CMOP cruise plan
Oregon & Washington Coasts and Columbia River, Plume & Estuary
R/V New Horizon, cruise #NEW090829A
Aug 29-Sept 11, 2009

A. **Area of Operations**: Pacific Northwest coastal ocean from South Oregon (44 N) to North Washington (48 N), Columbia River plume, Columbia River and its estuary (Figure 1).

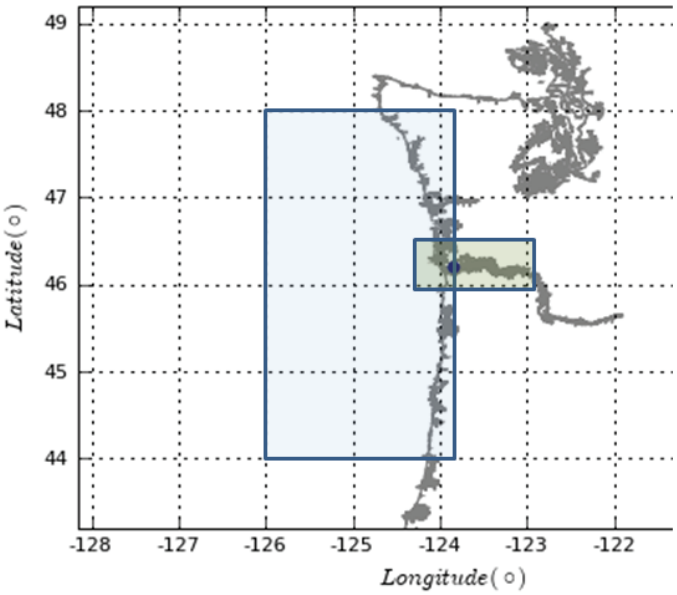


Figure 1. Map of area of operations. Blue box shows the coastal-offshore extent of our operations, while the green box indicates estuary and river sampling sites.

B. **Special notes/requests**:

1. We are aware that working in the estuary poses several challenges for operations. High current speeds mean that deploying a CTD rosette is only possible at slack tide or moderate currents; we plan to bring a CTD cage with weights to deploy within the estuary to capture samples throughout the tidal cycle and over depth. This will require swapping out the CTD hydrowire between the cage and the rosette two times (once from rosette to cage, once from cage back to rosette). Station keeping will be challenging due to swift currents. We will defer to the captain and crew to ensure safe operations.
2. We kindly request pumps for the conductivity-temperature-depth meter designed for use in fresh water.
3. We will be coordinating operations with the R/V Pt Sur during at least part of the cruise (Pt Sur dates = **Sept 1 – 14**).
4. There will be two days devoted to plume tracking; one of those surveys will occur in concert with Pt. Sur (C.S. Chris Wingard, OSU); both surveys will involve receiving coordinates approximately 12-18 h ahead of time.
5. We will have 3-4 incubators on the aft deck requiring flowing seawater.
6. If HighSeasNet is not reliable, we will request at least occasional access to traditional email exchanges; we are aware that we may incur additional costs for this service on a per-use basis.
7. We will have two instruments to be integrated into the ship's uncontaminated seawater flow-through system: (1) a flow-through flow cytometer (SeaFlow) and a reagent-based nutrient analyzer (Autonomous Profiling Nutrient Analyzer).
8. We kindly request copies of all calibration/information sheets for R/V *New Horizon* instruments.

C. Scientific personnel

Name	Institute	Role	Activity	Berth
Peterson, Tawnya	OHSU	Scientist	Chief Scientist, CTD, CH_4 oxidation	13
Prahl, Fred	OSU	Scientist	CTD, methane (GC)	15
Bender, Sara	UW	Graduate student	CTD, nitrogen use by phytoplankton	16
Blakely, Misty	UCSC	Graduate student	CTD, phosphorus cycling	20
Campbell, Vikki	OHSU	Technician	CTD, DNA/RNA	28
Durkin, Colleen	UW	Graduate student	CTD, silica cycling	17
Fortunato, Caroline	UMCES	Graduate student	CTD, BP, fixations	19
Gilbert, Melissa	OHSU	Graduate student	CTD, nutrient analyzer (APNA)	21
Law, Grant	OHSU	Technician	CTD; data management, data entry	18
Kahn, Peter	OHSU	Graduate student	CTD, primary production	23
Kass, Adena	OHSU/U Mich	Undergraduate student	CTD, chemistry, CDOM	31
Kuvaldina, Natalja	OHSU/Tallin Univ.	Graduate student	CTD, FlowCAM, phytoplankton fixations	24
Maier, Michelle	OHSU	Graduate student	CTD, FlowCAM, Electrasense	29
Manger, Josh	UCSD	Restech	Restech	27
McKibben, Morgaine	OSU	Graduate student	CTD, HABs	25
Morales, Yolanda	OSU/Treasure Valley Community College	Teacher	CTD, assist with chemistry or Electrasense	22
Reina, Justin	UW	Undergraduate student	CTD, Flow Cytometer (SeaFlow)	30
Prahl, Patrick	UO	Volunteer	CTD, chemistry, data entry	26
Wolhowe, Matthew	OSU	Graduate student	CTD, methane (GC)	
BERTHS FILLED				
BERTHS OPEN				

D. Watches

We need assistance from scientists in deploying and recovering the CTD - note that **5** people are required for this operation, 4 on deck and 1 running the computer and directing the winch operator. There will be only 1 shift for most of the cruise (with exceptions for time series); days will typically begin at 06:00 (Primary Production) and end between 6 and 9 pm. Note that while in the estuary there will be 30-min to hourly casts performed for 24 h for *Myrionecta rubra* sampling and methane time series. This will require an adjustment of schedules for those involved (see appropriate section for suggested teams).

Note that *New Horizon* requests that CTD handlers must be on deck when the CTD rosette is in less than 100 m of water. Work-vests are required during all deck operations, and sturdy shoes must be worn. You WILL get wet out on deck; rain pants or tall boots are suggested.

E. R/V *New Horizon* objectives and operations

I. Working Hypotheses:

Background. Biological productivity is greater along the Washington coast compared to the Oregon coast as viewed by satellite remote sensing and confirmed by in situ measurements. Recent work (eg. Hickey et al., in press) indicates that this is due primarily to the presence of plume waters present over the Washington shelf (Fraser and Columbia). The mechanisms responsible for enhancing primary production under this scenario are intuitive: enhanced buoyancy reduces deep mixing, thereby retaining cells in the euphotic zone. Nutrient supply either from rivers or from upwelling close to shore provides substrates for growth. However, the time scales of response by heterotrophic microbes and microzooplankton have not been well-characterized and the degree to which primary and secondary production are coupled is

not well understood. Moreover, the influence of dissolved organic substances (characteristic of land margin ecosystems) on microbial populations along river-influenced coastal margins remains poorly characterized. An inter-disciplinary team of scientists will therefore carry out an array of activities aimed at characterizing the dominant influences on the nature and function of microbial communities in a river and upwelling-influenced coastal margin.

Coast-Plume Hypotheses

H1. Inputs of dissolved organic substances (a) influence bacterial & primary production and (b) are related to the Columbia River estuary and plume outflow

H1a. Heterotrophic microbial productivity is most closely related to sources of organic nutrients, for example in the Columbia River plume. Rates will be higher off the coast of Washington compared to Oregon and will be related to water column stability.
Activity: measure rates of bacterial production and primary production along cross-shore transects off Oregon, Washington, and in the Columbia River estuary and plume.

H1b. Dissolved organic carbon compounds (as estimated by fluorescence characteristics of dissolved matter, colored dissolved organic matter, CDOM) will vary according to water mass (i.e. plume versus upwelled water) and may be related to rates of bacterial production.
Activity: Water will be collected and passed through combusted GF/F filters for on-shore analysis of 3-D CDOM fluorescence signatures of organic carbon compounds. The magnitude and spectral signature of the compounds will be compared across salinity gradients.

H1c. Gene expression patterns will reflect growth substrates such as dissolved organic carbon and nitrogen, and levels of organic matter (as estimated by particulate organic carbon/nitrogen and chlorophyll a).
Activity: Gene expression will be assayed by collecting large volumes of water and filtering through polycarbonate filters. Pyrosequencing of mRNA will reveal gene transcripts that will be related to functional enzymes responsible for microbial metabolism and growth.

H1d. Organic phosphorus utilization patterns differ among microbial taxa (e.g. dinoflagellates vs. diatoms) and therefore influence assemblage structure in plume-influenced waters.
Activity: Grow-out experiments will be conducted to determine the level of phosphorus limitation as indicated by alkaline phosphatase activity in different phytoplankton taxa.

H2. Microbial communities will be most complex (highest diversity) within retentive features such as Heceta Bank; since retention of coastal waters tends to be higher off Washington compared to Oregon, communities may different between the broad regions

H2a. Differences in microbial community structure will reflect differences in mixing versus retentive regimes.
Activity: determine assemblage structure of microbial populations (prokaryotes and eukaryotes) using broad-spectrum and specific genetic markers in regions with varying physical characteristics, for example Heceta Bank (retentive), coastal Oregon (advective environment), La Push or Grays Harbor (plume-influenced).

H2b. Toxin-production by harmful algae will be highest within retentive features such as Heceta Bank.

Activity: Algal assemblages will be assessed by microscopy and using the Flow Cytometer And Microscope, as well as by on-board analysis of PCR-amplified inter-transcribed spacer regions of the ribosome of *Pseudo-nitzschia* spp. Samples will be collected for particulate and dissolved toxin analysis (domoic acid, etc.).

H3. Fine-scale spatial variability in bacterial assemblage structure accompanies vertical density structure in the Columbia River plume.

Activity: Fine-scale vertical sampling in the plume (surface and base of plume, ca. 5 m) will be carried out using a pump system. Gene expression and the characteristics of microbial assemblages will be assessed relative to plume features such as tidal phase, wind-influence on plume dynamics, and plume volume.

Estuary Hypotheses

H1. *Myrionecta rubra* distributions in the estuary reflect a combination of behavioral adaptations and physical circulation features

H1a. *M. rubra* originates in oceanic waters and is concentrated in peripheral bays such as Baker Bay

Activity: Sample for *M. rubra* abundance over a 24-h period near the Astoria-Meglar Bridge (north channel) to ascertain which tidal phase and what water characteristics (e.g. salinity, dissolved organic nutrients) are associated with *M. rubra* dynamics.

H1b. *M . rubra* exhibits diel vertical migration patterns in the estuary.

Activity: 24-h sampling through the water column of *M. rubra* and cryptophyte abundance and environmental characteristics (nutrients, pigments, etc.).

H2. Methane concentrations reflect tidally-phased interactions between estuarine waters and the hyporheic zone, particularly near marshlands/wetlands.

H2a. As the tide transitions from high to low, drainage of the adjacent land masses provides a source of methane originating from sub-oxic waters.

Activity: Sample for dissolved methane gas over (1) a transect from Beaver Army Terminal to Astoria and (2) over an 18-h period while anchored near Cathlamet Bay

H2b. Methane oxidation rates in the estuary remove this source of methane before it reaches the sea.

Activity: Determine oxidation rates of methane (either fixed into biomass or oxidized to CO_2) by methanotrophs using a stable isotope tracer (13CH4) and 24 -48 h deckboard incubations.

H3. Microbial assemblage structure in the estuary reflects physical mixing as river waters interact with oceanic waters.

Activity: Water will be collected over a gradient in salinity (0, 5, 10, 15, 20, 15 PSU) and assemblages (both prokaryotic and eukaryotic) will be characterized.

II. Activities

1. **CTD casts and sensor-based measurements.** We will occupy a grid of stations over the Oregon and Washington continental shelf and slope (Fig. 1) to measure hydrographic (T, S, pressure), bio-optical (chlorophyll fluorescence, light transmission) and chemical (nitrate, phosphate, silicate, dissolved oxygen) parameters. An in situ nitrate sensor (ISUS) will be supplied by CMOP scientists and attached to the CTD rosette frame using stainless steel hose clamps. Based on operations in May 2009, the most feasible arrangement is for the ISUS itself to be attached to one side of the CTD-fluorometer package and the battery pack to be attached to the other side, below the Niskin bottles. We will combine in situ data with ADCP measurements of subsurface velocity to explore biophysical interactions.
2. **Water sampling.** We will collect water samples for DNA- and RNA-based microbial community analyses, measure uptake rates of carbon and nitrogen, and determine oxidation rates of methane by planktonic microbes. We will collect water samples for chemical analysis across environmental gradients in pelagic environments of the Columbia River, estuary, plume, and along established sampling lines on the Oregon and Washington coasts (Fig. 2). Some sampling will be coordinated with the R/V Point Sur to sample the Estuarine Turbidity Maxima (ETM) in the North and South Channels, and in the Columbia River Plume.
    a. **Chemical and biological measurements.** Microbiology samples (including DNA/RNA and chemistry) will be collected from three depths (surface [~2m], chl max [to be determined from the real-time profiles displayed on the lab computer during CTD casts], and bottom [bottom depth – 5 m]). Additional depths will be added to yield a more fine-scaled picture of vertical distributions of constituents of interest at select stations. These samples will be analyzed for a suite of chemical and biological concentrations (dissolved nutrients [nitrate, phosphate, ammonium, silicic acid], TDN/P, DOC, POC/N, SPM, Chl-a, HPLC pigments, flow cytometric cell counts, microscopic cell counts). Select samples will be analyzed for Colored Dissolved Organic Matter (CDOM) and dissolved CH_4 (at sites along a transect from outside CRmouth, into the estuary and upriver to Beaver Army Terminal (BAT)).
    b. **Rate measurements.** Most samples will be analyzed for bacterial production rate (using ^{3}H-leucine), and select samples will be analyzed for primary production rate (using $NaH^{13}CO_3$), nitrogen uptake rate (as $Na^{15}NO_3$, $^{15}NH_4Cl$, and 15-N labeled urea), and for biogeochemical rate processes (CH_4 oxidation [using ^{13}CH4-labelled gas] and NH_4 oxidation [using $^{15}NH_4Cl$]). BP measurements will be conducted in an isotope van. Other measurements will be incubated in on-deck incubators. Grow-out experiments will be conducted to evaluate the rate of silicification in microplankton using on-deck incubators and to determine the metabolic response by phytoplankton to nutrient amendments from differing coastal environments.
    c. **Phytoplankton assemblage structure, harmful algae, and phycotoxins.** We will collect water samples from the Niskin bottles to evaluate phytoplankton assemblages in near-real time using a Flow Cytometer And Microscope (FlowCAM). Based on the results of the assemblages viewed by this instrument, we will collect samples for the analysis of domoic acid, yessotoxin, and other algal toxins potentially present in the waters of the Oregon/Washington coasts.
3. **Surface flow-through system**. We will make continuous measurements of surface water chemistry with instrumentation attached to the continuous flow seawater system. These instruments will be ship-supplied fluorometer, flow meter, thermosalinograph, and oxygen sensor; and scientist-supplied nutrient analyzer (APNA) and flow-through cytometer ('SeaFlow').

4. **Plume Feature Tracking.** Conduct Feature Tracking exercises using the SWAP system and model-based CORIE forecasts to identify sampling locations in the Columbia River Plume. Ship will cast CTD at pre-determined locations/times to test model.
5. **Estuary ETM, methane, and *Myrionecta rubra* sampling.** Sample the Estuarine Turbidity maxima in the north and south channels of the estuary with a modified CTD attached to pump tubing that is paid out during each cast (coordinate with R/V Point Sur). We will conduct vertical profiles to evaluate the distribution of the bloom-forming ciliate, *Myrionecta rubra* that often forms red streaks in waters of the Columbia River estuary.

III. Tasks

1. CTD: Deploy CTD (4 people on deck); run Seabird software (in the lab). Choose sampling depths, collect water into 20-L carboys from bottom, middle, and surface, as well as collecting samples for nutrient profiles at selected stations *Training will occur at NH-10 Shakedown station; also, see attached sheet for general instructions for sample collection*
2. Chemistry: Dissolved and particulate chemistry (nutrients and organic matter)
    i. Nutrients (Nitrate, nitrite, ammonium, phosphate, silicic acid)
    ii. DOC (Dissolved organic carbon)
    iii. TSS (Total suspended solids)
    iv. POC/N (Particulate organic carbon/nitrogen)
    v. TDN/TDP (Total dissolved nitrogen, total dissolved phosphorous)
    vi. Pigments (Chlorophyll)
    vii. Methane sampling / onboard analysis (F. Prahl)
3. Fixations: Glutaraldehyde fixation, Flow cytometry fixation, FISH fixation
4. DNA/RNA: Collect DNA and RNA samples on Sterivex filters
5. BP: Bacterial Production measurements in radioisotope van
6. PP: Primary production (on-deck incubator)
7. CH_4 oxidation experiments (on-deck incubator)

IV. Detailed description of tasks/activities

1. CTD casts and sensor-based measurements.

We will be using **two types of CTD systems** during the cruise. Aug 29 – Sept 3 and Sept 8 – Sept 11) we will be using a large CTD rosette carrying 24 10-L Niskin bottles. This rosette will be equipped with several sensors for real-time analysis of water column conditions. These sensors are:

- CTD, Sea-bird 911plus with pressure sensor 401K-105
- Temperature sensor, Sea-Bird SBE3Plus
- Conductivity sensor, Sea-Bird SBE4C
- Pump, Sea-Bird SBE5T
- Altimeter, Benthos 916D
- PAR sensor QSP-200L
- Fluorometer, Seapoint, 6km, Chlorophyll
- Transmissometer, WetLabs, 25cm
- Oxygen Sensor SBE43
- ISUS Nitrate sensor (supplied by scientists)

On Sept 4 we will switch to using a small CTD Unit consisting of the stainless steel Sea-Bird CTD frame set in a scientist-provided metal cage. This cage has hydrodynamic flaps to keep the CTD

package from spinning in the strong currents of the estuary. Heavy weights are attached to the bottom of the cage, and the end of a length of pump tubing is attached to the leading end of the cage. Tubing will be clipped to the CTD wire every 3 meters during the cast. This tubing will be removed when water samples are not required.

2. Water sampling

Most water samples will be subsampled for:

DNA (OHSU)	1 sterivex, 4 ml RNAlater, Cha-seal, male luer plug (Zuber)
RNA (OHSU)	1 sterivex, 4 ml RNAlater, Cha-seal, male luer plug (Zuber)
DNA (UMCES)	2 sterivex, 2 ml DNA Extraction Buffer, Cha-seal, male luer plug (Crump)
POC/N	2 ashed 25mm GF/F filters (Crump)
SPM	2 pre-weighed GF/F filters, labeled petri plate (Crump)
Nutrients	1 nutrient bottle; Fill to shoulder (Needoba)
NH_4^+	1 50 ml plastic centrifuge tube, measured on board if possible (Needoba)
TDN/TDP	1 pre-extracted 30 ml bottle; add EXACTLY 20 ml of sample (provided by UMCES Horn Point Laboratory analytical services; Crump)
DOC	1 polypropylene 20ml scintillation vial; fill to shoulder (Crump)
CDOM	60 mL combusted amber glass bottle; fill to shoulder and refrigerate (Needoba)
Flow cytometry	1 5ml cryo tube, 0.1ml paraformaldehyde (provided by ?)
ChlA/phaeo	1 25mm GF/F filter, 2ml cryovial (Needoba)
Cell counts	1 glass 7ml scint vial, 0.25ml 25% pre-filtered glutaraldehyde (Crump)
Phyto counts	100 mL French squares preserved with Lugols iodine (Peterson)
FlowCAM	50 mL corning centrifuge tubes (Peterson)
BP	Bacterial production rate measured as ^{3}H-leucine incorporation rate

Select water samples will be subsampled for:

PP	Primary Production rate (Kahn)
CH4	Methane concentration (Prahl)
CH4 uptake	Methane uptake rate (Peterson, Prahl)
Nutrient expts.	Water incubated with nitrate, silicate, or nitrate+silicate amendments (Bender & Durkin)
DNA/RNA (UW)	DNA/RNA (Bender & Durkin)
FC	flow cytometry samples (Bender & Durkin)
Lugols	Lugol's fixed seawater (Bender & Durkin; Maier & Peterson)
PDMPO	silica staining (Bender & Durkin)
Fv/Fm	Photosynthetic parameters using a PhytoPAM fluorometer (Bender & Durkin; Blakely)
BioSi	Biogenic Silica (Bender & Durkin)
Urea	Urea concentration (Bender & Durkin)
Alkaline phosphatase	Blakely
Domoic acid	McKibben, Maier

Bacterial production measurements will be carried out in an isotope van. Other rate measurements will be set up in the wet lab and incubated in on-deck incubators.

3. Surface flow-through system

The surface water flow-through system on the *New Horizon* is currently equipped with a flow meter, thermosalinograph, fluorometer, and oxygen sensor. We plan to connect two devices to the surface flow-through system on the New Horizon. The first is a flow-through cytometer (SeaFlow). This cytometer can be loaded on the ship be two people. It sits on a lab bench and needs about 5 feet wide by 1 foot deep of space, although it can be squeezed into 1x3. It needs to be located somewhere near

the ship's flow-through line. We have plumbing for tee-ing into the on-board system. We would like to plumb it as close to the flowing line as possible to minimize the dead volume. The outflow from the cytometer is around 20 ml/min and can go into a sink or overboard. For more information please contact Jarred Swalwell (jarred@u.washington.edu). The second is an Autonomous Profiling Nutrient Analyzer (APNA) to be installed by J. Needoba (needobaj@stccmop.org, OHSU) and Missy Gilbert (OHSU graduate student).

4. Plume study (to be coordinated with Pt Sur)

a. We will choose locations for plume tracking based on the forecasts from numerical models. Communications from the bridge between the chief scientist on Pt Sur (Chris Wingard) and New Horizon are desirable. We will collect water at several stations during the daytime series with Niskin bottles. Weather-permitting, Pt Sur will deploy a Remus autonomous vehicle from the rhib in the plume area.

b. Feature tracking exercise independent of Pt Sur. We will collect water at 8 sites using a Pacer pump.

5. ETM studies (South Channel and North Channel)(Lead: Microbiology team)

Estuarine Turbidity Maxima in the North and South Channels of the estuary will be sampled. In the south channel, the ETM will be sampled between the Astoria-Meglar Bridge and Tongue Point (e.g. 46 12.46, -123.48), while in the North Channel the ETM will be sampled at NC-11 (adjacent to SATURN-01). We will cast the small CTD unit regularly during these series, and will collect water during one ebb tide and one flood tide per station. We will perform casts to detect the ETM using turbidity. We are targeting:

- Before ETM (bottom)
- Peak ETM (bottom and surface)
- Post-ETM (bottom)

6. Freshwater end member sampling and Columbia River transect (Lead: Prahl)

New Horizon will transit to Beaver Army Terminal (RM-53) to collect water and return to the estuary (Cathlamet Bay, RM-17) for a 15 h time series to examine methane concentrations. We will conduct CTD casts and collect water samples during the lesser ebb tide on Sept 4 (start at RM-53 at ~14:30 and travel to Youngs Bay) along a down-river transect (see Table 2 for locations) from Beaver Army Dock to Astoria, and during a time series at a station in the South Channel of the estuary at the outlet to Cathlamet Bay (RM-17), a large freshwater marsh. The river transect sites will be sampled at two depths (surface & bottom) over the course of a minor ebb tide IF POSSIBLE. This scheme will have to be negotiated with the Captain of the New Horizon to ensure safe operations in the channel.

7. Methane time series (Lead: Prahl) ** 24 h operations****

Beginning at ~19:00 on Sept 4, we will perform a time series sampling at a fixed location just off the channel leading to Saturn 4 (Mott Island Turning Basin; RM-17, in the anchorage area). The time series will begin once the river transect is complete. That time marks slack water before the major flood. Anchored at that location, we will evaluate how drainage of Cathlamet Bay, a freshwater marsh, impacts the methane signature in the main Columbia River Estuary. Sampling will be done **hourly** at surface and bottom depths over the course of ~15hrs (until ~11:00 on Sept 5). Sampling will consist of (1) dissolved methane concentrations (Fred) and (2) methane oxidation experiments (Tawnya).

We will need a CTD crew to help out with this 24-h sampling:
Team 1 (4-8): Colleen (CTD computer); deck ops: Grant, Patrick, Missy, Natalja
Team 2 (8-12): Misty (CTD computer); deck ops: Morgaine, Sara, Caroline
Team 3 (12-4): Tawnya (CTD computer); deck ops: Justin, Vikki, Adena

Table 2. Sampling locations for Columbia River methane transect; this is a target set of stations

Site	Lat	Lon
Beaver Army Terminal	46.180	-123.180
Upriver #4	46.150	-123.320
Upriver #3	46.540	-123.420
Upriver #2	46.255	-123.550
Upriver #1	46.225	-123.680
Tongue Point	46.220	-123.770
Astoria Port Docks	46.213	-123.830
Youngs Bay	46.205	-123.870
RM-17	46.210	-123.777

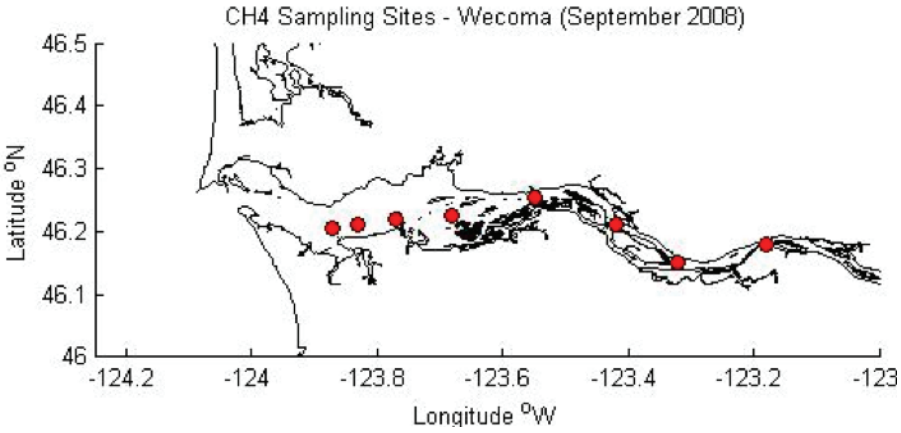
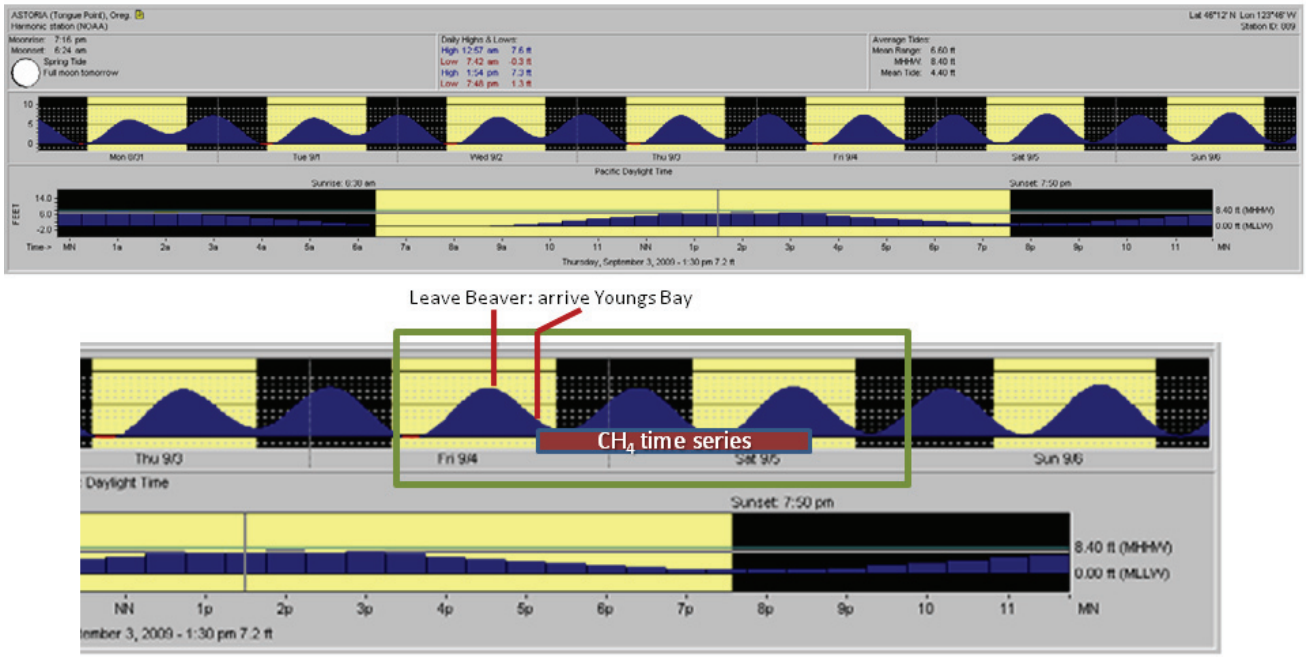


Figure 2. Sampling sites for Columbia River methane transect

Figure 3. Tides at Tongue Point, OR (Sept 1 – Sept 6). Columbia River transect begins from Beaver Army Terminal (RM-53) (indicated by first red line) and ends near Youngs Bay (indicated by second red line). Time series survey time span is indicated by the red rectangle (~17:00 09/04-14:00 09/05).



8. *Myrionecta rubra* time series (Lead: Herfort) **24-h operations**

This will be a **24 h time series** of observations at NC-11 beginning on Sept 6 at ~16:00. Water samples for DNA/RNA (2 Sterivex filters for each depth), nutrients (organic and inorganic), Fv/Fm, and chlorophyll will be collected using the Pacer pump. Water will be collected from throughout the water column (0, 5, and 10 m) to get good vertical profiles to assess vertical migration over the day-night cycle. Ideally, CTD casts will be performed every 30 minutes, and water will be collected every 2 h. Coordination with Pt. Sur is desired.

For the *Myrionecta* work, we will need **three teams** to tackle 24 h sampling. NOTE that people will have to carefully coordinate their sleeping schedules accordingly.

Team 1 (4-8): Sara (CTD computer); Sara (DNA/RNA) filtration; Patrick, Missy (chemistry); Natalja Kuvaldina (FlowCAM); CTD deck ops: Grant, Missy, Patrick, Natalja

Team 2 (8-12): Fred Prahl (CTD computer); Caroline (DNA/RNA); Adena Kass (chemistry); Michelle Maier (FlowCAM); CTD deck ops: Josh, Michelle, Adena, Caroline

Team 3 (12-4): Tawnya Peterson (CTD computer); Vikki (DNA/RNA); Tawnya Peterson (FlowCAM); Morgaine McKibben (chemistry), CTD deck ops: Missy, Vikki, Morgaine, Justin

9. Salinity gradient (Lead: Microbiology team)

Salinity gradients (from 0, 5, 10, 15, 20, and 25 PSU) will be sampled between 16:00 and 20:00 on Sept 7. We will do a series of casts using the Pacer pump until the desired salinities are found. (the 4-8 team from *Myrionecta* sampling will be primarily responsible for carrying out the salinity gradient work.

10. Characterizing communities - Environmental controls on river-to-ocean variability in bacterioplankton community composition (Lead: Fortunado & Crump)

Our goal is to describe broad-scale spatial variability in bacterioplankton community composition throughout the CMOP study region, identify environmental factors that explain this variability, and determine how diversity patterns relate to major coastal environmental gradients. This

study provides a first picture of how microbial communities are distributed spatially across the river to ocean gradient, and it has been used as a baseline of information for several other ongoing research projects.
Desired Sampling Scheme: Microbial baseline sample set.

11. Determining microbial community gene expression in Columbia River estuary, plume, and coastal ocean (Fortunado & Crump)

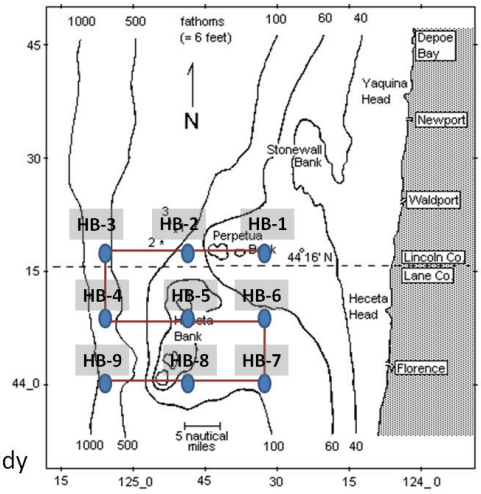
Microbial transcriptomics is a new and potentially powerful tool in microbial ecology. To fully understand the crucial role microbes play in a system, it is important to not only look at who is there, but also what they are doing. Determining the key functional genes being expressed in a microbial community through collection, pyrosequencing, and analysis of mRNA transcripts will help to elucidate the important ecosystem processes occurring across environmental gradients. The goal of this study, therefore, is to construct a library of transcripts from river to ocean in order to better understand the how microbial processes might change and the impact of this change on biogeochemical activity and overall ecosystem dynamics.

Desired Sampling Scheme: Water will be collected using two, 144 mm filter rigs (3 µm pre-filter, 0.22 µm filters) and a Geo-pump. The amount of water will vary, but ideally 10-20 L will be needed. Coastal ocean and estuary transcriptomic samples will be taken within the microbial baseline sampling set and do not require additional stations (although additional water outside amount allotted for baseline may need to be collected). The exact number of samples collected along coast and within estuary has yet to be determined, but will include surface, deep ocean, and salinity gradient (0,10, 20, 30).

Sampling within plume will require additional stations, allotted ship time outside of microbial baseline, and use of pump system for water collection. Collection of water at plume surface and below mixed layer using pump system. Ideally ~6-8 stations within plume would be required. Sampling could occur during scheduled plume feature-tracking exercises.

12. Heceta Bank survey

The ship will survey (with the flow-through system and with CTD casts – time permitting) the region around Heceta Bank (Fig. 4).



Heceta Bank study

Figure 4. Heceta Bank area survey.

Schedule

Aug. 27-Aug28: Load ship starting afternoon of Aug. 27

Aug 29: **Safety meeting 08:00; depart at 09:30 from Newport [6 micro samples]**

1. **NH-10** (micro) [shakedown station]; collect water for Suzanne DeLorenzo's experiments
2. Steam to CM-3 or 5 (do a cast) and steam toward CM-a until midnight; end up near the Columbia River mouth for 06:00

Aug 30: **Plume survey #1 (06:00)**

-Stations to be determined from model forecasts; 4 micro stations
-Depths will be surface (~1 m) and 5 m (or just below the plume) [2 depths]
-deploy and recover (after 8-10 h) Brightwater drifter, if it is close by
-8 micro samples (4 stations, 2 depths)

Station	Lat	Lon	Notes
Plume-1 (micro)*			
Plume-2			
Plume-3			
Plume-4			
Plume-5			
Plume-6			
Plume-7			
Plume-8			
Plume-9			
Plume-10			

*4 microbiology stations will be chosen depending on desired salinities

-**Steam first to CR-40 and then to CR-4 (overnight)**

Aug 31: **CR line (06:00) [*Pt Sur loading day*]: 12 micro samples**

Station	Lat	Lon	Notes
CR-4	46.167	124.077	
CR-7 (micro)	46.167	124.158	3 micro samples
CR-10	46.167	124.218	
CR-15 (micro)	46.167	124.333	3 micro samples
CR-20	46.167	124.452	
CR-25	46.167	124.557	
CR-30 (micro)	46.167	124.670	3 micro samples
CR-35	46.167	124.792	
CR-40 (micro)*	46.167	124.910	3 micro samples; filter 15 L per Sterivex for bottom water
CR-50	46.167	125.030	

-**Steam to WB line; do night survey of WB line from offshore to inshore; end up at GH-3**

Sept 1: **GH Line (06:00) [*Pt Sur sailing day: plume study*]: 9 micro samples**

Station	Lat	Lon	Notes
GH-3 (micro)	47.000	124.247	3 micro samples
GH-6	47.000	124.320	
GH-10	47.000	124.417	
GH-16	47.000	124.558	
GH-21 (micro)	47.000	124.695	3 micro samples
GH-26	47.000	124.813	
GH-36	47.000	125.062	
GH-46 (micro)	47.000	125.315	3 micro samples

-Steam to QR-29 (outer QR-line); then on to LP-62 and LP-52

Sept 2: **LP Line (06:00) [*Pt Sur: AUV first deployment; slow drop; underway surveys*]: 9 micro samples**

Station	Lat	Lon	Notes
LP-52 (micro)	47.917	125.928	3 micro samples
LP-32 (micro)	47.917	125.432	3 micro samples
LP-27	47.917	125.308	
LP-22	47.917	125.192	
LP-17 (micro)	47.917	125.083	3 micro samples
LP-12	47.917	124.958	
LP-9	47.917	124.875	
LP-6 (micro)	47.917	124.792	3 micro samples
LP-4	47.917	124.742	

-Steam to QR-3 (inshore) and WB-5

****Call Pt Sur to coordinate Sept 2 Plume Survey (5 pm)****

Sept 3: **Plume survey #2 (<u>in coordination with Pt. Sur</u>) [*Pt Sur: slow drops in morning*]: 9 micro samples**

-sites to be determined from model predictions (approximately 10 stations)

-**3 sites** for microbiology (3 depths) to be sampled with a CTD

Sept 4: **Beaver and Columbia River Transect: 2 micro samples**

Cross the bar at 07:30

1. Transit to Beaver Army Terminal [*switch hydrowire from CTD rosette to small cage*]
2. Do 1 CTD casts (2 depths)(end ~13:00) [1 surface, 1 bottom]
3. Transit to Youngs Bay via the following weigh points (IF POSSIBLE):

Site	Lat	Lon
Beaver Army Terminal	46.180	-123.180
Upriver #4	46.150	-123.320
Upriver #3	46.540	-123.420
Upriver #2	46.255	-123.550
Upriver #1	46.225	-123.680
Tongue Point	46.220	-123.770
Astoria Port Docks	46.213	-123.830
Youngs Bay	46.205	-123.870
RM-17	46.210	-123.777

*Consider deploying pump tubing in transit mode, with tubing attached to a stiff line hook at 1 m depth

4. Anchor at RM-17 for methane time series (anchorage spot, with Pt. Sur)
5. Begin hourly time series for methane concentrations and oxidation rates (18:30 – 11:00, 09/05) (CTD casts each hour, 2 depths)

Methane time series

Hour of sampling	Time	Complete/notes
0		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
1		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
2		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
3		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
4		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
5		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
6		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
7		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
8		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
9		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
10		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
11		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
12		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
13		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
14		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
15		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
16		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
17		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM
18		Nuts, [CH_4], CH_4 oxidation, DIC, CDOM

Sept 5: **Finish methane time series; South Channel ETMm [*Pt. Sur at NC-7*; *AUV ops in North Channel*]: 4 micro samples**

1. Complete methane time series (11:00)
2. South Channel ETM study (11:00 – 16:00); collect water using Pacer pump at 3 times:
   (1) prior to ETM (bottom water)[1]

(2) during ETM max (surface & bottom water)[2]
(3) following ETM (bottom water) [1]

3. Transit to NC-11; sample for methane on evening ebb tide using Pacer pump (2 depths);
4. Anchor at NC-11

Sept 6: **North Channel ETM; Begin *M. rubra* sampling [*Pt. Sur at NC-7*; *AUV ops in North Channel*]: 4 samples per time point [=48 micro samples]**

Cross bar at 09:00 for pump out and trash disposal

1. Survey plume area until slack tide occurs – enter estuary ~15:00 [*potential for N-S transect near plume mouth*]
2. 15:00 – 16:00: ETM survey (North Channel) [similar to that performed for South Channel; 4 time points]
3. 16:00 – 16:00 (09/07): *M. rubra* **24 h time series** at NC-11 (anchor)
   -coordinate with Pt. Sur (CTD casts downstream of NC-11 and SATURN-01)
   -CTD every 30 minutes; water collection from 0, 5, 10 m (and possibly surface bucket sample) every 2 h

Sept 7: **Complete M rubra sampling** (16:00) **and Salinity gradient [6 micro samples]**

1. 16:00 – 20:00: Salinity gradient (0, 5, 10, 15, 20, 25 PSU)
   -Salinity gradient sampled using Pacer pump [*switch hydrowire from pump to CTD rosette*]
2. Anchor at NC-11 overnight

Sept 8: **Transit to Heceta Bank; begin survey at 22:00, [*Pt Sur, NC-7-AUV ops, slow drop, anchor at NC-7*]: 9 micro samples**

***Drop-off of F. Prahl and C. Fortunato, pick up M. Wolhowe before 08:00 (*coordinate with Pt. Sur);* Cross bar at ~09:30; transit to HB-1

1. Do micro cast outside of river mouth
2. Perform survey around Heceta Bank (see Fig. 4)

Station	Lat	Lon	Notes
Micro cast-River mouth			3 micro samples
HB-1	44.333	124.500	
HB-2	44.333	124.750	
HB-3	44.333	125.000	
HB-4	44.250	125.000	
HB-5 (micro)	44.250	124.750	[~04:30]; 3 micro samples
HB-6	44.250	124.500	
HB-7	44.000	124.500	
HB-8	44.000	124.750	
HB-9 (micro)	44.000	125.000	[~10:30]; 3 micro samples

-Steam to SH-e (offshore extent of SH line)

Sept 9: **SH Line (13:00), [*Pt. Sur: leave estuary, sample plume with pump (Caroline), slow drops*]: 9 micro samples**

Station	Lat	Lon	Notes
SH-e	44.252	125.042	3 micro samples
SH-c	44.252	124.808	
SH-a	44.252	124.575	
SH-100	44.252	124.462	
SH-80	44.252	124.328	3 micro samples
SH-70	44.252	124.252	
SH-50	44.252	124.169	
SH-30	44.252	124.134	
SH-15	44.252	124.127	[*End at 01:00]; 3 micro samples

-**Steam to NH-55**

Sept 10: **NH Line (07:00), [*Pt. Sur: plume studies; AUV & slow drops*]: 12 micro samples**

Station	Lat	Lon	Notes
NH-55	44.652	125.367	3 micro samples
NH-45	44.652	125.117	
NH-35	44.652	124.883	
NH-25	44.652	124.650	
NH-20	44.652	124.528	3 micro samples
NH-15	44.652	124.412	
NH-10	44.652	124.295	3 micro samples
NH-5	44.652	124.177	[*End at ~23:00]; 3 micro samples

Sept 11: **Dock in Newport; [*Pt. Sur: plume studies; AUV & slow drops*]**

Cruise Plan Summary

Dates / Location / CTDs # / Maximum # of water samples for biology team / Coring site #

Date	Location	# CTDs	Water samples	Primary Prod.
8/27/09	Load Ship (afternoon 8/27-evening 8/28)	0	0	0
8/29/09	Depart at 09:30; NH-10 shakedown station, steam to CM – cast at CM-5	2	6	1
8/30/09	Plume survey #1; steam to CR-4	10	8	2
8/31/09	Columbia River line; steam to GH-3	10	12	3
9/01/09	Grays Harbor line (coordinate with glider)	8	9	3
9/02/09	LaPush line	9	12	3
9/03/09	Plume survey #2; coordinate with Pt Sur (slow-drop & Remus)	10	9	3
9/04/09	Estuary work; cross bar into estuary 07:00-08:00; transit to Beaver and Columbia River transect (methane)	10	2	1
9/05/09	Methane time series; South Channel ETM; transit to NC-11	21	4	1
9/06/09	Estuary work; Remus & *M. rubra* (North Channel)	20	52	1
9/07/09	*M. rubra* study; salinity gradient	22	6	1
9/08/09	Cross bar at 0930 (do cast), transit to Heceta Bank, begin survey	10	9	1
9/09/09	Finish Heceta Bank survey; Strawberry Hill Line	9	9	2
9/10/09	Newport Hydo Line	8	12	1
9/11/09	Dock in Newport, offload ship			
	Total		150	23

*Microbial process stations will typically collect water at 3-6 depths

CTD Cage
We constructed a cage to protect the CTD and other sensors during deployment in the Columbia River estuary. We attach a heavy weight to help get the package below the surface currents during strong ebb tides. The cage also has some hydrodynamic flaps to keep the package oriented properly when deployed.

Pump
We attach the ends of a rigid tube to the CTD cage and to an impeller pump on deck. This tubing is paid out as the cage is lowered, and clipped onto the hydrowire. Our maximum deployment depth in the estuary is approximately 25m.

Appendix A. **Notes for CTD operations**

CTD deck ops require a minimum of three tag liners and one person communicating with the winch operator. Inside, one person runs the computer to fire bottles at desired depths.

The person running the CTD from the lab will have a cast sheet to fill out (see Appendix C).

If you require or desire samples from a given cast, please make it known to the person running the CTD in the lab. There are 24 bottles on the rosette, so all of your water needs should be easily accommodated.

Note that once the CTD package is under water, New Horizon requires that ALL bottles must be fired prior to bringing it on deck.

Once the CTD comes back to the surface check the cast sheet to see which bottles go with which depths. For the microbiology samples, 4 bottles per depth will be used. The depths are: Bottom (5 m from the bottom), Middle (chl max), and Surface (~2 m). For each of these 3 depths, fill ONE 20-L carboy with water from 4 Niskin bottles. These carboys will be used to dispense water for chemistry and DNA/RNA analyses. Important: remember to shake the carboy before sampling; particles can settle when left over time, and ideally we want as homogenous a sample as possible.

Appendix B. Chemistry analyses: instructions for processing water samples

A filtration rack will be set up in the wet lab adjacent to the deck and CTD. In here the samples will be filtered for DNA/RNA as well as chemistry. Each constituent has a slightly different handling method. These are briefly outlined below.

When filtering for particulates, especially chlorophyll a and particulate carbon and nitrogen, make sure that the vacuum is less than 100 mmHg. We do not want the cells to burst, releasing all the good stuff we are trying to measure.

Please be diligent about recording each sample number in the yellow notebook; each cast and depth (e.g. Cast 10, Middle) is associated with a sample number. All parameters measured get this sample label. For example, say that Cast 10, Middle depth is Sample 433 – then chl, POC/N, SPM, etc. are ALL labeled sample 433.

Chlorophyll a

-Use GFF filters provided by Needoba
-we want these filters to be slightly colored, but not overly green; therefore, the amount filtered will vary depending on whether we are close to shore (lots of biomass) or away from shore (low biomas); close to shore, filter 280 mL (pre-measured bottles); for the offshore stations, volumes closer to 500 mL may be required.
-place the GFF filter into a 5 mL test tube and cap it; place in -80oC freezer until analysis. KEEP THESE IN THE DARK under aluminum foil.

FISH

- Fill falcon tube to 40 mL
- Add 1.6 mL of Formalin (37%)
- Incubate for 1 hour at room temp and store at -80 oC
- Collect 1 tube per sample, except for red water study where **2 tubes per sample** need to be collected

Particulate Carbon/Nitrogen

-use only combusted (ashed) GFFs
-filter 2 L onto a combusted filter (more in the ocean, less in the estuary)
-collect the filtrate for nutrient analysis
-store in either a Petri dish or in a 2 mL cryovial at -20^{o}C

SPM

-use 2 pre-weighed GFF filters in labeled petri plates
-filter as much onto these as possible (1-3 L)
-store at -20^{o}C

Nutrients

-use filtrate from POC/N analysis;
-collect filtrate into 30 mL acid-cleaned Nalgene bottles provided by the Needoba lab
-fill to shoulder
-store upright at -20^{o}C

TDN/TDP

-into 1 pre-extracted 30 mL bottle, dispense exactly 20 mL of sample (filtrate from POC/N analysis)
-store upright at -20^{o}C

Ammonium

-filter sample through combusted GF/F filter into orange topped centrifuge tube (fill to 40 mL)
-freeze immediately, upright

DOC

-into 1 polypropylene 20 mL scintillation vial, dispense ~18 mL (to shoulder) volume from POC/N filtrate

CDOM
-into one 40 mL combusted amber glass bottle, dispense ~37 mL (to shoulder) of filtrate run through glass filter funnel (we want to avoid any contact with plastics!)
-store upright at $4^{o}C$

Phytoplankton counts
-fill one bottle –gently- to nearly the top; add a dropper-full of Lugol's Iodine (until the liquid is 'tea colored')
-store in the cold room

FlowCAM
-into one 50 mL polypropylene centrifuge tube, gently dispense ~48 mL of liquid; store in the fridge

A work about LABELLING

For microbiology samples, you MUST use consecutive sample numbers (see the yellow book). The cruise will start with a given number – move on from there.

CTD casts will be labeled with the first cast being C001. ALL casts must be given a number, even those for which there is a problem.

Time: we will operate in UTC (GMT) unless otherwise specified.

For profile samples (non-microbiology), use the CAST NUMBER and BOTTLE NUMBER as identifiers for each sample. For example, for a profile of chlorophyll and dissolved nutrients, data might look as follows:

NH0829A, C010, Bot 12

Where NH0829A is the cruise identifier, C010 is cast 10, and Bot 12 is Niskin bottle number 12. Since all CTD files will be named according to the Cast and Bottle number, it is quite easy to integrate this information later on using this sample naming convention.

A **cruise spreadsheet** will need to be filled out during the cruise that carries all information present in the yellow book (volume filtered for each sample, sample number, etc.). It is VERY IMPORTANT that this gets filled out.

Appendix C- Example cast sheet. **New Horizon Sept 2009**

Date: Aug 30 Latitude: 45°25.15 Time start: 13:18

Station ID: CM-a Longitude: 124°50.23 Time end: 14:00

Cast Number: C010 Bottom depth: 1000 m

User initials	Bottle No.	Nominal depth	Time (GMT)	Temperature	Salinity
TP	1	100	13:25	8.3	33.8
MB	2	100	13:26	8.3	33.7
Micro	3	100	13:27	8.25	33.8
Micro	4	100	13:28	8.3	33.7
Micro	5	100	13:29	8.4	33.6
Micro	6	100	13:30	8.2	33.7
TP	7	50	13:35	10.4	32.8
MB	8	50	13:36	10.6	32.7
MB	9	25	13:37	12.1	32.6
FP/Morg	10	25	13:38	12.2	32.5
FP/Morg	11	25	13:39	12.3	32.6
FP	12	25	13:40	12.1	32.6
Micro	13	25	13:41	12.3	32.5
Micro	14	25	13:42	12.2	32.6
Micro	15	25	13:43	12.4	32.5
Micro	16	25	13:44	12.2	32.5
Micro	17	2	13:47	13.1	32.1
Micro	18	2	13:48	13.2	32.0
Micro	19	2	13:49	13.2	32.2
Micro	20	2	13:50	13.1	32.0
SB/CD	21	2	13:50	13.2	31.9
SB/CD	22	2	13:51	13.1	32.0
SB/CD	23	2	13:51	13.0	32.1
SB/CD	24	2	13:52	13.1	32.0

Notes: jellyfish tentacles on the CTD rosette frame

Cast Number:_____ Date:_____

Bottle No.	depth	Chl a	POC/PN	SPM	DNA/RNA (1)	DNA/RNA (2)	DNA/RNA (3)	DNA/RNA (4)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								

Cast Number:_____ Date:_____

Check off when completed

Bottle No.	depth	Nutrients	NH4	DOC	CDOM	TDN/TDP	FlowCAM	
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								

Appendix D.

01 LEVEL (UPPER DECK)

Room No.	Bunk No.		
01-5	13	Chief Scientist	Tawnya Peterson
01-2	14	Upper	Grant Law
01-2	15	Lower	Fred Prahl
01-3	16	Upper	Sara Bender
01-3	17	Lower	Colleen Durkin
01-4	18	Upper	Michelle Maier
01-4	19	Lower	Vikki Campbell
01-7	30	Upper	
01-7	31	Lower	Yolanda Morales

PLATFORM DECK (LOWER DECK) STARBOARD SIDE

Room No.	Bunk No.		
A1	20	Upper	Natalja Kuvaldina
A1	21	Lower	Caroline Fortunato
A2	22	Upper	Patrick Prahl
A2	23	Lower	Pete Kahn

PLATFORM DECK (LOWER DECK) PORT SIDE

Room No.	Bunk No.		
A8	24	Upper	Misty Blakely
A8	25	Lower	Morgaine McKibben
A10	26	Upper	Justin Reina
A10	27	Lower	Josh Manger
A14	28	Upper	Melissa Gilbert
A14	29	Lower	Adena Kass