



ECO HAB-PNW 5 CRUISE REPORT

**R/V Melville TUIM14VM
September 2-22, 2005**

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Area of Operations

Coastal Waters off Washington State and Vancouver Island

Itinerary

Depart Seattle, WA, September 2, 2005

Arrive Seattle, WA, September 22, 2005

Participating Organizations

NOAA/Northwest Fisheries Science Center
Romburg Tiburon Center, San Francisco State University
University of Maine
University of Washington
University of Western Ontario

Cruise Logistics (not onboard)

Nicolaus Adams, NOAA/Northwest Fisheries Science Center
Dr. Nancy Kachel, University of Washington

Personnel

Chief Scientist

Dr. Barbara M. Hickey, School of Oceanography, University of Washington

Principle Investigators

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Dr. Mike Foreman, Department of Fisheries and Oceans, Canada

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Dr. Vera Trainer, NOAA/Northwest Fisheries Science Center
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Amy MacFadyen, University of Washington
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Eric Roy, University of Maine
Michele Wrabel, University of Washington

Cruise Objectives and Sampling Scheme

The purpose of this cruise was to determine the physical, chemical and physiological conditions under which diatoms of the genus *Pseudo-nitzschia* (PN) produce the neurotoxin domoic acid (DA), and the ecophysiological conditions which promote cellular release of toxin to the surrounding environment. We attempted to observe the conditions under which toxic cells advect towards the

coast of Washington where they are consumed by shellfish. Such occurrences lead to closure of beaches to razor clam collection to avoid outbreaks of amnesic shellfish poisoning.

Sampling was organized around a comprehensive grid of stations, sampled repeatedly as environmental conditions changed. Continuous surface water measurements included: temperature, salinity and in vivo fluorescence, discrete surface samples for planktonic community analysis and species identification were collected with net tows. Property profiles were obtained with an instrumented rosette including a CTD (conductivity, temperature, depth) and additional sensors that measured in vivo fluorescence, photosynthetically active radiation (PAR), beam attenuation (light transmission), and oxygen concentration. During CTD casts discrete samples were collected with Niskin water samplers for chlorophyll, inorganic and organic nutrients, plankton species and community identification (via FlowCAM and flow cytometer analyses), and particulate and dissolved DA. A trace metal clean, underway sampling system was employed to collect subsurface samples that could be measured on board (e.g. iron), and to collect samples for multi-element determination (including copper) ashore. On-deck incubations of phytoplankton assemblages were conducted for growth, nitrogenous nutrition and grazing experiments, and shipboard analyses of the plankton were routinely conducted using both traditional (microscopic) and advanced (FlowCAM image and flow cytometric analyses) methods. Satellite-tracked drifters were released in the Strait of Juan de Fuca, near the Juan de Fuca eddy and off the coast of Washington. One drifter was followed, sampling the water properties with CTD and Niskin water samples at 3 hour intervals. The cruise was diverted to Neah Bay on September 13 to exchange personnel. The overall ship track and CTD stations are shown in Figure 1.

Operations

ADCP lines: ~3000 km

Flow-Through system track with T, S, FL sensors: ~3000 km

CTD casts: 204

Satellite-tracked drifter deployments: >10

Samples Collected

Chlorophyll samples: >200 stations and deck-board experiments (~2600 samples)

Cellular fluorescence capacity samples: 364 (DCMU-mediated F_v/F_m) samples

Inorganic nutrient samples: >150 stations and deck-board experiments (>2000 samples)

^{14}C Uptake (P vs. E) rates: 61 experiments (~1200 samples)

Heterotrophic Bacterial Productivity: 46 experiments (300 samples and controls)

Flow Cytometry samples (nanoplankton, cyanobacteria, bacteria): >125 Stations

HPLC Pigment samples: >125 Stations at 5 m depth
Dilution growth and grazing experiments: 12
Microplankton samples (preserved): ~130 survey samples and 360 dilution experiment samples
Nitrogen uptake rate experiments: ~300 ¹⁵N particulate samples
FlowCAM samples: ~ 600 samples, survey and experiments
Surface (~4 m) samples for Fe determination and samples for analysis of other bioactive trace metals (Zn, Co, Cu, Ni, Cd) ~130 samples
Particulate DA: 1040 samples (650 survey, 360 grow out experiments, 30 dilution experiments)
Dissolved DA: 1040 samples (650 survey, 360 grow out experiments, 30 dilution experiments)
Preserved net tow samples for scanning electron microscopy: 550 survey samples, 100 drift
Whole water samples for *PN* cell counts: 550 survey samples, 100 drift
DNA and RNA samples: 116 samples (4 depths at 29 stations)
Ammonium samples: ~ 600 discrete samples from profiles and experiments
Urea samples: ~300 discrete samples from vertical profiles and experiments

Cruise Summary

Introduction

The ECOHAB-PNW 5 cruise has provided an important contrast to the 2003 and 2004 fall cruises: diatoms of the *Pseudo-nitzschia* (*PN*) genus were relatively rare in the eddy, but were present in large numbers along the southern Washington coast. The cruise took place during a summer with anomalously late onset of upwelling-favorable winds (mid August). On our July cruise, our research team was able to measure the system in the reduced upwelling state. We were also able to follow its recovery in a strong, persistent upwelling period that lasted for ~2 weeks of the cruise period. We demonstrated furthermore that although local upwelling had not begun, the larger scale density field that is responsible for the shelf edge jet had upwelled, possibly due to remote forcing.

The study consisted of obtaining multi-disciplinary data from a large scale grid (Section 1), sampling water properties and plankton while following a drifter (Section 2), the deployment of surface drifters (Section 3), satellite imagery (Section 4), and on-board laboratory experiments using water/plankton collected at selected sites (Section 5).

The setting of cruise sampling events with respect to wind direction (upwelling or downwelling-favorable) is shown in Figure 2. For simplicity we have characterized the wind patterns into two periods: predominantly upwelling (1), and weak and variable but with more upwelling than downwelling (2). The primary grid was sampled in the second period (Survey 2). A less comprehensive grid sampling (Survey 1) occurred during the first period. Dates of the surveys

are also noted in Figure 2. All data sections and maps on the website are grouped into these two periods.

Over 200 water column profiles were obtained. Satellite imagery [sea surface temperature (SST) and chlorophyll] was limited during the first week of the cruise (one useable image). However, a number of very good surface fluorescence (Chl *a*) and turbidity images were obtained in the upwelling period during the second half of the cruise. Cruise activities were recorded in a sequential "Event" log (Table 1).

Our cruises to date have been highly successful—moreover we have been able to sample sufficiently different biological, chemical and physical conditions in our study area to allow a comparative analysis of various environmental factors and their effects on *PN*, growth, grazing, nutrient pathways and DA production. In particular, with the new information obtained during this cruise we have shown that:

- The Juan de Fuca eddy is more eddy-like (closed) during periods of downwelling winds.
Thus development of a large bloom in the eddy such as in September 2004 likely requires a substantial period of downwelling.
- Phytoplankton (including *PN*) and their associated toxins escape from the eddy primarily during periods of upwelling.
- In contrast with the nearshore coastal regions, the Juan de Fuca eddy region has substantial surface macronutrients (nitrate, silicate) even during extended periods with no coastal upwelling. Thus plankton in the eddy experience a different nutrient environment (N and Si are present at saturating concentrations for the uptake and growth of diatoms) than the plankton communities near the coast where sub-saturating concentrations are more prevalent.
- Community structure and the dominant species of *PN* have large interannual variability.

1. Regional Surveys (ECOHAB-PNW team)

Our large scale survey grid was designed to include areas influenced by the Strait of Juan de Fuca, the Juan de Fuca eddy region and the coastal upwelling region off the Washington coast (Fig. 3). Data collected on surveys included conductivity (C), temperature (T), light transmission, PAR, oxygen and fluorescence (FI) profiles, and bottle samples for phytoplankton biomass (chlorophyll *a*), whole cell fluorescent molecular probe assays, particulate DA, dissolved DA, FlowCAM and Flow cytometry samples, samples for scanning electron microscopy of *PN* species, plankton (including *PN* cell counts) and macronutrients, all at selected depths in the water column. Surface net tows for qualitative community assessment were taken at all survey stations. Water

samples containing *PN* were placed in medium for isolation and culturing in the laboratory. Underway data included T, S and FI pumped from a depth of about 4 m as well as ADCP current profiles from both 75 and 150 kHz RDI ADCPs.

In addition to survey data, a time series at a fixed location near the center of the eddy (EC) was obtained. The objective of the time series was to determine whether night time cooling was important in the formation of mixed layers at this location. The series was continued for about 24 hours (CTD 41-49). A CTD/nutrient transect was conducted along the Strait of Juan de Fuca on September 6 (Fig. 4a).

Lines were sampled in whichever direction made best use of ship time. CTD profiles were taken to 500 m where possible. For the second time in our ECOHAB-PNW studies, the northern line LD was sampled (also done in July). Several calibration stations were taken at the three mooring sites.

Chlorophyll *a* (size-fractionated samples: >5 μm and GF/F filters), particulate and dissolved DA and plankton samples (for both microscope and occasional molecular probe analysis) were taken near the surface (~0 m), 5 m and 10 m. DA samples were also taken at 15, 30 and 50 m at other stations, in particular, at the drifter A stations (Section 2). Flow cytometry and HPLC pigment samples were taken at 5 m depth. Macronutrients (nitrate + nitrite, silicate) were taken generally at the surface, 5 m, 10 m, 15 m, 30 m, 50 m, 100 m, 200 m, 500 m and ~5-10 m above bottom if the bottom was less than 500 m deep. In the Juan de Fuca Strait survey, samples were taken also at 150 m. Whole water samples (4 L) from these deep stations were concentrated through a 20 micron mesh and plankton were visualized through the FlowCAM. On transects, macro-nutrients were taken in most cases at the two stations closest to shore on a line and then every other station. Chlorophyll samples were taken at all stations at 3-4 depths (0, 5 10 m, and chlorophyll maximum depth).

The ISUS nitrate sensor data, mounted on the instrumented rosette can be used for vertical profiling, with appropriate calibration. The ISUS malfunctioned and no good data were obtained.

Upper water column iron samples were taken at selected stations (Table 1). On the primary grid, samples were made on roughly every other line in order to obtain the overall spatial pattern of iron distribution. These samples were obtained by flying the trace-metal sampler "FISH" below the surface (~4 m). Samples were taken as the ship approached station (within 10-min). Water was pumped for roughly 10 minutes (20 min prior to station location) to flush the lines thoroughly before samples were taken. In addition to FISH sampling for trace metals, all physiological measures and growth rates (phytoplankton and bacteria) were obtained from the FISH, as well as samples for deckboard incubation experiments. Physiological studies were made on several stations per grid line during the main survey.

The CTD data were partially edited onboard ship. Shipboard editing included replacing downcast data with upcast data if necessary. The shipboard data were used to construct the preliminary maps and sections appended to the report. Following the cruise, salinity calibration will be performed and more detailed editing completed (Hickey group). Although water property spatial patterns are likely robust, actual values may change slightly following the final editing which we hope to complete this fall. ADCP data require more extensive processing and will be provided later this year (Foreman group). Preliminary water property maps and sections obtained from CTD data are given on the ECOHAB-PNW website (T, S, O₂, Chl, FI maps at selected depths; T, S, density, FI, O₂ transects versus depth for all transects, 0-100 m and 0-500 m scales). Maps of relative abundance of *PN* at the surface are also included.

The CTD data are organized into two groups: Survey 1 (Sept. 3-Sept. 9) and Survey 2 (Sept. 14-Sept. 21). Grid stations sampled in each survey are shown in Figures 4a,b. Survey 2, which took place during variable winds, following a period of upwelling winds, was the only complete survey (Fig. 4b). However, sufficient CTD and underway sampling were done in the Survey 1 period to define the large scale patterns during downwelling reasonably well.

Underway data should be treated with caution. Water is pumped from about 4 m depth near the bow. Unfortunately the C sensor in the bow was down, although temperature functioned. A second set of sensors (SeaBird) were situated in the chemical lab. Water from the bow was pumped through the ship to these sensors and was used to calculate salinity. Temperature was too warm by about 1 degree and thus salinity is too high by about 0.5 psu. We performed a regression between CTD and underway salinity and this could be used to correct the data. For temperature, the user should use the data labeled SST (which should be the lower of the two choices).

One drift study was performed on the cruise—this was a drift in the coastal region during a period of upwelling (September 9-12) in an area with high concentrations of *PN* (drifter # 3938, deployed at station GH3). Note that on this cruise high *PN* numbers were only observed near the southern coast (two four plus stations were encountered). The drift lasted more than two days and was terminated only to transit to Neah Bay for personnel exchange. Samples (including iron and other micronutrients) were taken every 3 hours. An expendable drifter (# 60057) was deployed when the original drifter was recovered. This drifter, marking the toxic bloom, moved up and down the coast for several days in very shallow water (< 20 m), entered and left Willapa Bay, finally beaching on the coast just south of Grays Harbor. Another drifter was deployed in this area at GH1 on September 14 in very shallow water in the bloom area. It beached just north of Grays Harbor in a day and was recovered.

The RTC/SFSU flow-injection nutrient autoanalyzer malfunctioned due to contamination of Milli-Q water used for baseline and reagent preparation. Over

two days were spent troubleshooting the instrument. The problem was finally traced to the onboard Milli-Q water system maintained and owned by the R/V Melville which, despite having its deionizing filter cartridges replaced at the beginning of the cruise and one week into the cruise, began to produce increasing less volume of water and of poorer, unsuitable quality. After extensive troubleshooting by the SFSU research technician, the SIO Resident Technician, and assistance from the manufacturer, the problem was traced to an internal switching valve that had been incorrectly replaced prior to the ECOHAB cruise; total down-time was four days.

Some Preliminary Results:

The first survey clearly captured the coastal upwelling that was occurring during most of the sampling period (see web site surface maps). Saltier, colder water was observed along the mid to southern Washington coast. Highest fluorescence was observed in a band along the Washington coast, in the region where we performed Drift A. Maximum values were observed offshore—at stations 2 and 3, rather than at the coast. Offshore displacement of a plankton bloom is usually observed during strong coastal upwelling. Fresher water was also observed near the mouth of the strait. High macronutrients were observed in the eddy region. Nitrate was also high at the surface nearshore along the Washington coast in the vicinity of Drift A.

The second survey was made during variable wind conditions. Upwelling winds became stronger during the last 3 lines of the grid survey (LBC, LC, and LD). The eddy was much better developed at the surface than in the first period—with colder temperatures and also a good separation between the northern coast upwelling region and the eddy. In general, coastal waters were warmer than in the first period, a consequence of variable and weaker winds in the second period. Surface salinity showed a classic pattern of saltier upwelled water near the coast and a salty eddy, with a fresher Vancouver Island Coastal Current along Vancouver Island (perhaps our best pattern in 5 cruises). It is perhaps surprising that the upwelling pattern had not weakened significantly over the period of weak winds. Surface fluorescence during the period showed higher fluorescence in the region north and offshore of Barkley Sound and over much of the Washington coast. Highest values were observed on the southern coast, nearshore. The fluorescence pattern appears to have shifted southward and onshore since the first survey period.

PN cell numbers and particulate DA were extremely low in the eddy region during Survey 1 but high along the Grays Harbor transect and in Drift A. In general highest particulate DA was observed near the coast and on the Grays Harbor and Copalis Beach lines. Maximum numbers of *PN* were also observed near the Washington coast with moderate levels of DA. Molecular probe work indicated that *P. australis* and *P. multiseriis* were both present in the coastal upwelling region. Because the muD2 probe (cross reacts with *P. pseudodelicatissima*) did

not react with the small cell type observed, we believe that these small cells may again be *P. cuspidata*, the species responsible for record levels of offshore toxicity in September 2004.

2. Drift Studies (Amy MacFadyen, Barbara Hickey, Tom Connolly, Jim Postel; whole team for water/nutrient)

One drift study was performed (Drift A), following a water patch with a satellite-tracked Brightwaters surface drifter. To avoid regions of high current shear, no drogues were used—the drifters average the top meter of the flow field. Deployment and recovery times and deployment location are listed in Table 2. CTD profiles and bottle casts were taken at 3 hourly intervals. Nutrients were taken at the usual depths.

The drifter (#3938) was deployed at GH3 on September 9 in a region of very dense *PN*. The purpose was to study the development or decline of the bloom, which was toxic and very close to the coast. The drifter moved southeast in the nearshore coastal flow. In spite of the upwelling winds, the drifter moved onshore relative to the isobaths rather than offshore as we expected.

The drift was aborted at the end of September 12 to transit to Neah Bay to exchange personnel. An expendable drifter (#60057) was deployed in place of the CT drifter. This drifter moved up and down the inner shelf for another week, entering and leaving Willapa Bay, and finally beaching on September 19 on the coast just south of Grays Harbor.

3. Drifter Deployments (Amy MacFadyen, Tom Connolly, Jim Postel, Barbara Hickey)

Several Davis-type Brightwaters drifter were deployed to delineate patterns and speeds of surface flow in the eddy area, as well as to determine the ultimate fate of eddy water. Drifter deployment and recovery times and deployment locations are given in Table 2.

Data were stored at the University of Washington and were also available online on the ship as the ship had web access. Drifters record GPS location and water temperatures at 30 minute intervals during deployment periods. Several drifters will continue to collect data until about the end of October. All drifters were deployed at the surface (i.e. no drogues were used).

Four drifters were deployed at the beginning of the cruise (Figure 6): one (#3775) just inside the strait; a second (#3917) near the expected center of the eddy; a third (#3860) near the southwest edge of the eddy; and a fourth (#3974) just south of the eddy region on the outer shelf. All four drifters were deployed prior to any real time information about the eddy location. All four drifters escaped the eddy, a likely result of the upwelling-favorable winds and surface Ekman

transport, as we have observed on previous cruises. These drifters all moved south along the WA coast during the cruise. One was recovered (September 9, #3974) near the latitude of Grays Harbor. The drifter deployed at EH1 near the mouth of the strait moved around the eddy and west to the outer shelf and over the slope. It was recovered on September 17 (#3775) on the slope near the Ozette line (OZ). This drifter, originating from the mouth of the strait, was sampled twice for *PN* counts and DA. Currents over the slope were slow and variable. In general the shelf currents during the cruise were also weaker and more variable than seen on most other cruises.

A second round of drifter deployments was made on September 13, just prior to the primary grid survey. Drifters were deployed at EH1 (#3974), LAB3 (#60059), LAB6 (#60055) and LAB10 (#60058). During this period all drifters meandered in unexpected directions, indicating a weak and variable surface current pattern. Drifter 60055 headed east-southeast and beached on the northern coast on about September 20. The drifter deployed at the mouth of the strait headed onshore and then along the coast, entering Barkley Sound.

To study very nearshore flow direction under upwelling conditions, one additional drifter (#60054) was deployed at GH1 in less than 20 m bottom depth on September 14. It headed southward and onshore, beaching to the north of Grays Harbor.

Two drifters were deployed at the end of the cruise to monitor coastal flow during this traditional HAB period. One was deployed at EH1 (#3775), the other at LAB3 (#60056).

4. Satellite Imagery (Rick Stumpf, Dana Woodruff)

Satellite imagery during the cruise was provided by two groups who sent data to the ECOHAB-PNW ftp site—Dana Woodruff from Battelle Northwest Laboratory provided SST imagery and surface chlorophyll. Turbidity imagery was provided by Rick Stumpf at NOAA. The available imagery and an assessment of its quality are listed in Table 3. In general, because of the predominantly overcast conditions, few good images were obtained. However, one very good image was obtained on September 20 near the end of the grid survey.

5. Laboratory Analyses

Lessard Group (Evelyn Lessard, Brady Olson, Mike Foy, Michele Wrabel)

The main goal of this component of ECOHAB-PNW is to determine the role of grazers in *PN* population dynamics and DA production. We used the dilution technique to experimentally alter grazing rate and nutrient recycling to determine the effects of grazers on the net growth rate of the whole and size fractionated

phytoplankton community, specific species and groups of phytoplankton, and the production of dissolved and particulate DA. These experiments also provide estimates of the in situ growth rates of *PN* compared to other phytoplankton. We took FlowCAM and fixed samples to follow the in situ spatial and temporal changes in the microphytoplankton and protist grazing community in relation to *PN* and hydrography. We also conducted the third of a series of experiments to determine the relative importance of biotic (natural community of bacteria, protists) versus abiotic (organism-free water, light) factors in the degradation of dissolved DA in seawater.

On this cruise, we performed the following:

1. *12 dilution growth and grazing experiment*: In these experiments, we followed changes in $<5\ \mu\text{m}$, $>5\ \mu\text{m}$ and total chlorophyll, particulate DA, dissolved DA, *PN* species, and macronutrients (including ammonium). Samples were also preserved and processed onboard for microscopic enumeration of major phytoplankton species later in the laboratory. Chlorophylls were analyzed onboard as well as macronutrients (measured by Cochlan's group), dissolved and particulate DA (measured by Trainer's group), and cyanobacteria (measured by Trick's group). Twelve microplankton dilution experiments were conducted during this cruise. A series of four experiments were performed during the Drift A near the southern coast where *PN* was relatively abundant and somewhat toxic. Of the twelve experiments, only seven had significant *PN* abundance, and none of these were in the eddy region, in strong contrast to Sept 2005.
2. *Dissolved DA degradation experiments*: We also conducted the third of a series of experiments to determine the relative importance of biotic (natural community of bacteria, with and without protists) versus abiotic (organism-free water, light) factors in the degradation of dissolved DA in seawater (in collaboration with Trainer). These experiments were motivated by our observations of significant changes in ambient dDA during short term (24h) dilution experiment incubations. In the degradation experiments we followed changes in added dissolved DA with time in the following treatments: whole seawater, $<0.2\ \mu\text{m}$ seawater, $<0.8\ \mu\text{m}$ seawater, as well as a control with no DA added. The three experiments have been conducted with different communities on three ECOHAB cruises, providing us with a comparative study of different microbial communities and ambient DA levels.
3. *Ammonium regeneration rates*: In collaboration with Cochlan, Brady Olson performed four experiments using the ^{15}N -labeled nitrogen to determine rates of ammonium regeneration rates of the $<200\ \mu\text{m}$ size fraction in unmanipulated controls and as a function of iron availability (DFB and iron addition treatments).
4. *High frequency abundance estimates of PN and other plankton with the FlowCAM*: Discrete FlowCAM samples from multiple depths from Niskin

bottles, as well as surface samples from the Fe pump, were taken from selected transects during the first and second surveys. All initial and final samples from the dilution experiments were also analyzed with the FlowCAM. The data files were stored and will be edited and calibrated in the lab to obtain quantitative counts. At selected stations, replicate fixed samples were taken for microscopic enumerations and calibration of the FlowCAM. During surveys, the FlowCAM proved particularly useful (in addition to the surface net tows) for a quick assessment of *PN* abundance and community composition at the surface and at depth. In addition, the FlowCAM was used to analyze below the pycnocline, in chlorophyll maxima, and at the benthic boundary layer to determine if *PN* and other plankton had sedimented or been subducted to deeper subeuphotic locations. FlowCAM samples were also run for time-zero and time-final samples of incubation growth experiments of Liza McClintock (Trick group).

5. *Vertical profiles of micro- and nanoplankton*: We took preserved plankton samples at nineteen stations on the surveys for microscopic determination of autotrophic and heterotrophic nanoplankton, and heterotrophic/mixotrophic dinoflagellates and ciliates.
6. *DNA and RNA samples for exploratory work with identification and gene expression of PN species*: Michele Wrabel, a first-year UW graduate student supported by the UW's Pacific Northwest Center for Human Health and Ocean Studies, was invited to participate in this cruise to assist the Lessard group and to collect DNA and RNA samples for use in her future molecular research on *PN*.

b) RTC/SFSU Research Group (William Cochlan, Julia Betts, Julian Herndon, Maureen Auro, Regina Radan, Jessica Schneider)

The primary objective of this component of ECOHAB-PNW is to examine the relationship between elevated concentrations of the pennate diatom *PN* and its toxin DA, and ambient concentrations of macro-nutrients and phytoplankton biomass. In addition mini bioassays (grow-out experiments described below) were conducted to determine the relationship between copper, iron and DA production. At each station of the survey sampling grid, size-fractionated phytoplankton biomass levels were estimated from chlorophyll *a* (Chl *a*) concentrations determined using *in vitro* fluorometry (aboard ship) after extraction for 24 h with 90% acetone. Chl *a* samples generally were collected at three depths (0, 5, 10 m) and, at an additional depth corresponding to the chlorophyll maximum layer, when present. Size-fractionated biomass estimates were conducted as follows: total planktonic community was collected on Whatman GF/F filters (nominal pore-size of 0.7 μm), and cells $>5 \mu\text{m}$ in size were collected on Poretics silver membranes. At every second station, dissolved inorganic nutrients were collected at 0, 5, 10, 15, 30, 50, 100, 200 m and near bottom and analyzed using appropriate colorimetric methods for determination of

nitrate plus nitrite, and silicate with a Lachat Instruments QuickChem 8000 Series Flow Injection Automated Ion Analyzer. Both Chl *a* and nutrients were determined at the two most shoreward stations of each sampling line. Vertical profiles of dissolved inorganic nutrients were also determined at the drifter stations, during time-series experiments to estimate convective vertical mixing (in association with Hickey), and at a series of 6-7 vertical stations in the Strait of Juan de Fuca. Samples from the Juan de Fuca transit and grow-out experiments were also collected for ammonium (analyzed onboard using a very sensitive fluorometric method) and urea (frozen for analysis ashore using a spectrophotometric method), in addition to the standard inorganic nutrients. Dissolved nutrients were determined at the beginning (time-zero) and end (time-final) of all of the dilution experiments performed by Lessard's research group.

A series of 20 shipboard incubation experiments (termed 'survey grow-outs') were designed to assess the role of trace metal (Cu and Fe) availability on the growth of *PN* and *DA* production. These multi-day experiments were conducted with water collected from the surface mixed layer (~ 4-5 m) using the trace-metal clean sampling system (FISH; Wells Group) at stations throughout the sampling grid, with particular emphasis in regions previously found to harbor elevated concentrations of *PN* and *DA*. This is the second such extensive survey of the ECOHAB-PNW study area, and it also will provide estimates of the spatial and temporal variability of autotrophic and heterotrophic productivity in relation to the physical and chemical water mass properties of the study area. During all grow-out experiments, samples were analyzed onboard for bacterial and picoplankton abundance by the University of Western Ontario team using flow cytometry (Becton Dickinson, FACSCalibur), and will be used to generate specific rates of bacterial productivity from the bacterial protein synthesis estimates (³H-leucine method). Photosynthetic-irradiance (P-E) curves were generated from short-term (1h) ¹⁴C uptake experiments using photosynthetrons at the initiation of all survey grow-out experiments, at selected times during the drifter experiments and large volume grow-out experiments, and at the termination of continuous culture experiments (in association with Lisa Pickell, Univ. Maine); these results will be used to describe the efficiency and capacity of phytoplankton photosynthesis with respect to light intensity. Phytoplankton biomass estimates (as previously described) were determined for all metal and macronutrient treatments at the initiation and termination of each incubation experiments. These measures, together with draw-down rates of macronutrients, will be used to estimate the growth response (including *DA* production) of the phytoplankton community to copper and iron amendments. Other biological measurements conducted during the grow-out experiments included: microscopic taxonomy, sinking rates (Trick Group), total and dissolved *DA* (Wells and Trainer Groups), trace metals (Wells Group), and cellular fluorescence capacity (CFC; as measured using the inhibitor DCMU). Two, large volume (8-L) multi-day grow-out experiments were conducted to assess the differential growth and toxicity of *PN* assemblages as a function of nitrogenous nutrient source (nitrate, ammonium and urea). These experiments will discern if anthropogenic N sources, associated with agricultural

and human activities, promote either the growth or toxicity of *PN* in the study region.

Expected Results:

1. *Dissolved Inorganic and Organic Nutrients*: Approximately 50% of the samples for automated analysis were conducted onboard and final, nutrient concentrations made available. This enabled working maps of inorganic nutrients to be developed that helped guide further sampling strategies. The remainder will be available by January 15, 2006 using automated (nitrate + nitrite, silicate) methods. All ammonium samples were analyzed onboard, and the frozen samples collected for manual (urea) colorimetric analysis will be conducted by December 15, 2005. Inadequate clean water supply aboard the R/V Melville precluded complete analysis of all inorganic samples collected during the 3-week cruise.
2. *Phytoplankton Biomass*: Over 95% of the survey grid samples, drifter profiles and onboard deck experiments were analyzed onboard, and are currently available in draft form. Samples collected during the final days were frozen and later analyzed ashore at Univ. Washington by the Hickey group. All results are currently available.
3. *Photosynthetic Efficiency*: Radio-isotope samples (^{14}C) were prepared on board for liquid scintillation counting ashore at RTC; P-E curves and photosynthetic parameters should be generated by December 15.
4. *Cellular Fluorescence Capacity*: All samples analyzed onboard and are available in draft form.
5. *Bacterial Productivity*: Radio-isotope samples (^3H) were prepared on board for liquid scintillation counting ashore at RTC; rates should be generated by January 1, 2006.
6. *Nitrogen Uptake*: Samples for particulate nitrogen (PN) and (^{15}N) analysis by will be run at RTC during December 2005/January 2006, provided adequate mass spectrometry time is available. Nitrogen (nitrate, ammonium urea) rates are expected in early 2006.

c) University of Western Ontario Research Group (Charlie Trick, Liza McClintock, Benjamin Beall)

Our contribution to the ECOHAB project is two-fold: 1) to provide flow cytometric analysis (FCM) and HPLC pigment analysis to characterize the community assemblage; and 2) to provide experimental evidence of factors that either increase the competitive ability of *PN* or increase the level of DA per cell. Samples for FCM were collected at all survey stations at 5 m depth and at 5 m and 30 m in the Strait of Juan de Fuca survey. HPLC samples were collected at the 5 m depth throughout the grid and Strait of Juan de Fuca survey. This will

allow for quantitative analysis of bacteria, cyanobacteria, and nanoplankton communities, complemented by pigment analysis to characterize the phytoplankton assemblage, which will be performed using HPLC isolation-and-characterization methods. This method uses the presence or absence of the taxon-specific pigments (often referred to as the "minor or accessory" pigments) in relation to the ubiquitous photosynthetic pigments (chlorophyll) to describe the phytoplankton community structure. Our analysis by HPLC will establish the composition of the communities before and after the presence of the diatom communities, thus serving as an important oceanographic descriptor. These samples will be analyzed within ~ 1-2 months since they preserve poorly. Maps of reconstructed photosynthetic communities will be available soon thereafter.

In our second major contribution to the cruise mandate, the personnel from the Cochlan, Wells and Trick labs carried out deckboard incubation growth experiments. All labs offered their expertise to the common goal of all growth experiments (biomass formation, nutrient drawdown measurements, DA analysis (particulate and dissolved), community structure changes, bacterial and phytoplankton productivity and photosynthetic efficiency and capacity). The overall foundation of these grow-out experiments was aimed at elucidating the factors that influence the initiation, formation and/or maintenance of *PN* blooms or DA levels (either cellular or extracellular). The working hypothesis for this set of experiments was that *PN* benefits from producing DA because DA serves as an iron and/or copper chelator. Thus in the presence of macronutrients (either in upwelling sites or in the areas of high nutrients associated with the Juan de Fuca eddy) DA would act as an iron chelator, ensuring that the cells would have a supply of iron as iron concentrations diminish, either through colloid formation or utilization. Alternatively DA could serve as a copper chelator, reducing the levels of cupric ion to less inhibitory levels, allowing *PN* to fully utilize the macronutrients and grow effectively.

d) Trainer Group (Vera Trainer, Kathi Lefebvre, Keri Baugh, Shelly Nance, Sheryl Day, Brian Bill, Anthony Odell, Deborah McArthur, Anne Mataia, Marc Suddleson, Jessica Hendrickson, Stephanie Moore)

At each survey and drift station, samples were routinely taken at 0, 5, 10 m for measurement of particulate and dissolved levels of DA, whole cell counts of *PN*, enumeration of *PN* size classes, and scanning electron microscopy for species determination in selected samples. A net tow was taken at every station to rapidly determine the presence or absence of *PN* and their relative abundance. At drifter A stations, depth profiles of cells and toxins were done at some of the following depths: 0, 5, 10, 20, 30, 50 m.

1. *Particulate DA*: 1 L of seawater was filtered through a Nucleopore HA filter (0.45 micron pore size). Filters were minced in 5 mL distilled water with a thin metal spatula and sonicated for 2 h in a bath sonicator to lyse cells. An aliquot of each sample was analyzed using a receptor binding assay in

- 96-well plate format using a multiwell harvester and Top Count scintillation counter. The receptor binding assay tests the displacement of [³H]kainate by DA in a sample from a cloned glutamate receptor. Each plate of samples is compared to known DA standards analyzed on the same plate. Endogenous glutamate was digested prior to sample analysis using glutamate dehydrogenase.
2. *Whole cell hybridization assay*: Approximately 15 mL sample was filtered and fixed with saline-ethanol for 2 hours. Then specific *P. australis* (auD1) *P. multiseriis* (muD2) and *P. pungens* (puD1) probes (fluorescein labeled) were incubated with samples from stations with abundant *PN* (assessed by surface net tows) taken at several depths. Fluorescence intensity was compared to uniC (positive universal species control) and uniR (negative control) probes. Positively labeled cells on each filter were counted using fluorescence microscopy. Slides were kept in the dark for cell counting in our land-based laboratory.
 3. *Dissolved DA*: These samples were filtered through a 0.45 mm syringe filter and refrigerated until analysis. Selected samples from grow out experiments were tested using a commercially available enzyme-linked immunosorbent assay (ELISA) with picomolar sensitivity (Beacon Analytical System). This ELISA was developed using antibodies produced at NWFSC, therefore kits can be produced by Beacon at great savings (\$100 per kit) over the Biosense ELISA kits (>~300 per plate).
 4. *PN culturing*: At stations throughout the cruise where *PN* cells were present, a drop of sample was placed in f/2 medium for isolation and culturing upon return to the lab. *PN* cells will be allowed to grow in artificial seawater medium and growth and toxin production will be determined for several isolates. This will allow us to understand the relative levels of dissolved and particulate toxin each species is contributing to our cruise samples. Monoclonal isolates from the eddy and nearshore regions will be used to assess the genetic diversity among certain *PN* species using microsatellite DNA markers. This information will be used to make a preliminary determination of the relationship between *PN* populations in the eddy and nearshore regions (Nicolaus Adams, Master's thesis).
 5. *Fish exposure studies* Kathi Lefebvre): The goal of this project is to determine if dietary exposure to toxic *PN* causes neurotoxic effects in planktivorous anchovies. Anchovies were brought onboard the research vessel and were exposed to various *PN* blooms collected from Particulate DA was analyzed by filtering 1 L seawater through a Nucleopore HA filter (0.45 micron pore size). Filters were minced in 5 ml distilled water with a thin metal spatula and sonicated for 2 h in a bath sonicator to lyse cells. An aliquot of each sample was analyzed using a receptor binding assay in 96-well plate format using a multiwell harvester and Top Count scintillation counter. The receptor binding assay tests the displacement of [³H]kainate by DA in a sample from a cloned glutamate receptor. Each plate of samples is compared to known DA standards analyzed on the same plate.

Endogenous glutamate was digested prior to sample analysis using glutamate dehydrogenase. sampling stations off the coast of Washington and Canada. Exposure conditions were fully characterized for cell density, community structure, and toxicity. Exposure media was exchanged daily to maintain water quality. The following samples were taken at the beginning and end of each renewal; size-fractionated chlorophyll measurements, formaldehyde and Lugol's fixed samples, 0.2 and 0.8 stained slides, silicate, nitrate-nitrite, NH₄⁺, urea, particulate DA, and dissolved DA. Daily behavioral observations were also performed throughout the exposure. No neurotoxic symptoms were observed. Control and exposed fish were also sampled from exposure and control tanks every 24 to 48 hours. Tissues were dissected into viscera and muscle for analysis for the presence of DA. After the 10-day continuous exposure experiment, a 24-hour grazing experiment was also performed to quantify grazing rates of anchovies on ecologically realistic algal communities. This project was funded by an Oceans and Human Health grant from the Northwest Fisheries Science Center. The work was a collaborative effort with ECOHAB-PNW.

6. *Vibrio sample collection* (collaboration with NOAA West coast center in Oceans and Human Health): Surface seawater was filtered onto 0.22 µm filters, placed in Petri dishes, and frozen for characterization of potential *Vibrio* interactions with phytoplankton along our survey lines. This work will be done using PCR techniques at the land-based laboratory by Mark Strom's group at the NOAA OHH center.

e) Wells Group (Eric Roy, Peggy Hughes, Lisa Pickell, Kathleen Hardy)

The University of Maine component of the ECOHAB-PNW cruise had two primary goals: to collect seawater samples from the study area for trace metal analysis and to optimize, and to field test a flow injection based method for iron analysis. Roughly 130 surface samples from the survey station grid were collected underway, through a trace-metal clean, sampling fish. The collected samples will be later analyzed at the University of Maine by high resolution Inductively Coupled Plasma Mass Spectrometry to observe spatial and temporal variability of trace metals, and to serve as means for comparison with the shipboard iron method.

The shipboard method for iron provided preliminary iron concentrations to guide various grow-out experiments conducted by the Cochlan and Trick research teams. As a general pattern, total iron concentrations were high (1-2 nM) at nearshore stations and decreased with distance offshore, then increased again farther offshore. The highest iron concentrations, for the most part, were found on the GH line, with nearshore stations as high as 5 nM, while the Juan de Fuca eddy had some of the lowest concentrations (0.3 nM).

6. Outreach

In collaboration with NOAA's West coast center for Oceans and Human Health, Deborah McArthur participated in leg 1 of the cruise and published several online newsletters titled "Sea Times". These are now linked to the outreach page on the ECOHAB-PNW website and inform the public of experimental design, the lives of scientists onboard the ship, and the importance of the ship's crew in helping to make our science operations run smoothly.

Acknowledgements

We would like to thank the captain and crew of the R/V Melville for their support and extra effort that made the September 2005 cruise successful, in particular we thank the SIO marine technician. We also thank our shoreside support team: Jack Wekell and Susan Geier. Nicolaus Adams and Nancy Kachel deserve our special thanks for handling cruise leader responsibilities before and after the cruise. This research was supported through the Ecology and Oceanography of Harmful Algal Blooms program by National Oceanographic and Atmospheric Administration/Coastal Ocean Program Award No. NA17OP2789 and National Science Foundation Award No. 0234587.

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