Title: Improving predictive biogeochemical models through single cell-based analyses of marine plankton physiological plasticity, genetic diversity and evolutionary processes

Conveners: Michael Lomas, Ben Twining, Ramunas Stepanauskus

Organizing Committee: to be determined if proposal is successful. Suggested members are: Ben

van Mooy, Sarah Fawcett, Alison Taylor, Mick Follows, Adrian Marchetti

1. Scientific Summary:

The global ocean is currently undergoing significant changes, from the acidification of surface seawater to expansion of mid-water oxygen minimum zones to changes in vertical stratification and nutrient inputs. Understanding these present changes and prediction of their impact on marine plankton-driven biogeochemical processes requires detailed information at the level of the individual organism, such as their physiological traits, characteristics, rate processes and plasticity to respond to environmental change. 'Omics' measurements can provide detailed information on taxonomic diversity, presence/absence of particular genes and if they are transcribed and translated, but these methods have limited ability to provide quantitative information on cellular characteristics and rate processes. Indeed, our current understanding of the relative importance of taxonomic diversity versus physiological plasticity in biogeochemical cycling of organic elements remains inadequate. Recent progress in various single cell and population-level analytical techniques offers an opportunity to bridge this knowledge gap.

With this OCB Scoping Workshop proposal, we seek to bring together people with complimentary interests in observing and/or modeling specific traits and physiological characteristics and rates in marine plankton. The objective of this workshop is to summarize our current state of knowledge of physiological observations in single cells and taxonomic populations from the environment, identify impediments to filling this knowledge gap (including data handling and analysis), and discuss future research areas.

2. Scientific Justification:

An important goal of marine biogeochemists is to quantify the rates at which elements cycle through the ocean's diverse microbial assemblages. Traditional views, such as phytoplankton being producers and bacteria being consumers, or that ecosystem function can be resolved by defining plankton functional groups, have been found to be overly simplistic. Indeed, environmental metagenomics is discovering new and important microbial metabolisms and diversity at an accelerating rate. Many nutritional strategies previously attributed to one microorganism or functional group are also carried out by other groups. New analytical and modeling approaches will be required to tease apart which organisms are doing what, at what rates, and how this translates to ecosystem-level processes.

Ecosystem and biogeochemical models are our primary method to make integrated projections of the future state of the ocean. However, a major challenge is incorporating planktonic biodiversity in a manner other than descriptive functional groups (e.g, diatoms, N2 fixers, calcifiers) that, in the light of recent discoveries, may not be adequate. In part this challenge

exists because we lack the needed direct observations on physiological characteristics and rates to improve our understanding, and the detailed understanding of how much biodiversity needs to be included to maximize model utility. That is, do we need to model 2, 20, or 200 'species' as representative of a functional group to understand the role of that functional group?

In the open ocean it has been presumed that the environment selects for ecological fitness. However, it is becoming increasingly clear that while there is taxonomic diversity (i.e., genetically determined variability) within a taxa, there is substantial plasticity in physiology and traits, also modulated by the local environment, that likely serves as an equally important control point on biogeochemical cycling. For example, single cell genomics studies have discovered the prevalence of genome streamlining, metabolic specialization and genetic microdiversity among bacterioplankton, suggesting that diversification rather than physiological plasticity may be the predominant mechanism of their response to environmental changes. Evidence is accumulating that demonstrates different lineages of marine plankton have fundamentally different elemental ratios, yet cellular quotas and ratios can also vary in unexpected ways across environmental gradients within lineages. Similarly, it is increasingly observed that the response of taxa to the same environmental stress is related to their prior environmental 'conditioning' and genetic content. Recent evidence has shown that discrete planktonic lineages in situ have more efficient metabolic capabilities (e.g., lower nutrient uptake saturation parameters for specific macronutrients) than those same lineages in culture, and these uptake parameters change with environmental conditions. This type of information needs to be captured for successful integration into new ocean ecosystem models (e.g., DARWIN Project models), and we are beginning to see reports of direct in situ observational information from natural populations at this level of biological resolution.

Despite this growing body of knowledge and use of more complicated models, our understanding of the relative importance of taxonomic diversity versus physiological plasticity and the relevant level of biodiversity to include (or not) remains inadequate. The overarching goal of this scoping workshop is to fill this substantial gap in our understanding.

We propose an OCB scoping workshop around two interrelated broad themes:

- 1) Taxon-specific physiological responses of marine microbes and phytoplankton in the current and a changing environment;
- 2) Interactions and feedbacks between plankton physiological plasticity and taxonomic diversity and the impact on global ocean biogeochemical cycles

The focus of this workshop is to synthesize information that employs single cell methods (e.g., single-cell X-ray fluorescence, NanoSIMS) and methods that resolve discrete populations (e.g., flow cytometric sorting and secondary analyses), identify unique biological traits at these levels, and determine what those traits mean to ocean biogeochemistry now and in the future. The success of this workshop will come from bringing together scientists with a broad set of skills and points of view, with the overarching focus on the use of single cell-level analyses in ecosystem-level models.

We suggest breakout groups related to the above themes centered on the following questions.

- 1) What are the ranges and controls on taxon-specific nutrient uptake rates, elemental quotas and standing stocks?
- 2) Do taxonomic diversity and physiological plasticity have similar or different impacts on ocean biogeochemistry, particularly the production and export of particulate organic matter from the surface ocean?
- 3) What roles do taxonomic diversity and physiological plasticity play in governing the response of planktonic communities to environmental stressors, for example, oxygen minimum zones, ocean acidification, ocean warming, stratification and changing nutrient concentrations?
- 4) What type of single cell-specific and population-level data is required for the mechanistic understanding and predictive modeling of marine biogeochemistry? How does it compare to the data collected now? Can we recommend improvements?

Each breakout group will be tasked with collating information on several points to further enhance the continuity between groups and relationships to the broader themes. Specifically, each group will 1) summarize current state of knowledge; 2) identify limitations to forward progress on the topic; and 3) describe appropriate and suitable sampling platforms and technologies, and 4) propose strategies for moving the field forward.

3. Objectives and Products:

The proposed workshop aims to provide opportunities for the ocean science community to formulate a forward-looking vision on future research directions role of genetic diversity, physiological plasticity and evolutionary processes in marine biogeochemical cycles. The workshop will be used as a forum for community input and review of data published in this rapidly developing field, as well as a venue to develop new approaches to building future research directions.

The workshop will result in several tangible products:

- **1)** A formal workshop report to the OCB Office in the form of a white paper that communicates the vision and forward-looking priorities for this research.
- 2) An article for EOS highlighting the workshop conclusions and vision at a high level.
- **3)** A detailed review publication in *L&O*: *Review, Frontiers in Aquatic Microbiology,* or similar journal.
- **4)** Collaboration with BCO-DMO to make available the synthesis data set on all 'quality controlled' published data that can be used as the contextual basis for future research and a baseline for evaluating change in the future.
- **5)** Identification of key topics and possible speakers for a special session at the 2015 Aquatic Sciences meeting, potentially to include a town hall meeting to discuss the outcomes of the meeting and future research avenues.

4. Workshop Logistics:

Workshop size will be restricted to 50 people to keep discussions focused and allow appropriate size for breakout groups (12-14). We plan a balanced attendance from senior

researchers to graduate students. We plan on inviting ~10 researchers to give plenary talks through the meeting, to be the discussion leaders for the breakout groups and/or members of the organizing committee. These invitees will be tasked with giving a broad and insightful tutorial talk on a topic germane to the breakout groups. The remaining participants will be split between active researchers (~20), post-doctoral scholars (~15) and graduate students (~5). The rationale for the balance it to get different points of view but also to engage more junior scientists in the dialogue that we envision will result in new collaborative programs and proposals. The process for selecting non-invited participants will be via completion of an online short-answer survey. We will ask potential participants to answer questions such as "why do you wish to participate?", "how does your past research relate to the objectives of the workshop?", and "how will participation enhance and advance your future research objectives?" The answers to these questions will be evaluated by the conveners and organizational committee so as to provide the broadest level of community participation possible.

The workshop will be held in mid-May (tentatively) in East Boothbay, Maine, hosted at the Bigelow Laboratory for Ocean Sciences. A proposed scenario, pending agreement on dates, is that participants would travel to Maine on Monday, with the formal meeting commencing Tuesday morning and going through Thursday afternoon, with participants departing on Friday. The general structure of the workshop will include a plenary session with two talks related to the breakout session topic of the day each morning. Speakers will be asked to provide a short outline of their talk highlighting the main themes and their ideas on future directions prior to the meeting that will be shared with all participants. Following the breakout groups discussion, all participants will return to plenary where a presenter from each group will summarize the answers discussed by each group. At the end of the workshop, the plenary talks, written summaries for each breakout group, and any other workshop materials will be posted on the website for reference and to stimulate thinking about future research directions by participants. The last afternoon of the workshop will be devoted to synthesis of the workshop conclusions and drafting of the workshop summary report.

5. List of proposed attendees and roles:

| Name | Affiliation | Role | |
|----------------------|----------------------|-------------------------------------|--|
| Michael Lomas | Bigelow Laboratory | Convener | |
| Ben Twining | Bigelow Laboratory | Convener/Speaker (BG#1) | |
| Ramunas Stepanauskas | Bigelow Laboratory | Convener | |
| Ben van Mooy | WHOI | Organizing Committee | |
| Sarah Fawcett (PD)* | Princeton University | Organizing Committee/Speaker (BG#2) | |
| Alison Taylor | UNC-Wilmington | Organizing Committee/Speaker (BG#3) | |
| Adrian Marchetti | UNC-Chapel Hill | Organizing Committee | |
| Michael Follows | MIT | Organizing Committee/Speaker (BG#4) | |
| Adam Martiny | UC-Irvine | Breakout Group #1 leader | |
| Craig Carlson | UC – Santa Barbara | Breakout Group #2 leader | |
| Barney Balch | Bigelow Laboratory | Breakout Group #3 leader | |

| Nick Record | Bigelow Laboratory | Breakout Group #4 leader | | |
|-----------------|------------------------|---------------------------|--|--|
| Sallie Chisholm | MIT | Speaker (BG#4) | | |
| Mike Landry | Scripps Speaker (BG#2) | | | |
| David Hutchins | U. Southern California | Speaker (BG#3) | | |
| Edward Laws | LSU | Speaker (BG#1) | | |
| Heather Benway | WHOI-OCB | OCB Office Representative | | |
| Scott Doney | WHOI-OCB | OCB Office Representative | | |

^{*} PD = post-doctoral scholar

6. Preliminary Budget and justification:

| People | Expense | \$ Cost /pp | \$ Total | Comments |
|-----------------------|------------------|-------------|----------|----------------------------|
| External Participants | airfare | \$500 | \$15,000 | 30 organizers, invited |
| | | | | speakers, students, post- |
| | | | | docs |
| 4 nights | Hotel | \$125 | \$20,000 | 40 external participants |
| 4 days | Meals | \$65 | \$10,400 | 40 external participants |
| Bigelow attendees | Meals | \$50 | \$2,000 | 10 participants |
| Services | | | | |
| Bigelow Site Fee | Conference fee | | \$6,000 | Includes breakout rooms |
| Transportation | from/to Portland | | \$1,950 | 3 trips/day on arrival and |
| | to BBH | | | departure days |
| | from/to Hotel | | \$3,000 | 2 trips/day |
| Supplies | | | \$ 1,000 | Name badges, |
| | | | | photocopies, notebooks, |
| | | | | etc. |
| Total Meeting | | | \$59,350 | |

For this workshop we are planning on participation of 40 scientists not resident at Bigelow Laboratory for Ocean Sciences, and approximately 10 scientists from Bigelow Laboratory. Of this, 10 will be invited speakers/organizers/breakout leads, 10 other faculty, 15 post-docs and 5 senior graduate students. The reason for the diversity is to build a diverse community at the meeting. Based upon experience, flights will likely be ~\$500 each and we propose to pay airfare for invitees, post-docs and students (total of 30). All meals for non-Bigelow participants will be covered, for Bigelow attendees, only lunch and dinner will be covered. Housing will be provided at Fisherman's Wharf hotel in Boothbay Harbor.

Conference Fees are budgeted at \$40/person/day. This covers wireless access, additional cleaning staff time, IT support for web page application step, time for person coordinating travel, etc. Portland International is the nearest major airport and transportation will be provided from/to the airport, as well as a travel to/from the hotel twice each day of the workshop. Lastly, \$1,000 is budgeted for meeting supplies.