deployment:** If possible, a subsequent dive within the seasonal sampling event should return the species, and associated clean sediment, to their aquarium as soon as possible.

Table 2: Anticipated number of dives for the invertebrate field growth study. Subject to change.

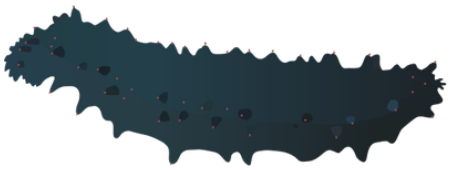
Task	Location	Date	No. of dives
Sediment collection	Hangar Cove	January	1-2
Species collection	Hangar Cove, South Cove	January	2-3
Mesocosm installation	Hangar Cove, Backbay Lagoon	February-March	10 (3 mesocosms per dive)
Repeated sampling: Autumn	Hangar Cove, Backbay Lagoon	June	6 dives *
Repeated sampling: Winter	Hangar Cove, Backbay Lagoon	September	6 dives *
Repeated sampling: Summer	Hangar Cove, Backbay Lagoon	December	6 dives *

**1 suction sampling dive at each site for bivalves; 1 collection dive for hand collecting mobile fauna at each site; 1 dive at each site to return all organisms*

ii) Collect year- round growth rates and physiological information from tagged *Heterocucumis steineni*

Overview: *Heterocucumis steineni* is an important suspension feeder in Antarctic benthic communities. Field growth rates are currently scant for many invertebrates. The aim of this study is to collect a large cohort, of adults ranging in size, with the aim of following their grates rates throughout the season. This project will develop a novel PIT tagging technique which has never been used in Antarctica before. Field data will be combined with laboratory experiments (dietary analyses, metabolism and faceable egestion) with the overall objective of obtaining a seasonal energy budget for this sea cucumber species. This holistic approach aims to improve our understanding of *H. steineni*'s role and contribution to the food web.

Obtain 20-30 *Heterocucumis steineni* for laboratory trials (late Dec- early January). Joe and Emma will collect these in anticipation of my arrival. The purpose of this is to monitor PIT tag retention rates.



Gold standard: Successfully PIT tag 120 *H. steineni* and repeatedly sample 50 individuals, 4 times across a 12 month period.

Bronze standard: PIT tag 50. Repeatedly sample 10 individuals at each interval and do not conduct a re-deployment dive.

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1) **Collection:** Collect ~125 *H. steineni* individuals from Hangar Cove during the summer (March). Sampling should cover as much of a large range as possible to mitigate the impact on the population. Insert PIT tags via the oral tentacle in aquaria and leave in holding tank for a few weeks.

2) **Deployment:** Deploy the individuals at **Hangar or South Cove** (March-April) and utilise the pre-existing concrete blocks to constrain ranging behaviour and aid with successful relocation.

Alternative option is to choose a site in conjunction with the RMAsst collections and build a 'sea cucumber nursery' for the cucumbers.

3) **Repeated sampling:** Each season ~2 dives will be conducted at Hangar/South Cove with a transponder. This will enable re-location of 50 tagged *H. steineni*. *H. steineni* will be returned to the lab and 25 individuals will be sacrificed for gut content analyses. The remaining 25 live *H. steineni* will be held in the laboratory for 3 days to perform faecal egestion and respirometry. A subsequent dive should aim to re-deploy the 25 live cucumbers back to their 'nursery'. The purpose of this is to replenish the long-term cohort and obtain seasonal measures from specific individuals.

Table 3: Anticipated number of dives for the *H. steineni* field growth study. Subject to change

Task	**Location**	**No. of cucumbers**	**Date**	**No. of dives**
Collection for test purposes	*Pending*	30	December-January	*Joe and Emma*
Experimental collection	Hangar or South Cove	125	March	2
Deployment	Hangar or South Cove	125	April	1
Repeated sampling: Autumn	Hangar or South Cove	50	July	2 (x1 dive for re-location and x1 dive for re-deployment)
Repeated sampling: Winter	Hangar or South Cove	50	October	2
Repeated sampling: Summer	Hangar or South Cove	50	January	2

4) **Water sampling:** Collect replicate water samples with a Niskin bottle in conjunction with each seasons sampling. This could be obtained during subsequent boating trips/ RMasst sampling. Samples should be collected below the surface to quantify Particulate Organic Matter (source of organic matter). 5-10 replicates.

The purpose of this is to add further dimension to the seasonal dietary study of *H. steineni,* an important benthic suspension feeder, and quantify seasonal fluctuations in organic matter content.

iii) Measure the *in situ* degradation rates of macroalgae species

Overview: The purpose of this experiment is to establish the degradation pathways of mixed-species composition of algae detritus and explore their functional role in the food web. This will be achieved by installing a field experiment of degrading detritus in mesh bags on the seabed and collecting the mobile herbivore grazers which are attracted to this organic material. This experiment will run across a ~56 day period which will allow questions to be explored about the community succession of grazers, the palatability of macroalgae as it degrades and the sequestration of macroalgae detritus into the sediment. As glaciers retreat, more available space for macroalgae colonisation on different substrate (eg. muddy sand) which may serve as blue carbon sinks.

1) **Collection:** Collect 500g of each of the following seaweed: *Iridaea cordata, Palmaria decipiens, Desmarestia menziesii, Plocamium cartilagineum.* Already agreed with Joe and Emma that they will try and collect the macroalgae prior to my arrival. Algae will be maintained in aquaria for 14 days and enriched with stable isotopes.

2) **Deployment:** Deploy 40 mesh bags of algae (**30 x 60 cm** basket size, **0.5 cm** mesh size, each containing 50g).

a) Individually fix (re-usable cable ties) the mesh bags onto a heavily weighted chain on muddy/sandy substrate. Target a site that experienced ice-scoured depressions as candidate sites where macroalgae deposition may occur. To be decided after a preliminary survey of macroalgae deposition sites/ using ROV data from Ben Robinson.

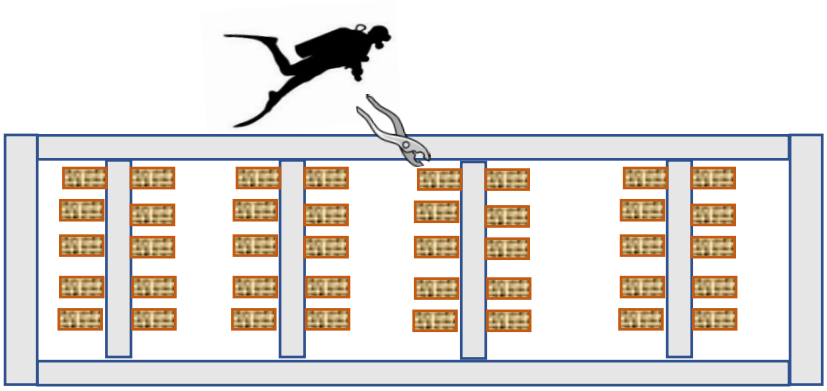
Table 4: Candidate sites for macroalgae deployment experiment

Site	**Characteristics**
Backbay Lagoon	Patches of bedrock, used for sediment sampling in the past
South of Killingbeck Island	Site of macroalgae deposition -recommended by Dave Barnes
Cheshire Island	Deep depression for potential macroalgae accumulations. One of few sites with macroalgae communities
South Cove	Less ice impact, more exposed bedrock. Sediment patches towards the north end.

b) Plan B: Fix bags onto existing chain rings in the rock and collect sediment samples from adjacent sites.

c) Plan C: Build a square structure using hollow, pierced drainpipes with 4 modular units. Affix 10 mesh bags to each pipe section with cable ties. At 2-week intervals, divers can remove a section ¼ of the experimental system, isolate each individual detrital cadge with a fine mesh bag to capture macrofaunal community, affix to a rope and give line signals for topside to hoist the sample (Fig.1).

Figure 1: Schematic of algal deployment structure. Grey units represent drainpipes. 10 mesh macroalgae bags are attached to 4 individual detachable units.



Description of a single sampling period

3) **Macroalgae collection:** At 2-week intervals, the diver will remove a ¼ section of the experimental unit. Each mesh bag will be carefully isolated using fine mesh bags (175 µm, **45 x 60 cm**) to contain the whole grazer community. Ropes will be lowered from the RIB and fixed to the structure using carabiners and shackles. FFM communication will signal to topside to hoist the structure.
4) **Sediment sampling: Two proposed methods:**
   - **a)** After the structure has been hoisted, five **20 cm** sediment cores will be collected directly below the degrading mesh bags of algae (n = 20).
   - **b)** If the site experiences little sediment accumulation, Five **5cm** sediment cores will be collected instead.

Sediment will be dried and analysed for enriched isotopic signatures. The core will be sectioned (Fig. 2) for subsequent identification of infaunal communities to determine their role in carbon-drawn down processes.

Figure 2: Schematic of the sediment core

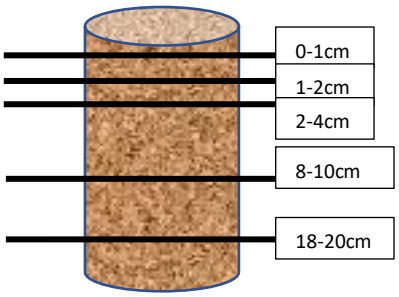


Table 5: Anticipated number of dives for the macroalgal degradation experiment. Subject to change.

Task	**Location**	**Date**	**No. of dives**
Macroalgae collection	South Cove	December/January	*Joe and Emma*
Deployment	Deployment site	February	1
Retrieval of 10 mesh bags	Southern Lagoon, Killingback Island	~14 days later	1

Retrieval of 10 mesh bags	Southern Lagoon, Killingback Island	~28 days later	1
Retrieval of 10 mesh bags	Southern Lagoon, Killingback Island	~42 days later	1
Retrieval of 10 mesh bags	Southern Lagoon, Killingback Island	~56 days later	1