Mesoscale Iron Fertilization Effects on Plankton Community Structure, Phytoplankton Growth and Zooplankton Grazing

Overview:

This SOFeX component is a collaborative study involving M. Landry, B. Bidigare and co-workers at the University of Hawaii and S. Chisholm and Z. Johnson at MIT. The specific objectives of our research are:

1. To determine population abundances and community biomass structure, including bacteria, phytoplankton and zooplankton, and their temporal and spatial responses to mesoscale iron enrichment;
2. To estimate taxon-specific growth rates and cellular fluorescence, pigment and physiological responses of phytoplankton to iron enrichment; and
3. To assess grazing rates of micro- and mesozooplankton, and evaluate the capacity of the grazer community to control or modulate the phytoplankton response to iron fertilization.

Objective 1 will be determined using a combination of complementary approaches for different sizes of organisms and functional groups:

Fraction Analyzed	Method	Samples
Total phytoplankton	Fluorometric Chlorophyll	170-280 ml, filtered
Group-specific autotrophs	HPLC Pigments	1-2 liter, filtered/frozen
Autotrophic pico- & microplankton	Large volume FCM, ship	5-10 ml, live
Bacteria and picophytoplankton	Dual-beam FCM, lab	1 ml, preserved/frozen
Auto- & heterotrophic nanoplankton	Epifluor. Microscopy	20-250 ml, preserved
Heterotrophic microplankton	Inverted Microscopy	100-500 ml, preserved
Mesozooplankton populations	Microscopy - Dissecting	Net tows, preserved
Mesozooplankton biomass	CHN Analyses	Net tows, screened/frozen

Objectives 2 and 3 will be met from rate inferences from dilution experiments (phytoplankton growth, microzooplankton grazing and pigment adaptations), physiological indices from flow cytometry and background irradiance gradient single turnover fluorometer (BIG-STf), and assessments of mesozooplankton grazing from gut pigments and degradation products**.**

Phytoplankton Pigments and Biomarkers

Total (GF/F) and size-fractioned (>5 and >20 μm) particulate samples were collected for HPLC pigment analyses by vacuum filtration. Target analyses include chlorophylls, carotenoids, bacteriopigments, and their diagenetic alteration products. At each station, samples were collected from the euphotic zone for generating pigment profiles (100, 47, 30, 16, 10, 5, 1, and 0.1% E_o) (23 profiles) and size-fractioned pigment distributions (47, 30, and 16% E_o) (16 independent analyses). Transect and survey samples were also collected to map vertical and horizontal variations in pigment structure in the northern (1 survey) and southern patches (2 surveys). Initial (n = 3) and final (n = 13) HPLC pigment samples were collected from each of the dilution experiments performed aboard the R/Vs MELVILLE & REVELLE. Five “diatom soup” samples (200-2000 μm) were also collected from 3 inside and 2 outside Southern Patch stations for the determination of biomarker:biovolume:C:N:Si:Th ratios.

Particulate samples were stored under liquid nitrogen prior to shipment (dry ice) to the University of Hawaii for subsequent analysis. It is anticipated that all HPLC analyses will be completed within 6 months of collection.

Large-volume (85-130 L) particulate samples were collected at 15 stations using a Challenger Oceanic *in situ* pump fitted with a 142 mm GF/F filter manifold. Samples were collected from the upper mixed layer (20-30 m) at each of the inside and outside stations occupied during SOFeX. Particulate samples will be used for the determination of phytoplankton biomarker (chlorophylls, carotenoids, bacteriopigments, phaeopigments, sterols, fatty acids and phytol) concentrations and carbon isotopic compositions. CHN and bulk isotopic analyses will also be performed on punches prepared from these filters. Paired seawater samples were also collected prior to or shortly after each pump cast for the determination of [DIC] and $\delta^{13}C_{DIC}$. Particulate samples were stored under liquid nitrogen prior to shipment (dry ice) to the University of Hawaii for subsequent analysis. Biomarker analyses should be completed within 9 months of collection.

Flow Cytometry and Microbial Diversity

Samples were taken for flow cytometry and genetic diversity in order to assess how microbial community structure changes in iron fertilized waters. Both provide information on the absolute and relative abundance of different microbial populations, including both phytoplankton and bacteria during bloom development. In addition, the flow cytometry data will be able to distinguish between increases in cellular chlorophyll or increases in the number of cells. This will be important in determining whether the increases in total chlorophyll biomass in the patches were due to a stimulation of the ambient phytoplankton microbial community or if rare species increased in dominance. Flow cytometry data from initial and final dilution experiment samples will help determine population-specific grazing estimates.

Two complete profiles of flow cytometry data were taken at each hydrostation -- one from the TM and one from the CTD rosette. In addition, flow cytometry samples were run on two additional TM casts that corresponded to special casts for primary production and growth rate/grazing rates. Flow cytometry samples were also run for the vertical transect measurements as well as several of the surface water patch surveys. In total, nearly 1000 samples were analyzed. Identical samples that were preserved and frozen will be analyzed later in the laboratory for bacteria populations.

Over 125 large-volume samples from the mixed layer (typically 30 m) were filtered for genetic diversity at every major station occupation and on two of the vertical transects. These will later be extracted and amplified using the polymerase chain reaction (PCR) protocol to determine eukaryotic and prokaryotic diversity from the size of DNA fragments after restriction enzyme digests (T-RFLP). This technique is highly sensitive and has been successfully used with microbial communities in other heterogeneous environments.

In collaboration with the primary production team from Duke University, we also used a single-turnover fluorometer to assess properties of photosystem II. Similar to the fast repetition rate fluorometer (FRRf), the background irradiance gradient single turnover fluorometer (BIG-STf) can measure the photochemical conversion efficiency (Fv/Fm) and the functional cross section ($_{PSII}$) of photosystem II. In addition, the BIG-STf measures these properties over a range of background light levels. Thus, just as a photosynthesis-irradiance curve (P-E) curve can provide information on the efficiency and capacity of photosynthesis, BIG-STf data can determine the role of photosystem II in regulating the total photosynthetic

response. BIG-STf curves were generated at every major station for mixed layer samples. These samples were taken in conjunction with photosynthesis-irradiance samples to facilitate comparisons. From these samples, over 30GB worth of BIG-STf data has been archived for later analysis. BIG-STf data will help to resolve if increases in biomass-specific primary production from iron addition are due to an absolute increase in photosynthetic efficiency or due to an increase in photosynthetic capacity of PSII. BIG-STf data can also be used to assess photoacclimation of the phytoplankton. Comparisons between photosynthesis-irradiance curves and Fv/Fm-irradiance curves generated from the BIG-STf will clarify the role that iron plays in regulating PSII and photosynthesis.

Microscopical Analyses

A complete depth profile (to the 0.1% I_o light level) was taken for nanoplankton and microplankton abundance and biomass (by epifluorescence microscopy) at each of the 17 stations occupied. At selected stations, an additional profile was taken to estimate the variability of the community on successive casts. Nanoplankton (20 - 50 ml) samples were preserved with paraformaldehyde and stained with proflavin and DAPI, then filtered onto black 1-µm Poretics filters. Similarly, microplankton (100 - 250 ml) samples were fixed with 1% formaldehyde, stained with proflavin and DAPI, then filtered onto black 8-µm Poretics filters. These filters were mounted on glass slides with immersion oil. The freshly prepared slides were examined on shipboard with a Zeiss Image Analysis System (Zeiss Standard microscope, ZVS 3-chip CCD video camera, and Image Pro Plus Software), and 30 - 40 random fields of each preparation were recorded to a compact disk.

Preliminary results indicate that the phytoplankton communities were quite distinct in the north and south patches. In the north patch, the OUT stations were dominated by nanoplankton and <20-µm dinoflagellates, with few diatoms present, whereas the IN stations had more diatoms (dominated by *Pseudonitzschia* chains) as the patch matured. In the southern patch, both the OUT and IN stations were dominated by centric diatom species (notably *Corethron*, several *Chaetoceros* spp. and *Rhizosolenia* spp., and *Asteromphalus*). The most striking difference between the IN and OUT stations was the buildup of chloroplasts in IN station phytoplankton cells as the experiment progressed.

Additionally, samples were taken at the depth corresponding to a light intensity of ~30% I_o for assessment of heterotrophic microplankton (i.e., ciliates and dinoflagellates) abundance and biomass and diatom taxonomy. These microplankton samples (500 ml) were preserved in two ways for shore-based analyses by inverted microscopy (Utermöhl method). Acid Lugols preservation was used for quantitative enumeration of ciliates, whereas borate-buffered formaldehyde preservation was used for enumeration and identification of diatoms and dinoflagellates. Additional samples were taken at the start of each dilution experiment, to define the grazing community in these incubations.

Shore-based analyses of these microscopy samples will result in enumeration of nanoplankton and microplankton within their respective size categories as functional groups: auto- and heterotrophic (non-dino) flagellates, auto- and heterotrophic dinoflagellates, centric and pennate diatoms, auto- and heterotrophic ciliates, and metazooplankton. Cellular biovolumes will also be calculated using cell dimensions and appropriate geometrical shapes. Cell carbon contents will be calculated from biovolumes using appropriate conversion factors. It is expected that all analyses will be completed within 9-months.

Phytoplankton Growth and Microzooplankton Grazing

Dilution experiments were conducted at each IN and OUT station to assess community and taxa-specific growth and mortality rates of autotrophic populations. Water was routinely collected from and incubated at a light intensity of ~30% I_o, which was generally close to the mean light level in the "mixed" layer. Including those from the R/V REVELLE, 10 one-day incubations were successfully completed for the Northern Exp. (IN:OUT ratio = 1:1) and 17 two-day incubations were completed for the Southern Exp. (IN:OUT = 2:1). Shipboard fluorometric Chl analyses suggest phytoplankton growth rate range of 0.4-0.6 d^{-1} in the N. Patch and 0.1-0.3^{+} d^{-1} in the S. Patch. Median growth rates were 0.46 and 0.22 d^{-1}, respectively, with highest rates closest to the start of the fertilization, particularly in the S. Patch. In both areas, losses to microzooplankton grazing accounted for ~1/3 of phytoplankton growth rates. These ***very preliminary results will likely change appreciably*** as we account for the influences of cellular pigment changes, which were very apparent in microscopical observations. The gradual, but substantial, increases in cellular pigment content of cells in the iron enriched patches clearly bias the interpretation of cell and carbon-specific growth rates, and pigment change artifacts must also be investigated for the OUT-patch bottle incubations. We will attempt to unravel the three interrelated rates (cell growth, pigment adaptation, grazing mortality) underlying the net *in situ* Chl *a* changes using the combination of HPLC pigments and FCM (cell abundances and fluorescence). In addition, pigment and carbon-based growth rate estimates will be constrained by the results of contemporaneous incubations for pigment-labeling (VIMS) and14-C uptake (DUKE). Grazing rates estimates for component populations will be determined by flow cytometry and taxa-specific pigments. Microscopy samples will be used to assess net growth rates of identifiable auto- and heterotrophic populations and to determine the relationship between microzooplankton grazing impact and the biomass of heterotrophic protists.

Mesozooplankton Biomass and Grazing

Mesozooplankton were collected quantitatively at night using a 200μm mesh plankton net. Although much of the material was saved for later species identification, dry weight, and elemental analysis, distinct differences in species composition and abundance were noted between northern and southern stations. The northern community was very diverse in composition, consisting of ostracods, medium to large (0.5 to >2mm) copepods, thecosomate and gymnosomate pteropods, and small krill (*Thysanoessa* spp.). In contrast, the southern mesozooplankton community was dominated by large (1 to >2mm) copepods (*Rhincalanus gigas*, *Calanus propinquus*, and *Calanoides acutus*) and large krill (*Thysanoessa* spp). Salps (*Salpa thompsonii*) were observed in both northern and southern stations, but were not collected quantitatively. The abundance of mesozooplankton, including krill, was much lower at southern stations than at northern stations, perhaps only reaching 1-5 copepods per m^3. Although variation in mesozooplankton biomass and species composition within northern and southern stations was observed, this did not appear to be related to sampling at IN vs. OUT patch locations. Characterization of mesozooplankton biomass and species composition at different spatial scales will continue as collected samples are processed in the laboratory.

Grazing of phytoplankton in fertilized vs. non-fertilized locations was assessed using net collection of mesozooplankton. Mesozooplankton collected quantitatively using a 200-μm mesh plankton net were flash frozen for later gut pigment analyses. These analyses, which determine the concentration of chlorophyll degradation products contained in zooplankton guts, can be used to assess the amount of phytoplankton ingested by mesozooplankton. The zooplankton collected for gut pigment content included copepods, krill (*Thysanoessa marcrura*) and some salps (*S. thompsonii*). Although differences in gut pigment content between IN and OUT patch waters remains to be determined, it is apparent that krill and large copepods (Family Calanidae) are the dominant grazers of phytoplankton in the south, whereas

thecosomate pteropods, and likely krill and some copepods are the dominant grazers of large phytoplankton in the north. Food selection experiments were performed using mesozooplankton collected with a 333-μm mesh large (30L) cod end net. These experiments can be used to derive a rate of "clearance" of phytoplankton taxa from the water column by mesozooplankton. Although removal of phytoplankton chlorophyll by copepods was observed during the experiments, low copepod abundance (at least in the southern patch) is likely to preclude a significant role for mesozooplankton in removal of phytoplankton biomass at both patch sites as noted previously during SOIREE.