Pump it Up- workshop report

Sept. 28th (Cape Cod Resort) and Sept. 29th (WHOI Quissett Campus), 2017

Summary

A 2-day workshop was conducted to trade ideas and brainstorm about how to advance our understanding of the ocean's biological pump. The goal was to identify the most important scientific issues that are unresolved but might be addressed with new and future technological advances. The timing was intended to spur new collaborations and identify the most promising advances that could be developed into proposals either for internal support, such as the Oct. 15th WHOI Technology Awards, or for the Oct. 16th planning letter deadline for NOPP, or other programs at federal agencies or with private funding sources.

The format included short "chalk talks" (no slides with a white board) as well as brainstorming on day 1 and breakout groups and synthesis of ideas on day 2. This report will summarize who attended, the talk topics, and provide a short synopsis of what came out of the Day 2 breakout groups who were charged with moving from motivating issues, to key scientific questions, and then the technological challenges and engineering solutions to address the science questions.

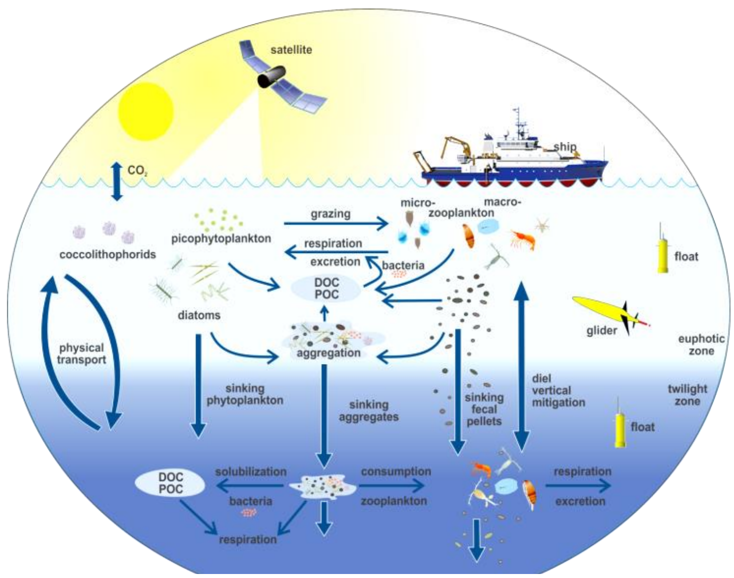

Figure 1 Depiction of biological pump pathways and measurement platforms

Day 1

Twenty-five participants joined in discussion at the Cape Cod resort (list below) from all 5 WHOI Departments and including 5 non-WHOI attendees. The 20 chalk talks were presented using a small white board to convey to the group their individual interests. The experience using a white board vs. powerpoint slides proved very engaging, with some speakers including props to "show and tell" their inventions and one using a video screen to show results from an in-situ study of fluid and particle motion associated with larvaceans in Monterey Bay.

Attendees, affiliation and chalk talk topic

Name	**Dept/Inst**	**Chalk talk topic**
Ken Buesseler	MCG	Particle flux and sinking rates
Melissa Omand	URI	Particle properties and rates using small, cheap Lagrangian platforms
Heidi Sosik	BIO	Population dynamics and community structure using plankton imaging systems
Scott Gallager	BIO	Benthic-pelagic coupling studies with controlled "cube" experiments
Meg Estapa	Skidmore	Global controls on biogeochemical flux with distributed profiling floats
David (Roo) Nicholson	MCG	Biogeochemical patchiness seen in O2 variability in surface/subsurface
Dana Yoerger	AOPE	Lagrangian platforms and key ways to track and image particle motions
Virginia Edgcomb	MGG	Getting at rates of biogeochemical transformations and metabolisms using in-situ experiments
Annette F. Govindarajan	BIO	AUV for sampling and genetic biodiversity assessments of metazoans
Alex Frank	MCG	POM-DOM lability and quality
Kevin Ulmer	Seaquester, Inc.	Biotech and particularly genomics, including microfluidics for understanding TZ biodiversty
Jon Hunter	MCG	Microscale POM Degradation tracked by single particle nano-lipidomics
Gareth Lawson	BIO	Zooplankton, micronekton and active carbon flux: bio-acoustics and related approaches
Kakani Katija	MBARI	Animal-fluid-particle interactions in the sea and how to measure them using PIV
Amala Mahadevan	PO	Thinking about scales of export and building simple models for predictions
Matthieu Dever	PO	Tracking of particle trajectories and sources in models
Hilary Palevsky	MCG	Importance of understanding vertical variability in NCP and export
Allan Adams	MIT	From high end photography to low cost camera systems as tools waiting to be exploited
Jason Kapit	AOPE	Repackaging optical technologies for in-situ biogeochemical studies
Mike Jakuba	AOPE	CLIO as a new tool for biogeochemically-clean sampling from an autonomous vehicle
Tristan Horner	MCG	Particulate trace metals as mesopelagic process indicators
Jim Bellingham	AOPE	Lessons from biological and physical process experiments
Yuehan Lu	U Alabama	
Chris Rauch	AOPE	
Eyal Wurgaft	MC&G	

Day 2. Breakout groups

On day 2, two groups were formed around the overlapping themes of:

<u>**Group 1**</u>

a) Tackling Patchiness and Scales of the biological pump: Distributed observing with low cost platforms and sensors
b) Particle Sinking Speeds

<u>**Group 2**</u>

c) AUV sampling
d) Biodiversity in the mesopelagic: observing complexity and particle-organism interactions and transformations using imaging, bio-acoustics, and genomics

Each group was tasked to discuss:

1. Motivation/big picture reasons to study
2. Key science questions
3. Challenges in answering those key questions
4. New technologies that can help address key questions.

The following summary integrates the key points from both groups:

1. Motivation, big picture

- Importance of the biological pump for the ocean C cycle and its role in climate
- Need to know how the biological pump will change due to – climate, ocean acidification, plastic pollution, overfishing
- Urgency - overfishing – recent estimates of a much larger mesopelagic fish biomass (Irigoien et al.) needs to be confirmed but commercial interests are advancing
- open ocean aquaculture/ranching (tuna ranching)
- Naval applications – marine mammals
- Policy – high seas/international waters
- Biochemical pathways; possible pharmaceuticals from marine organisms
- Geochemical cycling- trace metals/micro nutrients and proxies in seafloor sediments
- Education, training, and public outreach – to further science goals and capture the public's attention (cool animals etc)
- Basic/pure science, so much we don't know, last frontier

2. Key science questions

2a. Regarding biological pump variability, key questions include:

- How patchy is the biological pump in space?
- How quickly does the biological pump vary?
- What are the processes that drive this variability?

2b. Regarding particle sinking speeds, key questions include:

- What are particle sinking rates?

- What are the associated elemental fluxes that are important for geochemical mass balances?
- What are the controls on particle sinking rates – physical mechanisms, biological/community structure, particle morphologies, chemical composition/density?

2c. Regarding biodiversity in the mesopelagic, key questions include:

- Can we characterize POM, substrates, transformations; history (source) and residence times, link particle abundances to biological processes and physical properties?
- What are the implications for processes that shape C and other nutrient cycling of environmental shifts (e.g., O2, temp, pH, currents) associated with climate change?
- In carbon models, what are the relevant variables, carbon fluxes; deep ocean carbon budget – more respiration than can be accounted for so where does the missing carbon/energy come from?
- What are the predictive powers of models - need better understanding of different processes that affect carbon cycling before we can predict; what is their relative importance and under what conditions?
- What organisms (all domains of life) are present at different depths and locations? How do communities and the rates of their transformations of carbon and other nutrients change with conditions, depth, and time?
- Are there barriers to community connectivity?
- What are the basic ecological interactions, life history; implications for fisheries?
- How will the biological pump adapt to changing conditions?
- Can we link biodiversity (microbes to whales) to chemistry, fluxes?
- Quantify rates of active carbon flux by zooplankton and micronekton
  What are the roles of signaling molecules and regulation of degradation of captured POM; quorum sensing?

3. Challenges

3a. Under the topic of biological pump variability and scales, the challenges include

- Trade-off between lots of platforms vs. how fast they sample through fields
- Illumination is as important as cameras
- Small/cheap vs. multi parameter AUV's
- Long term- biofouling will be an issue (less so if disposable/short duration systems)

3b. Under the topic of particle sinking rates challenges include:

- Fluid motion vs. particle motion vs. vehicle motion- how to separate these?
  - This topic generated significant discussion and possible solutions to resolve background particles from sinking one's through combinations of reduced vehicle motion to data processing, adding reference sinking beads, etc.
- Passive floats- isopycnal to reduce motions
- Materials, drag will be important in vehicle design to keep Lagrangian
- Need precise measurements of flow or not? Essentially can we correct for flow cheaply? Not likely?

- Camera systems are becoming small, cheap, disposable
- Reference particles/beads needed at least for testing our ability to quantify sinking rates in-situ
- Need systems that can measure sinking velocities in range of 10 m/d to 100's m/d;
- Sinking particles are rare (few per cubic meter; few in traps over several days) so hard to quantify

3c. Under the topics of biological diversity and processes, challenges include

- Contamination – biological and chemical
- Conducting in situ studies of rates of microbial processes. Need development to provide for adequate experimental replication of incubation studies (4-12 chambers). In-situ analyses require monitoring environmental parameters during the experiment (temperature, oxygen, etc.) inside the incubation chambers; stirring of chamber contents. Developments are required to implement optimal sensors for doing this on a cabled or a moored instrument.
- Biofouling
- Scaling up – operationally (sampling) spatially and temporally – Different instrument platforms are required for different goals -pumping and preserving large volumes of water vs. high-resolution spatial sampling on an AUV, vs. moored vs. cabled sample collection, collection of whole water vs. filtered water samples, etc. Instrumentation should be affordable.
- Combined application of acoustics (including new broadband methods), nets (large, to sample sparse, fragile, and agile micronekton adequately), and optics
- Connect metagenome, transcriptome, and imaging
- Maximal information per effort, operationally – data for chemists, biologists; linking relevant metadata for people looking at data from other perspectives/disciplines; getting the most information out of your data; (solution - interdisciplinary planning)
- Informatics – need experts in informatics to handle big data; metagenomics/metagenetics; Joint Genome Institute expertise (operated by Dept of Energy); informatics infrastructure; high performance computing; education/training in BOTH science and informatics; reluctance by NSF to train in informatics because they end up going to industry; not enough in science
- Funding – diversify sources

4. New technologies and advances

4a. New technologies and advances that can help us capture spatial scales include:

- Modularity will be important for rapid development
- Cameras – low cost; generic; 3D PIV (particle image velocimetry)
- Illumination- LED, etc. is becoming easier and cheaper
- Disposable- what is the cost? ARGO was considered upper end cost- about $20K, but ideally many of the systems discussed would be <$100-200
- Affordable instruments for replicated in situ incubation studies of microbial processes
- AUV-mounted high-resolution (hundreds of samples) water sampling to get information on distribution of microbial diversity and processes, and information on time-sensitive events (e.g. blooms).
- Off the shelf parts are now available cheaply from other technologies (i.e. phones)
- New materials being developed to deal with biofouling- "slips" technology at MIT

- Use larger/complex AUV's to ground truth cheap/small devices
- Can we build a generic low cost camera module with laser & camera
- Imaging across many size scales can be done with good bulk optical properties and ways to ID particles that are already being developed

4b. New technologies and advances that can help address key questions related to particle sinking rate include:

- Camera systems – 3D PIV; slit camera; particle tracking
- Lighting is important, but will need to be cycled on/off
  - don't want to attract animals; power consumption issues
- Reference particles/beads & technology for dispensing (vortex generator)
  - Bead coatings for enhanced visualization
- Small passive floats are being developed for multi depth & space deployments
- Larger complex instruments for multi-platform comparisons
- Velocity of platform through water – sensors available to do this cheaply
- Schlieren imaging- low cost/in situ method of imaging motions

4c. New Technologies and advances related to biodiversity can be broken down into:

- Autonomous samplers such as SUPR samplers to link particles, biology
  - SUPR samplers – need to prevent contamination; metal and biotic; the requirements may be orthogonal; quantify contamination
  - CLIO samplers
  - SID samplers for moored or wired sampling for in situ incubation studies and for filtering water samples and preserving them in situ or for collecting and preserving whole water samples.
  - operations – on deck – metal tools, people; clean room on the ship
  - inexpensive single use filter holders that are prepped on shore to reduce need for handling on ship; injection molding for mass production of filter holders
  - Preservatives- Different preservatives for different downstream applications; but some preservatives destroy the sample for other applications – is there a one size fits all preservative? Probably not; preserve different particles in different ways; - Clio – RNALater but other preservatives can be used; one preservative type per filter holder; but multiple filter holders
  - Consider Sterivex filters (currently used for microbes)
  - Adaptive sampling; relative to sensed data, images
  - Combine with zooplankton/micronekton sampling as some these organisms may not be adequately sampled by AUVs (although they may potentially leave an environmental DNA signature).

- Isolating and characterizing particles
  - Isolate particles – currently done manually and is very labor intensive; can it be automated? And the individual particles preserved? Monitor the particle while it is being handled;

- Laser capture microdissection – take material (particle) and specifically remove things that you optically identify for subsequent molecular analysis;
- nanotechnology
- See and measure particles in situ, and then measure the rates; and then give data to physical oceanographers; how representative are samples, for scaling up
- In situ, multi-parameter imaging flow sorter with micro encapsulation and preservation; imaging, classification; other optical parameters – scattering; chlorophyll content; can do 1000s of particles of second; get from the microfluidics community; micron-sized to mm sized; "mesofluidics"; depends on inlet aperture; could be done in mesopelagic
- SOMAmer Slow Off-rate Modified Aptamer (modified nucleotides to measure specific analytes); challenge in applying to seawater

- Genetics/genomics
  - Metagenetics (which targets one or a small number of marker DNA regions) for biodiversity assessments (i.e., DNA barcoding; metabarcoding; environmental DNA analysis)
  - Metagenomics – comparisons of whole organism genomes and identification of genes of interest
  - Metatranscriptomics – comparisons of expressed genes (mRNA)
  - Benchtop and in-situ, real time DNA sequencing using Nanopore technology; incorporate into an AUV - existing technology (i.e., from the ESP) can help on the sample processing engineering; match capabilities (i.e., sequencing results and analysis; targeted DNA markers) to AUV capabilities

- In-situ incubation studies
  - Software and engineering adjustments to current designs to allow for replicated studies – e.g., 4-8 chambers instead of 1-2
  - Moored deployment platforms

Concluding comments regarding the Pump it Up workshop

The workshop brought together a diverse group of WHOI and outside chemists, biologists, physical oceanographers, and engineers with the intent of stimulating new collaborations and research directions for tackling knowledge gaps in our understanding of the biological carbon pump. Technological advances, many of which are spearheaded by WHOI personnel, in wide-ranging disciplines including AUV sampling and particle tracking, optical and acoustic sensing, genetics, and microfluidics are providing new opportunities to address key questions such as the temporal and spatial scales of biological pump variability, particle transformations, and particle sinking rates, and the biological, chemical, and physical factors that control these processes. Several challenges were identified including sampling platforms constraints (quantity vs. sampling speed), bringing down the cost of sampling and sensing AUVs (and increasing their number to enhance coverage and replication), developing and improving *in-situ* experimental chambers, maintaining contamination-free parts and work areas for highly sensitive chemical and genetic analyses, and obtaining the informatics expertise and infrastructure required for analysis of big data.

Looking ahead at funding opportunities, the workshop considered WHOI internal funding opportunities, such as the upcoming Innovative Technology Awards (Oct. 15), as well as the Catalyst program, Translational Research awards and possible new Research Opportunity funding. Outside of WHOI, an upcoming NOPP deadline (Oct. 16, moved to Oct. 23rd) was seen as a possible target for contributing larger, sustained efforts (3yr) that was of interest to several participants. The OTIC program at NSF and other agencies (ONR) and Foundations (GBMF) are also possible avenues for support.

Overall, given the many compelling and urgent motivations (e.g., climate change, exploitation of resources), it is hoped that the cross-fertilization of ideas from the workshop stimulates new collaborations, proposals, and research directions. From discussion at the workshop summarized in this report, and post-workshop progress on new collaborative projects, we think we made significant progress in achieving these goals. The increased attention and motivation for study of the biological pump is leading to new opportunities and programs at the national and international level, for example NASA's recent support of EXPORTS, and NSFs investments in new mesopelagic instrumentation, via the MRI and OTIC processes.

We should take advantage of this broader interest and use internal support at WHOI for funding risky new technologies that in the past have led to larger externally funded projects. This can be done by increasing internal funding opportunities and/or focusing existing ones on directed calls along specific research themes, such as the biological pump. In addition, WHOI could consider adding Postdoctoral Fellowship support that is specific to research themes, such as this one. This would allow us to bring in a new generation of engineers and scientists to work with existing staff on some of the key questions identified in this workshop. Such appointments serve as an important pathway on to the WHOI staff that would allow us build and maintain leadership in this field for decades to come. This pathway also enhances external collaborations by bringing in the best new scientists and engineers from other Institutions, and after postdoctoral level training, seeding the community with WHOI trained personnel. Understanding the ocean's biological pump takes interdisciplinary science and cutting edge technologies and we are well poised to make considerable contributions to this field.