Sampling Requirements from the CANON Sampling Workshop Apr. 15/16 2010

A set of questions was determined prior to the workshop and 1 to 2 engineers participated in each of the five case study breakout sessions. See below for the questions/answers gathered at the workshop.

Case Study #1: Phytoplankton blooms/HABs (Sosik/Scholin):

1) **Briefly summarize the research topic and goals.**
   a) Identify and quantify specific species in space and time.
   b) Include the ecological context in which the bloom occurs.
   c) Look for the unique opportunity to do something different.
   d) Goals are well encapsulated in ECOHAB project.

2) **Briefly summarize the role of water sample acquisition in this research.**
   a) We require discrete samples for this work. Many properties are too complicated and expensive to measure in-situ (e.g. copper, iron, nickel, nutrients). We don't always know what we're looking for, and even when we do, we need to ground truth in-situ measurements.

3) **What is the target measurement of the water sample (organisms, dissolved material, particulate material)?**
   a) The target is organisms - the community of viruses to bacteria to "target species", but probably not including fish.

4) **Are there multiple target measurements?**
   a) Yes, we need community context of metals and nutrients in addition to target species. Big challenge is stabilizing samples on instrument for analysis later. We want to co-locate measurements to ensure they're from the same parcel of water. This could be done by replicating ports or by taking a large sample and splitting it later.

5) **Where should samples be acquired (environments, depth(s))?**
   a) Context dependent. Sometimes it's more important to sample in a strategic place (e.g. harbor entrance) that sees a lot of water moving past even if it doesn't help constrain the model. Then you sample in more places to locate the source. Sometimes integrative organisms, such as barnacles, can show HAB byproducts before cells are seen. Depth varies, e.g. in Monterey Bay you need to constantly adjust your depth in water column in response to internal waves.

6) **When should samples be acquired?**
   a) We want to sample all of the time for research, but in specific times and places for resource management.

7) **Time of year, time of day.**
   a) Time of year - seasonal, time of day - all.

8) **Temporal resolution (frequency of sampling)**
   a) Context dependent. For some, once a day is plenty; for others, every few hours may be required.

9) **Duration of sampling program (time-series requirements)**
   a) Resource management requires ongoing sampling.

10) **What determines when a sample should be acquired (fixed schedule, response to environmental conditions, etc.)?**
   a) You want a combination of strategically located integration along with adaptive sampling at other places when things get interesting. Need to sample autonomously based on prevailing conditions as opposed to a fixed schedule.

11) **Does sample collection need a moving platform or occur at a fixed location, both?**
   a) Need both moving and fixed platforms. Ideally a platform could stay in one place until commanded to go elsewhere. Nice to have synoptic sampling at an array of sites. Technology can't be so expensive that you can only afford one sampler. Be careful not to adaptively over-sample a small, interesting area while you miss the big picture.

12) **What methods will be applied to the sample?**
   a) Cytometry (optical) and DNA analysis (molecular probes).

13) **What volume of whole water is needed per sample?**
   a) Cytometry - 15 ml/hr, Toxins - 500 ml, Alexandrium - 1 liter, HABs in Monterey Bay - 1 liter. All samples on the order of 1 liter. No great need for whole water; filtrate is okay.

14) **What are the requirements for sample purity? (Must each sample be acquired in a pristine container?)**
   a) Samples must be preserved with fixative agent. Stabilized particles highest priority, then absorbed DOM on resins, and lowest priority for whole water. Would also like to acquire mass of concentrated cells that can be pushed intact downstream, i.e. pre-concentration followed by manipulation.

15) **Must the sampling system avoid sample exposure to specific materials?**
   a) Target organisms are not very sensitive to materials used in collection system. Peek, stainless steel, buna, glass, teflon, all okay. Must be much more careful when sampling for trace metals or nutrients.

16) **How rapidly must sample collection occur (sip, slurp or gulp)?**
   a) Gulping is more desirable than sipping, but both are needed at some time. Gulping fits with traditional methods of collection in this work (e.g. Niskin bottles). Sipping gives greater temporal and spatial resolution, but must be careful about only sampling from within boundary layer.

17) **Must the sample be processed immediately? If not how do you maintain integrity (preservation, filtration, reagent addition)?**

a) Integrity preserved by concentration and then fixation. Can accumulate, then preserve, as opposed to zapping during collection.

18) How soon following acquisition must a sample be analyzed to be useful (i.e. if there is a HAB knowing the organism after a month does not help)?

a) Need analysis within hours to a day. A couple of days at the most when required by sampling system, such as long-range AUV.

19) Is there a need to archive samples? If so, at what stage of processing?

a) Yes, samples are archived as they are acquired.

20) What contextual data are required for the sample data (e.g. in situ CTD and chlorophyll fluorescence, etc.)? What spatial and temporal resolutions are required for the contextual data?

a) Need traditional contextual measurements such as CTD, transmissometry, spectral backscatter, fluorescence, etc. But there's a good chance that other things not measured now may turn out to be important, e.g. ammonia, turbulence.
b) Poor performance of some sensors, such as nutrient sensors, may drive need to return whole water samples.

Case Study #2: Thin Layers (Donaghay/Ryan):

1) **Briefly summarize the research topic and goals.**
   a) **To understand the organization, dynamics and distribution of plankton in thin layers.**
   b) **Characterize the processes that go on inside these layers.**
   c) You may be interested in finding a different in concentration of 0.5%
   d) Early criteria was that if something appears in two profiles for more than two hours above some level, that is a thin layer. That means they were spatially and temporally coherent.
   e) **The gradients are the intereseting parts**
   f) **Track the evolution of these thin layers**
   g) Identifying the structure (these layers can be 10cm to 3m thick and up to 10km wide and at varying depths including very shallow and very deep)
   h) Really interested in the rates? Yes definitely
   i) These are huge production sources.
   j) Move horizontally at up to 20-30 cm/s and vertically up to 2 cm/s
   k) Would like high frequency sampling through the gradient.

2) **Briefly summarize the role of water sample acquisition in this research.**
   a) Multiple species? Who's there?
      i) Health?
      ii) Activity
      iii) Trophic linkages
      iv) Chemical environment
         (1) Nutrients
         (2) Allelopathy
         (3) Interactions
      v) Biological interaction

3) **What is the target measurement of the water sample (organisms, dissolved material, particulate material)?**
   a) phytoplankton, zooplankton, Microbe identification, Hydrogen sulfide

4) **Are there multiple target measurements?**
   a) Would like to high frequency sampling through the gradient.

5) **Where should samples be acquired (environments, depth(s))?**
   a) before and after the layer
   b) higher resolution through both gradients
   c) in the layer
   d) control samples needed in order to know the gradient that you're sampling

6) **When should samples be acquired?**
   a) At the peak of the layer and immediately adjacent water.

7) **What determines when a sample should be acquired (fixed schedule, response to environmental conditions, etc.)?**
   a) Purely by location in the layer and environmental conditions

8) **Does sample collection need a moving platform or occur at a fixed location, both?**
   a) Moving through/with the layer. XY fixed, but Z moving.
   b) The ideal would be to have a vertical view of the layer and moving with it.
   c) Both

9) **What methods will be applied to the sample?**
   a) Methods to discover species makeup, health, activity, trophic linkages, environment chemical analysis
   b) Microscopic examination/enumeration
   c) Particle characterization

10) **What volume of whole water is needed per sample?**
   a) 10ml to 1L

11) **What are the requirements for sample purity?**
   a) This is critical as the only water that is in the sample is from the location of interest. This is important if you are trying to implement some type of sample buffering scheme.
   b) Must the sampling system avoid sample exposure to specific materials?
      i) Must be careful of materials and reactions with samples as can affect results. (e.g. glass)
   c) Must each sample be acquired in a pristine container?

12) **How rapidly must sample collection occur (sip, slurp or gulp)?**
   a) Assuming you can target the proper spot, the acquisition must be quick because things are moving rapidly (gulp).
   b) Use constant flow-through for sampling.
   c) But this must be done without perturbing the surrounding environment during the same (driving through the layer).

13) **Must the sample be processed immediately? If not how do you maintain integrity (preservation, filtration, reagent addition)?**
   a) Yes, further decisions will have to be made based on in-situ analysis
   b) Sometimes things will need to be filtered and preserved.

14) **How soon following acquisition must a sample be analyzed to be useful (i.e. if there is a HAB knowing the organism after a month does not help)?**
   a) In situ, but is there a time constraint (TBD on the time).

15) **Is there a need to archive samples? If so, at what stage of processing?**
   a) Yes, but it is not practical to do it all analysis back at shore, so do as much as possible out there (in situ). There will always be a need to do some stuff back on shore (look for unexpected critters) so yes.
   b) You are going to take one sample and split it out to several processes.
   c) Micro Photographic/Video archiving has been very valuable.

16) **What contextual data are required for the sample data (e.g. in situ CTD and chlorophyll fluorescence, etc.)? What spatial and temporal resolutions are required for the contextual data?**
   a) Chemical

b) Internal Waves
c) Currents
d) Control samples

Case Study #3: Open Ocean Eddies (Church/Worden):

1) **Briefly summarize the research topic and goals.**
   a) Eddies are major zones of new production and export in the oligotrophic ocean. We want to track eddies through their life-cycle - maybe 6 months duration or so.
   b) Major species diversity and production appears to occur near eddie boundaries and especially at eddie-eddie boundaries, where shears and physicochemical gradients are greatest.
   c) Eddy formation is closely correlated with climatic conditions, hence changing climate could affect eddie formation and associated biological communities. Eddies are an obvious research target, since they can be readily sensed. Eddies are typically 150-200 kilometers in diameter, and frequently (always?) occur in cyclonic-anticyclonic pairs; this structure dominates the sampling strategy.

2) **Briefly summarize the role of water sample acquisition in this research.**
   a) It is problematic - and in some cases impractical - to definitively identify micro-organism species and other genetic markers in situ - hence the need for take-home water samples.

3) **What is the target measurement of the water sample (organisms, dissolved material, particulate material)?**
   a) Need to know species, nutrients, primary producers, secondary consumers, microbial/bacteria consumers, physical fields (salinity, temperature, current), rate measurements (primary production, nitrogen fixation...), respiration, export, mortality

4) **Are there multiple target measurements?**
   a) Yes

5) **Where should samples be acquired (environments, depth(s))?**
   a) Samples should be acquired within and between eddie and counter-eddie, always in the photic zone (< 300 meters depth).
   b) Mobile sampling platform would first sample along yo-yo transect of eddie pair to determine physical extent.
   c) Platform would then concentrate in the region between the eddie pair, as this is expected to contain the most biological diversity.

6) **When should samples be acquired?**
   a) Need knowledge of the time resolution of the eddy. More research and sampling is needed to understand Open Ocean Eddies before we can answer specific sampling questions. The answers also depend upon the specific science question being asked.

7) **Time of year, time of day**
   a) Dusk-dawn sampling may be requested for metabolic studies.

8) **Temporal resolution (frequency of sampling).**

a) See (6) above

9) Duration of sampling program (time-series requirements)

a) Want to sample an eddie throughout its lifetime - from one to six months.

10) What determines when a sample should be acquired (fixed schedule, response to environmental conditions, etc.)?

a) Sampling is largely dependent on spatial location rather than time. Most samples would be taken in the shear zone between gyres, in the photic zone.

11) Does sample collection need a moving platform or occur at a fixed location, both?

a) Mobile platforms needed.

12) What methods will be applied to the sample?

a) HPLC, oxygen isotope analysis, qPCR, incubation (in situ), flow cytometry (scanning and imaging).

13) What volume of whole water is needed per sample?

a) Some samples consist of "whole" seawater, perhaps with reagents added; these include nutrients,
   i) trace elements, DOC, gases (N2O, O2).
   ii) Need ~10s of ml per whole seawater sample.
   iii) Overall, want ~100 to ~1000 seawater samples per eddy.
   iv) Other samples are filtered residue; these include nucleic acids, organic carbon, particulate metals, DNA

14) What are the requirements for sample purity?

a) TBD

15) Must the sampling system avoid sample exposure to specific materials?

a) TBD

16) Must each sample be acquired in a pristine container?

a) TBD

17) How rapidly must sample collection occur (sip, slurp or gulp)?

a) TBD

18) Must the sample be processed immediately? If not how do you maintain integrity (preservation, filtration, reagent addition)?

a) Need at least 30 days sample preservation ("RNA Later" can preserve RNA for about a month, at least in colder water)

19) How soon following acquisition must a sample be analyzed to be useful (i.e. if there is a HAB knowing the organism after a month does not help)?

a) Not applicable

20) Is there a need to archive samples? If so, at what stage of processing?

a) TBD

21) What contextual data are required for the sample data (e.g. in situ CTD and chlorophyll fluorescence, etc.)? What spatial and temporal resolutions are required for the contextual data?

a) Density field (CTD), backscatter, chlorophyll fluorometry, NO3, pH, CH4/NH3, PO4, dissolved gases, O2, imaging flow cytometer, acoustic. Need highly-resolved temporal/spatial data in-situ, with some "confirmatory" water samples. Scanning flow cytometry.

Case Study #4: Low Oxygen (Letelier/Chavez):

1) **Briefly summarize the research topic and goals.**
   a) Goal 1: Are microbial diversity (and abundance) and oxygen correlated (in OMZs)?
   b) Goal 2: How does oxygen modify microbial processes/rates (in OMZs)?
   c) Goal 3: How do we measure/quantify the role of changing OMZs on air-sea greenhouse gas fluxes?
   d) Goal 4: How do we measure/quantify the role of OMZs on the biological pump?

2) **Briefly summarize the role of water sample acquisition in this research.**
   a) For Goal 1: to determine correlation of microbial diversity / abundance and oxygen.
      i) Waters samples (~1L in < 1 hr) to determine microbe abundance and diversity – need standard contextual information (T, S, O2, pH, optical backscatter)
      ii) Water samples (~50 ml < 1 minute) to determine abundance and contextual chemistry (nutrients)
   b) For Goal 2: How 02 modifies microbial process/rates
      i) Sample ~1L in < 1 hr for microbe transcriptomics 4-8 times per day
      ii) Sample ~1L in < 1 hr and return to lab in ~12 hours
      iii) Sample ~1L in < 1 hr and "incubate" in situ for 1-24 hours
      iv) Budget chemistry (nutrients, O2, carbon) in a moving "box" – need many 50 ml samples preserved.
   c) For Goal 3: How to measure/quantify the role of changing OMZ on air-sea greenhouse gas fluxes
      i) Large scale inventories of nitrate deficits and N2O – need fleets of autonomous systems (floats, gliders, AUVs) with standard contextual information (T, S, O2, pH) and ~ 50 ml for contextual chemistry (N2O, NO2, N2/Argon)
   d) For Goal 4: How to measure/quantify the role of OMZ's on the bio pump
      i) Collect sinking material at a fixed (i.e. sediment trap) and for rates return to laboratory in ~12 hours or incubate "in situ"
      ii) Collect sinking material from a mobile device and "as above"

3) **What is the target measurement of the water sample (organisms, dissolved material, particulate material)?**
   a) organisms
   b) contextual information (T, S, 02, pH, optical backscatter)
   c) sediment

4) **Are there multiple target measurements?**
   a) Yes,
      i) Example: nano molar resolution of O2

5) **Where should samples be acquired (environments, depth(s))?**
   a) air-sea interface
   b) omz interface (50 – 150 m depth)
   c) at points down thru the omz

d) at the bottom
e) Sampling along an iso-therm or isopycnal

6) When should samples be acquired?
a) TBD

7) Time of year, time of day
a) TBD

8) Temporal resolution (frequency of sampling)
a) TBD

9) Duration of sampling program (time-series requirements)
a) TBD

10) What determines when a sample should be acquired (fixed schedule, response to environmental conditions, etc.)?
a) TBD

11) Does sample collection need a moving platform or occur at a fixed location, both?
a) If cost was not an issue and possible to deploy a large number (>100) sediment traps, an array of strings of traps at levels down thru the water column would solve the sampling problem. Resolution down thru the water column (25m?) is TBD, resolution of across the OMZ is TBD.
   i) This is not cost effective. Typically you can afford 1 or 2 of them and this greatly challenges the sampling.
b) Also, you have very large/sharp gradients. Trying to sample at fine resolutions would require flexibility. Vertical gradients (chemical and biological) - can be measured from the ship - the trick is to follow the 'patch' of interest horizontally which is not addressed by the ship.
c) Autonomous vehicles optimize the characterization of the 3-dimensional field which leads to ideal sampling.
d) The desire is to 'follow' a population of bacteria. Can you follow the organism over time and measure the processes. CTD casts have the problem of sampling a different population.
e) The size of the patch to be followed is TBD.

12) What methods will be applied to the sample?
a) Difficult to tell hypoxic vs anoxic with the sensors (O2 precision issue)
b) There is gene expression for sulfide reduction but not detected sulfide
c) Water mass budgeting - need multiple samples. If we had to budget carbon, nitrogen, oxygen. o2, pc02, pH, total CO2, nitrate, (can't do nitrite NO2), NO2 needs innovation or sample return, phosphate as in indication of remineralization, trace metals....
d) Need physical measurements. Gases - nitrous oxide, nitrogen / argon would help determine rate processes.

13) What volume of whole water is needed per sample?
a) For transcriptomics: 4 to 8 times a day. At $10,000 per sample, there is a cost issue. Processing is done in a lab. 1 Liter, in about 30 minutes. for shipboard processing, 15 minutes (faster is better)

14) What are the requirements for sample purity?

a) Sample integrity must be preserved (this needs further clarification)

15) Must the sampling system avoid sample exposure to specific materials?

a) TBD

16) Must each sample be acquired in a pristine container?

a) TBD

17) How rapidly must sample collection occur (sip, slurp or gulp)?

a) TBD

18) Must the sample be processed immediately? If not how do you maintain integrity (preservation, filtration, reagent addition)?

a) TBD

19) How soon following acquisition must a sample be analyzed to be useful (i.e. if there is a HAB knowing the organism after a month does not help)?

a) TBD

20) Is there a need to archive samples? If so, at what stage of processing?

a) TBD

21) What contextual data are required for the sample data (e.g. in situ CTD and chlorophyll fluorescence, etc.)? What spatial and temporal resolutions are required for the contextual data?

a) T, S, 02, pH, optical backscatter

22) Notes and questions

a) How big are the OMZ (size range horizontal and vertical),
   i) How much variation across the OMZ – variation in temperature, o2, n2, n2o,
   ii) What variations are interesting (02, temp, s, ...)
b) How fast do the OMZ's change in size?
c) What happens to the envelope zone around the OMZ?
d) When the OMZ shrinks what happens (chemically, biologically. …)
e) Can a single device give rate measurements?
f) Is there a necessary requirement for gross oversampling to determine the proper sampling frequency (temporal and spatial)
g) Do we want/need dead cell detector / sensor to see live/dead ratios and rates
h) What is the role of far field acoustics with near field imaging
i) What preservation methods to preserve morphology

Case Study #5: Zooplankton Layers (Checkley/Vrijenhoek):

1) **Briefly summarize the research topic and goals.**
   a) Areas of study:
      i) Zooplankton as fish food (is food source fo fish changing over time
      ii) Role of zooplankton in the Carbon cycle (eating particles)
      iii) Zooplankton movement (population dynamics)
   b) Broader impact of zooplankton research
      i) indicators of ecosystem health
      ii) integrated ecosystem assessment: key species in food web
      iii) gene flow
      iv) community interactions/trophic transfer

2) **Briefly summarize the role of water sample acquisition in this research.**
   a) Water sampling is used to collect or 'sample' the zooplankton inorder to identify:
      i) the species
      ii) ecosystem functional group (e.g. grazer)
      iii) specific organism structures (i.e. body parts)
      iv) indicator species
   b) In addition, water sampling is used to relate the zooplankton sample to it's immediate environment

3) **What is the target measurement of the water sample (organisms, dissolved material, particulate material)?**
   a) Organisms, classified by size. Each size may require a different sampling strategy. The size classes are:
      i) microzooplankton (10-200 µm)
      ii) mesozooplankton (200 µm-10 mm)
      iii) macrozooplankton (10 mm-10 cm)
      iv) megazooplankton (>10 cm)
   b) NOTE: Whole organisms are preferrable but there are cases where fragments and/or biopsied samples are useful for taxonomic and/or genomic identification. Multi-resolution sampling (e.g. collecting both meso and micro zooplankton) may be required in order to examine trophic relationships.

4) **Are there multiple target measurements?**

5) **Where should samples be acquired (environments, depth(s))?**
   a) Everywhere...the consensus of the breakout group was that sample collection is a 2 step process:
      i) An initial survey, perhaps using sensors such as acoustics or images, is required to 'sniff' the environment to determine if sample collection is appropriate.
      ii) If the initial survey meets some mission criteria then 'sampling' should be undertaken

6) **When should samples be acquired?**
   a) See #5

7) **Time of year, time of day**
   a) See #5

8) **Temporal resolution (frequency of sampling)**
   a) This would be determined by the specific scientific experiment conducted during a mission. For low density organisms, towed nets may have issues with temporal resolution since they may need to integrate over a long time span in order to collect enough material for analysis.

9) **Duration of sampling program (time-series requirements)**
   a) Not specified

10) **What determines when a sample should be acquired (fixed schedule, response to environmental conditions, etc.)?**
   a) See #5

11) **Does sample collection need a moving platform or occur at a fixed location, both?**
   a) Both

12) **What methods will be applied to the sample?**
   a) Genomic analysis
   b) Taxonomic identification
   c) Organism counts via cytometry/microscopy

13) **What volume of whole water is needed per sample?**
   a) The sample volume depends on both zooplankton density and zooplankton size. Volume estimates are:
      i) microzooplankton - 1-2L
      ii) mesozooplankton - >2L
      iii) macrozooplankton - >>2L
      iv) megazooplankton – TBD

14) **What are the requirements for sample purity?**

15) **Must the sampling system avoid sample exposure to specific materials?**

16) **Must each sample be acquired in a pristine container?**

17) **How rapidly must sample collection occur (sip, slurp or gulp)?**
   a) This depends on target species. Would be determined by specific science mission and density of zooplankton population
   b) Aggregated groups of micro and mesozooplankton could be collected via gulp.

18) **Must the sample be processed immediately? If not how do you maintain integrity (preservation, filtration, reagent addition)?**
   a) This was not specified in the meeting BUT zooplankton predation/grazing/senescense might continue in the sample container and could effect the results (depending on the experiment's objective)

19) How soon following acquisition must a sample be analyzed to be useful (i.e. if there is a HAB knowing the organism after a month does not help)?

a) See #18

20) Is there a need to archive samples? If so, at what stage of processing?

21) What contextual data are required for the sample data (e.g. in situ CTD and chlorophyll fluorescence, etc.)? What spatial and temporal resolutions are required for the contextual data?

a) *in situ* CTD
b) Acoustics (for classfiying population, density, population structure and patch size)
c) Imagery/Video (Cytobot, Video Plankton Recorder (VPR), Video)
    i) The type of imaging depends on target size. Microplankton might require a in situ cytometer (e.g. cytobot), while megaplankton could be identified from a still camera or video
d) Bioluminescence detector