Marine National Facility

CSIRO

RV *Investigator* Voyage Summary

Voyage #:	**IN2016_V04**		
Voyage title:	Influence of temperature and nutrient supply on the biogeochemical function and diversity of ocean microbes		
Mobilisation:	0800 Sydney, Tuesday, 30 August 2016		
Depart:	1400 Sydney, Wednesday, 31 August 2016		
Return:	1200 Brisbane, Thursday, 22 September 2016		
Demobilisation:	0800 Brisbane, Friday, 23 September 2016		
Voyage Manager:	Hugh Barker	**Contact details:**	Hugh.Barker@csiro.au
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Principal Investigators:	Mark Brown Martin Ostrowski Iain Suthers April Abbott (Integrated project: Geochemistry of ocean sediments)		
Project name:	Supplementary project: Natural iron fertilisation of oceans around Australia: Linking terrestrial dust and bushfires to marine biogeochemistry		
Affiliation:	University of Tasmania	**Contact details:**	Andrew.Bowie@utas.edu.au

Objectives and brief narrative of voyage

Scientific objectives

Our major scientific objective was to resolve how changes in seawater temperature and nutrient concentrations, linked to shifting oceanographic circulation in eastern Australia, influences the diversity of microbial communities and the key biogeochemical transformations (C, N, P, Si, Fe and S) they mediate.

We also wanted to understand the broader ecosystem implications of potential changes to the base of the food web. Our microbial investigations were therefore coupled with zooplankton, larval fish and marine megafauna observations.

Our voyage track allowed us to integrate a third objective which was to collect sediment cores to examine water-sediment geochemical processes and the historical record of plankton at a long-term coastal time-series site, Port Hacking.

The final objective was to collect aerosol particles under clean conditions to examine natural iron fertilisation of oceans around Australia, a potentially important process that influences microbial community composition and function.

Voyage objectives

1. Collect samples to characterise the diversity and function of microbial communities in the relatively warm EAC, against the relatively cool water of the Tasman Sea and adjacent shelf waters.

2. Conduct perturbation experiments to experimentally test the role of temperature and nutrients (particularly N and Fe) in microbially mediated biogeochemical transformations.

3. Assess the links between microbial biomass, size structure and carbon production with higher trophic levels (zooplankton, micronekton and cetaceans-seabirds) in a frontal eddy(ies) relative to adjacent shelf and EAC waters through coordinated sampling.

4. Sample sediments to examine water-sediment geochemical processes and historical record of plankton.

5. Sample aerosols to study natural iron fertilisation of oceans around Australia (Supplementary Project).

Results

1. Sample collection

We successfully archived RNA and DNA (to examine microbial function and diversity, respectively) from 40 CTD stations (see submitted Excel file) encompassing the following:

- Three ocean transects at 36.25 °S, 32.29 °S, and 28 °S spanning coastal to Tasman Sea waters;
- Several eddies and frontal features;
- Shelf water at the Port Hacking National Reference Station;
- The EAC (during the drift study) between Coffs Harbour and Newcastle.

These water masses represent a gradient of temperature and nutrients (Figure 1) and will therefore allow us to explore the drivers of microbial diversity and abundance in this region.

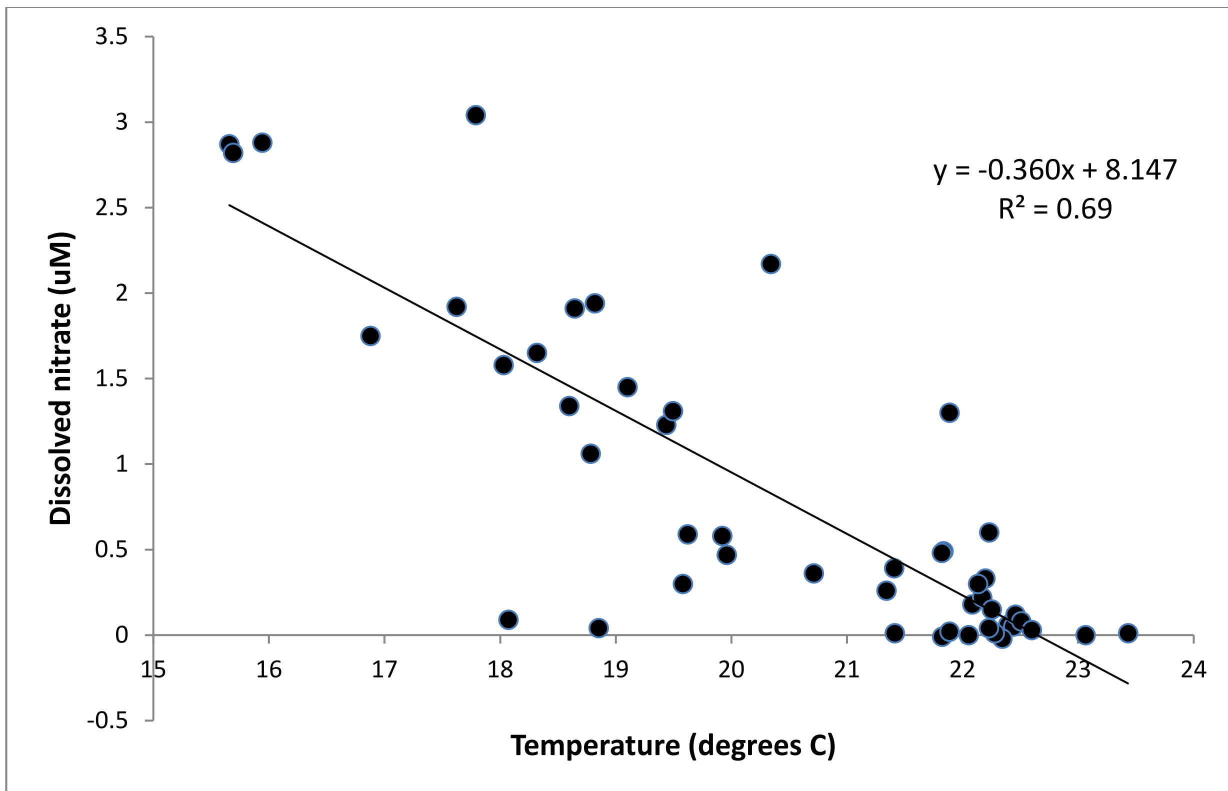


Figure 1. Relatively warm water is oligotrophic in the eastern Australian region. There is a strong negative correlation between surface water temperature (defined as upper 10 m) and its dissolved nitrate concentration. Plot contains data from 48 of 55 CTDs for which we have preliminary hydrochemistry data.

At each station, water (from the surface, Chl-a maximum, and below the Chl-a maximum) for RNA and DNA analysis was filtered onto a 0.2 µm membrane / cartridge filter. In addition, researchers from UTS used a gravity filtration device to prepare samples of larger eukaryote cells. Three litres of seawater from the surface and Chl-a maximum was pre-filtered through a 100µm mesh before filtration onto an 11µm mesh. Concentrated water obtained from backwashing the 11µm mesh was filtered again onto an 8.0 µm TETP membrane filter. Filters were stored immediately at -80 °C for subsequent DNA analysis, aiming to detect toxic dinoflagellates (Contact: Rendy Ruvindy).

Viruses were also collected to study the composition and distribution of the Australia's Viriome in relation to different water masses. Being the most abundant organisms in the marine environment, viruses have a considerable influence on microbial ecology and the ocean's biogeochemical cycles. Viral–induced mortality can influence the flux of nutrients in the oceanic microbial food web and also alter the species composition through horizontal gene transfer. A total of 37 samples were collected from 31 different stations. At each station, 20 litres of surface seawater were filtered through 0.2 µm followed by an hour incubation with iron chloride (John, Seth G., et al. 2011), then viruses were recovered with a 0.8 µm PC filter (1 filter each 10 L). Filters were kept at 4 degrees until further analysis. Deep samples were collected from the 2 deep (>4000 m) CTD casts at 4000, 3000, and the ~1800 m oxygen minimum. DNA will be extracted at Macquarie University and the viral metagenome will be sequenced to identify viral composition.

Microbial RNA/DNA samples will be analysed according to the extraction and sequencing procedures developed in the Bioplatforms Australia Marine Microbes Project (http://www.bioplatforms.com/marine-microbes/), allowing us to maximise data comparison opportunities.

In situ pumps. In addition to CTD sampling, microbial samples were also collected using in situ pumps (ISPs). ISPs were deployed at 14 stations (Appendix 1) and captured high volume samples (400L) at 1 to 3 depths, that will be used for meta-omic analyses (of genes, proteins). Given that only 2 to 4 L of water was captured on filters from the CTD, the ISPs provided an unprecedented amount of material for microbial investigations, without the need for long hours of filtering in the lab.

Monitoring of the functional genes expressed will provide detailed insight into the range of metabolic processes contributing to biogeochemical cycling in the ocean. In tandem, meta-proteomics will help determine the relative abundance of metabolic pathways, macromolecular complexes, such as photosystems, and help identify sensitive indicators of ecosystem status, e.g., by determining the taxon-specific abundance of nutrient transporters and stress response genes.

Community gene expression is highly dynamic over the day-night cycle, and in response to changes to environmental conditions, such as light and temperature. Using in situ pumps minimises perturbation while maximising the collection of microbial biomass (including bacteria, eukaryotes and viruses actively infecting both).

Samples were filtered onto 0.45 µm pore size PES membrane without any prefilter. Filters were cut in two and snap frozen in liquid Nitrogen within 30 min of the end of pumping. Loading and unloading of the pump heads was undertaken in the trace metal clean container according to standard operating procedures.

The pump program set a limit of 30-40 min of pumping at near maximum flow rates.

Sample volume: 400 liters

Initial flow rate: 7000 ml/min

Minimum flow rate: 3000 ml/min

Time limit: 40 minutes

Pump data period: 1 minutes

Countdown timer: 00:12:00 [HH:MM:SS]

Microplankton net sampling. After each CTD, a 20 µm mesh net was deployed vertically from the side of the ship to collect larger phytoplankton from 10-20 m deep. Representatives of the toxic, bloom forming *Alexandrium* group, and the nitrogen fixing organism *Trichodesmium* were being targeted, as well as chain-forming diatoms.

- One fraction (~40 mL) was fixed in Lugols solution and stored in the dark at 4 °C for microscopic characterization of the plankton diversity (contact: Penelope Ajani, UTS).
- Two further fractions (50 mL in duplicate) were filtered onto 5 µm nitrocellulose membrane filters, and filters were immediately stored at -80 °C to allow for subsequent DNA analysis to characterize plankton diversity (contact: Malwenn Lassudrie, UTS).
- Additionally, live samples were taken at a few CTD stations, pre-filtered through 100 µm mesh, diluted in filtered seawater and stored in temperature-controlled incubators for further isolation of phytoplankton cells back on land (contact: Penelope Ajani, UTS).
- Isolations of single cells/aggregates/colonies were also made on board using net tow samples and CTD water. These were brought back to the lab and placed into controlled temperature incubators in an attempt to establish new cultures for ongoing investigations (contact: Justin Ashworth, UTS).

Drift study. The EAC has a significant impact on the ecology of eastern Australia, and is well known for transporting organisms southward. However we know almost nothing about how EAC microbial communities, adapted to warm tropical conditions, change in structure or function as they are advected into temperate regions. Therefore, in the second week of the voyage, we undertook a drift study, collecting samples from within the EAC over three consecutive days. Microbial communities were sampled using the CTD-rosette sampler, the trace metal rosette (TMR) as well as the in situ pumps (ISP). A total of 15 CTDs were undertaken, to capture samples in the early morning, during the middle of the day, in the late afternoon and at night; the schedule of our sampling program is found in Appendix 3. After each Classification cast (i.e., CTD profile to 1000 m), a vertical phytoplankton net tow (20 µm) and two vertical net tows for zooplankton (200 µm, with 0.4 and 4 mm comprising the neck) were conducted.

Process experiments (CTD26, CTD34) conducted along the way measured important biological processes such as grazing of key phototrophs, carbon fixation (13C), nitrogen fixation and nitrogen uptake (15N) as well as changes in microbial composition in response to warming. Figure 2 shows the geographic location of the drift study, and a summary plot of CTD temperature profiles from within the EAC compared to CTDs conducted outside the EAC.

We were very pleased with the success of this study – as far as we know this was a world first. The vessel was an excellent platform for obtaining near real-time locations from the drifters (although more frequent updates than every 2-3 h would be a significant improvement) and deploying our sampling equipment including the EZ and neuston nets, as well as vertical haul nets. The combination of novel sampling methods and molecular techniques will result in new insight about the dynamics of these critical microbial assemblages and better understand their impact on southern Tasman Sea waters.

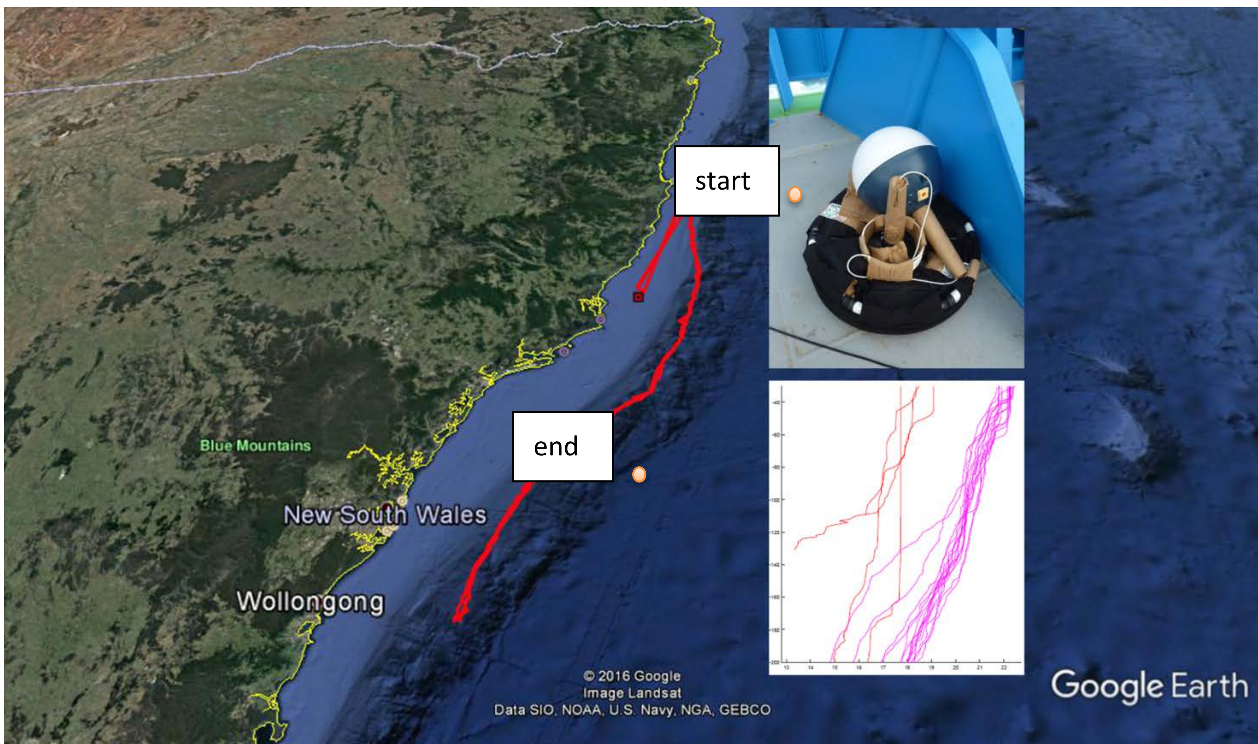


Figure 2. Drift trajectory of microbes in the EAC. Three surface drifters as shown in the upper right were released off Coffs Harbour (latitude: 30 ˚ 38.4'; longitude: 153 ˚ 37.0) on 12 September 2016. They travelled ~300 km southward during the 3-day drift study. CTD profiles (CTD26 to 40) from within the EAC are plotted in comparison to other CTD profiles (in red), clearly showing the relatively warm and consistent thermal structure of this western boundary current relative to other water masses sampled during the voyage (plots made by K. Malakov, CSIRO).

2. Biogeochemical and biological process measurements

Numerous biogeochemical rate and biological process assays (e.g., picoplankton grazing) were conducted on board, involving incubation of microbial communities in either the deckboard incubators or the laboratory. Carbon, Nitrogen, and Sulphur transformations were quantified, with details of CTD and depths of water for incubations detailed in Appendix 1. Another important component of our biogeochemical investigations was the continuous underway measurements of photosynthetic electron transfer (see below).

Rates of autotrophic carbon fixation. Primary productivity assays were undertaken using both 13C and 14C. Large volume (4L) whole community 13C net primary productivity assays were conducted in deckboard incubators for 24 h (joint incubations with 15N-nitrate, 15N-ammonium and 15N-urea; see below).

Additional small volume 14C gross primary productivity assays were conducted in the isotope laboratory (see further detail below regarding conversion of high resolution measurements of primary productivity in units of electron transfer rates to carbon uptake rates).

Rates of bacterial production. These measurements were undertaken at 40 of 55 CTD stations, at multiple depths (surface, chlorophyll a maximum, and below chlorophyll a maximum).

Rates of Nitrogen fixation and Nitrogen uptake. Biogeochemical transformations of Nitrogen were investigated at 8 stations representing coastal, EAC and Tasman Sea water masses.

CTD #	Date	Water mass type
8	02.09.16	coastal
13	06.09.16	coastal
21	08.09.16	Tasman sea green
26	13.0916	EAC
34	15.09.16	EAC
41	17.09.16	Tasman sea green
47	19.09.16	EAC
55	21.09.16	Tasman sea north

N fixation assays were coordinated with N-uptake assays so that rates of Nitrogen fixation by diazotrophs can be compared with rates of dissolved nitrate, dissolved ammonium and dissolved urea uptake by the whole microbial community. We have some preliminary data from a previous voyage (IN2015_V03) which shows that N-fixation is a source of N to upper ocean microbes of potentially the same magnitude as upwelling (vertical nutrient diffusion). This voyage will allow us to examine whether the demand for dissolved N in surface waters can be met by N-fixation.

For N-uptake assays, microbes were captured on 0.3, 2.0 and 10.0 µm filters to compare accumulation of N between different size classes. N fixation rates were measured in two size classes: whole community and < 10 µm. Uptake will also be compared across N sources (inorganic nitrate, ammonium, or organic urea), to examine preferences for new and regenerated N amongst the microbial community.

Rates of Sulphur transformation. Sulphur transformation in response to biochemical and physical changes along the Australian east coast was determined by measuring dimethylsulphide (DMS) and dimethylsulphoniopropionate (DMSP) (total and dissolved) concentrations by gas chromatography across three depths and along four latitudinal transects (CTD 14-22; CTD 2-5; CTD 42-46; CTD 49-53 from south to north) and the drift study (CTD 27-40).

Measurements were made using a Gas Chromatograph (GC) (Shimadzu 2010 Plus model) with Flame Photometric Detector (FPD). The GC-FPD was calibrated at the beginning of each transect (e.g. Figure 3), and air blanks and DMSP standards were run every ~ten samples.

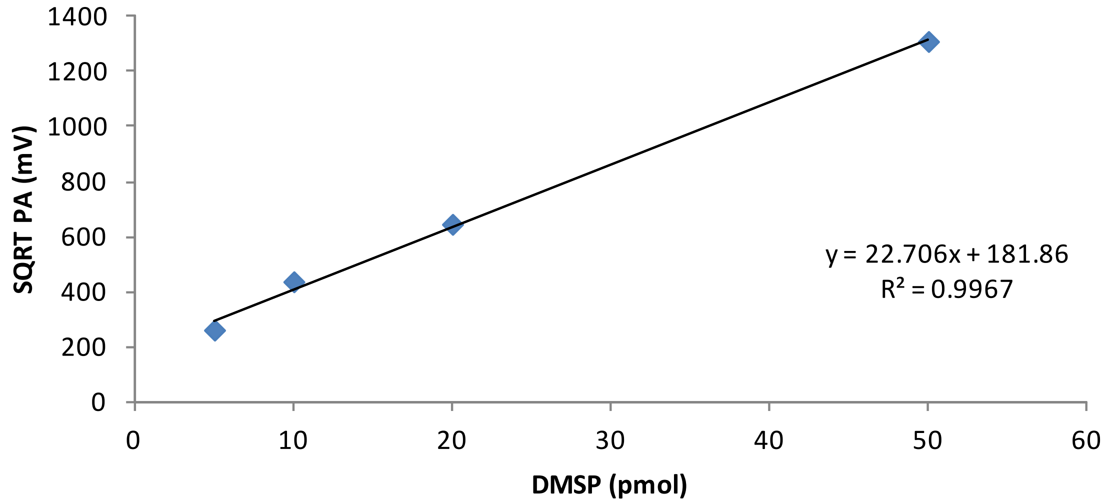


Figure 3. Example of a five-point calibration curve conducted on the Shimadzu GC-FPD using a concentration range of DMSP standards that were converted to DMS by alkaline treatment. DMSP quantities in pmol were plotted against the SQRT of the peak area (PA) obtained by peak integration.

In each measurement, DMS and DMSP were the dominant, if not the only, sulphur species present in seawater. They were consistently found at greater concentrations in surface water than in deeper water (Figure 4).

In addition, the sulphur composition of pore water from two cores (100 and 1500 m) was determined across twelve and nine depth intervals, respectively. Other sulphur compounds were detected in pore water, although formal identification of these sulphur compounds will be conducted at a later date, based on their relative retention time to DMS. When time allowed, DMS and DMSP were also measured from the underway seawater source.

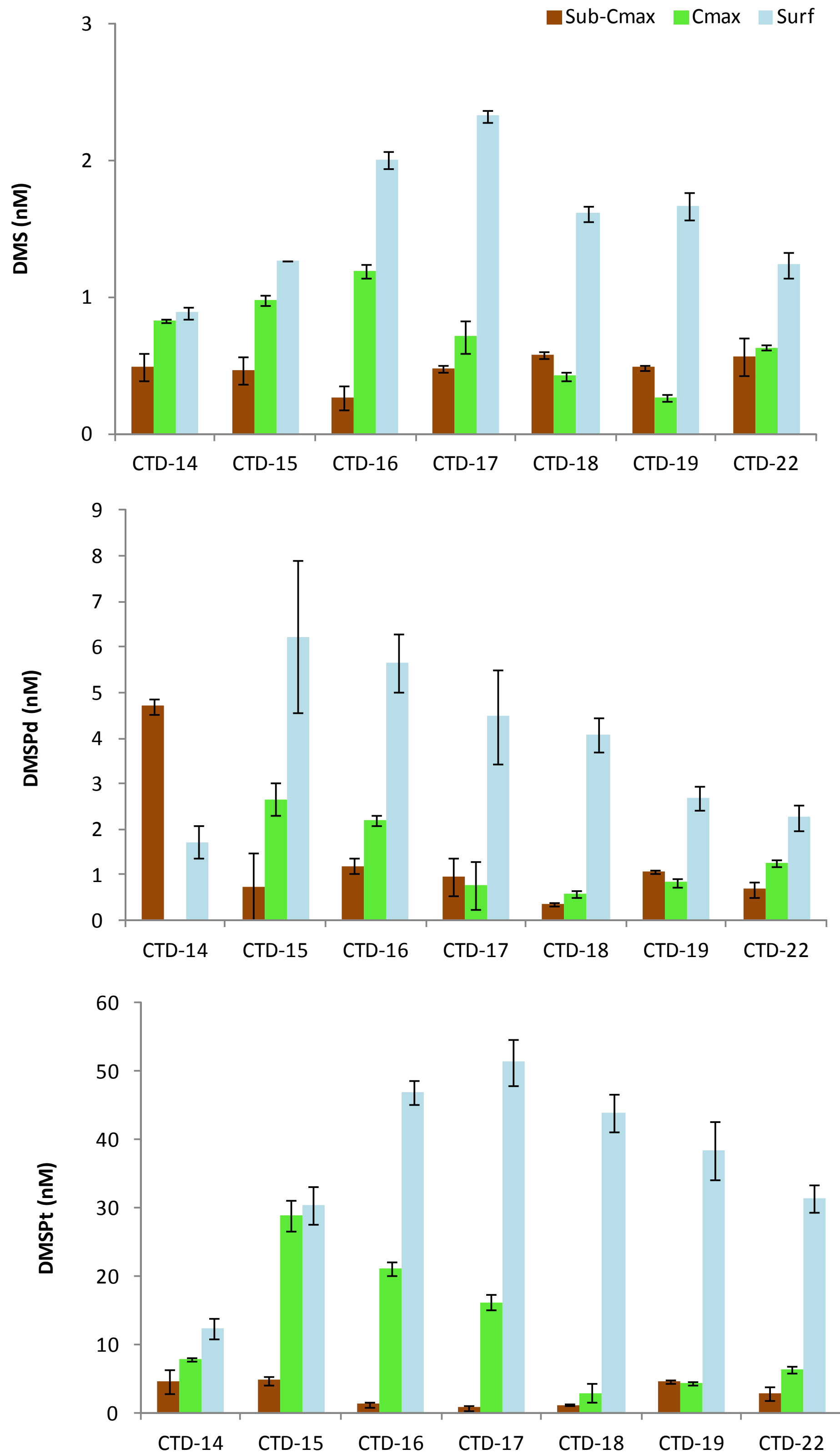


Figure 4. Example of the data obtained by Gas Chromatography for DMS, total DMSP ($DMSP_t$) and dissolved DMSP ($DMSP_d$) across three depths: below chlorophyll maximum (Sub-Cmax), Chlorophyll maximum (C-max) and surface (Surf) seawater along the most southerly transect of IN2016-V04.

Multiple factor analyses including temperature, fluorescence, dO_2, salinity, light intensity and nutrient concentrations will be conducted to determine which of these biochemical factors are driving the sulphur transformation.

Biological processes. The biological processes quantified during the voyage included viral lysis (12 experiments) and grazing of picoplankton (4 experiments).

Dilution experiments to quantify viral lysis were carried out according to Landry (Landry et al. 1995) and Warden (Warden et al. 2003). Filtered seawater (0.2 um) was added to different concentrations of unfiltered surface seawater in order to decrease the probability of encounter between predators and their prey. In parallel, another dilution series was performed using 30kDa filtered water and unfiltered water, in order to identify the rates of viral lysis. Samples for flow cytometry analyses were collected at time zero and after 24 h, and cryopreserved for subsequent analysis.

Cryopreserved samples will be counted using flow cytometry to enumerate bacteria, cyanobacteria, photosynthetic eukaryotes (and viruses) in each replicate. In situ growth, grazing and virus killing rates will be inferred from regression analysis of each dilution series.

For grazing studies, local isolated cultures of picocyanobacteria were labelled with ^{13}C sodium bicarbonate and then inoculated in seawater at the same ratio of natural populations, previously counted with flow cytometry. Samples were incubated 48 hours, in 4L bottles, in the deckboard incubators and then filtered through a 0.45 µm PES filter to recover the DNA. Filter were then frozen in liquid N2 and stored at -80 ˚C.

DNA will be extracted from cryopreserved samples and resolved on caesium-chloride gradient to identify the isotopically enriched DNA. Heavy 18S and 16S regions will be amplified and sequenced to understand which organisms are actively grazing on cyanobacteria.

High spatial resolution phytoplankton physiology and productivity mapping. In order to improve satellite determinations of phytoplankton productivity (currently based on spatially and temporally sparse in situ measurements), state-of-the-art multispectral Fast Repetition Rate fluorometers (FRRfs) were used to continuously (1 data point min^{-1}) measure phytoplankton physiological parameters to enable new high-resolution productivity estimates (specifically of "electron transport rates"). A key step towards this goal was generation of a novel algorithm to correct for background instrument fluorescence contamination that otherwise biases the physiological parameter retrieval (Figure 5); this exercise has never before been undertaken for marine underway FRRf data; approximately 70 samples were collected throughout the voyage track (to cover the broad range of phytoplankton biomass encountered).

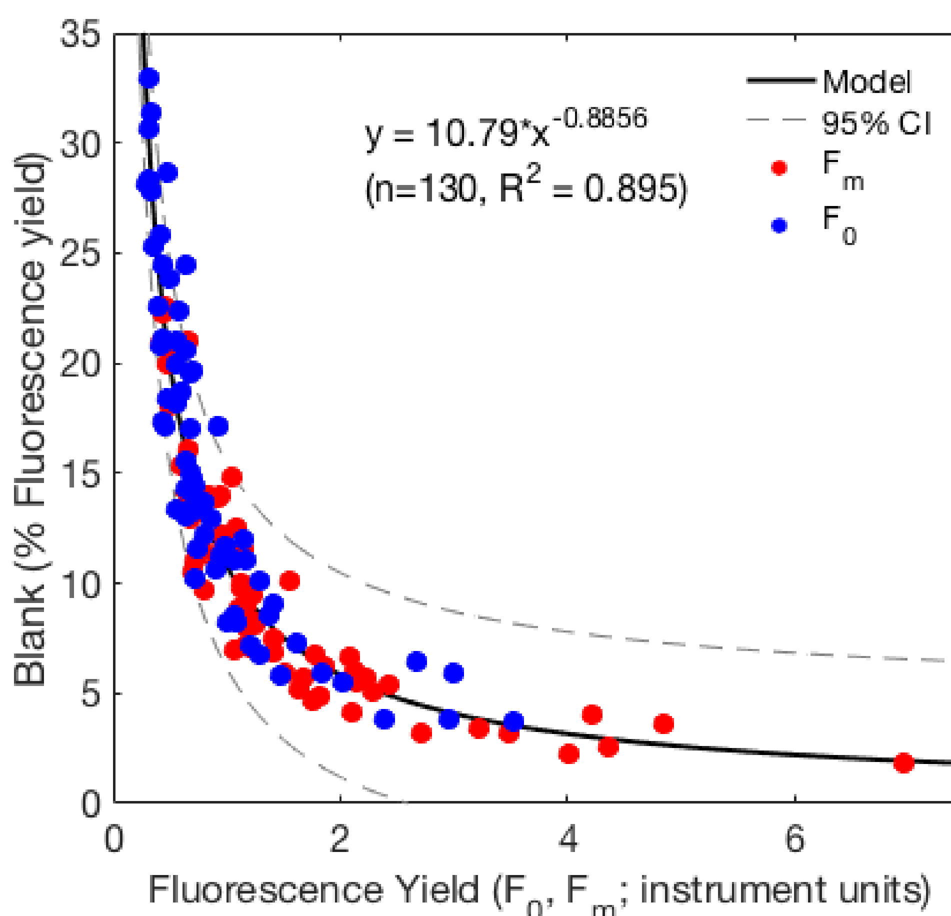


Figure 5: Background fluorescence correction for underway measurements of electron transport rates. Scatter plot of % contribution of background fluorescence (non-biological) to FRRf minimum (F_0) and maximum (F_m) fluorescence yields (discrete samples collected during daily instrument cleaning and maintenance). Power function model applied to this trend explains nearly 90% of the data retrieved, and thus a robust algorithm that can then be applied to all along track data (see next figure for example of application).

This algorithm will be applied to the continuous underway data (see example in Figure 6 which shows the different trends with and without application of the background fluorescence correction).

Continuous underway FRRf data was collected in this way for three different colour combinations: “blue” (450nm), blue-green” (450nm+530nm) and “blue-orange” (450nm+624nm). These different combinations will enable quasi-discrimination of different pigment groups (chromophytes, chlorophtes and cyanobacteria) and their contribution to physiological signatures (and biomass changes) along track. Using this approach, we generated approximately 90,000 underway FRRf data acquisitions (of which half can be used for daytime productivity analysis and the other half for night time ‘relaxed’ photophysiological trends in relation to hydrochemistry and phytoplankton taxonomic structure).

A range of additional data was collected (~ 70 samples) from discrete surface water samples to support the high-resolution productivity exercise:

a) *Steady state electron transport-light response curves*. Along-track physiological data (Figure 4) is applied to algorithms that retrieve the rate of light absorption (σ), the light saturation intensity (E_K) and therefore the maximum electron transfer rate (ETR^{max}); in order to verify the accuracy and precision of retrieval of these parameters, parallel incubations were made to directly measure the 'photosynthesis light response' to yield these parameters in more detail/resolution. These light curves will enable correction for along-track parameterisation (e.g. as a result of prolonged dark acclimation in the underway system) where necessary.

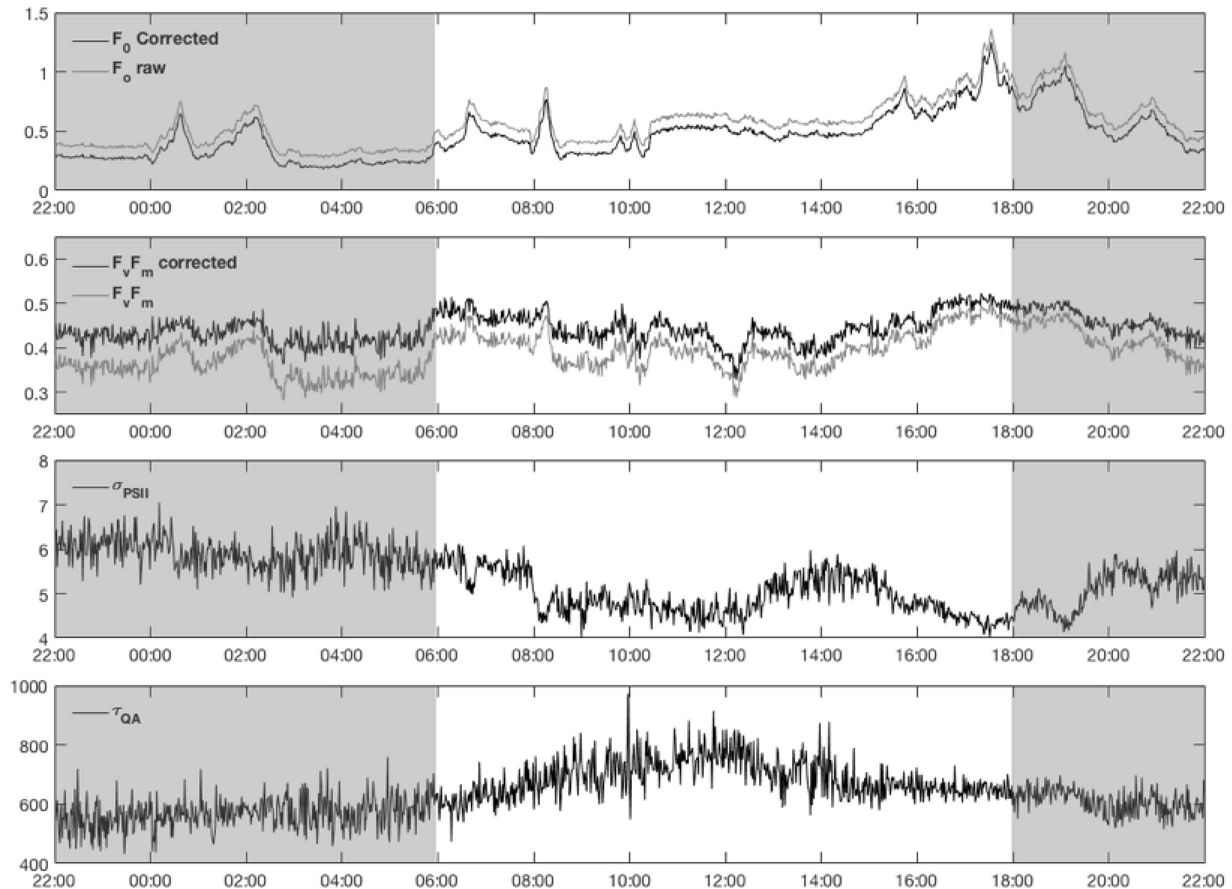


Figure 6. Example of underway data delivered by FRRf FastOcean (450nm LED only) continuously throughout the voyage, for 22:00 17th Sept to 22:00 18th Sept (grey shaded areas indicate approximate night). The minimum fluorescence yield (F_0, instrument units) serves as a proxy for chlorophyll biomass. Whilst distinct fluorescence quenching is apparent during the day (general reduction in PSII photochemical efficiency (F_v/F_m, dimensionless), σ_{PSII}, nm^2) and increase in PSII electron turnover time (τ_{QA}, μs)), patchiness can be seen relating to differences in phytoplankton taxa, physiological state and/or environmental condition. Note also the application of the correction factor to F_0 and F_v/F_m; in the latter case, F_v/F_m appears lower in especially low biomass regions giving the incorrect) perception of 'stress'.

b) *Inter-comparison of ETR and C-uptake rates*. In order to convert high resolution measurements of productivity in units of electron transfer rates to carbon uptake rates, "dual incubations" were conducted; here, time integrated 14C-uptake rates were performed within the optical head of an FRRf also measuring electron transfer rates. Such data has not been previously collected in Eastern Australian waters and the high throughput approach employed here will be used to develop an additional algorithm to predict the conversion of electrons to carbon uptake (ETR:C) from physico-chemical parameters (temperature, nutrients, chlorophyll content), to enable future broader scale implementation of FRRf technology to monitor productivity.

An additional novel aspect of this work is to determine the extent to which (ETR:C) can be predicted as a function of phytoplankton community structure (determined by parallel samples for flow cytometry and digital imaging) and to a first order 'master trait' of cell size. In the latter case, a key focus was size fractionated chlorophyll measurements collected in parallel. Figure 7 shows the spread of data collected throughout the voyage, whereby the majority of waters were dominated by cells 2-10 µm, but with ~ 20% of all samples dominated by >10 µm cells and 10% samples dominated by <2 µm cells.

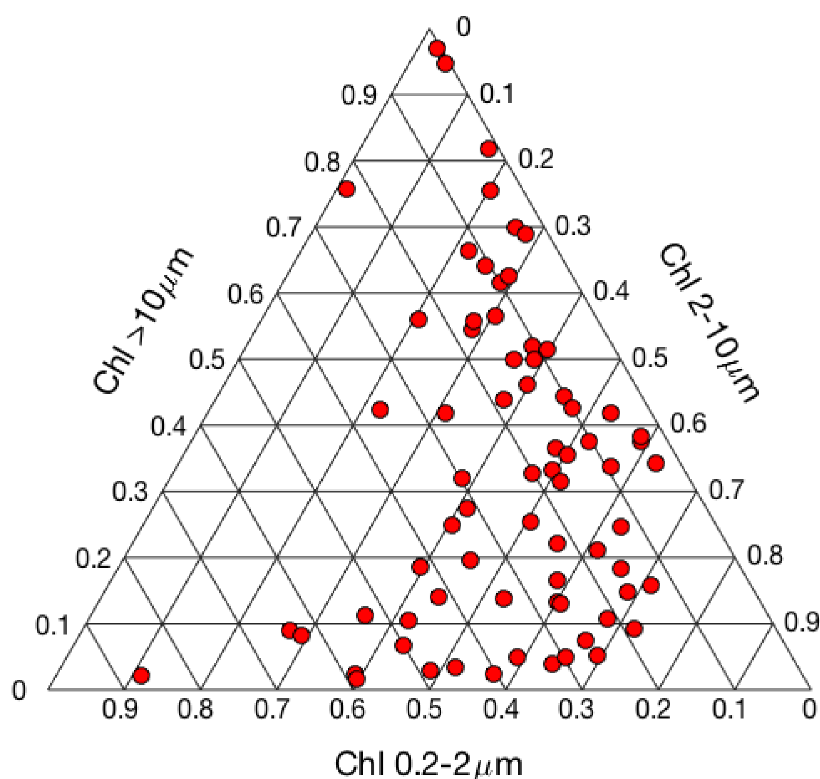


Figure 7. Ternary plot of proportion total chlorophyll a separated into three size classes, <2 µm, 2-10 µm and >10 µm cells (n=71). Data were predominantly from surface waters where upper layers well mixed (but ~ 15 data points from subsurface chlorophyll maxima are included) throughout the voyage until 17th September. Additional samples (~ 10) were sampled in highly oligotrophic waters 18-19th September (and expected to be predominantly <2mm size class) but extracted chlorophyll samples were not examined at the time of plot preparation.

Together this unique data set will next be used in several ways to reach the objective of mapping high-resolution productivity: (i) ETR^{max} (CO_2^{max}) E_K and will be examined (binned) relative to physico-chemical conditions (predominantly SST) to (re)-evaluate existing regional models of productivity based on environmental dependency of photosynthesis-light relationship parameters (and re-evaluate existing paradigms as to the scale of variance of productivity terms; and (ii) Underway PAR data (corrected for surface reflection and attenuation) is applied to the continuous records of the light-response parameterisation for retrieval of instantaneous electron transfer rates (which is then converted to carbon); these data can then be used to evaluate broad (regional scale) measures of ocean colour and better parameterise ocean-colour products that relate to productivity (where the FastOcean collects absorbance and production data at wavelengths that are also remotely sensed).

Perturbation experiments. In addition to biogeochemical rate estimates, we also conducted four perturbation experiments with microbial communities collected from coastal water, the Tasman Sea (south and north) and the EAC. We incubated water collected at two depths (surface and at the bottom of the mixed layer) to understand whether phototrophic diazotrophs have different responses compared to heterotrophic diazotrophs.

Due to the iron (Fe) requirement of diazotrophs and other key microbial groups (e.g., diatoms), water was collected using the trace metal rosette (TMR) and bottles were only opened in a HEPA filtered laminar flow cabinet, with samples being withdrawn using acid-washed pipette tips.

The experimental design (Figure 8) involved keeping control bottles at ambient temperature and light (i.e., same conditions as in situ), and heating the remaining bottles (+3 °C above ambient) with three levels of nutrient amendment: no addition, 0.5 nM Fe (III) added daily, and 0.5 nM Fe (III) plus 1.0 µM nitrate, 0.1 µM phosphate and 0.08 µM silicate added daily. Nitrate:phosphate ratios were designed to match ratios observed in east coast waters > 100 m deep (~10). Our rationale for the 12.5 ratio of nitrate to silicate was two-fold: to moderate diatom growth in our incubations, and to simulate N:Si ratios in f/2 medium.

All bottles were sampled daily (T0, T1, T2, T3) for dissolved nutrient concentration, flow cytometric enumeration of phototrophs (Prochloroccus, Synechococcus, picoeukaryotes), as well as bacteria and viruses, and assessment of the physiological status of phototrophs using a Fast Repetition Rate Fluorometer. Control bottles were sampled daily to estimate Dissolved Inorganic Carbon (DIC), and samples for DIC, size fractionated Chl-a and RNA (0.2 and 5.0 µm) were collected from all bottles at T3.

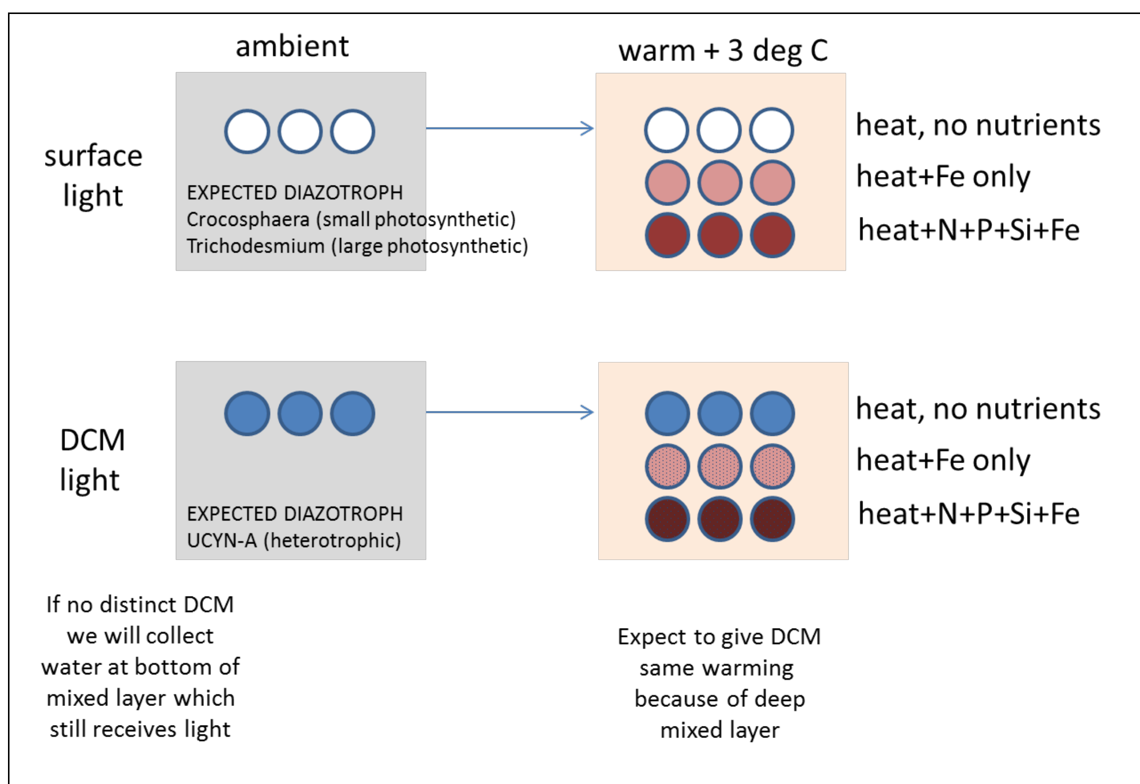


Figure 8. Diazotrophs are expected to increase in abundance in a warmer ocean, provided they have enough iron (Fe). This experimental design tests whether short-term warming over 3 days increases the abundance of diazotrophs in the microbial community. Different forms of nutrient enrichment (none, with added Fe(III) and with added Fe(III) plus nitrate, phosphate and silicate) are designed to determine the most favourable nutrient conditions for maximum diazotroph abundance.

3. Trophic links

We sought to understand trophic linkages by coordinating microbial sampling with observations and samples of higher trophic levels. Zooplankton and larval fish were targeted with net sampling, Laser Optical Plankton Counter (LOPC) measurements on the Triaxus and the ship's EK60 multibeam echosounder (particularly 38 kHz that targets fish swim bladders and 70 kHz that targets gelatinous zooplankton). Visual and photographic observations were made of megafauna such as seals, dolphins, whales and seabirds.

In addition to understanding the impact of mesoscale and frontal eddies on the development of larval fish, the southern NSW region near Montague Island became a focal point for trophic investigations, not only because it is an area of high biological significance (seal colony, penguin breeding ground), but also because an emerging research direction is to understand how the biological oceanography in this region influences the marine foodweb (see Carroll et al. 2016 Scientific Reports. 6:22236; doi:10.1038/srep22236).

Description of **zooplankton and larval fish** sampling gear and mesh:

- EZ net (MOCNESS) 1 m^2 mouth area, and 500 µm mesh, with Seabird37; transmissometer, PAR, 2 inside and outside digital flow meters, (and 61 cm bongo nets as back-up, which were not used); initially towed at 3 knots, changed by 3 September to 2.5 knots.
- 20 cm diameter, 100 µm mesh nets inside Net1, Net2, and later Net3 of the EZ.
- Neuston net (an EZ net on a frame with depressor and mechanical flowmeter
- A vertical haul from 50 m for most EZ nets with an “N70” net – 70 cm diameter (area=0.385 m^2) with 0.2, 0.4 and 4 mm mesh. Over 50 m depth samples 19.2 m^3 during the up-cast at 1 m/s; as used before on 2008, 2009, 2010, 2014, 2015 voyages.

The foodweb team had three main goals:

1) To assess the growth and mortality of larval fish between the shelf and coastal waters to test the novel hypothesis that the offshore waters including frontal eddies of the EAC are a nursery ground for commercial fish;
2) To quantify the diel vertical migration and “carbon pump” of zooplankton down to 500 m depth, in northern and southern waters of the western Tasman Sea; and
3) To opportunistically sample larval lobster (phyllosoma).

These goals will complement the sampling planned for September 2017 (IN2017_v04).

Nearly 25 shallow EZ net tows (to 50 m deep to achieve goal 1) and 50 neuston tows were completed, finding many larval sardine (*Sardinops sagax*), mackerel (*Scomber australasicus*) and scad (*Trachurus novaezelandiae*). Approximately a third of these were sorted at sea due to the stability of the vessel. Another 10 deep EZ net tows were collected to meet goal 2. For goal 3, over 20 larval lobster were obtained and stored in cold alcohol for genetic identification and genetic analysis of their gut contents.

A surprising finding was the abundance of green slime in the nets (tentatively identified as *Thallasiosira* sp.) at 36 °S off Montague Island (Figure 9). This was an extensive bloom that stretched from coastal inshore waters to 200 nm eastward in the Tasman Sea and completely dominated the plankton (zooplankton and larval fish abundance was very low). Given the virtual absence of any other planktonic organisms in these waters, we speculate that the efficiency of carbon transfer to higher trophic levels in the bloom was very low - carbon appeared to be held in the cells themselves, and their surrounding mucilage (extracellular polysaccharide), effectively ‘trapping’ carbon at the base of the foodweb.

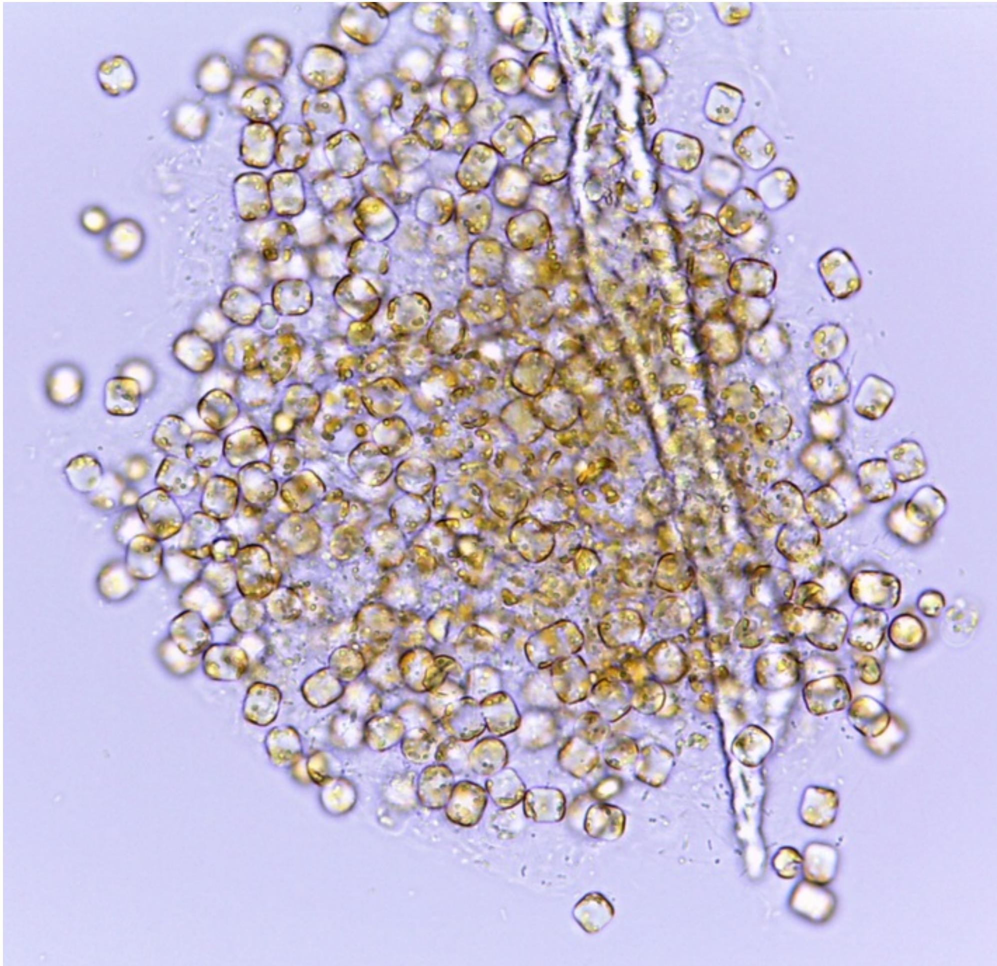


Figure 9. The 'green slime' alga, nominally identified as Thalassiosia sp. that formed an extensive bloom in southern NSW during the voyage. Molecular identification is underway to confirm its identity. Photo: Justin Ashworth.

In comparison, there was relatively high abundance of zooplankton and larval fish in the Sydney eddy, with some green slime; compared to the low abundance of phytoplankton and zooplankton off the Gold Coast at 28 °S. The western Tasman front (Mullaney & Suthers 2011) off Sydney is indeed a productive nursey area, downstream of the EAC separation zone.

The Triaxus enabled collection of zooplankton abundance, biomass and size spectra data (alongside CTD, fluorescence, oxygen) across different water masses. A total of 11 deployments (with the LOPC) were conducted and provided excellent coverage from the surface to ~200 m. Plots of the first deployment across the Sydney eddy are provided in Figure 10.

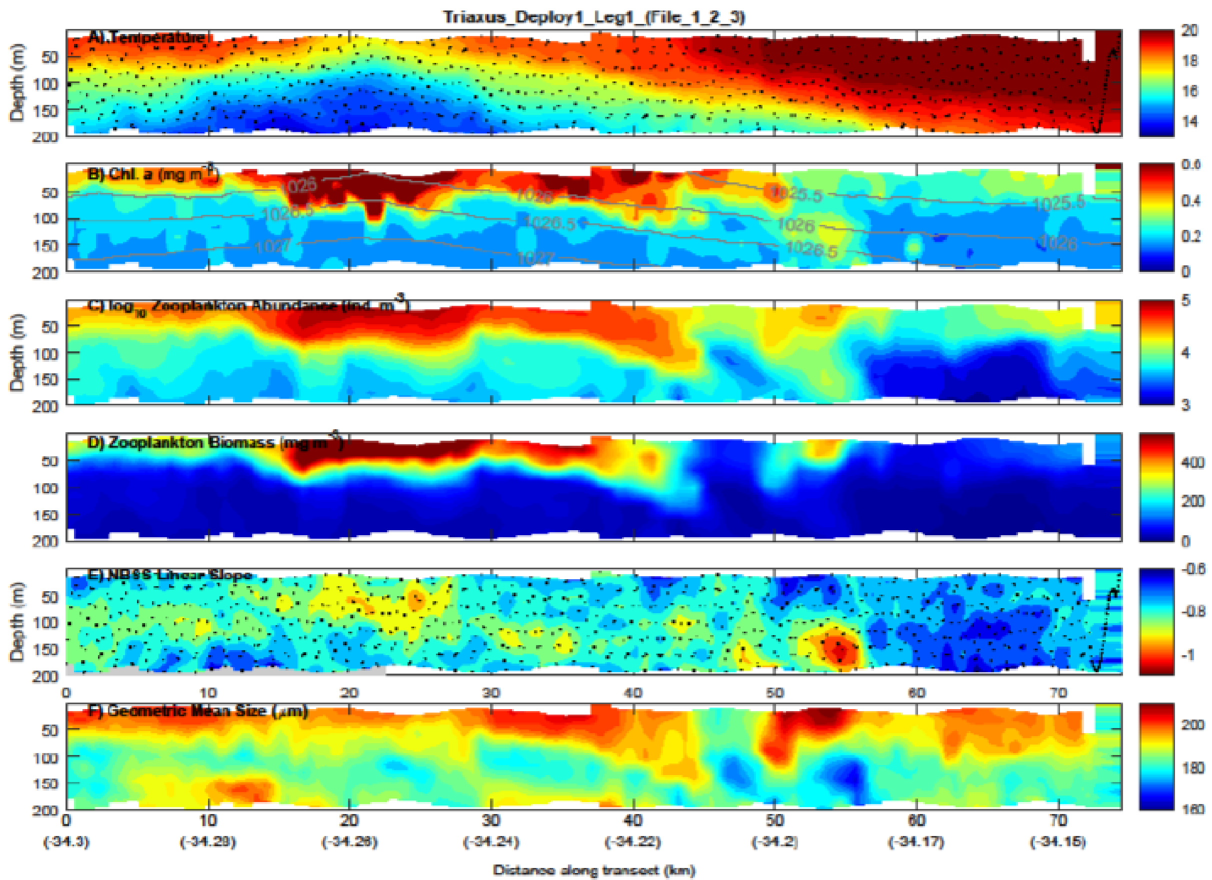


Figure 10. Enhanced plankton biomass within a cyclonic (cold-core) eddy. Gradients of temperature, chlorophyll a (proxy for phytoplankton biomass), zooplankton abundance, zooplankton biomass, slope of the normalised biomass size spectrum (NBSS) and the mean size of zooplankton individuals along a transect spanning the Sydney eddy (left hand side) to the EAC (right hand side). Plots: Zoe White (UNSW).

The ship's echosounder was also used to examine fish and zooplankton distributions. Interestingly, we observed a gradient of fish abundance in the EK60 data north and south of Montague Island that provides a useful starting point for examining fish abundance across other spatial or oceanographic gradients (Figure 11).

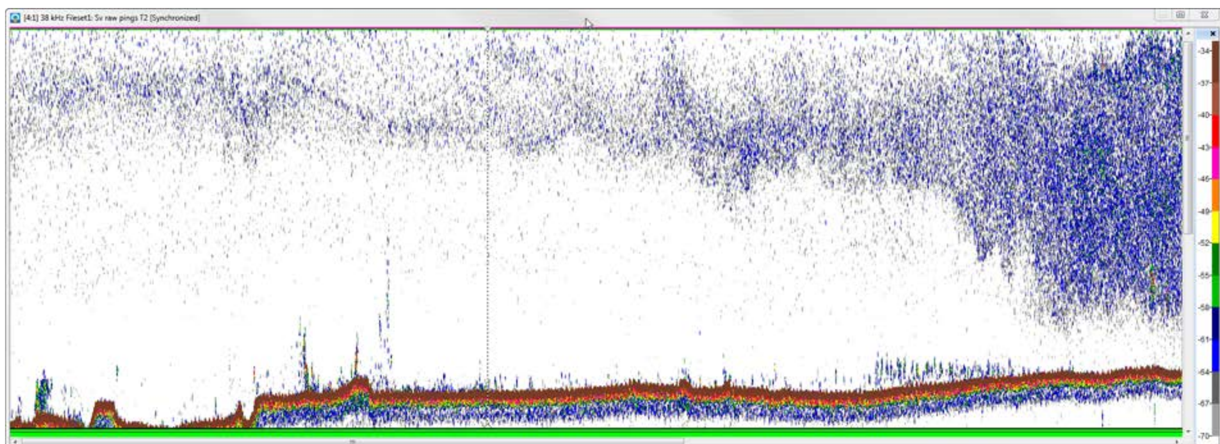


Figure 11. Fish abundance (derived from 38 kHz signal from EK60 echosounder) along a transect spanning waters south (left hand side) to north of Montague Island.

Cetacean, seal, bird observations in relation to the biological oceanography.

Description of observation gear used on the Observation Deck.

- Binoculars
- SLR Camera with 150/600mm lens
- Rangefinder to estimate distance
- Protractor to measure angle of observation
- GPS to mark way points

Our aims were to use the data obtained from our visual surveys of marine mammals and seabirds to produce spatial distribution models using the oceanographic variables obtained from the underway system. Our survey effort spanned a total of 183 hours over 22 days. We identified 2 whale and 4 dolphin species with approximately 524 individuals.

The most common species observed was the humpback whale (Figure 12), due to their southward migration following the conclusion of their breeding season. Almost all observations were taken while inshore where the water depth was less than 200 m. Very few cetaceans were sighted in much deeper waters (> 1000 m) which was surprising. We noticed that humpback whales were very active at dawn as the sun appeared above the horizon. There was a lot of breaching/ aerial displays. The 12th September was the busiest day as we transited from Sydney up to Seal Rocks on the way up to Coffs Harbour. We had a total of 38 sightings throughout the day. Maps of whale and dolphin sightings are found in Appendix 5.

Figure 12. Charismatic megafauna. Humpback whale and common dolphins observed during the voyage. Images: Ricardo Alvarez Pacheco.

4. Sediment sampling

The KC Denmark multi-corer model 70.000 6 x ø100mm (Figure 13) was deployed at a total of six stations between 4 to 19 September. Samples were collected at three of the six stations with 6 out of 9 successful deployments (water depths between 150 m and 4500 m). A 66% success rate for multicore deployments is at least on par, or slightly higher, than PI Abbott’s past multi-coring voyages. A total of 32 cores were sampled (between sites at 150 m, 1500 m, and 2600 m) with 4 disturbed cores discarded.

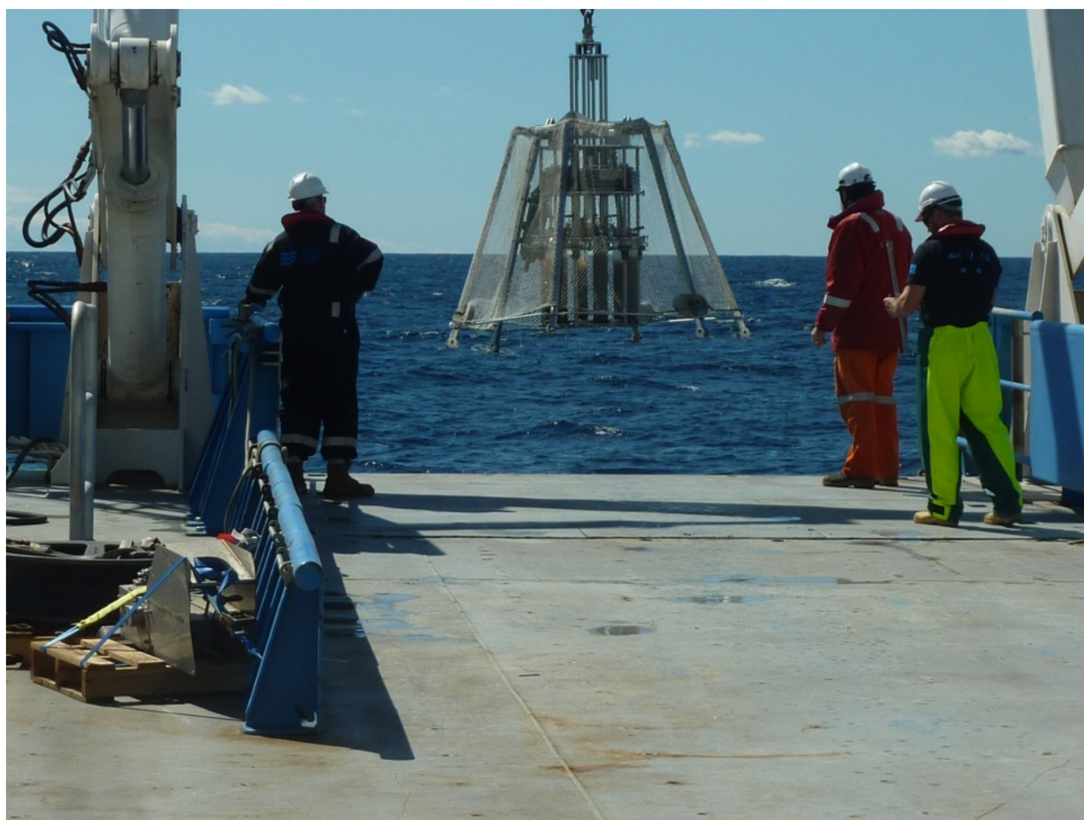


Figure 13. Multicorer being brought back on deck after a successful sediment retrieval from Port Hacking.

The first station was off Port Hacking (-34.12821 °S, 151.23619 °E), specifically prioritized as a site for a historical record of phytoplankton. Two successful deployments resulted in 11 cores for analyses, with 1 core discarded due to poor recovery. These were morning deployments with few clouds and calm seas. Three cores were taken in aluminium tubes, capped with aluminium foil, and remained intact for transport back to Sydney (Gustaaf Hallegraeff, Penelope Ajani, Martina Doblin). Of the remaining 8 cores in polycarbonate tubes, 1 was sectioned with samples split between collaborators Shauna Murray, Leanne Armand, Martin Ostrowski, and Simon George for microbial, DNA, biomarker, and plankton studies. The other 7 were sectioned in anoxic conditions (N_2 environment) into 85 mL centrifuge tube for pore fluid collection. The sediment for 6 of these cores was bagged and frozen, the 7th was allocated to Shauna Murray. The cores from this site were largely bioturbated, with burrowing shrimp found to core bottom (~35 cm). The sediment seemed largely bimodal, split between clay and sand sized particles. H_2S was apparent starting from a depth of between 12 and 15 cm. Pore fluid was sampled for 24 intervals. Aliquots of pore fluid were provided to Liz Desacheux and Joey Crosswell for on-board DMS and DIC analysis, respectively.

The second attempt was made the evening of 8 September in 4500 meters of water off Narooma (36°55.9995 S, 153°55.774 E). No samples were collected as the corer misfired and time limitations prevented a second deployment. An attempt at this depth takes about 3 hours on the winch with a deployment speed of 60 m min^{-1}. The corer appeared to have landed on the bottom but evidently had already been triggered in the water column. The false trigger may have been the result of the winch stopping ~50 m before the corer hit the bottom. The false trigger was only 2-3cm, as it was only a partial trigger (5 of 6 gates triggered and the niskin bottle didn't fire).

In an attempt to prevent future false triggers, the bottom gate trip wires were extended by 10 cm. The trade off being that short cores (less than 10 cm) were not possible, but since the target was >20 cm it was considered more important to prevent early trips. An additional 113 kg of weight was added to the legs of the coring frame to minimize jostling in the water column.

After these alterations, our third attempt resulted in two successful recoveries in 1500 m of water off Jervis Bay (34°49.760 S, 151°20.057 E) despite a night deployment in 20 knot winds. We were able to section 11 of the 12 cores for pore fluid, the twelfth was too disturbed and had to be discarded. Life was apparent at the surface of most cores (potentially hydroids). Overall, the sediment at this site was fine silt and clay with pockets of sand sized grains. Very challenging material to cut through for sectioning, and the sandy pockets were generally wetter than the fine grain matrix. There was no detectable H_2S presence. Sediment from 1 core (post-centrifuge and pore fluid extraction) was sub-sampled for Shauna Murray. All other centrifuged sediments and core bottoms (below last pore fluid interval) were stored in zip lock bags and frozen. Pore fluid was sampled for 18 intervals. Aliquots of pore fluid were provided to Liz Desacheux and Joey Crosswell for on-board DMS and DIC analysis, respectively.

The fourth site resulted in no cores. This site was a 1500 m attempt off Port Macquarie (-31.56122 S, 153.37365 E) on the morning of 14 September in moderate conditions. The corer appeared to operate correctly, and the niskin bottle indicated the corer did in fact trigger at the bottom (based on dissolved oxygen, temperature, salinity, and DIC). The failure to collect cores was likely due to a hard substrate at that location.

Later that afternoon, we successfully collected 10 cores from 2 deployments at 2600 m off Crowdy Bay National Park (-31°95568 S, 153°30344 E) towards the edge of a submarine canyon. Deployment occurred in strong (30+ knot) winds. We lost 2 cores total (slipped before recovery), one from each deployment, but 10 cores were in good condition. The second deployment broke a spring on the gate closure for core 8 which had to be repaired before our 6th deployment. The sediment here was similar to the Jervis Bay site, but largely lacked the sand-sized patches. As at Jervis Bay, hydroids were intact on the surface of the core. Pore fluid was sampled for 18 intervals. Aliquots of pore fluid were provided to Joey Crosswell for on-board DIC analysis. Again, no H_2S was detected. Sediment below pore fluid intervals was subsampled for collaborators Leanne Armand, Martin Ostrowski, and Simon George for microbial, DNA, biomarker, and plankton studies from two cores. All centrifuged sediments and core bottoms for the 8 other cores were stored in zip lock bags and frozen.

On 19 September we did a single deployment in 4600 m off Surfer's Paradise (-28.15491 S, 154.88172 E). The corer false triggered about half way down; niskin bottle sample indicated the multi-corer fired around 2200 m based on water temperature and DIC. Winds had calmed to 15 knots before deployment and no obvious disturbances were observed that would account for the early trigger. Time limitations prevented a second attempt. Mud on the corer frame confirmed it did land in soft sediment, but had already triggered.

In summary, sediment cores were successfully retrieved from three sites.

Successful Sites

- Port Hacking
  - 4th September 2016
  - 11:00 am back-to-back deployments
  - **150 m**
  - -34.12821 S, 151.23619 E
  - 3 Aluminium tubes to bring back (Penny, Martina, Gustaaf)
  - 7 cores processed for pore fluid
  - 1 core sectioned (Malwenn, Leanne, Simon)
  - 1 core disturbed, dumped
  - calm, sunny

- Jervis Bay*
  - 11th September 2016*
  - **1553 m**
  - 2 deployments, 1st recovery 23:57
  - 11 cores processed for pore fluid
  - 34°49.760 S, 151°20.057 E
  - 1 core disturbed, dumped
  - 20 knot winds, dolphins

- Crowdy Bay*
  - 14th September 2016*
  - **2662 m**
  - -31°95568 S, 153°30344 E
  - 10 cores processed for pore fluid
  - 2 core disturbed, dumped
  - high winds (35 knots), rainy
  - have swatch image

<u>Additional Deployments</u>

- Narooma
  - 8th September 2016
  - **4500 m attempt**
  - 36°55.9995 S, 153°55.774 E
  - corer mis-fired (5 gates triggered, 1 gate remained open niskin bottle didn't fire.. had to have only gone 2-3 cm)
  - winch stopped about 50m before bottom, possible trigger

- Port Macquarie*
  - 14th September 2016
  - **1500 m attempt**
  - -31.56122, 153.37365
  - corer seemed to function properly, but no sample-potentially hard bottom

- Surfers Paradise*
  - 19th September 2016
  - **4600 m attempt**
  - -28.15491, 154.88172
  - Corer triggered on the way down, temperature and DIC from niskin bottle suggest corer fired at 2200 m; sediment stuck to core frame suggests fine reddish colored clay soft sediments

**Cables extended by 10 cm, additional weight for these deployments*

113.4 kilos added to legs

10cm to lower gate trip wires

Starting at:

full 610 kilos (corer plus 30 x 10kg top weights)

<<Investigator Aug 2016 << KCMulticorer_70.000.pdf

KC Denmark multi-corer model 70.000 6 x ø100mm

For assessment of past presence of plankton at Port Hacking, the following was undertaken:

- 1 whole core, stored at -80 °C, was brought back to the lab for further analysis of the presence of toxic phytoplankton (contact: Penelope Ajani, UTS)
- Samples of core #1 from 6 different depths (10g in triplicate), for further analysis of presence (DNA) and germination of toxic dinoflagellates (replicate samples stored at -80 °C (x 2) and 4 °C (x1)) (contact: Shauna Murray, UTS).
- Samples of core #3 (sediment remaining after centrifugation for pore water), from all depths (several grams), diluted in filtered seawater and preserved in Lugols solution (4 °C in the dark) for further characterization of the phytoplankton species present (contact: Malwenn Lassudrie, UTS).

5. Aerosol sampling

This objective aimed to quantify atmospheric wet and dry deposition of iron into the ocean. Such iron input impacts ocean health through its influence on microbial composition and activity, and is critical to include in models forecasting marine primary productivity.

An atmospheric sampling system for the collection of air particles was installed in the ship's aerosol lab on transit voyage INV2016_T02. This system (Figure 14), consists of vacuum pumps (Thomas Sheboygan 2107CD18), volume meters (DiTGM ML-2500) and filtration systems (Savillex PFA). The manifold is connected to air intake lines fed from the sampling nozzle located ~10 m above sea level on the foremast at the bow of the vessel. Samples were collected on 47mm diameter Whatman 41 cellulose filters in Savillex PFA filtration holders housed within a laminar flow hood (AirClean Systems) to avoid contamination from dust. The system is automatically activated by a wind sector and speed control switch (pump controller) to ensure 'clean' air sampling only from the forward sector (270° to 90° starboard), avoiding air impacted by the ship's exhaust. The system is capable of running up to 4 flow lines in parallel to enable sampling for replicates or different parameters. Filters were changed approximately daily, depending on the aerosol loading and amount of time the pumps were on. Samples were stored frozen and returned to the shore-based laboratory for further experiments and analyses.

Fifty-six filters were collected during INV2016_T02 and INV2016_V04 including exposure blanks of 1 min, 5 min and 24 hours.

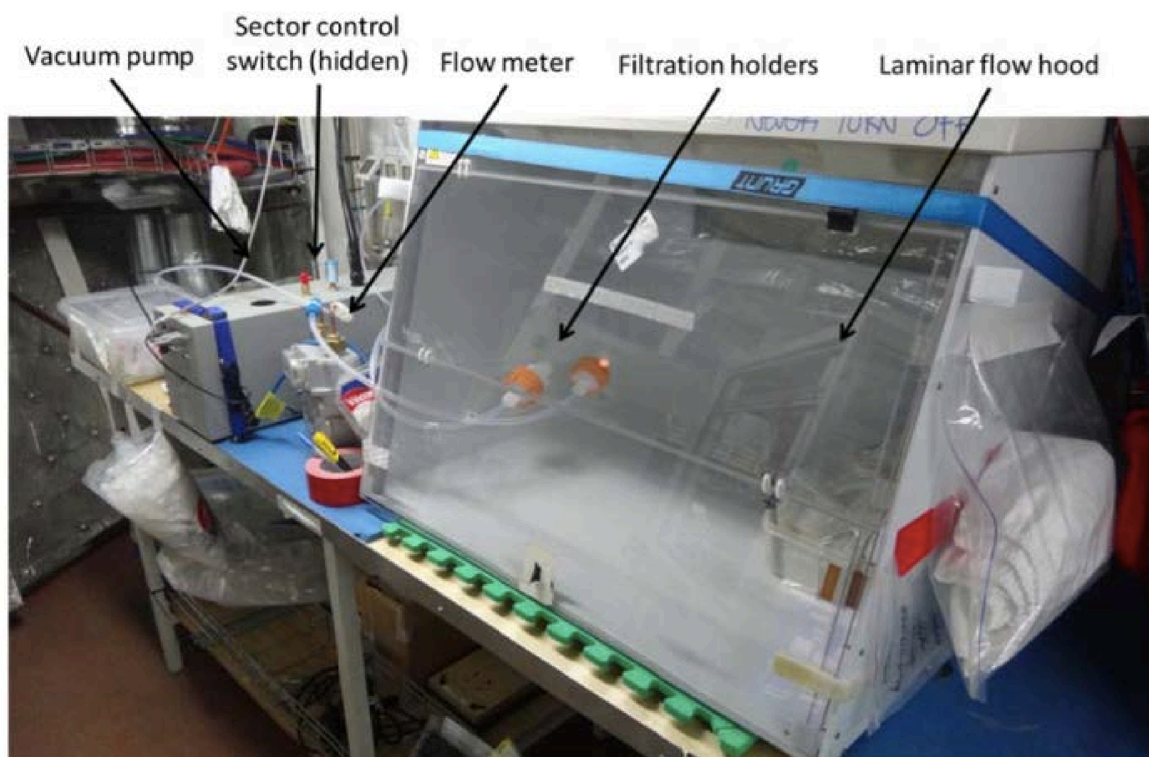


Figure 14. Aerosol sampling system installed in the ship's aerosol laboratory. The large instrument in front of the sector control switch is a nephelometer and is not part of the aerosol sampling manifold.

We also opportunistically collected rain event water samples using a Dual Chimney Precipitation Sampler (N-Con Systems model 00-127) to quantify the trace metal wet only deposition. The sampler was installed in front of the bridge, on the upper 5th deck near the hand rail (Figure 15). This location seemed to be optimal for minimising contamination from the ship's exhaust and sea spray. Two 1L LDPE bottles were placed in the cavity of the sampler which is quilted and well-sealed to keep temperature constant and to limit contamination when the lid is closed, respectively. The rain sampler is equipped with a motion sensor on its ocean side (Figure 15) that automatically opens the sampler's lid when 5 drops of water pass through it. A data logger records the lid motion in order to track the samples. Samples were processed in the trace metal container TMC02 on the back deck after collection. One rain event collection is sub-divided in 4 different samples when more than 250mL is collected in total. Two samples are directly stored in the fridge while the remaining two are filtered onto a 0.2 µm polycarbonate filter and acidified with 1 µL/L distilled HCl before being stored in the fridge. Back in the land-based laboratory, rain water samples from each storage method will be tested for determination of trace elements and major ions.

Figure 15. N-CON TM00-127 automatic rain sampler installed on the 5th deck during INV2016_V04.

Supplementing the aerosol and wet precipitation samples, were samples collected from the TMR. Samples were collected in small LDPE bottles, filtered to 0.2um with PALL accropak filtration set and acidified with 2uL/mL distilled HCl for seawater depth profile trace element analysis. Bulk seawater samples were also filtered and stored in carboys in a fridge for later experiments in the land-based laboratory.

Sea water samples collected are reported in the table below.

Table 1: Seawater samples for trace metal analyses collected using the TMR during the voyage.

Sample type	TMR #	Date/time (local)	Location	Depth (m)	Processing
Sea water	TMR 002	6/9/16 6.20	36° 15.185 150° 17.750	10 and 20	filtered + fridge
Sea water	TMR 004	8/9/16 14.04	36° 58.8241 153° 52.7743	15-25-65-120-250	filtered + acidified + fridge
Sea water	TMR 005.2	14/9/16 14.45	31° 47.7845 153° 19.0436	15-40-60-120-250-500	filtered + acidified + fridge
Sea water	TMR 005.2	14/9/16 14.45	31° 47.7845 153° 19.0436	15	filtered + fridge
Sea water	TMR 006	16/9/16 06.50	33° 00.63342 152° 54.2487	15-30-60-120	filtered + acidified + fridge
Sea water	TMR 006	16/9/16 06.50	33° 00.63342 152° 54.2487	15	filtered + fridge
Sea water	TMR 007	17/9/16 05.15	32° 28.2959S 153° 29.1615	10-60	filtered + acidified + fridge

Sample type	TMR #	Date/time (local)	Location	Depth (m)	Processing
Sea water	TMR 009	19/9/16 05.25	29° 09.2776S 154° 28.7107	10-25-40-60-100-250	filtered + acidified + fridge
Sea water	TMR 009	19/9/16 05.25	29° 09.2776S 154° 28.7107	10	filtered + fridge

Voyage Narrative

Our voyage operations proceeded as follows, with microbial sampling and megafauna operations occurring during daytime hours (nominally 0500 to 1800) and zooplankton and larval fish sampling at night (1800 to 0200). The ship's underway systems were operating continuously, but between 0200 and 0500 (where possible) were targeted towards collection of bathymetric data in areas that had not previously been surveyed.

The voyage began with sampling in what appeared to be a cyclonic eddy southeast of Sydney (Figure 16).

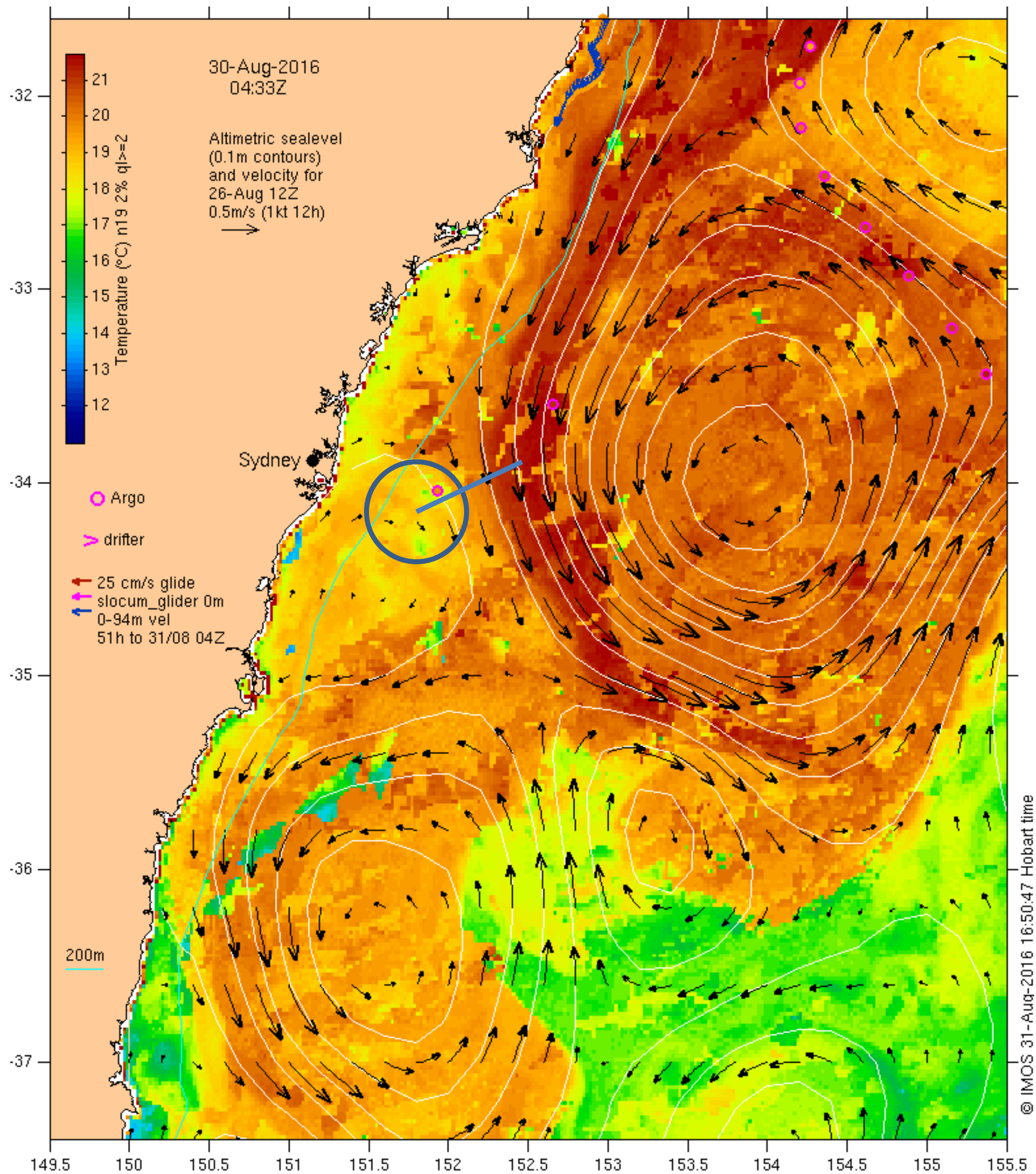


Figure 16. Satellite image of sea surface temperature at the outset of the voyage, showing a large warm core eddy east of Sydney, with a smaller cyclonic feature to the southwest (circle). The blue line indicates our initial transect.

We then transited northward towards Newcastle and Port Stephens where we further sampled frontal eddy features. From there, we travelled southward to Port Hacking, a coastal time series station where we did our first successful multicorer deployment. Next, our focus was to examine the southern NSW foodweb, conducting a preliminary north-south transect at Montague Island before undertaking a long cross-shelf transect into the Tasman Sea, through an extensive phytoplankton bloom.

We returned to the coast where we spent another day surveying the area north and south of Montague Island before transiting to Coffs Harbour for the start of the drift study.

After three days of drifting, we transited northward again, to complete another cross-shelf transect into the Tasman Sea, bisecting the East Australian Current. After another northward transit, we attempted to deploy nets in the Evans Head region but it was too windy.

The final part of our final was focussed in northern NSW where we conducted another cross-shelf transect.

A general schedule of our sampling is shown in Table 2.

Table 2. Daily schedule of activities conducted on the voyage.

Date	**Day**	**Activity**
30-Aug	Tue	Mobilisation
31-Aug	Wed	Departure from Sydney; test cast to 1000 m in eddy
1-Sep	Thu	Sydney eddy CTD transect
2-Sep	Fri	Process station Stockton Beach; EZ nets overnight
3-Sep	Sat	Port Stephens
4-Sep	Sun	Port Hacking multicorer deployment
5-Sep	Mon	Transit to Montague Is
6-Sep	Tue	CTD transect offshore
7-Sep	Wed	Triaxus across eddies and further CTDs
8-Sep	Thu	Tasman Sea process station - in the bloom; multicorer deployment
9-Sep	Fri	Deep (4000 m) CTD and transit back to coast
10-Sep	Sat	Montague Island survey; multicorer deployment
11-Sep	Sun	Re-sample Sydney eddy
12-Sep	Mon	overnight multicorer deployment; transit to Coffs Harbour
13-Sep	Tue	Start of drift study
14-Sep	Wed	drift
15-Sep	Thurs	drift
16-Sep	Fri	End of drift study; transit to Sugarloaf Point; Triaxus transect offshore
17-Sep	Sat	CTD transect offshore to inshore through the EAC
18-Sep	Sun	Transit to Evans Head
19-Sep	Mon	Process station in oligotrophic water; transit to Surfers Paradise 4000 m site
20-Sep	Tue	Surfers Paradise CTD transect
21-Sep	Wed	Deep CTD cast and process station; Triaxus transect back to coast
22-Sep	Thurs	Arrival in Brisbane
23-Sep	Fri	Demobilisation

Summary

This voyage met all sampling goals. The drift study, encompassing three diel cycles, was the first of its kind. As such, this drift study will form the centrepiece of a high-impact manuscript from the PIs once microbial analyses are complete. Only minor setbacks in sampling were experienced, including a few net deployments that could not be completed due to bad weather (high winds) and three failed multi-corer deployments. Of the corer deployments that did not retrieve sediment samples, one of the three was possibly due to the absence of soft sediment rather than a triggering issue. The large volume pore water sampling from the successful multi-corer deployments for neodymium isotopic analysis is the first of its kind in the southern hemisphere.

On board experiments (i.e. biogeochemical assays, warming and nutrient enrichments) were relatively successful. The presence of the large diatom bloom covering much of the southern part of the domain (in both coastal and Tasman Sea waters) overwhelmed the microbial community which may have masked treatment effects during at least one of our experiments. We were very pleased to achieve 2 to 3 biogeochemical assays in a single water mass, because previously we have mostly been limited to one experiment per water mass.

During the voyage we fully utilised the underway data collection systems (ADCP, multi-beam echosounder, thermosalinograph etc), with Grafana (underway visualisation software) being a significant tool to help determine our sampling locations (Figure 12). The high resolution measurements of DIC, particle size distribution (LISST), $\delta^{13}C\text{-}CO_2$ and electron transport rate from both pulse amplitude modulated fluorometry and fast repetition rate fluorometry were highly successful, demonstrating the immense potential for additional underway datastreams to be brought online by researchers.

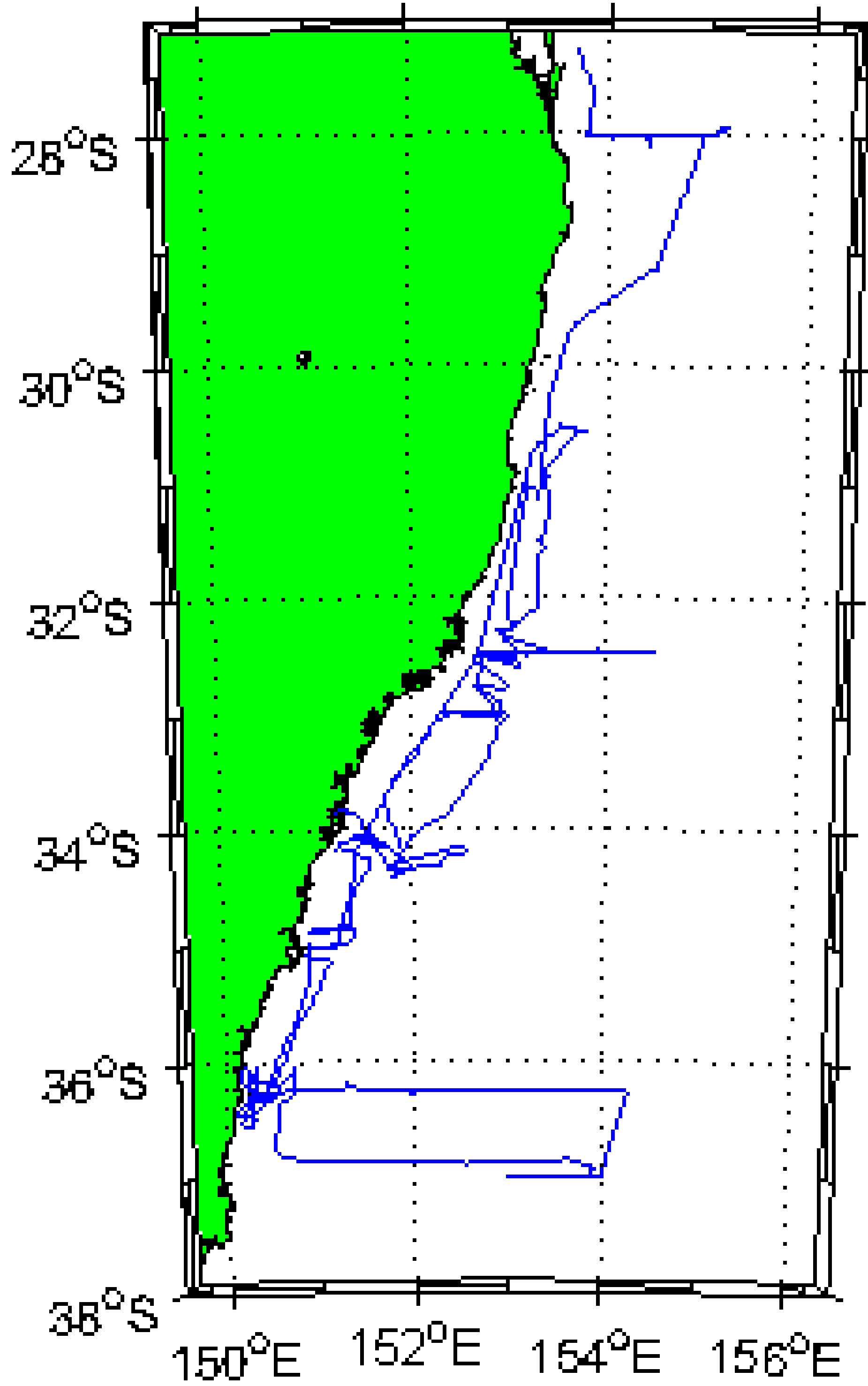
The Triaxus was deployed 11 times, expanding our data collection capacity beyond 55 CTDs, and adding a vast volume of depth profiles in the study region. The insight we will draw from LOPC estimates of plankton size spectra will be second to none.

Our observations of whales and other megafauna was no doubt enhanced by the voyage coinciding with the migration season, but was also influenced by our coastal focus. Participants commented that they rarely see such megafauna on oceanic voyages.

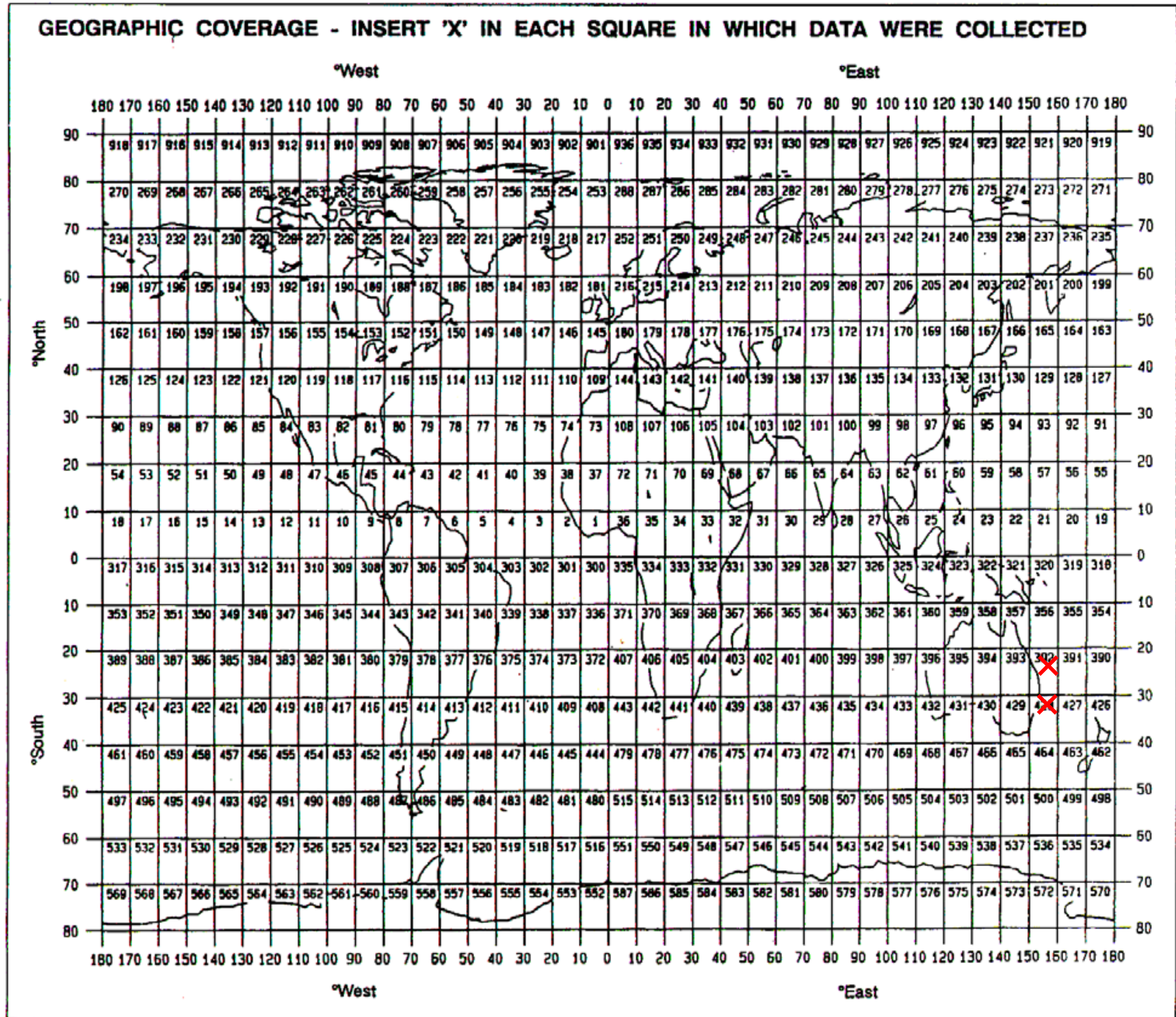
All of these are positive signs that the scientific outcomes of the voyage will be achieved, but there is a large amount of lab work and data processing yet to be completed.

Figure 17. Illustrative example of how the underway system was used to target specific water masses for sample collection.

Voyage Track



Marsden Squares



Moorings, bottom mounted gear and drifting systems

Item No	PI See page above	APPROXIMATE POSITION						DATA TYPE enter code(s) from list on last page	DESCRIPTION
		LATITUDE			LONGITUDE				
		deg	min	N/S	deg	min	E/W		
1	Iain Suthers	30	31.92	°S	153	31.40	°E	D06	Surface drifter #1 (145577) deployed at beginning of EAC drift study
2	Iain Suthers	30	31.92	°S	153	31.40	°E	D06	Surface drifter #2 (145594) deployed at beginning of EAC drift study
3	Iain Suthers	30	31.92	°S	153	31.40	°E	D06	Surface drifter #3 (145602) deployed at beginning of EAC drift study

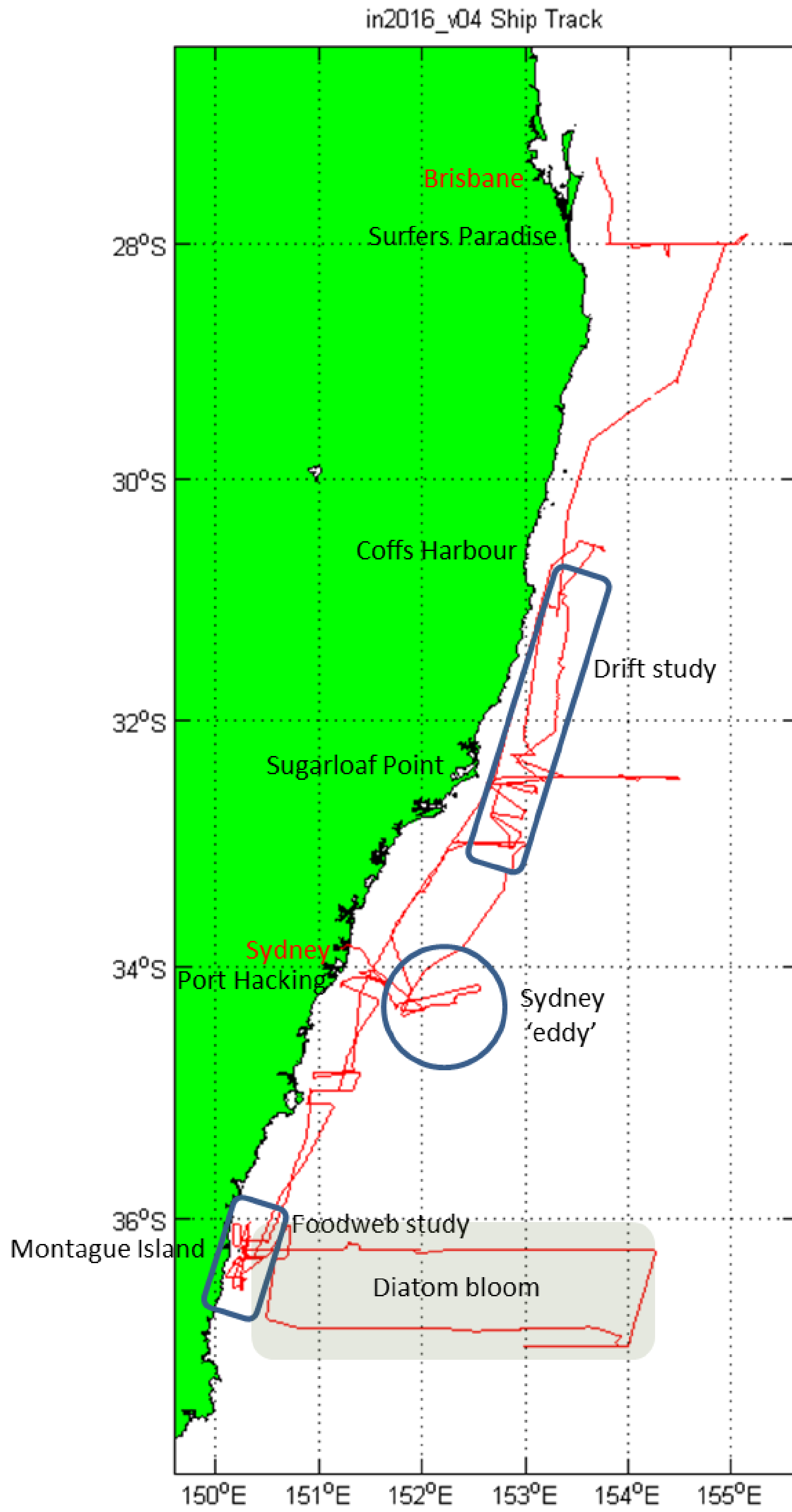
Summary of Measurements and samples taken

This information has been submitted to MNF as a separate Excel file.

Curation Report

This information has been submitted to MNF as a separate Excel file.

Track Chart



Personnel List

	Name	Organisation	Role
1.	Hugh Barker	CSIRO MNF	Voyage Manager
2.	Brett Muir	CSIRO MNF	SIT Support
3.	Nicole Morgan	CSIRO MNF	SIT Support
4.	Amy Nau	CSIRO MNF	GSM Support
5.	Dave Watts	CSIRO MNF	GSM Support
6.	Christine Rees	CSIRO MNF	Hydrochemistry
7.	Stephen Tibbens	CSIRO MNF	Hydrochemistry
8.	Pamela Brodie	CSIRO MNF	DAP Support
9.	Karl Malakov	CSIRO MNF	DAP Support
10.	Mark Lewis	CSIRO MNF	Mechanical Support
11.	Jason Fazey	CSIRO MNF	Mechanical Support
12.	Martina Doblin	UTS	Chief Scientist
13.	Allison McInnes	UTS	Post-doc
14.	Leonardo Laiolo	UTS	PhD student
15.	Marco Alvarez	UTS	PhD student
16.	Kun Xiao	UTS	Flow cytometry operator
17.	Mark Brown	UNSW	Alternate Chief Scientist
18.	Kirianne Goosen	UTas	PhD student
19.	Elisabeth Deschaseaux	UTS	Post-doc
20.	Marco Gardinia	UTS	PhD student
21.	Martin Ostrowski	MacU	Principal Investigator
22.	Amarantha Focardi	MacU	PhD student
23.	Rebecca Darcy	MacU	MRes student
24.	Joey Crosswell	UTS	Post-doc
25.	Justin Ashworth	UTS	Research fellow
26.	David Suggett	UTS	Principal Investigator
27.	David Hughes	UTS	PhD student
28.	April Abbott	MacU	Research scientist
29.	Iain Suthers	UNSW	Zooplankton team leader
30.	Zoe White	UNSW	MSc – OPC and Triaxus
31.	Gary Truong	UNSW	PhD– cetaceans and seabirds-1
32.	Chris Stanley	UNSW	MSc - larval fish, lobster
33.	Ricardo Alvarez Pacheco	UNSW	PhD– cetaceans and seabirds-2
34.	Luvia Garcia	Uni Auckland, New Zealand	Student
35.	Malwenn Lassudrie-Duchesne	UTS	Visiting Fellow UTS
36.	Deepa Varkey	UTS	Postdoc
37.	Morgane Perron	UTas	PhD student
38.	Bonnie Laverock	UTS	Postdoc
39.	Matt Holland	UNSW	prospective PhD student

Marine Crew

Name	Role
John Highton	Master
Gurmukh Nagra	Chief Mate
Adrian Koolhof	Second Mate
Tom Watson	Third Mate
Chris Minness	Chief Engineer
Sam Benson	First Engineer
Mike Sinclair	Second Engineer
Ryan Agnew	Third Engineer
Shane Kromkamp	Electrical Engineer
Gary Hall	Chief Caterer
Emma Lade	Caterer
Keith Sheppherd	Chief Cook
Matt Gardiner	Cook
Graham McDougall	Chief Integrated Rating
Dennis Bassi	Integrated Rating
Paul Langford	Integrated Rating
Rod Langham	Integrated Rating
Peter Taylor	Integrated Rating
Chris Dorling	Integrated Rating
Matt McNeill	Integrated Rating

Acknowledgements

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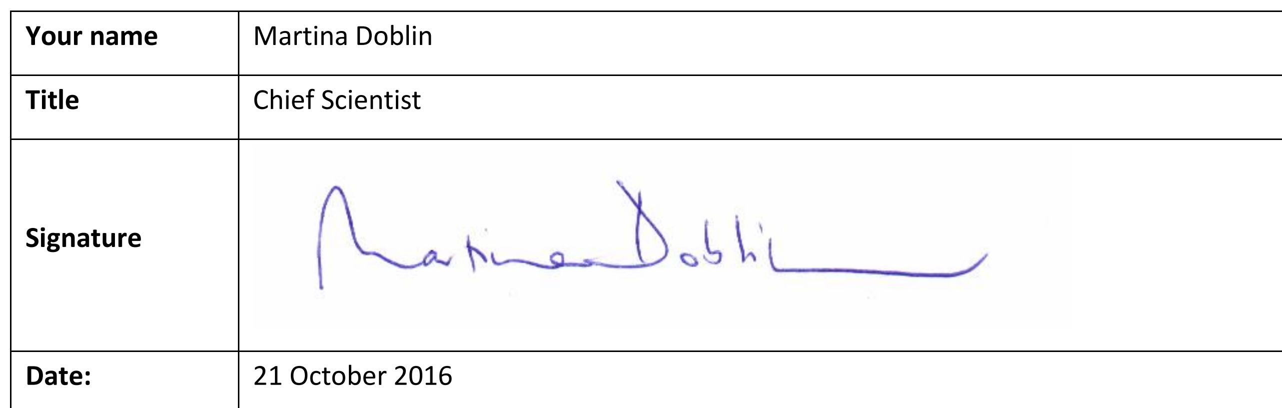
Signature



Your name	Martina Doblin
Title	Chief Scientist
Signature	
Date:	21 October 2016

List of additional figures and documents

Appendix 1 Summary of in situ pump deployments

Appendix 2 CSR/ROSCOP Parameter CodeS

Appendix 3 Sampling schedule of EAC drift study

Appendix 1 Summary of in situ pump deployments

ISP	Name	Preceding CTD	Date	Latitude	Longitude	Depth
ISP001_10m	PH4	11	4/09/2016	34˚07.639S	151˚14.091E	10 m
ISP002_10m		12	5/09/2016	36˚19.6399	150˚16.6755	10 m
ISP002_40m		12	5/09/2016	36˚19.6399	150˚16.6755	40 m
ISP003_10m	WC Eddy	15	6/09/2016	32˚12.8759	151˚12.1230	10 m
ISP003_52m	WC Eddy	15	6/09/2016	32˚12.8759	151˚12.1230	52m
ISP004_10m	Cold Tasman	22	8/09/2016	36˚58.9916	153˚52.9731	10 m
ISP004_65m	Cold Tasman	22	8/09/2016	36˚58.9916	153˚52.9731	65 m
!SP005_10m	drift #1	27	13/09/2016	30˚42.2468	153˚53.5890	10m
ISP005_50m	drift #1	27	13/09/2016	30˚42.2468	153˚53.5890	50m
ISP006_10m	drift #2	28	13/09/2016	30˚51.6121	153˚24.177	10m
ISP006_30m	drift #2	28	13/09/2016	30˚51.6121	153˚24.177	30m
ISP007_10m	drift #3		14/09/2016	30˚59.1824	153˚22.8051	10m
ISP007_40m	drift #3		14/09/2016	30˚59.1824	153˚22.8051	38.8m
!SP008_10m	drift #4	*30	14/09/2016	31˚29.4825	153˚21.21	10m
ISP008_40m	drift #4	*30	14/09/2016	31˚29.4825	153˚21.21	38.8m
ISP009_10m	drift #5	31	14/09/2016	31˚39.9360	153˚18.8267	10m
ISP009_40m	drift #5	31	14/09/2016	31˚39.9360	153˚18.8267	38m
ISP010_10m	drift #6	33	14/09/2016	32˚00.1258	153˚17.5195999999	10m
ISP010_55m	drift #6	33	14/09/2016	32˚00.1258	153˚17.5195999999	55m
ISP011_10m	drift #7	36	15/09/2016	32˚34.7278	153˚06.7772	10m
ISP011_40m	drift #7	36	15/09/2016	32˚34.7278	153˚06.7772	40m
ISP012_10m	drift #8	38	16/09/2016	32˚46.1663	152˚58.5720	10m
ISP012_55m	drift #8	38	16/09/2016	32˚46.1663	152˚58.5720	10m
ISP013_10m	sugarloaf transect Tasman	43	17/09/2016	32˚28.90498	154˚05.7794	10m
ISP013_36m	sugarloaf transect Tasman	43	17/09/2016	32˚28.90498	154˚05.7794	36m
ISP013_100m	sugarloaf transect Tasman	43	17/09/2016	32˚28.90498	154˚05.7794	100m
ISP014_120m	EAC	48	19/09/2016	29˚10.1290	154˚28.5365	120m
ISP014_10m	EAC	48	19/09/2016	29˚10.1290	154˚28.5365	10m
ISP015_10m	East of EAC	51	20/09/2016	28˚01.3003	154˚21.3021	10m
ISP015_65m	East of EAC	51	20/09/2016	28˚01.3003	154˚21.3021	65m

Appendix 2 CSR/ROSCOP Parameter CodeS

	METEOROLOGY
M01	Upper air observations
M02	Incident radiation
M05	Occasional standard measurements
M06	Routine standard measurements
M71	Atmospheric chemistry
M90	Other meteorological measurements

	PHYSICAL OCEANOGRAPHY
H71	Surface measurements underway (T,S)
H13	Bathythermograph
H09	Water bottle stations
H10	CTD stations
H11	Subsurface measurements underway (T,S)
H72	Thermistor chain
H16	Transparency (eg transmissometer)
H17	Optics (eg underwater light levels)
H73	Geochemical tracers (eg freons)
D01	Current meters
D71	Current profiler (eg ADCP)
D03	Currents measured from ship drift
D04	GEK
D05	Surface drifters/drifting buoys
D06	Neutrally buoyant floats

	MARINE BIOLOGY/FISHERIES
B01	Primary productivity
B02	Phytoplankton pigments (eg chlorophyll, fluorescence)
B71	Particulate organic matter (inc POC, PON)
B06	Dissolved organic matter (inc DOC)
B72	Biochemical measurements (eg lipids, amino acids)
B73	Sediment traps
B08	Phytoplankton
B09	Zooplankton
B03	Seston
B10	Neuston
B11	Nekton
B13	Eggs & larvae
B07	Pelagic bacteria/micro-organisms
B16	Benthic bacteria/micro-organisms
B17	Phytobenthos
B18	Zoobenthos
B25	Birds
B26	Mammals & reptiles
B14	Pelagic fish
B19	Demersal fish
B20	Molluscs
B21	Crustaceans
B28	Acoustic reflection on marine organisms

D09	Sea level (incl. Bottom pressure & inverted echosounder)
D72	Instrumented wave measurements
D90	Other physical oceanographic measurements

	CHEMICAL OCEANOGRAPHY
H21	Oxygen
H74	Carbon dioxide
H33	Other dissolved gases
H22	Phosphate
H23	Total - P
H24	Nitrate
H25	Nitrite
H75	Total - N
H76	Ammonia
H26	Silicate
H27	Alkalinity
H28	PH
H30	Trace elements
H31	Radioactivity
H32	Isotopes
H90	Other chemical oceanographic measurements

B37	Taggings
B64	Gear research
B65	Exploratory fishing
B90	Other biological/fisheries measurements

	MARINE GEOLOGY/GEOPHYSICS
G01	Dredge
G02	Grab
G03	Core - rock
G04	Core - soft bottom
G08	Bottom photography
G71	In-situ seafloor measurement/sampling
G72	Geophysical measurements made at depth
G73	Single-beam echosounding
G74	Multi-beam echosounding
G24	Long/short range side scan sonar
G75	Single channel seismic reflection
G76	Multichannel seismic reflection
G26	Seismic refraction
G27	Gravity measurements
G28	Magnetic measurements
G90	Other geological/geophysical measurements

	MARINE CONTAMINANTS/POLLUTION
P01	Suspended matter
P02	Trace metals
P03	Petroleum residues
P04	Chlorinated hydrocarbons
P05	Other dissolved substances
P12	Bottom deposits
P13	Contaminants in organisms
P90	Other contaminant measurements

Appendix 3 Sampling schedule of EAC drift study

EAC drift		Quick-Dip (Surf/DCM)	TMR	Classification CTD (1000m)	IS P
Tues 13 September	12a m				
DAY 1	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8	Quick-Dip (Surf/DCM) CTD26			
	9		Leo - DCM/Surf		
	10			CTD27	
	11				
	12p m				
	1				
	2				
	3		Morgane - battery failed		
	4			CTD28	
	5				
	6				
	7				
	8				
	9				
	10			CTD29	
	11				
Wed 14 September	12a m				
DAY 2	1				
	2				
	3				
	4				
	5				
	6			CTD30	
	7				
	8				
	9				
	10				
	11			CTD31	
	12p m				

EAC drift		Quick-Dip (Surf/DCM)	TMR	Classification CTD (1000m)	ISP
	1				
	2				
	3				
	4			CTD32	
	5				
	6				
	7				
	8				
	9				
	10			CTD33	
	11				
Thurs 15 September	12am				
DAY3	1				
	2				
	3				
	4				
	5	Quick-Dip (Surf/DCM) CTD34			
	6			CTD35	
	7				
	8				
	9				
	10				
	11			CTD36	
	12pm				
	1				
	2				
	3		Morgane - 500		
	4			CTD37	
	5				
	6				
	7				
	8				
	9				
	10			CTD38	
	11				
Friday 16 September	12am				
DAY 4	1				
	2				
	3				
	4				

EAC drift		Quick-Dip (Surf/DCM)	TMR	Classification CTD (1000m)	IS P
	5			CTD39	
	6				
	7				
	8				
	9				
	10				
	11			CTD40	