

In situ MRV design and protocol

In compliance with Puro.Earth [Marine Carbon Fixation and Storage methodology](#)

Operations and Monitoring Overview	1
Example deployment specification	3
Operation area	4
Vessels and personnel	5
Monitoring equipment	6
Sampling locations	10
Sampling intervals and frequency (Temporal and spatial sampling frequencies)	14
Sampling details and frequency:	15
• Water sampling	15
• Substrates sampling	16
• Sinking rate analysis and imaging	16
Table 1: Variables to be measured in the water and the temporal and spatial sampling frequencies.	17
Table 2: Variables to be measured in the collected substrates	20
Table 3: Description of the sampling methodology, procedures and/or analytical methods that will be used	21
Table 4: Description of the information each parameter conveys categorized by their purpose within the MCFS methodology	29
Environmental risk monitoring and mitigation	35
Table 5: The major environmental risks, their monitoring plan, and the specific mitigation responses	36
Information management and data quality control	42
Data management system	42
Appendix A. Example schedule planned for January 2026 deployment	44
Appendix B. Required Scientific equipment	46
Appendix C. List of calibrated equipment	47
Appendix D: Sampling and Fixation protocols; November 2025	48
Seawater sampling	48
Substrate sampling	52
Lab subsampling and fixation procedures for seawater samples	53
Lab subsampling and Fixation procedures for substrate samples	56



Operations and Monitoring Overview

The project achieves carbon dioxide removal (CDR) under the Marine Carbon Fixation and Storage (MCFS) methodology by deploying a prepared substrate into a defined marine area to promote photosynthetic biomass growth in the substrates while floating in the surface waters. This biological process draws dissolved inorganic carbon (DIC) from the upper ocean, creating a deficit that drives atmospheric CO₂ uptake through sea–air gas exchange. The resulting organic carbon along with the substrate that it is fixed to are transported and sinks to deep, stratified waters, where they remain isolated from the atmosphere over long timescales.

The operations are designed to deliver a controlled and environmentally responsible removal activity that ensures the safe execution of deployment, the eligibility of the removal pathway under MCFS criteria, and the accurate quantification of durable carbon storage. All activities, ranging from substrate handling and deployment to oceanographic observation and post-deposition assessment, are structured to minimize ecological impact while maintaining full traceability of actions and data.

The monitoring plan provides the framework through which all operational and environmental data are collected, analyzed, and reported. Its objectives are to ensure:

- i) the safety of the removal activity, through the continuous assessment of environmental conditions and potential risks;
- ii) the eligibility of the removal activity, by demonstrating adherence to methodological requirements for substrate characteristics, ocean conditions, and storage durability;
- iii) the precise quantification of CORCs, through integrated measurement and modelling of carbon fixation, sinking dynamics, and long-term isolation.

Together, these elements establish a transparent, science-based system that ensures the carbon removal is measurable, reportable, and verifiable in alignment with the MCFS methodology.

This monitoring plan provides the overarching framework for all deployments to be conducted within the Area of Operation (AOI) for the duration of activities. While general in scope and not limited to a single operation, the plan outlines procedures and protocols based on the first scheduled deployment in January 2026, which serves as a representative example of the monitoring approach. Subsequent deployments within



the AOI will follow this framework, with operational details and monitoring parameters refined and optimized based on experience and lessons learned from the initial campaign.

Example deployment specification

The first planned deployment in January 2026 serves as the operational example, including both free-floating and controlled platforms to track fixation and sinking processes. While exact substrate quantities may vary in subsequent deployments, the overall operational timeline is expected to follow a similar structure and duration for all future campaigns within the AOI.

- Timeline: January 2026
- Amount of Substrate: 100-200 tons
- Location: NZ open water HNLC (deployment location approx. 46.6S, 172.7-172.8E - transect over ~10km)

Anticipated Duration and timeline (detailed timeline for the planned January 2026 operations available in *Appendix A*):

Day 1-9: Mob at Wellington port

Day 10: Transit from Wellington port to control site

Day 11: Control site measurements

Day 11: Transit to deployment site

Day 12: measurements at deployment site (pre deployment)

Day 13: Deployment of 100-200 tons of substrate into open ocean

Deployment of in situ platform (total of 5) and variation pens (total of 2)

Deployment of drifters alongside the free-plume substrate

Days 14-31: monitoring of open ocean plume

Day 32 (End of export phase): platform substrate release and estimate of sinking rates. Retrieval of variation pens samples; retrieval of platforms

Day 33-34: sampling at sinking site

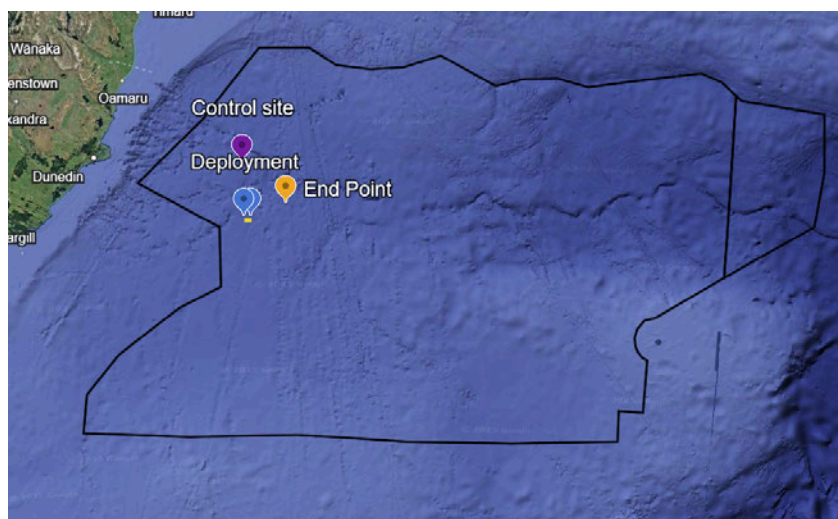
Day 35: Transit to control site; sampling at control site

Day 35-37: Transit to Wellington, NZ

Day 37-38: Arrival and demob at Wellington, NZ

Operation area

All projects will be conducted within a defined Area of Operation (AOI) in the Southern Pacific Ocean within New Zealand's exclusive economic zone (EEZ), selected based on oceanographic stability, depth, and suitability for long-term carbon storage. Figure 1a presents the general location of the AOI, along with an example of the deployment area, control site, and sinking site, illustrating their spatial relationships and monitoring coverage, for the first deployment planned for January 2026. Figure 1b provides a detailed view of the deployment and control sites, showing an example of a single (pre-deployment) modelled scenario for the substrate trajectory from surface release through the fixation and export phases to the point of sinking initiation and final deposition along the seafloor within the designated settlement area.



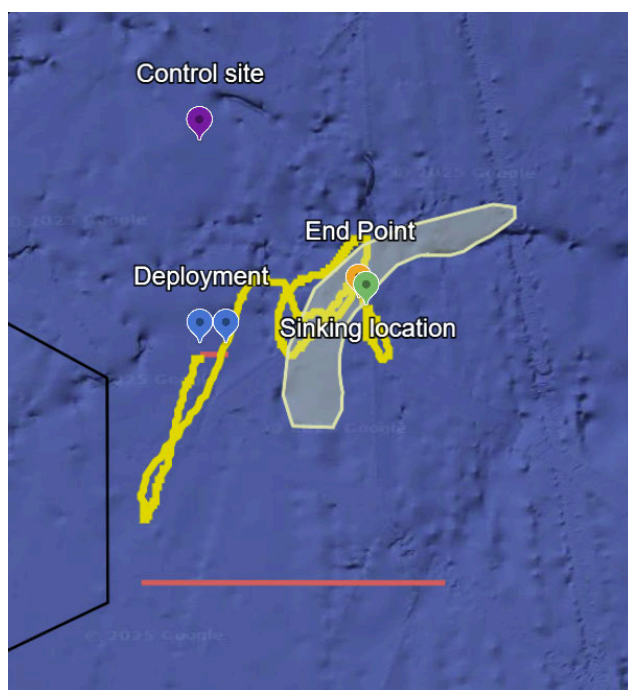


Figure 1: A. Map of the Area of Interest (AOI) polygon **B.** Example of a specific deployment (January 2026) deployment and sinking sites. The bottom orange line indicates a 50 nautical mile (NM) scale. The yellow trajectory illustrates a single modelled scenario for the plume's flotation, starting at a deployment trajectory of ~4 NM (short orange line between deployment sites; approx. 46.6S, 172.72E to 46.6S, 172.83=> ~8NM) and concluding at the “End Point” after 18 days, where the plume has sunk. The light green polygon outlines the sunken plume's estimated area along the seafloor, which is approximately 1,200 km².

Vessels and personnel

All planned operations will utilize two marine vessels: a deployment vessel, responsible for transporting and releasing the substrate at the designated site, and a monitoring and research vessel, which will operate alongside and conduct all oceanographic, biological, and environmental monitoring activities described in this plan. This two-vessel configuration allows simultaneous execution of deployment and data collection, ensuring continuous observation and documentation throughout each operational phase. For the January 2026 operations two marine vessels will be utilized:

1. [Seaworker](#) (MRV and science vessel); 9-11 science members:

[GigaBlue](#):

- Dr. Natasha Belkin- Chief scientist, Head of operations
- Oded Ezra- ROV pilot
- Hillel Wittler - Marine Engineer
- Danielle Zelinger- Marine biologist, data manager

GIGABLUE

[NIWA](#): scientific crew members (Operators: ADCP, echosounders, drifters, CTD + rosette sampling, fish pump- Iron free sampling, pens+mesocosms sampling, MOCNESS) x4

9(2)(b)(ii) - Drone Chief Pilot

2. [Rangitata](#) (Deployment vessel):

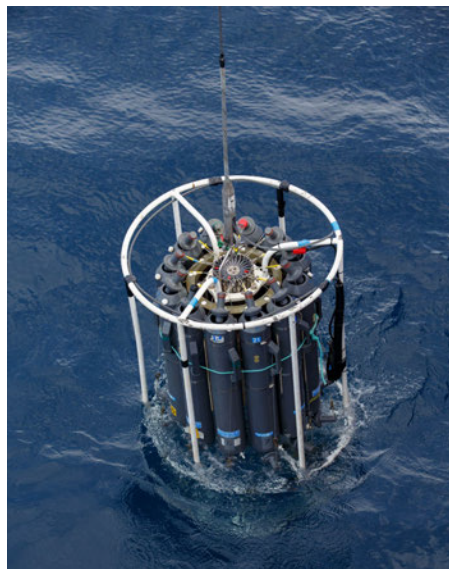
[GigaBlue](#): (Josh (Kernohan) + 3 operation members)

TBD

Monitoring equipment

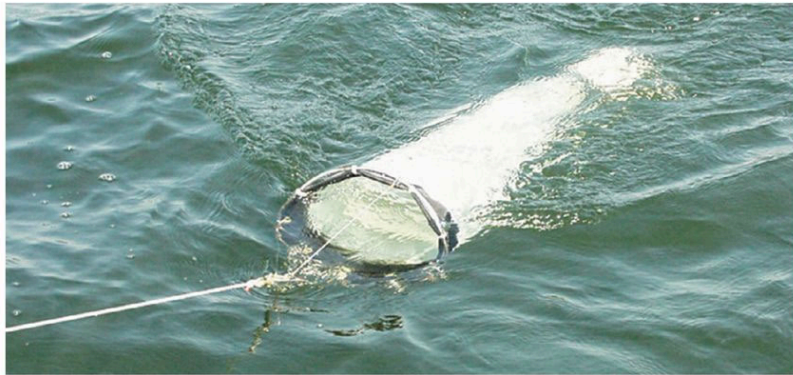
The following equipment is planned to provide comprehensive monitoring coverage across all project objectives, including the quantification of carbon removals, verification of eligibility criteria, and ongoing assessment of environmental and social safeguards as well as potential risks of reversal. The instrumentation listed represents the configuration planned for the January 2026 deployment, which serves as the reference case for this monitoring plan. Future deployments within the AOI will use this same suite of instruments, or equivalent systems providing comparable functionality and accuracy, with the final configuration refined and optimized based on lessons learned from the first deployment.

1. A [Seabird 911plus CTD](#) rosette equipped with a 12-bottle [Seabird 32 carousel water sampler](#) and integrated oceanographic sensors (or equivalent) is used to obtain vertical profiles of temperature, salinity, dissolved oxygen, and other key parameters, as well as discrete water samples for chemical and biological analyses.

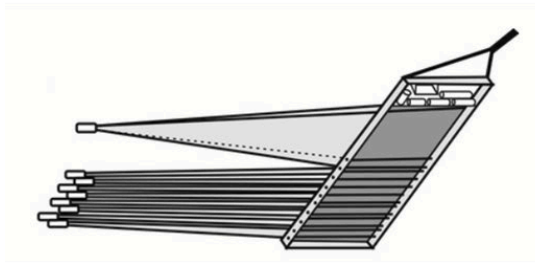


GIGABLUE

2. A plankton net with a 1 m diameter opening and 350 μm mesh size is used to collect suspended particulate and biological material during the fixation phase, enabling analysis of biomass composition, abundance, and carbon content in surface and mid-water layers. The 1-meter diameter creates a large mouth area for collecting a significant volume of water.

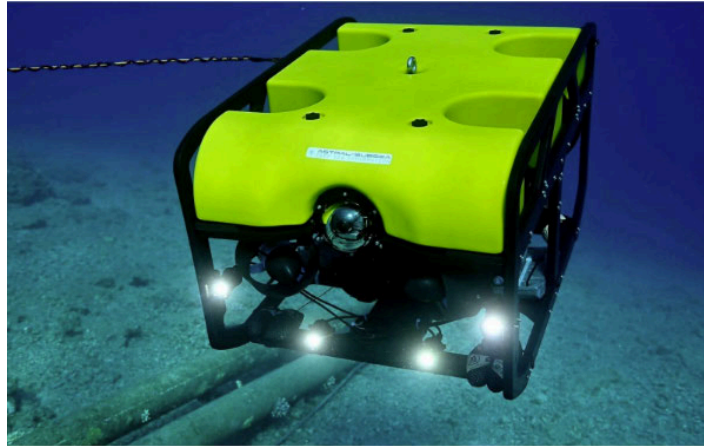


3. A [MOCNESS](#) (Multiple Opening/Closing Net and Environmental Sensing System) is deployed to collect depth-resolved biological and substrate samples, allowing assessment of vertical distribution and substrate size composition throughout the water column.



1. An [Astral subsea](#), next-generation Survey-Class underwater remotely operated vehicle (ROV) developed for professional subsea inspection and intervention missions. [AstralROV](#) is utilized for visual inspection and sinking velocity measurement throughout the water column.

GIGABLUE



4. A pole-mounted [Simrad EK80 scientific echosounder](#) used to monitor subsurface substrate dynamics during deployment, fixation and export phases. The pole is configured with the 38 kHz transducer in the upper position and the 120 kHz transducer below, allowing detection of plume structure and vertical movement throughout the water column.



GIGABLUE

5. GPS drifters (Pacific Gyre [Microstar](#) and [Stokes](#) models) are deployed at the surface to track near-field surface current patterns during and after substrate release, providing data for Lagrangian model validation and assessment of particle dispersion and plume drift.



6. A DJI [Matrice 4E](#) drone is used to capture aerial imagery and video of deployment activities and surface plume dynamics, supporting operational documentation, spatial mapping, and visual correlation with in-water drifter and sensor data.



7. Passive Acoustic Monitoring (PAM) - A marine hydrophone array will be deployed to a depth of 20m at the deployment site prior to substrates deployment. The vessel's engines will be shut down for 20 minutes to allow for passive listening. The recorded data will then be sent to a marine mammal acoustics expert for rapid analysis to confirm the absence of marine mammals in the vicinity of the deployment site.
8. Optical and Synthetic Aperture Radar (SAR) Satellite Imagery - Within 24 hours of deployment, both optical and SAR satellite imagery will be acquired for the deployment location and projected plume

trajectory. These images will be analyzed to detect signals from substrates on the ocean surface. If the acquired signals are sufficient to detect the plume, additional satellite footage will be scheduled for the subsequent days of the operation's fixation phase.

Sampling locations

1. **Laboratory:** Prior to deployment each batch of the pristine substrate will be analyzed and characterized based on the MCFS requirements. These analyses include: mass per unit, volume per unit, mass fraction of carbon per unit and total solids, determination of the share of organic carbon of the Substrate material, diameter, density, stability, containment mechanism, floating duration and sinking rate, micronutrient concentration, PTE's (including heavy metals, PAH's, PFA's, pesticides), phytoplankton growth rates, nutrient leaching, and anion sorption. See the "Substrate characterization report" for details, protocols, and results.
2. **Control Site:** This site needs to be geographically proximate to the deployment and sinking site, yet it must remain unaffected by substrate disturbances (for the January 2026 operation this is planned at 46S, 172.72E).
3. **Deployment site:** This site is in the open ocean and is a free-floating plume. The pristine substrates (80-100 tons for January deployment) will be released over a transect of ~4NM during 12-24 hours from the deployment vessel. GPS drifters will be released every 1-2 hours during the deployment operation. The substrates are expected to float in the uppermost 10 centimeters, with their trajectory determined by surface currents and winds. As the plume floats, over time, the substrate concentration will decrease, and its size will expand (see the Lagrangian model description for more details). The picture below was taken over a plume formed in 2024 in a small scale experiment as an example of a 3 days "old" free-floating plume.

GPS drifters will indicate the plume's location throughout the flotation period. The monitoring vessel will continuously accompany the plume, collecting water and substrate samples (sampling details provided below). Daily drone flights, weather permitting, will inspect and locate the plume if it deviates. Additionally, light aircraft will perform flights at the operation area on several occasions to inspect and help locate the plume.

GIGABLUE

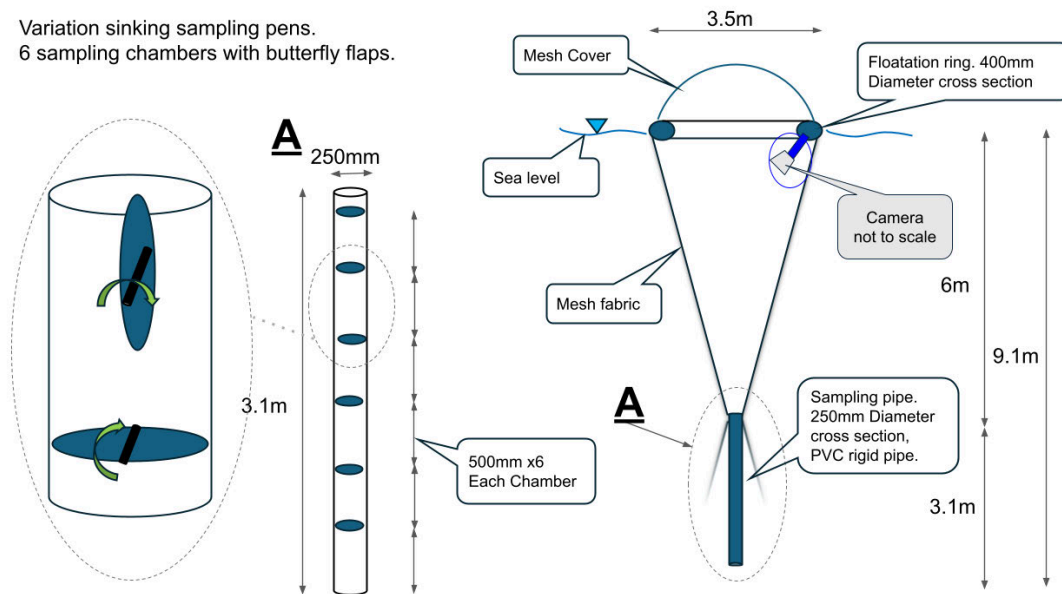


4. **Deployment site:** In Situ platforms will be deployed in a flower-like shape consisting of 5 x 12.7 m diameter inflatable pens with an underwater net (8 m depth), each containing up to 1 m³ of substrate (~20 kg). The pens platform (pictured below, 2024) will be deployed alongside the free-floating plume. Navigation buoys equipped with lights, AIS, and GPS will be attached to the platform for easy location tracking. This platform will float freely with surface currents. The pens act as a backup in case the free-floating plume is lost or overly diluted, leading to a failure in substrate sampling during the flotation period. Once the operation concludes (when most of the substrates sink), the entrapped substrates within the net will be released to sink freely, while an ROV tracks their descent to measure sinking velocity.



5. **Deployment site:** Variation Pens will be included as will to assist in characterizing the floating duration and sinking variability. The central ring of the flower will contain 2 x 3 m diameter rings with underwater net (8 m depth) and cod end divided into 6 compartments, allowing the collection of 6 samples (scheme below). Each variation pen holds 300g of pristine substrates. Two pens, designed to evaluate floating time variation with autonomous "trapping" mechanisms, will be placed in the central ring of the previously described pens platform. These autonomous pens will collect sunken substrates at predefined intervals during the flotation period. They will be recovered from the water at the end of the operation and the amount of substrates in each sample will be counted. The samples collected from these pens will then be used to determine the relative proportion of substrates that have sunk at each time point from the total deployed.

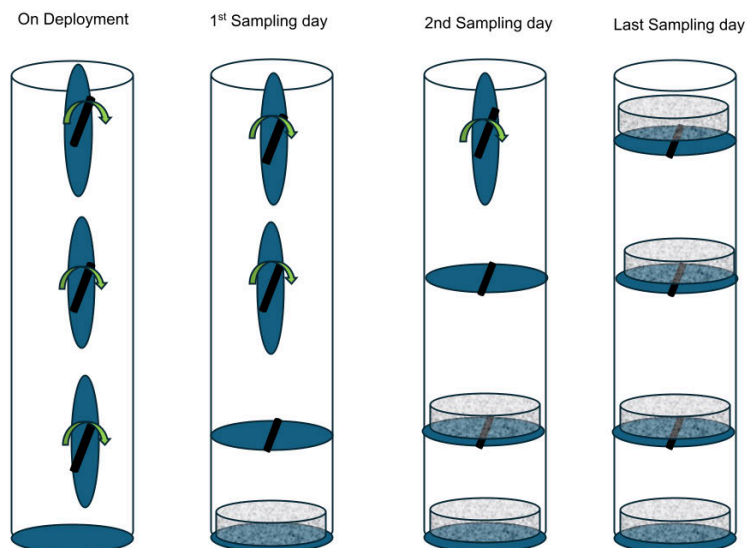
Variation sinking sampling pens.
6 sampling chambers with butterfly flaps.



Principle of sampling.

Drawing not to scale.

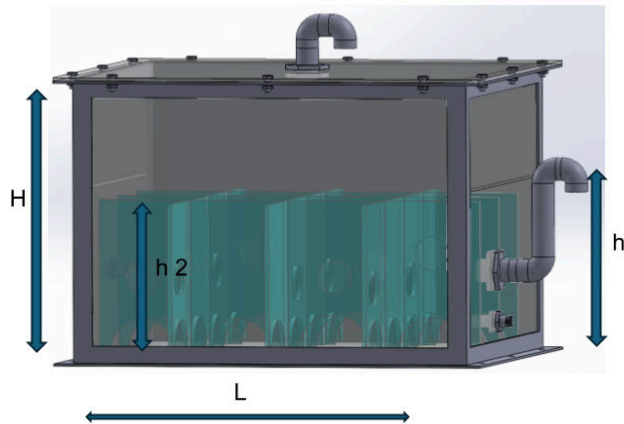
We have 6 chambers
6 sampling days.



6. **On-board incubations:** for carbon fixation rates and remineralization rates (mesocosms). Upon the MRV vessel five 250L transparent containers will be linked to a continuous surface water circulation system, with an intake flow of approximately 0.25 m³/hour (4 L/min). Three of these containers will be used as incubators for ¹³C carbon fixation analysis to measure primary production. In addition, remineralization rates will be determined by incubating retrieved substrate sub-samples in filtered seawater in a gas-tight dark bottle. The incubation will be within the surface water circulation to keep

GIGABLUE

ambient temperature conditions. Oxygen consumption will then be measured and converted into surface remineralization rates.



Tank water capacity 0.15-0.25 m³

H- Tank height – 500mm

h – Water level 300-400mm

h2 – Wave breaker height =

h-300mm

L -Tank base size– 700-800 mm

Sampling intervals and frequency (Temporal and spatial sampling frequencies)

A – Background properties of the water column prior to substrate deployment will be measured at (overall 3 casts minimum):

- the control station (x1).
- at the deployment site (x2).

B – During the Floating/Fixation phase:

- Three Surface water samples per day at least every 3 days.
- Three floating substrates sampling (surface plankton net tows) per day at least every 3 days.

C – Final fixation phase:

- 2x euphotic zone water samples per day at least every 3 days (days 10-18).
- 3x floating substrates sampling (surface net) per day at least every 3 days.

D – Export phase:

- 2x full water column profiles of CTD + sensors (CTD/MOCNESS sensors).
- substrate samples for CHN and TS analysis, every 3 days at minimum.
- Substrate sampling from below the euphotic zone (as much as possible - with MOCNESS and ROV).
- Substrate sinking velocity evaluation from ROV.

E – Variation pens at the end of operation

- Variation in substrate sinking time evaluated by “variation pens” by autonomous sampling of substrate from pens.

F – Post-operations phase (overall 3 casts at minimum):

- 2x full water column profiles at the sinking area.
- 1x full water column at control station x1.

Sampling details and frequency:

● Water sampling

- ❖ Equipment: Rosette equipped with Go-flow bottles, CTD with DO, Chl-fluorescence, turbidity (backscatter), and PAR sensors will be used to measure and collect seawater samples between the surface and ~15 m above the bottom depth (1000-2000m).

Water sampling will occur in several locations, several times:

- a. Control site location, pre-deployment:
 - i. 1 CTD profile cast of the entire water column.
 - ii. Water samples to be taken in the euphotic zone.
- b. Deployment site prior to substrate deployment:
 - i. 2 CTD profile casts.
 - ii. Water samples to be taken in the whole water column (10 depths).
- c. Fixation phase (floating period):
 - i. Photic zone sampling every 3 days. (8-10 sampling events, 2 casts per sampling event).
- d. Export phase (sinking period):
 - i. CTD x2 casts every 3 days.
- e. Sinking site, post sinking:
 - i. 2 CTD profile casts.
 - ii. Water samples to be taken in the whole water column (10 depths).
- f. Control site location, post-operation:
 - i. 1 CTD profile cast of the entire water column.
 - ii. Water samples to be taken in the euphotic zone.

Water will be sampled and analysed for the parameters in Table 1.

- Substrates sampling

- **Substrate Pre-Loading and Measurement:** Prior to loading the substrate onto the deployment vessel, each batch of substrate is weighed when loaded to the container using calibrated load cells or weighbridges to determine the total mass ($M_{\text{substrate}}$) in accordance with MCFS methodological requirements. Representative samples are collected during loading to determine moisture content, enabling calculation of the dry weight. All measurements are performed immediately prior to sailing to ensure temporal proximity to deployment.
- **Open ocean, free plume -** The primary focus will be on the free plume particles and photic layer water.
 - 3x floating substrates sampling (surface net tows) per day at least every 3 days. The substrates will be sampled by plankton net surface tows.
 - Substrates will be sampled while sinking using MOCNESS at different water layers (up to 1000m depth).
 - Substrates will be sampled while sinking using the ROV with a dedicated sampler skid from the plume on different days during the export phase (estimated to begin around day 7 and last until day 18, and at the end of the export phase (estimated to be day 19) for the particles released to sink from Pens the ROV only.
- **Pens -** Surface sampling of substrates will be used as a comparison to open ocean sampling and a backup in case the plume is hard to track.
- **Variation pens -** autonomous sampling of the fraction of substrate every 2-5 days. Samples will be retrieved from the cod end at the end of the operation for unit counting.

- Sinking rate analysis and imaging

- Echosounders will be used to locate sinking particles when possible and evaluate the substrates' sinking rate,
- ROV will be used to visualize, evaluate the sinking rates, and sample substrates as deep as possible.

Substrates will be sampled and analysed for the parameters in Table 2.

Table 1: Variables to be measured in the water and the temporal and spatial sampling frequencies.

Parameter/Variable	Sampling mechanism type	Rationale/Objective	Temporal and spatial sampling frequencies: A-Control Site B-Baseline (pre-deployment) C-Fixation D-Export E-Verification
Currents in the upper water column	ADCP hull /pole-mounted	Plume trajectory	A- Euphotic zone B- Euphotic zone C- Euphotic zone D- Euphotic zone E- Euphotic zone
Temperature (T)	CTD	Physical characteristics	A- Full water column B- Full water column C- Euphotic zone D- Full water column E- Full water column
Salinity (Sal)	CTD	Physico-chemical characteristic	A- Full water column B- Full water column C- Euphotic zone D- Full water column E- Full water column
Dissolved Oxygen (DO)	CTD mounted sensor	Chemical characteristic	A- Full water column B- Full water column C- Euphotic zone D- Full water column E- Full water column
Photosynthetically active radiation (PAR)	CTD mounted sensor	Available light characteristics, photic zone definition	A- Euphotic zone B- Euphotic zone C- Euphotic zone D- Euphotic zone E- Euphotic zone
Chlorophyll a (Chl.a)	CTD mounted fluorometer sensor	Algal biomass proxy	A- Euphotic zone B- Euphotic zone C- Euphotic zone D- Euphotic zone E- Euphotic zone

GIGABLUE

Turbidity (Tur)	CTD mounted sensor	Physical characteristic	A- Full water column B- Full water column C- Euphotic zone D- Full water column E- Full water column
Inorganic macronutrients (NO ₃ , PO ₄ , SiOH)	Water samples	To test if there is detectable nutrient depletion, and make sure we're at HNLC	A- Euphotic zone B- Full water column C- Euphotic zone D- Not required E- Full water column
Micronutrients (Fe and Mn)	Water samples	To make sure we're at HNLC	A- Surface B- Surface C- Surface D- Not required E- Surface
Carbonate system (DIC)	Water samples	DIC is an integral part of the carbonate system of the oceans (two out of three)	A- Euphotic zone B- Full water column C- Euphotic zone D- Not required E- Full water column
Carbonate system (TA)	Water samples	TA is an integral part of the carbonate system of the oceans (two out of three)	A- Euphotic zone B- Full water column C- Euphotic zone D- Not required E- Full water column
Particulate Organic Carbon (POC)	Water samples	The amount particulate organic carbon in the water column	A- Euphotic zone B- Full water column C- Not required D- Not required E- Full water column
Total organic carbon (TOC)	Water samples	The amount total organic carbon in the water column	A- Euphotic zone B- Full water column C- Not required D- Not required E- Full water column
¹³ C Carbon fixation rate	Water samples	Evaluate the background NPP	A- Euphotic zone B- Euphotic zone C- Euphotic zone D- Not required E- Euphotic zone

GIGABLUE

Chlorophyll a	Water samples	Algal biomass proxy	A- Euphotic zone B- Euphotic zone C- Euphotic zone D- Not required E- Euphotic zone
Ultraphytoplankton abundance (FCM)	Water samples	<60 um algae+cyano counts	A- Euphotic zone B- Full water column C- Not required D- Not required E- Full water column
Microphytoplankton abundance microscopy (Lugol)	Water samples	Algal community composition	A- Euphotic zone B- Euphotic zone C- Not required D- Not required E- Euphotic zone
Bacterial abundance (FCM)	Water samples	Bacterial counts	A- Euphotic zone B- Full water column C- Not required D- Not required E- Full water column
Characterization of the phytoplankton and bacterioplankton communities and biodiversity (DNA)	Water samples	Community composition DNA 16S+18S (Cawthron)	A- Euphotic zone B- Full water column C- Not required D- Not required E- Full water column
CH ₄ , N ₂ O	Water samples	To ensure no methane and N ₂ O production	A- Surface B- Surface C- Surface D- Not required E- Surface
Algal toxins	Water samples	It will be analyzed if potential toxic algae/cyano were found in the DNA analysis	A- Euphotic zone B- Euphotic zone C- Euphotic zone D- Not required E- Euphotic zone
DMSP	Water samples	Surface water DMS production potential in phytoplankton	A- Surface B- Surface C- Surface D- Not required E- Surface

Table 2: Variables to be measured in the collected substrates

Parameter/Variable	Sampling mechanism type	Rationale/Objective	Sample measurement locations: A-Control Site B-Baseline (pre-deployment) C-Fixation D-Export E-Verification
TS	Substrates	The amount of biomass on the particles	A- Not required B- Not required C- Surface D- Surface E- Not required
CHN	Substrates	The amount of C on the particles	A- Not required B- Not required C- Surface D- Surface E- Not required
Remineralisation rate	Substrates	Surface water remineralisation rate from respiration	C- Surface x3 D- Surface x3

Table 3: Description of the sampling methodology, procedures and/or analytical methods that will be used. More details for sampling on board in [appendix D](#).

Monitored parameter	Sampling methodology	Procedures and/or analytical methods
Currents in the upper water column	Acoustic Doppler Current Profiler (ADCP), pole or hull-mounted faced down from the research vessel. Turned on during the operation to collect current data in the area of operation.	Calibrated electronic sensor measurement, following Lhermitte and Serafin, 1984. The Acoustic Doppler Current Profiler (ADCP) measures water current velocity across depths using the Doppler effect. It transmits sound pulses that scatter off suspended particles moving with the current, causing a Doppler shift in the returning wave. By measuring this frequency shift with multiple slanted acoustic beams, the ADCP calculates the true three-dimensional water velocity (u, v, w) within discrete depth layers, creating a vertical current profile.
Temperature (T)	Conductivity, temperature, and depth (CTD) instrument, mounted on the rosette sampler deployed from the surface gradually to 10-15m above the seabed. The rosette sampler, a circular frame equipped with multiple Niskin bottles, is deployed from the vessel via a cable. As the instrument package descends through the water column, the CTD continuously transmits real-time data on depth, temperature, and salinity back to the ship. This allows operators to send an electronic command to close the stoppers of a specific Niskin bottle at an exact, designated depth, thereby trapping a discrete volume of water. Once the rosette is recovered on deck, these collected water samples are transferred to	Calibrated electronic sensor measurements. The Conductivity, Temperature, Depth (CTD) is the standard technique in oceanography for determining the essential physical properties of seawater. The instrument measures electrical conductivity (which is directly related to salinity), temperature (in degrees Celsius), and pressure (which is converted to depth) simultaneously as it is lowered through the water column. The CTD provides a highly accurate, continuous vertical profile of these three parameters.

GIGABLUE

	the laboratory. Subsequent analysis of these samples for biogeochemical parameters that are outlined below.	
Salinity (Sal)	Conductivity, temperature, and depth (CTD) instrument, deployed from the vessel on a cable to 10-15m above the seabed.	Calibrated electronic sensor measurements. The Conductivity, Temperature, Depth (CTD) is the standard technique in oceanography for determining the essential physical properties of seawater. The instrument measures electrical conductivity (which is directly related to salinity), temperature (in degrees Celsius), and pressure (which is converted to depth) simultaneously as it is lowered through the water column. The CTD provides a highly accurate, continuous vertical profile of these three parameters.
Dissolved Oxygen (DO)	Dissolved oxygen optode mounted on the CTD instrument, deployed from the vessel on a cable to 10-15m above the seabed.	Calibrated electronic sensor measurements. The Dissolved Oxygen (DO) sensor, connected to the CTD, is an optical sensor for dissolved oxygen measurements (optode). This sensor relies on the principle of luminescence quenching. In this process, a light source excites a fluorescent dye embedded in the sensor foil. The oxygen gas present then quenches—or reduces—either the intensity or the decay time of the emitted light. The oxygen concentration is determined by the degree of quenching, with a stronger quenching indicating a lower concentration. This mechanism allows the optode to deliver fast, high-resolution vertical profiles of dissolved oxygen.
Photosynthetically active radiation (PAR)	A photosynthetically active radiation (PAR) sensor mounted on the CTD instrument, deployed from the vessel on a cable to 10-15m above the seabed.	Calibrated electronic sensor measurements. The Photosynthetically Active Radiation (PAR) sensor is integrated into a CTD package to quantify the light intensity available for photosynthesis in the water column. This light

GIGABLUE

		spans the 400 to 700 nanometer (nm) spectral range and is measured as Photosynthetic Photon Flux Density. The sensor operates on the photoelectric sensing principle. It employs a light-sensitive photodiode that converts absorbed light into an electrical current. To ensure accuracy, the photodiode is covered by an optical filter—to exclude non-PAR wavelengths—and a diffuser—to provide a correct "cosine response," for accurate readings regardless of the light's angle. By recording the rapid, exponential decrease of light with increasing depth as the CTD is lowered, the PAR sensor allows for the determination of the euphotic zone depth.
Chlorophyll a (Chl.a)	Chl-a fluorescence sensor mounted on the CTD instrument, deployed from the vessel on a cable to 10-15m above the seabed.	Calibrated electronic sensor measurements. The Chlorophyll-a (Chl-a) fluorometric sensor on a CTD package measures phytoplankton biomass. It works by shining a blue LED light, which Chl-a molecules absorb and re-emit as red fluorescence (around 685 nm). A photodetector measures this fluorescence intensity, which is proportional to the Chl-a concentration, providing a continuous, real-time vertical profile of phytoplankton biomass.
Turbidity (Tur)	Turbidity sensor mounted on the CTD instrument, deployed from the vessel on a cable to 10-15m above the seabed.	Calibrated electronic sensor measurements. The turbidity sensor on a CTD package quantifies the cloudiness or haziness of water resulting from suspended material. Its operation is primarily based on the nephelometric method: a light source (typically an LED) illuminates the water, and a photodetector measures the intensity of light scattered by the particles, usually at a 90-degree angle. This measured scattered light intensity is directly correlated with the concentration of suspended solids, such as silt, clay, or phytoplankton. The

GIGABLUE

		sensor provides a continuous vertical profile, which is valuable for identifying sediment plumes, resuspension events, and zones with elevated particle concentration.
Inorganic macronutrients (NO ₃ , PO ₄ , SiOH)	Seawater samples 250 ml are pre-filtered (GFF). Stored at -20°C.	A filtered sample will be colorimetrically analyzed for nutrient concentrations. Methods: Ammonia: SEAL Method G-327-05 Rev 8. Based on K��rouel & Aminot, (1997) Nitrate+Nitrite: SEAL Method G-172. Based Armstrong et al., (1967) and Grasshoff (1983) DRP: SEAL Method G-297. Based on Murphy & Riley, (1962) DRSi: SEAL Method G-177. Based on Grasshoff and Kremling (1983).
Micronutrients (Fe and Mn)	Water samples from the dedicated trace metals clean pump tubing. Each filtered sample (0.2 ��m) will be acidified with quartz-distilled HCl to a final concentration of 0.025 M. Samples stored at 4°C.	Subsequently in the analytical lab, trace metals in the seawater samples will undergo preconcentration using a seaFAST system (Elemental Scientific Inc.). Elemental analyses will then be conducted by sector-field inductively coupled plasma mass spectrometry (SF-ICP-MS; Nu Instruments AttoM), following the methodologies outlined in Wuttig et al. (2019).
Carbonate system (DIC)	Seawater samples (250 mL) will be collected in pre-ashed Schott bottles and preserved with approximately 100 ��l of saturated HgCl ₂ solution from a dropper bottle. Fixed samples stored at RT.	The samples will undergo colometric analysis according to the protocols established by Dickson & Goyet (1994).

Carbonate system (TA)	Seawater samples (250 mL) will be collected in pre-ashed Schott bottles and preserved with approximately 100 µl of saturated HgCl ₂ solution from a dropper bottle. Fixed samples stored at RT.	Total Alkalinity (TA) will be analyzed by automated potentiometric titration and derived using a least squares optimization technique with an accuracy of <1.0 mmol kg ⁻¹ based on CRMs (Dickson et al., 2007; Currie et al., 2011)
Particulate Organic Carbon (POC)	Collect 2L of seawater in a clean Nalgene bottle, then filter it using pre-combusted GFF filters and freeze. Filters stored at -20°C.	In the lab, filters will be dried, fumed over concentrated 12 M HCl to remove inorganic carbon and analysed for POC and PON by Flash 2000 elemental analyser using a MAS 200 R autosampler (Thermo-Fisher Scientific, Bremen, Germany).
Total organic carbon (TOC)	Water samples collected from the CTD bottle casts using the Joint Global Ocean Flux Study (JGOFS) recommended procedures (JGOFS, 1996).	Acidified samples will be analysed in the Total Organic Carbon analyser (TOC).
¹³ C Carbon fixation rate	Water samples collected from the CTD bottle casts using the Joint Global Ocean Flux Study (JGOFS) recommended procedures (JGOFS, 1996). Additions of 99 at% ¹³C sodium bicarbonate will be made to 4 (2 light and 2 dark) x 2L incubation bottles to approximately 5% of ambient DIC concentrations. Bottles will be incubated for 24 h using simulated in situ deck incubations with ambient light and surface water temperature. Deeper water samples bottles will be shaded to reach 100%, 66%, 30%, and 2% from ambient light. At the end of incubation, bottles will be kept cold and dark and filtered onto ashed (5 h at 450 °C) Whatman GF/F filters, as soon as possible under gentle vacuum to minimise cell disruption. Filters	Filters will be dried, fumed over concentrated 12 M HCl to remove inorganic carbon and analysed for POC, PON and ¹³C. Stable Isotope Mass Spectrometry (IRMS) analyses will be carried out on a DELTA V Plus continuous flow isotope ratio mass spectrometer linked to a Flash 2000 elemental analyser using a MAS 200 R autosampler (Thermo-Fisher Scientific, Bremen, Germany). https://niwa.co.nz/scientific-tools-data/stable-isotope-analytics/stable-isotope-analytical-facility#plus Carbon uptake rates followed the formula derived by Collos and Slawyk (1985). Natural abundance values for nitrogen and carbon will be taken from analysis of unenriched plankton samples from each depth.

GIGABLUE

	will be rinsed with 0.2 µm filtered seawater prior to frozen storage at -20 °C.	
Chlorophyll a (Chl-a)	Water samples collected from the CTD bottle casts using the Joint Global Ocean Flux Study (JGOFS) recommended procedures (JGOFS, 1996).	500 mL water samples will be filtered onto 25 mm GF/F filters. Filters will be folded, placed in 15 mL Falcon tubes with 10 mL of 90% acetone, vortexed, and stored overnight in the dark in a refrigerator. A Turner 10AU fluorometer, calibrated with pure spinach Chla (Merck) and using 436 nm excitation and 680 nm emission filters, will measure Chl-a content before and after acidification. This filter and light source (430-440 nm) combination minimizes pigment interference (Welschmeyer, 1994).
Ultraphytoplankton abundance (FCM)	Seawater samples will be pipetted into 1.5 mL cryovials, then fixed with 25% glutaraldehyde to a concentration of approximately 0.5%. These samples will be snap-frozen in liquid nitrogen and stored at -80°C.	Before analysis, samples will undergo filtration through a 35 µm mesh strainer to eliminate larger particles. Phytoplankton will be identified based on chlorophyll-a and picoerythrin autofluorescence. A Becton Dickinson Accuri C6 Plus flow cytometer, equipped with appropriate laser and detector settings, will be utilized for sample analysis. Specific fluorescence signals will be detected as follows: SSC vs FL3 and FL3 vs FL2 for eukaryotic picophytoplankton, and SSC vs FL2 for prokaryotic picophytoplankton. Prepared samples will be processed through the flow cytometer at a consistent flow rate of 100 µl/min, with a nominal sample volume of 100 µL. Data acquisition will involve recording the fluorescence intensity and scatter properties of individual cells. The identification and quantification of phytoplankton and bacterial populations is based on their fluorescence and scatter characteristics.

GIGABLUE

Microphytoplankton abundance microscopy (Lugol)	Samples for taxonomic analysis in seawater will be collected by transferring 250 mL of seawater into plastic bottles and adding 1.25 mL Lugol's iodine (1% final concentration). Stored at RT.	Samples will be settled in measuring cylinders, allowing a minimum of 0.5 hours per millimeter of settling column depth. The supernatant will then be siphoned off, and the remaining 10 mL will be mixed and transferred to Utermöhl chambers for resettlement. Phytoplankton will then be enumerated using a Leica DMI3000B inverted microscope at 100x to 600x magnification, following Edler & M. Elbrächter (2010). Abundant phytoplankton (exceeding 100 cells per liter) will be identified to genus or species level where feasible, counted, and categorized into algal 'Division/Group' (e.g., diatoms, dinoflagellates). Further taxonomic classification, including Phylum, Order, Family, Genus, and Species, will be applied where possible.
Bacterial abundance (FCM)	Seawater samples will be pipetted into 1.5 mL cryovials, then fixed with 25% glutaraldehyde to a concentration of approximately 0.5%. These samples will be snap-frozen in liquid nitrogen and stored at -80°C.	Before analysis, samples will be filtered through a 35 µm mesh strainer to remove larger particles, and stained with SYBR Green II fluorescent dye (Molecular Probes Inc.) at a concentration of 10^{-4} of the stock solution, followed by a 10–15 minute incubation in the dark. A Becton Dickinson Accuri C6 Plus flow cytometer, configured with appropriate laser and detector settings, will be used to analyze the samples. Specific fluorescence signals will be detected as follows: SSC vs FL1 for bacteria. Prepared samples will be run through the flow cytometer at a consistent flow rate of 100 µl/min and a nominal sample volume of 100 µL. Data acquisition will involve recording the fluorescence intensity and scatter properties of individual cells. The identification and quantification of bacterial populations based on their fluorescence and scatter characteristics.

Characterization of the phytoplankton and bacterioplankton communities and biodiversity	1 L seawater samples will be filtered through a Sterivex™ (0.2 µm) using a peristaltic pump. The Sterivex units will then be flash-frozen in liquid nitrogen and stored at -80°C.	DNA will be extracted from each sample and amplified using PCR with 16S bacterial, 18S eukaryote, 18S haptophyte, and 28S dinophyte primers. The resulting sequences will undergo standard processing including truncation, quality filtering, denoising, and removal of potential chimeric sequences. Taxonomic classification will be performed using Amplicon Sequence Variants (ASVs). For 18S eukaryote and 18S haptophyte datasets, the Protist Ribosomal Reference database (PR2) (Guillou et al. 2012) with the Ribosomal Database Project (RDP) classifier (Wang et al. 2007) will be used. For 18S haptophyte, a blast search will be conducted against the National Center for Biotechnology Information (NCBI) nt database with a max target hit of 1 and a 99% similarity threshold. For the 16S bacterial dataset, taxonomic classification will utilize the CyanoSeq (Lefler et al. 2023) and Silva v138 (Quast et al. 2012) databases via RDP. The 28S dinophyte dataset will be classified using a blast against the NCBI database and a custom dinoflagellate blast database. All results will be parsed into a table using the phyloseq package (McMurdie and Holmes 2013). Potential contamination will be addressed by examining sequences in laboratory and field negative controls. Putative contaminants will be removed using the microDecon R package (McKnight et al. 2019) with default settings. Data analysis will include estimating and ordinating community dissimilarities and Bray–Curtis dissimilarity matrices using principal coordinate analyses (PCoA) with the R packages phyloseq and microViz (Barnett et al. 2021).
---	---	--

		To identify pathogens and harmful microalgae, taxonomic assignments will be compared against the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (https://www.marinespecies.org/hab/index.php) and the Multiple Bacterial Pathogen Detection database (MBPD; Yang et al. 2023).
CH ₄ , N ₂ O	Sample taken directly from CTD bottle, into glass bottles with a gas-tight cap with silicone septa and crimp. Gas samples fixed with HgCl ₂ and stored at RT.	Gas chromatography is used to separate the gases. Methane (CH ₄) is measured using a Flame Ionization Detector (FID), while Nitrous Oxide (N ₂ O) is measured using an Electron Capture Detector (ECD).
Algal toxins	Seawater samples will be collected into 500ml plastic bottles. Stored at -20°C.	If DNA analysis reveals a significant increase in potentially toxic algae, the relevant toxin sample will be analyzed for the presence of specific toxins. Toxins in the relevant sample will be concentrated on an adsorbent. The concentrated extract will then be analyzed by LC-MS. Depending on the specific toxin associated with the detected algae, toxins will be retained on a reversed-phase sorbent. For suspected polar toxins (e.g., saxitoxins), activated carbon will be required.
DMSP	Seawater samples will be collected into 50ml glass bottles. Samples alkalized and stored at RT.	DMSP concentrations will be measured using a cryogenic purge-and-trap/gas chromatography system, as outlined by Turner et al (1990).
Variable	Sampling methodology	Procedures and/or analytical methods
TS	Plankton net/ net on a pole. Stored at -20 °C.	The total solids (TS i.e. total dry solids) method measured as described in EPA Method 1684. TS are quantified by measuring the residue of a sample after it has been dried at 105 °C to drive off all water.

GIGABLUE

C	Plankton net/ net on a pole. Stored at -20 °C.	Carbon content will be measured by an elemental analyzer. C content will be normalized to the total mass of the sample
Remineralisation rate	Plankton net/ net on a pole.	Substrates will be incubated in BOD bottles with FSW for 4-8 hours, measuring dissolved oxygen (DO) at both the start and end of this period. The organic carbon content of the substrates will be determined using an elemental analyzer. Remineralization rates will then be calculated from the decrease in DO concentrations and the carbon content, following the methodology described by McDonnell et al., 2015.

Table 4: Description of the information each parameter conveys categorized by their purpose within the MCFS methodology.

These three categories are: Eligibility (ensuring the site and substrate meet the required criteria), Quantification (contributing to the calculation of Carbon Removal Certificates), and Safety (addressing environmental and social risks or guaranteeing durability).

Physical Oceanography and Circulation Parameters	Eligibility	Quantification	Safety	How evaluated
Temperature (°C), Salinity (psu), Density (kg m⁻³)	General site characterization (e.g., assessing water column stratification, thermocline/pycnocline, and mixed layer depth) prior to deployment.	Temperature (Tz) is a necessary input for modeling the remineralization rate (R) [6.1.7, 6.1.8], which is used to calculate Sinking Efficiency (SEproject) [6.1.7].	Profiles are used to assess water mass stratification and movement, which affect the long-term durability of stored carbon. Colder temperatures favor carbon storage efficiency.	In situ measurement
Current Profile euphotic zone / Seawater Velocities (m s⁻¹)	Site characterization and assessment of deep ocean circulation patterns necessary to ensure durable storage (200+ years) [9.3.7].		The Lagrangian particle tracking model is used to predict Substrate trajectories and seabed deposition for accurate placement of the sinking location. Modeling predicted trajectories ensures the Substrate plume avoids sensitive benthic ecosystems and designated control sites.	Currents are modeled Surface currents are measured in situ for verification
Surface water retention time (months)	Area of Interest (AOI) characterization [9.3.7].	Verifies the Air-Sea Gas Exchange (AS) efficiency factor used in calculating the gross carbon stored (Cstored).	Confirms that the carbon deficit created in the surface water has sufficient time to draw down atmospheric CO2 before subducting, ensuring	Model

			net-negativity.	
Deep water trajectory (n/a)	Demonstrates that the water mass and stored carbon will remain isolated from the atmosphere for at least 200 years [9.3.7].	Component of Ventilated Fraction (Fvent) calculation, which quantifies long-term losses (Closs) due to deep water circulation returning DIC to the surface.	Addresses reversal risk and permanence requirements (Section 4.3) by confirming that the deep water masses maintain isolation from the atmosphere.	Models
Sinking Rate (m/hr)	The Substrate must demonstrate a minimum sinking velocity of 20 m/hr. Measurement ensures the rate is fast enough to minimize contact time with the mixed layer [9.6.14].	Directly used as the sinking speed (w) input for the remineralization rate (R) calculation, which determines the project sinking efficiency (SEproject) term in Cstored quantification [6.1.7, 6.1.8].	Rapid sinking minimizes contact time with the shallow water column, reducing the amount of organic matter that is respired (remineralized) before reaching the storage depth.	In situ measurement
Photosynthetic Active Radiation (PAR) ($\mu\text{mol photon m}^{-2} \text{s}^{-1}$)	Used to determine the depth of the euphotic zone (0.5% surface PAR depth) [9.3.7, 9.3.9].	The depth of the euphotic zone (dphot) is required for NPP depths decision for sampling.	The depth of the euphotic zone (dphot) is required in modeling calculations related to the nutrient robbing assessment (e.g., fractional DIC uptake, 4.5.2 - Equation 4.3).	In situ measurement
Turbidity (NTU)	General physical characteristic used for water column baseline characterization [9.3.7].		Used to monitor the physical characteristics of the water column throughout the activity.	In situ measurement
Organic Carbon Quantification Parameters in the water	Eligibility	Quantification	Safety	How evaluated
^{13}C carbon fixation rate (NPP) ($\text{g C m}^{-2} \text{day}^{-1}$)	Required for environmental baseline characterization [9.3.7].	Baseline Net Primary Production (NPPbaseline) [6.2.1, 6.2.2].	Used to monitor and model the impact of the MCFS activity on	In situ sampling

			natural phytoplankton growth rates (μ) to detect and mitigate local nutrient-robbing risks [4.5.2].	
Total Organic Carbon (TOC/POC,DOC) ($\mu\text{mol L}^{-1}$)	Required for environmental baseline characterization [9.3.7].		Used to monitor and model the impact of the MCFS activity on natural phytoplankton growth rates (μ) to detect and mitigate local nutrient-robbing risks [4.5.2]. POC and TOC are monitored to assess risks related to biodiversity changes, food web disruption, and potential impacts on benthic ecosystems from decomposition products.	In situ sampling
Substrates Organic Carbon Quantification Parameters	Eligibility	Quantification	Safety	How evaluated
Total Solids (TS) (g DW unit⁻¹)	Required for characterizing the Substrate material properties prior to deployment (TS ₀) [9.3.3, 10.4.4].	Measured and used in conjunction with carbon content (C/TS) ₀ to calculate the net organic carbon accumulated on the Substrate (C _{org}), a key component of C _{fixed} [6.1.6].		Lab for pristine substrate In situ sampling
C content (C g⁻¹ DW)	Required for characterizing the Substrate material properties prior to deployment [9.3.3, 10.4.4].	Measured and used in conjunction with carbon content (C/TS) ₀ to calculate the net organic carbon accumulated on		Lab for pristine substrate In situ sampling

		the Substrate (Corg), a key component of Cfixed [6.1.6].		
Remineralisation rate (d⁻¹)		Directly used as the non-sinking remineralization rate of substrate after microalgal growth (d0) input for the remineralization rate (R) calculation, which determines the project sinking efficiency (SEproject) term in Cstored quantification [6.1.7, 6.1.8].		In situ sampling, respiration measurement on board.
Chemical Composition Parameters	Eligibility	Quantification	Safety	How evaluated
Dissolved Oxygen (DO) (μmol L⁻¹)	Required for environmental baseline characterization [9.3.7].	DO profiles (O2z) are input for modeling the remineralization rate (R) [6.1.7, 6.1.8], necessary for quantifying Sinking Efficiency (SEproject).	Monitored in the whole water column to assess the risk of oxygen depletion (hypoxia) caused by organic matter degradation [4.5.5]. Assessed in the water overlaying the sediments by box model simulations of DO consumption in deep water.	In situ measurement Box model in deep water
Inorganic macro-nutrients (N, P, Si) (μmol L⁻¹)	Core eligibility criteria for HNLC deployment regions (e.g., Nitrate > 8 μM, Phosphate > 0.5 μM) [3.7.5]. Required for water column baseline characterization [9.3.7].	Used in calculations (Equations 4.4 - 4.7) to assess nutrient uptake and potential depletion and estimate remote/far-field impacts which may necessitate deductions from Cbaseline (Nutrient Robbing) [6.2.8].	Monitored to avoid nutrient robbing (depletion of local macronutrients below intra-seasonal minimums) [4.5.1].	In situ sampling

Trace metals (Fe, Mn) (nmol L ⁻¹)	Core eligibility criteria for HNLC deployment regions (Iron < 0.2 nM) [3.7.5, 9.3.7].		Monitored to verify that the micronutrient supply in the Substrate does not result in trace element leaching.	In situ sampling
Carbonate system (DIC, pH, TA)	Required for environmental baseline characterization [9.3.7].		pH and TA are monitored to assess the risk of detrimental changes to the carbonate system (e.g., pH exceeding the tolerated range of 7.5-8.5 for marine organisms) [4.5.9].	In situ sampling for TA and DIC, pH is calculated from the carbonate system equations
CH₄, N₂O (nmol L⁻¹)			Monitored to ensure that the project does not lead to enhanced production of these potent greenhouse gases, typically associated with low oxygen conditions and phytoplankton bloom [4.5.7].	In situ sampling
Dimethylsulfoniopropionate (DMSP)			Monitoring serves research into potential co-benefits of MCFS, as DMSP is a precursor to DMS, which can have climate cooling effects [4.5.15]. Used to monitor for potential adverse effects, such as the attraction of grazers, which could reduce CDR efficiency.	In situ sampling

GIGABLUE

Biological and Ecosystem Parameters	Eligibility	Quantification	Safety	How evaluated
Chlorophyll a ($\mu\text{g L}^{-1}$)	Core eligibility criteria for HNLC regions (Chlorophyll a < 1 $\mu\text{g/L}$) [3.7.5]. Required for environmental baseline establishment [9.3.7].		Monitored to ensure that Substrate deployment does not trigger an excessive bloom that could lead to subsequent environmental risks.	In situ sampling
Community Composition / Biodiversity	Required for environmental baseline characterization [9.3.7].		Monitoring composition is necessary to detect potential Harmful Algal Blooms (HABs) [4.5.13] and assess general food web disruption risks [4.5.11].	In situ sampling
Ultraplankton / Microphytoplankton / Bacterial Abundance (cells L^{-1})	Required for environmental baseline characterization [9.3.7].		Monitored to ensure that the operation does not significantly change the bottom of the food web.	In situ sampling
Algal Toxins (ng L^{-1})			Monitored if a specific algal bloom was detected, in case species monitoring indicates a risk of toxin production [4.5.14].	In situ sampling

Environmental risk monitoring and mitigation

From a monitoring perspective, environmental risk management focuses on early detection, continuous observation, and verification of any potential environmental effects associated with the deployment and operation phases. The monitoring program is designed to track key physical, chemical, and biological indicators that could signal adverse impacts on the marine environment, including changes in water column oxygen levels, nutrient availability, plankton community composition, and benthic conditions at and around the deposition site. These parameters are measured at both the deployment and control sites to distinguish natural variability from project-related effects. A comprehensive analysis of environmental risks and mitigation approaches is outlined in the Environmental Impact Assessment (EIA) and Environmental and Social Safeguards Questionnaire. Table 5 outlines the major environmental risks, their monitoring plan, and the specific mitigation. The management strategy for these risks relies heavily on Anticipation, Avoidance, and Minimization through careful site selection, predictive modeling, and operational safeguards.

- Anticipation (Site Selection): The Area of Interest (AOI) was refined to avoid known marine mammal and seabird hotspots, especially for 'Threatened' species, and to avoid specific sensitive benthic habitats like seamounts and areas modeled to have high suitability for Vulnerable Marine Ecosystems (VMEs).
- Avoidance (Design): Substrate materials were tested and selected to be non-toxic, biodegradable (cellulose), and designed to hold micronutrients in a stable, low-leaching oxide form, thereby avoiding ecotoxicity risks.
- Minimization (Operational Control): The deployment method involves limiting the particle mass to the minimum required and deploying them across a transect to reduce surface and seabed concentrations. The rapid sinking rate (1.5 cm/s) minimizes the duration of exposure in the water column.

These measures ensure that the predicted residual adverse environmental effects from the operation are expected to be low to negligible, even when assessed against conservative, worst-case scenarios.

Table 5: The major environmental risks, their monitoring plan, and the specific mitigation responses

Environmental Risk	In-situ Monitoring / Assessment plan	Mitigation Hierarchy Response / Required Action
Nutrient Robbing and Changes in Phytoplankton Growth Rate	Modeling assessment: Nutrient uptake and its impact on natural phytoplankton growth rate (μ) was assessed using pre-determined parameters (μ_{max}, K_n; see modelling report for details). Global circulation models used to assess far-field depletion risks (water mass connectivity; see modelling report for details). In-situ monitoring: Pre-deployment, during fixation phase and post-operation monitoring: Measurements of inorganic macro-nutrients (N,P,Si) and ^{13}C carbon fixation rate (NPP-background productivity).	Avoidance/Minimization through site selection and operational planning. The deployment approach (Substrate application rate and distribution area) will be designed to avoid reducing local macronutrient concentrations below the established AOI intra-seasonal minimums (See climatology values in the marine characterization report). If evidence for far-field depletion is found, the associated loss in carbon-fixation potential will be quantified and deducted as the baseline removal (Cbaseline).
Oxygen Depletion (Hypoxia) and GHG Biogeochemistry	Modeling assessment: Sediment-Water Interface Model (multi-box model) applied to simulate DO consumption at the seafloor and bottom water layer (for details see Oxygen depletion model report). Modeling demonstrates that decreased DO is not expected to be reduced by up to 10%, such a reduction does not cause increased CH_4 or N_2O production.	Avoidance/Minimization through site selection and operational planning. The deployment approach (Substrate application rate and distribution area) will be designed to avoid a decrease in deep water DO concentration in excess of a 30% threshold, due to organic matter remineralization. The low mass of particles (current EIA up to 1,000 tonnes total) and organic matter expected to reach the ocean floor ensures that the scale of the activity is many orders of magnitude smaller than large-scale simulations that predict significant acidification/deoxygenation risks.

Phytoplankton bloom enhanced N₂O, CH₄, and DMS production at the surface water	In-situ monitoring: Concentrations of Dissolved Oxygen (DO), Nitrous Oxide (N ₂ O), Methane (CH ₄), and Ammonium (NH ₄ ⁺) and DMSP monitored in the surface water.	Avoidance/Minimization through operational planning. The deployment approach (Substrate application rate and distribution area) will be designed to avoid a decrease in surface water excessive phytoplankton bloom. Monitoring serves research purposes, which allows adjustment of substrate application rate in future deployments if necessary.
Changes in Carbonate System Chemistry	In-situ and lab monitoring: Pre-deployment assessment in the lab on the substrate materials (Substrate characterization report). Surface water Carbonate System parameters are measured pre-deployment, during fixation phase, and post-operation (DIC and TA are measured, pH and pCO ₂ calculated by the carbonate system equations).	Avoidance/Minimization through substrate design and operational planning. The deployment approach (Substrate application rate and distribution area) will be designed to ensure that pH changes remain within the tolerated range of most marine organisms, specifically between pH 7.5 and 8.5. Assessments during deployment serve research purposes, which allows strategic adjustments in substrate composition in future deployments. pH levels (Substrate characterization report). Monitoring informs adjustments to future deployment rates.
Changes in ocean chemistry	In-situ and lab monitoring: Pre-deployment assessment in the lab on the substrate materials (Substrate characterization report). Phosphate concentrations are measured pre-deployment, during the fixation phase, and post-operation.	Avoidance/Minimization through substrate design and operational planning. Substrate design is tested and characterized in the lab (Substrate characterization report). Assessments during deployment serve research purposes, which allows strategic adjustments in substrate composition in future deployments.

Biodiversity Changes and Food Web Disruption	In-situ monitoring: Changes in carbon parameters and biologically relevant parameters (phytoplankton growth rate, abundance, TOC, POC, and DIC) are monitored and compared to baseline. Microbial community composition and dynamics (using DNA sequencing) are tracked at deployment and control sites. Thresholds: Monitored parameters (phytoplankton growth rates, abundance and/or TOC) shall not exceed AOI intra-seasonal variability.	Avoidance: No introduction of foreign phytoplankton species will be applied. Comparison of the relevant parameters before and after the operation. If changes pose a significant risk to the ecosystem adjustment of the substrate's future application rate will be applied.
Harmful Algal Blooms (HABs) and Toxin Production	Lab monitoring: Incubation experiments using seawater samples and particles tested the microbial community composition for the presence of known HAB species (DNA sequencing). The presence of HAB species was minimal across all samples, and the proportion of HAB organisms was lower in treated samples than in the controls. In-situ monitoring: Microbial community composition is monitored for known HAB species (DNA sequencing and microphytoplankton microscopy). If a significant increase in potentially harmful species is detected, specialized analysis of Algal Toxins in surface water is conducted.	Avoidance/Minimization through substrate design: The design and preliminary testing confirm that the substrate components and activity do not favor the proliferation of pathogens or HABs.

Physical Harm to Larger Organisms	Assessment from existing scientific literature: Risk assessment based on feeding behaviors (e.g., filter feeders, surface feeders like petrels and shearwaters) and historical data on non-food item ingestion. Fisheries existing data to be collected and analyzed to characterize representative species' habitats and feeding strategies. In-situ monitoring: Before operations, Passive Acoustic Monitoring (PAM) with a hydrophone array will confirm the absence of marine mammals at the deployment site. Log all large marine animal sightings, including time, coordinates, and video footage (drone or vessel).	Avoidance/Minimization through site selection, substrate design, and operational planning: Assessments inform the operational planning to minimize risk. The release location was refined to avoid known seabird hotspots, particularly those containing 'Threatened' species, thereby reducing the likelihood of encountering the densest plume. Monitoring for fauna presence will be carried out prior to release, and substrates are deployed across a transect to reduce surface concentration. Ingestion risk is low to negligible because the cellulose material is soft, compressible, biodegradable, and is likely to be digested or excreted by fauna. Substrate does not contain toxic materials (Substrate characterization report).
Benthic Organism Smothering	Assessment from existing scientific literature: Scientific literature is analyzed to assess the vulnerability of local organisms in the AOI. Modeling assessment: The Lagrangian model is used to estimate the spatial distribution and concentration of Substrate particles to estimate seabed deposition density.	Avoidance/Minimization through site selection, substrate design, and operational planning: The trial area AOI refinement criteria specifically require the avoidance of marine seamounts (biodiversity hotspots) and areas with high modeled habitat suitability (> 5% probability of occurrence) for Vulnerable Marine Ecosystems (VMEs) such as corals and sea pens. Operational planning ensures sufficient dispersion so that the Substrate density on the seabed does not create a consistent layer and remains within the lower end of deep-sea deposition tolerances. The low deposition density, large grain size, and porous structure of the biodegradable particles reduce the risk of smothering or localized deoxygenation, allowing interstitial water flow. Smothering is likely only

		to occur at 3 mm thickness or above.
Ecotoxicity from Trace Metals (Fe/Mn) and Contaminants (PTEs)	Lab monitoring: Acute aquatic toxicity tests are performed on the substrate material against sensitive organisms (green microalga and blue mussel). ICP-MS Analysis: Detailed analysis determined elemental concentrations of metals, metalloids, and non-metals in the material. The material has to be classified as non-toxic at all tested concentrations. All contaminant concentrations are modeled to be well below applicable marine water quality guidelines (ANZG 95% DGVs) for 1-hour and 24-hour dispersion scenarios.	Avoidance/Minimization through substrate design: The substrates are composed primarily of cellulose, which is biodegradable. Micronutrients are incorporated in oxide form to minimize dissolution and leaching. Selection of materials that are inert and carry minimal toxicological risk to marine organisms.
Bioaccumulation and Biomagnification	Assessment from existing scientific literature: Evaluation of the pathways for uptake of trace metals (Fe, Mn) by plankton, invertebrates, fish, and mammals. Risk is low due to the very low concentration of metals in the substrate, the evolved regulatory mechanisms in marine life, and the fact that the material (cellulose) is hydrophilic (water-attracting), making it less likely to penetrate lipid membranes or accumulate in fatty tissue, unlike hydrophobic microplastics.	Mitigation (Co-Benefit): If accumulation of Fe/Mn were to occur, it could be potentially beneficial given that iron and manganese deficiencies have been reported in lower trophic levels in the region.

Turbidity and Visibility Reduction	Lab monitoring: Elutriation tests assessed turbidity levels following particle immersion. Experiments show a negligible increase in turbidity (from 1.3 NTU to 1.9 NTU) after 24 hours, even at high concentrations. (substrate characterisation report)	Avoidance/Minimization through substrate design and operational planning. Substrate design is tested and characterized in the lab (Substrate characterization report). Substrates are designed for rapid sinking (~1.5 cm/s, reaching 1,300 m in ~24 hours), which minimizes the duration of suspension. Effects are expected to be localized to the deployment site and limited to the first few days due to rapid surface dispersion.
---	--	--



Information management and data quality control

All operational, environmental, and analytical data collected during the project will be recorded, documented, and uploaded throughout the pre-deployment, deployment and post-deployment phases. Data from instruments, sensors, and field logs will be entered into a centralized data management system designed for shared access by the science and operations teams. Water and substrate samples will be labelled immediately upon collection with sample number, date, time, depth, and location, and stored properly onboard (in accordance with the expert laboratory requirements) until transfer to onshore laboratories. Analyses of these samples will be performed post-operation under standardized laboratory protocols to ensure consistency in measurements and analysis.

All digital data, including CTD and echosounder profiles, ROV and drone footage, will be logged and reviewed daily. Once a day, all field and sensor data will be uploaded to the data management system, where both the science and operations teams will conduct coordinated quality control (QC). Geolocation data from vessels and drifters will be shared in real time, and all visual documentation, including photos and videos, will be uploaded to the same system.

The quality control procedure is integrated directly into daily field and data management activities. All measurements and observations are reviewed by both the science and operations teams daily. This includes cross-checking sensor outputs, verifying sampling logs, and confirming calibration records. Data completeness and consistency are checked against operational notes and instrument metadata to identify and correct any discrepancies before the next day's deployment. Laboratory analyses conducted post-operation will undergo the same review process, comparing analytical results to field data to ensure internal consistency. Through this ongoing, collaborative review process, the project maintains high data accuracy and reliability across all stages of collection, processing, and reporting.

Data management system

A designated data manager onboard will oversee the daily acquisition, organization, and secure upload of all datasets to the designated cloud-based data repository. This includes physical and biogeochemical measurements from CTD and ADCP casts, echosounder outputs, ROV recordings, drone



footage, and real-time model forecasts. Vessel and drifter geolocation data will be securely streamed and archived continuously throughout operations.

- ADCP
- CTD casts
- Daily logs
- Drone
- EK80
- Lagrangian- forecasts
- ROV
- Samples lists

Screen dump of folder list of cloud based project data management system

Daily operational logs and reports will be prepared onboard and reviewed by the science and operations teams. Water and substrate samples will be labelled onboard immediately upon collection, recorded in the sampling database, and preserved for laboratory analysis following established protocols. This integrated system ensures that all monitoring, modelling, and verification datasets remain auditable and available for third-party review and future verification cycles.

[illegible]

Screen dump of sample inventory

Appendix A. **Example** schedule planned for January 2026 deployment

([See the adjacent gantt](#)):

** Note that the detailed schedule is subject to change will be finalized adjacent to deployment

—

Leaving Wellington on November 21th (after mobilization and sea test of 8-9 days)

1. Transit from Wellington to Deployment site (Figure 1; approx. 44 hours, 350NM)

23-24/11/2025 (Floating [-1] - control+2x background):

2. Control station sampling (approx: 46S, 172.7E, 23/11) CTD + sensors and seawater samples collection using the rosette equipped with 12x10L Niskin bottles.
 - a. CTD+sensors full water column + photic layer water samples (4 depths) including NPP sampling for incubation.
 - b. Surface water sampling with a TM clean fish pump.
 - c. ADCP measurements of the currents.
3. Deployment site (trajectory approx: 46.6S, 172.72E to 46.6S, 172.83=> ~8NM, 21/11) CTD+sensors profiling and seawater sampling using the rosette equipped with 12 or 24 Niskin bottles.
 - a. Full water column CTD cast + water sampling including NPP. X2
 - b. Surface water sampling with a TM clean fish pump. X2
 - c. ADCP measurements of the currents.

25/11/2025 (Floating 0 - deployment 24-36h):

4. Before deployment of substrates, deploy hydrophone on cable and tow it at ~20 m. Make sure shut down engines for 20 minutes listening. Send/share for analysis on shore to make sure no marine mammals in the proximity.
5. Deployment of free substrates by deployment vessel (100 tonnes - by Rangitata).
6. Deployment of 5 pens + 2 Variation pens with substrates inside, for floating time variation (Fsunk), by the Seaworker.
7. A drone will document the operations of the deployments.
8. Initiation of the on-board mesocosm experiments.

26/11-13/12/2025 (Fixation phase- Floating days 1-19):

9. Sampling the plume with Rosette+CTD: water samples including NPP every 2-3 days (photic layer) and particles from surface water.
10. Plankton net surface tows (every 2-3 days, on the same day with CTD).
11. Sampling the plume with MOCNESS sampling every time it's possible (every 2-3 days).
12. Sampling water and substrates from 3 pens (in case plume lost every 3 days) and mesocosms.

GIGABLUE

13. Measuring oxygen consumption by substrates retrieved from the surface water for remineralization rates.
14. The vessel steams between the drifters' location (indicating the free-plume location), and ADCP data collection is performed while steaming if possible in the current setup.
15. Drone observation of the free plume, observe birds/marine mammals in relation to plume.
16. Transit over the free plume collecting echo sounders data- to test sinking particles detection every 2-3 days, if detection reached- measurement will serve for sinking speed calculation.
17. ROV deployment to observe the plume (every 2-3 days). If sinking phenomena observed, follow sinking substrates for sinking speed measurement.
18. Steam to the pens location if the plume was lost.
19. ADCP measurements of the currents.
20. Drone observation of the pens (when possible, reach the pens-depends on the distance).

14/12/2025 (Day 20- sinking velocity from pens):

21. Sample substrates from pens.
22. Release the particles from the pens to sink and track and sample them as deep as possible with the ROV.

15-17/12/2025 (post-operation verification):

23. Sinking site CTD+sensors profiling and seawater sampling using the rosette equipped with 12 or 24 Niskin bottles.
 - d. Full water column cast, including NPP sampling for incubation. X2
 - e. Surface water sampling with a TM clean fish pump. X2
24. Collect the pens onboard.
25. Collect drifters onboard.
26. Control station (approx: 46S, 172.7E) CTD+sensors profiling and seawater sampling using the rosette equipped with 12 or 24 Niskin bottles.
 - a. CTD+sensors full water column + photic layer water samples (4 depths), including NPP sampling for incubation.
 - b. Surface water sampling with a TM clean fish pump.

17-18/12/2025:

27. Transit back to Wellington (approx. 38 hours, 300NM).

19-20/12/2025:

28. Demobilisation.

Appendix B. Required Scientific equipment

Equipment	Model - supplied previously by NIWA
Rosette+CTD+DO+PAR+Fluo+backscatter	Seabird 911 plus with 12 or 24 (preferred) bottle carousel
CTD+DO+PAR for pens	2 x Seabird 37 SMP-ODO
Scientific Echo-sounder	EK80 38/120 kHz
Vessel Positioning Systems	Hipap 352 - ?
20 x GPS Drifters	Pacific Gyre Microstar + Stokes MetOcean
2x Navigation buoys with light, radar reflector & AIS	Exact model unknown, similar to JFC NAV01 Ø600mm Navigation Buoy
Fish pump (TM clean)	For trace metals sampling
GoPros or other buoy-mounted cameras + housings	
MOCNESS	
DO optode - data logger - sensor that fits BOD bottles.	
Workboat	
Echosounder calibration equipment	

LABS:

- Wetlab setup for sample processing (as per 2024 voyage) - ALL lab equipment (Including: Filtration rigs with pumps for: POC, Chl/nut, peristaltic for DNA, 13C)
- Portable fluorometer for Chl.a analysis.
- DO optode with 12 BOD bottles (for respiration measurements of substrates)
- Dry Lab: CTD/electronics/ROV/MOCNESS connection
- Iron-free seawater supply for mesocosms
- -80 Freezer
- -20 Freezer
- 4C refrigerator
- Dewar with Liquid N2 for snap freeze
- Bottles and other consumables suitably prepared (including, where required, acid washing) to collect water and particle samples as agreed in the sampling plan.

GIGABLUE

OTHER EQUIPMENT:

Equipment	Model	Owner
ROV		Gigablue
ADCP	Nortek Signature VM 250 ADCP	Gigablue+Seaworks
Drone		9(2)(b)(ii)
Plankton net	Double trip mechanism 350um	Gigablue+NIWA
Mesocosm containers		Gigablue+Kernohan
Pens+ Var pens		Gigablue+Kernohan
X Drifters with acoustic signal detection capabilities		Gigablue+NIWA
X “stokes” drifters		Gigablue+NIWA

Appendix C. List of calibrated equipment

Evidence of calibration will be provided adjacent to deployment and/or analysis

	Specifications	Calibration date	From
CTD			NIWA
DO			NIWA
Chl a Fluorometer			NIWA
Backscatter			NIWA
PAR			NIWA
Scientific Echo-sounder	EK80 38/120 kHz		NIWA
ADCP	Nortek Signature VM 250 ADCP		STR
MOCNESS			NIWA
DO optode for BOD			NIWA

Appendix D: Sampling and Fixation protocols

Seawater sampling

1. Rosette - profiles

Before sampling, prepare 10 plastic baskets/sets (one for each depth). Prepare all samples tubes, mark with the cryo tags, check all labels are written in the log table.

In each set, place all the labeled sampling bottles/tubes for each depth.

- Start by sampling the gas samples (CH₄, N₂O, O₂-if sampled), sampled at the surface only.
- 4-10 L acid-cleaned plastic bottles (later in the lab subsampled for: Nutrients - prefiltered, TA, DIC, TOC; POC, Chl a - filtered; FCM)
- 1L for Community composition- DNA (Surface sample duplicate)
- 0.3L bottle for Microphytoplankton abundance (Euphotic only)
- 0.5L bottle for Toxins (Euphotic only)
- 5x 2L for NPP (Euphotic only- 2 bottles light, 2 bottles dark 1 natural abundance)
- Sample triplicate Surface SW for trace metal analysis from the “fish pump” only. Prefilter the samples.

At the control, background and post-operation stages, full casts will be sampled, and during the fixation stage, euphotic casts will be sampled. Evaluated sample numbers are outlined in Tables 1+2.

Substrate sampling

In general, we would like to sample a large number of substrate per sample to increase the representativeness of the sample (especially for TS and CHN).

1. Particle sampling with a net on a pole/plankton net. Collect approx. 2L of particles and place them in a plastic jar/box/bag.
2. Divide particles for each analysis (as detailed below).
3. Keep the extra particles aside and freeze.

GIGABLUE

Fixation procedures for seawater samples

Metals (micronutrients)- sampled from surface by the Fish pump

1. Sample 3L surface water to a dedicated trace metal clean 3L container.
2. Use a 50ml syringe and a syringe/Sterivex filter.
3. Filter 125 ml into acid-washed LDPE bottles (rinse 3 times with filtered sample water before filling). If Sterivex filters, mark filters from each location to further use for DNA sample collection.
4. Store samples at 4C.

Gas analyses

Dissolved oxygen – sample taken directly from CTD bottle

1. Attach silicone tube to CTD bottle and place to bottom of sample bottle
2. Rinse glass bottle 3x
3. Fill to overflowing
4. Fix with oxygen fixation chemicals – manganese chloride & sodium hydroxide/sodium iodide solution.
5. Cap with glass stopper
6. Shake the bottle well before storing
7. Store at room temperature (RT) in fish bin

CH₄ & N₂O – Sample taken directly from CTD bottle

1. Attach silicone tube to CTD bottle and place to the bottom of the sample bottle
2. Rinse 125ml glass serum bottle 3x
3. Fill to overflowing
4. Fix with 1 drop (50ul) HgCl₂.
5. Cap with silicone septa and crimp
6. Store at room temperature (RT) in fish bin
7. Release pressure in bottles by placing syringe needle into septa when samples at RT

Salinity – Sample taken directly from CTD bottle

1. Rinse glass bottle 3x
2. Fill to overflowing
3. Cap and store upside down in a wire rack

TA – Sample taken directly from CTD bottle

1. Place the tubing at the bottom of the sample bottle
2. Rinse 500ml plastic bottle 3x

GIGABLUE

3. Fill to overflowing. Remove the sample tube.
4. Fix with 1 drop (50ul) HgCl₂.
5. Parafilm lid, store in fish bin at RT.

DIC – Sample taken directly from CTD bottle

1. Place the tubing at the bottom of the sample bottle
2. Rinse a 500ml Schott glass bottle 3x
3. Fill to overflowing. Fix with 1 drop (50ul) HgCl₂.
4. Parafilm lid, store in fish bin at RT

DMSP

1. Fill 50ml seawater into glass bottle
2. Add 2 pellets of NaOH
3. Cap and store at RT

TOC – Sample taken directly from CTD bottle

1. Fill EPA glass vial with 20ml of sample water after rinsing it.
2. Freeze at -20C.

POC

1. Filter 1-2L seawater on a 25mm precombusted GFF filter (Pressure lower than 150 mmHg).
2. Rinse funnel x 2 with filtered seawater.
3. Place the filter in a 6-well plate. Make sure marked properly.
4. Freeze at -20 °C.

Chl a & Nutrients

1. Filter 1-1.5L on a 25mm precombusted GFF filter (Pressure lower than 150 mmHg) for Chlorophyll.
2. Rinse funnel x 2 with filtered seawater.
3. *At the same time:*
4. Place 250ml plastic bottle in the filtration rig under the filter funnel.
5. Rinse 250ml bottle twice after ~50ml of seawater has been filtered.
6. Continue filtering until the bottle is full. Remove from the filtration rig, pour out ~50ml of water from the bottle, and cap.
7. Parafilm lid and freeze at -20 °C in the fish bin.

Chla fluorometry:

8. Place the filter in a 15ml falcon tube. After all daily samples are in the vials, add 10 ml 90% acetone. Keep in -20C in the dark overnight.

GIGABLUE

9. Centrifuge tube.
10. Transfer acetone to 8ml borosilicate glass vial.
11. Read the fluorescence. (IF we have the fluorimeter on board-calibrated).
12. Acidify with 180ul 0.1N HCl and read fluorescence again (to get phaeopigments)

FCM (Ultraphytoplankton & bacteria)

1. Fill 50ml Falcon tube with seawater passed through a 100um mesh filter.
2. Place 1.5 ml of sample water in a 1.8 ml cryotube, sample duplicate (one phyto and one bacteria).
3. Fix with 30ul of 25% Glutaraldehyde SEM grade.
4. Snap freeze in liquid nitrogen.
5. Keep in -80C.

Microphytoplankton abundance

1. Fix the 200-300 ml with Lugol (final concentration 1%).
2. Keep in 4C.

Community composition- DNA

1. Filter triplicate 1 L on Sterivex (previously used by TM sampling).
2. Place it in Sterile Whirl-Pak sample bags (or Falcon tube). Freeze at -20 °C.
3. On every sampling day, filter 1 L of DDW for blank. Treat the same as a sample.

Toxins

1. Collect seawater to 0.5L plastic bottles.
2. Freeze the 0.5L water sample.
3. Keep in -20C.

NPP – 13C method (for more details see dedicated protocol)

1. **Prepare SS:** Make up pre-weighed ¹³C spike (0.34g in 50ml Falcon tube) about an hour before spiking using DI water. Make up **to 20 mls**. Gently mix the solution and do not shake or allow to degass.
2. Fill 20 L from each depth to dark carboys (or 2x10L whatever available) through 200um mesh. In the lab, rinse 4x 2L incubation bottles and fill each with 2L (use a 2L measuring cylinder).
3. Spike all 4 bottles with 1 ml of 13C SS.
4. For each depth, shade the 2 bottles to the original depth's PAR levels, and the other 2 to total darkness (prepare in advance).
5. Incubate all bottles in the transparent incubation container with circulating surface seawater for 24h.
6. Filter 2L on pre-combusted GFF filters for natural abundance (on POC rig not 13C rig), add blanks. Write down the volume filtered. Place in a 6-well plate- Label *13C natural abundance*.

GIGABLUE

7. After incubation of 24h: Filter spiked bottles on a pre-combusted GFF filter (On a dedicated filtration rig).
8. Place each filter in a 6-well plate. Label up the lid and the base of each plate as these are separated during the drying process before isotope analysis.
9. Label the plate well- **13C enriched**.
10. During filtration, add GFF blanks with FSW priming water.

Fixation procedures for substrate samples

TS/TVS and CHN analyses:

1. Take a batch of ~100 substrates (± 20 units), spread them on a tray so you can count individuals (tray needs to be smooth or covered with a plastic bag; particles need to be separate so individuals can be detected), take a picture of the tray (this will be utilized later by counting person or image analysis for substrates accurate count).
2. Store the batch in a zip-lock plastic bag or plastic box, mark and freeze.
3. Take a triplicate of ~100 units (± 20 units) batches from each pen for each analysis.
4. In the lab, the batch will be dried (TS) at 105°C for 2h, weighed (CHN samples will be sent for analysis), and for TVS ashed (at 550°C for 1.5h), weighed.

Remineralization evaluations- respiration measurements

1. Take 5-20 particles from the sample and place them in a BOD bottle filled with FSW to the top.
2. Measure the DO concentration in the bottle using an optode. Top up with FSW to overflow and seal without bubbles.
3. Incubate in the dark with surface seawater temperature for 4-6 hours.
4. Measure the DO at the end of the incubation (4h - write the exact times).
5. Retrieve the particles from the BOD bottle and place them in a 6-well plate (For CHN analysis).
6. Mark and freeze.