NF1802: summary of activities conducted May 2018, NOAA Ship Nancy Foster

- ✓ 141 stations, 2 cycles
- ✓ 3,700 BFT, 568 specimens measured
- ✓ 59 CTD casts. Double sensors on the CTD were temperature, oxygen, salinity, chlorophyll-*a* and CDOM, conductivity, PAR.
- ✓ 8 Photosynthetically-active radiation (PAR) casts
- ✓ Continuous measurements of currents (ADCP) throughout survey
- ✓ Continuous measurement of flow through (TSG)
- ✓ 5 SVP Drifters were deployed
- ✓ 364 Phytoplankton Pigments by fluorometric Chl*a*
- ✓ 171 High-performance liquid chromatography samples
- ✓ 25 Ring net tows (integrated to 135 m)
- ✓ 125 Size-fractioned samples for zooplankton biomass and grazing
- ✓ 55 Samples for microbiota of larval fish guts
- ✓ 12 Sea water samples for bacterial genetic sequencing
- ✓ 48 Growth and Grazing Rate Profiles (8 experiments x 6 depths)
- ✓ 27 ring net tows (100 + m)
- ✓ 2 Deckboard Incubations for NO_3^- $\delta^{15}N$ assimilation isotope effect studies (33 samples collected)
- ✓ 21 Nutrient Profiles for NO_3^-, NH_4^+, and PO_4^{3-} concentration and NO_3^- isotope analyses ($\delta^{15}N$ and $\delta^{18}O$)
- ✓ 77 samples collected for euphotic zone nutrient concentration and isotopic analysis supporting NO_3^- and NH_4^+ uptake rate estimates
- ✓ 187 samples collected for water column nutrient concentration and isotopic composition analysis supporting $\delta^{15}N$ budget calculations
- ✓ 216 Phytoplankton abundance estimates (live shipboard, flow cytometry)
- ✓ 282 Picophytoplanton and bacteria abundance estimates (flow cytometry)
- ✓ 100 Phytoplankton taxa, biomass and abundance estimates (microscopy)
- ✓ 50 Microzooplankton (ciliate/dinoflagellate) abundance, biomass estimates (microscopy)
- ✓ 84 Nitrogen-fixing organism abundance, biomass, isotope determinations
- ✓ 120 net tows (25m): 111 Bongo-90, 9 S25, & 17 Mini-bongo net tows
- ✓ 17 Mesozooplankton and 17 microzooplankton samples
- ✓ Live sorted 111 plankton samples

PI Landry, summary of accomplishments

Growth and Grazing Rate Profiles *(8 experiments x 6 depths = 48 determinations)*
Two-treatment dilution experiments were conducted over two experimental cycles to determine rate profiles (subsurface to 110 m depth) of phytoplankton growth and microzooplankton grazing. All bottles were incubated under ambient conditions of temperature and light for 24 h on the drift array. Community and population-level analyses will be done using samples collected for Chl *a*, flow cytometry and HPLC pigments.

Phytoplankton Pigments *(364 fluorometric Chla, 171 HPLC samples)*
Samples (2.2 L) were collected for phytoplankton pigments from 10 hydrographic casts, 8 profiles of grow and grazing rate experiments and 2 shipboard dilution experiments. Replicate samples (285 mL) for fluorometric analysis were filtered, extracted 24 h in 90% acetone and analyzed for chlorophyll a and phaeopigents on shipboard. Samples (2-2 L) for analysis by high-pressure liquid chromatography were filtered, frozen in liquid N_2 and will be analyzed on shore for group-specific chlorophylls and carotenoid accessory pigments.

Mesozooplankton Biomass and Grazing *(5 x 25 = 125 size-fractioned samples; 25 preserved samples)*
25 oblique net tows were taken from the surface to 135 m with a 1-m rig net, integrating standing stocks through the euphotic zone. Half of the tow (Folsum splitter) was preserved with formaldehyde, and half or a quarter was size-fractioned through a series of nested filters (5, 2, 1. 0.5 and 0.2 mm Nitex mesh). Each fraction was concentrated on a pre-weighed 0.2 mm Nitex filter, washed with isotonic ammonium formate to remove sea salt, and frozen in a -80°C freezer. In the lab, the samples will be weighed for wet weight (WW) and subsampled in replicated for gut pigment/WW analysis to estimate mesozooplankton grazing impact on phytoplankton. The remaining filter will be dried and reweighed (DW:WW and total DW by calculation), and then ground to a powder for elemental analysis (C:DW, N:DW, isotopes).

Microbiota of larva fish *(53 + 12 = 65 samples)*
A total of 53 larval fish that appeared to have empty stomach and intestines were sorted from the nighttime Bongo tow samples: bluefin tuna (n=20), skipjack tuna (n=16) and dolphin (mahi mahi; n=17). The larvae were preserved in different ways to analyze the composition and spatial distribution of bacteria within the gut and how they change as the larvae grow. A total of 23 larvae were preserved in RNA Shield and stored at -20°C. Their stomach and intestines will be dissected out and the DNA (16S rDNA) extracted, amplified by PCR in triplicate with specific Illumina 16S primers and sent for Illumina sequencing. To compare gut microflora to bacteria in the environmental community, 2.2-L water samples were collected at 5 and 20 m on six occasions. These samples were pre-filtered through a 3 µm filter and collected a 0.1-µm filter and preserved similarly to the larval fish. A total of 22 larvae were prepared for Fluorescence *In Situ* Hybridization (FISH) by rinsing in cold PBS, preserving in 4% paraformaldehyde in PBS for ~6 h at 4°C, then cryoprotecting in PBS containing 25% sucrose and 25% TissueTek for ~6 h, and finally freezing at -20°C in TissueTek. In the lab, the stomach plus intestines and gills will be sliced into fine cross sections (histology) and hybridized with

bacterial probes targeting different bacterial taxa. Particular attention will also be given to the distribution of possible bacteria symbionts associated with nitrogen waste recycling in the gut and gills, and on chitin degraders in the gut. Samples will be imaged with epifluorescence and confocal microscopy. Finally, 8 larval fish were stored at -80°C in 25% glycerol for possible isolation of putative fish symbionts.

PI Knapp, lab-based accomplishments

Nitrate concentration measurements have been completed on:

-33 of 33 samples collected for NO_3^- $\delta^{15}N$ assimilation isotope effect study

-11 of 77 euphotic zone samples for NO_3^- and NH_4^+ uptake rate estimates

-72 of 187 samples collected for NO_3^- $\delta^{15}N$ budget calculations

Phosphate concentration measurements have been completed on:

-63 of 187 samples collected for NO_3^- $\delta^{15}N$ budget calculations

Ammonium concentration measurements have been completed on:

-14 of 77 euphotic zone samples collected for NO_3^- and NH_4^+ uptake rate estimates

PI Selph, accomplishments

Phytoplankton & Bacterial Abundance *(216 live & 282 preserved flow cytometry samples)*
Samples (15 mL) were collected for live ship-board determinations of phytoplankton abundance from 14 hydrographic casts, 8 profiles of grow and grazing rate experiments and 2 shipboard dilution experiments. Samples (2 mL) were also collected for preserved picoplankton analyses from the same casts/experiments. Both initial and final samples were taken for all experiments.

Phytoplankton taxa, abundance and biomass *(8 experiments x 6 depths x 2 size fractions = 96 determinations; 2 experiments x 1 depth x 2 size fractions = 4 determinations)*
Microscope slides were made from each of the growth/grazing experiments to determine the phytoplankton taxa, abundance, and biomass in the incubations. Two slides were made per depth, one for smaller phytoplankton (0.8-20 µm, 50 mL sample) and one for larger phytoplankton (8-200 µm, 450 mL sample). Slides were also made for the 2 deckboard incubation experiments.

Microzooplankton (Ciliate) abundance and biomass *(8 experiments x 6 depths = 48 determinations; 2 experiments x 1 depth = 2 determinations)*
Samples for microzooplankton abundance and biomass, principally ciliates and dinoflagellates, were taken for each experiment, which involved preserving ~125 mL of sample with 6 mL of acid Lugols. These samples will be analyzed with inverted microscopy in the shore-based laboratory after settling.

Nitrogen-fixing organism abundance: Trichodesmium *(7 profiles x 6 depths x 2 size classes= 84 determinations)*

Samples were collected from surface (5-50 m) water, to determine the abundance, biomass, and stable isotopic composition of *Trichodesmium*, and any other co-occurring nitrogen-fixing organisms (e.g., diatoms with nitrogen-fixing symbionts). 6 L of water was filtered through 8 µm filters and preserved with formaldehyde, then frozen for later counting. Parallel samples were filtered onto 20 µm filters (~30 L each) and frozen live for subsequent isotope and biomass determinations.

Photosynthetically-active Radiation (PAR) casts *(8 profiles)*
A profiling PAR sensor, Biospherical Instruments QSP-2300, was attached to the CTD rosette frame and measurements of in situ PAR were made near local apparent noon for 8 profiles, corresponding to the days when experimental rate determinations were made. Data were used to estimate the proper depths

PI Stukel, accomplishments

On the NF1802 Cruise, the goal of the Stukel Lab (with participants Mike Stukel, Tom Kelly, and Taylor Shropshire) was to quantify phytoplankton production and nitrogen utilization and particle export from the euphotic zone. We conducted a total of 48 in situ $H^{13}CO_3^-$ uptake experiments (in triplicate with a dark control bottle) to measure primary productivity throughout the euphotic zone. These measurements were conducted on two 4-day Lagrangian experiments. We also conducted 48 in situ $^{15}NO_3^-$ uptake experiments (in duplicate) to quantify nitrate utilization by the phytoplankton community. To further assay phytoplankton nitrogen cycling, we conducted 6-hour nitrate and ammonium uptake experiments in deckboard incubators at three light levels (12 experiments each in triplicate for both nitrate uptake and ammonium uptake). These experiments were paired with N_2 fixation incubation measurements made by the bubble dissolution method (also 12 measurements made in triplicate). In addition to these phytoplankton incubations, we deployed sediment traps at 3 depths (50 m, 120 m, and 200 m) on each of the Lagrangian experiments. These sediment traps were deployed for 4 days with typically 8 PIT tubes per depth. After recovery the tubes were processed and filtered for an array of measurements: carbon, nitrogen, isotopes of carbon and nitrogen, C:^{234}Th ratio, chlorophyll a, phaeopigments, and genetics. The genetic samples were paired with genetic samples taken from the water column. We also made a total of 102 measurements of the isotopic composition (^{13}C and ^{15}N) of particulate organic matter suspended in the water column.

PI Lamkin, accomplishments

Bongo-90cm (111 tows x 2 nets = 222 plankton 0.5L jars)
Targeted bongo-90cm tows in favorable larval bluefin tuna habitat were carried out obliquely to 25m. Previous studies indicate that this 25m (surface) depth bin is most commonly associated with larval bluefin tuna in both the GOM and in the Mediterranean spawning grounds. In addition to extracting bluefin tuna from plankton samples, other co-occuring tunas and other top larval predators were removed (skipjack tuna, yellowfin/black fin tuna, mahi-mahi).

Ideally, the same larval bluefin tuna will be used in multiple studies including compound specific stable isotope analysis, age and growth, gut content analysis.
We sorted the right Bongo *in situ*, and processed 737 specimens Scombrids, with 640 BFT assigned developmental stage, and measured. Ethanol measurements of additional lab-sorted BFT are ongoing (n=538) for subsequent experiments.
S25 net tows were carried out at BFT positive stations during leg 2 to collect larger number of BFT for collaborative work.

Mini-bongo net tows (17 tows x 2 nets = 34 samples)
The mini-bongo has two meshes, one with 200 micron mesh targeting mesozooplankton and one with 50 micron mesh targeting microzooplankton. The mesozooplankton was fractioned in two samples, one preserved in ethanol for community analysis and the second was frozen. The 50 micron sample was filtered through a 200 micron mesh filter and then frozen. These samples will be analyzed for bulk stable isotope analysis of nitrogen and carbon to continue baseline measurements conducted in 2014, and in 2017. Larval bluefin were also concurrently collected to ascertain the signatures of the different trophic levels.