

Nutrients, chlorophyll a, and flow cytometry counts of bacteria, Synechococcus, and photosynthetic eucaryotes from 13 cruises on into the Northeast Pacific from 2001-2003 as part of the U.S. GLOBEC program (NEP project)

Website: <https://www.bco-dmo.org/dataset/2457>

Version: 1

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Project

» [U.S. GLOBEC Northeast Pacific](#) (NEP)

Program

» [U.S. GLOBal ocean ECosystems dynamics](#) (U.S. GLOBEC)

Contributors	Affiliation	Role
Sherr, Barry	Oregon State University (OSU-CEOAS)	Principal Investigator
Allison, Dicky	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract

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Coverage

Spatial Extent: N:44.6517 E:-124.1 S:41.9 W:-126.05

Dataset Description

Description: Also see corresponding CTD data files. Data is from Niskin bottle sampling on cruises in 2001, 2002, and 2003. Data are final. Data are organised by year, cruise, transect, station. Station numbers are included for reference to other cruise data.

E. B. SHERR, NEP-GLOBEC Flow Cytometry data on abundance of heterotrophic bacterial cells: total, high nucleic acid, and low nucleic acid, on abundance of coccoid cyanobacteria, and on abundance of small eukaryotic autotrophic cells.

Publications based on this data set:

Sherr, E.B., Sherr, B.F., Wheeler, P.A. 2005. Distribution of coccoid cyanobacteria and small eukaryotic phytoplankton in the upwelling ecosystem off the Oregon coast during 2001 and 2002. Deep-Sea Research II 52:317-330

Sherr E.B., Sherr B.F., Longnecker K. 2006. Distribution of bacterial abundance and cell-specific nucleic acid

Methods & Sampling

Sample collection: During each of 13 GLOBEC NEP-LTOP survey cruises, from March 2001 to September 2003, 4 to 8 stations were sampled along a transect line off Newport, Oregon to 160 kilometers offshore. In March and September 2001, and in April, July and September 2002 and 2003, three to four additional transect lines to the south were also sampled ([Figure 1.](#)). At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.

Data Processing Description

Flow Cytometry Methods:

For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 degrees C until flow cytometric analysis was performed. Duplicate subsamples of seawater for nutrient analyses were collected in acid-washed Nalgene™ 60 ml HDPE bottles and immediately frozen at -20 degrees C for nutrient analysis. For determination of chlorophyll-a concentration, subsamples of whole seawater were vacuum filtered onto 25 mm Whatman GF/F filters, and the filters stored frozen in glass Vacutainer™ tubes (-20 degrees C) until processed on shore.

Sample analysis

Nutrient and chlorophyll-a: Analysis of nutrient and chlorophyll-a concentrations during the GLOBEC cruises were carried out by the NEP-LTOP service group; results are available as a data report (Wetz et al. 2004). Analyses for concentration of nitrate plus nitrite, phosphate and silicic acid (silicate) were performed using a hybrid Technicon AutoAnalyzerII(tm) and Alpkem RFA300(tm) system following protocols modified from Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999); while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.).

Flow cytometric analysis of cell abundances: In the laboratory, samples were thawed and kept on ice in a dark container until run on a Becton-Dickinson FACSCaliber flow cytometer with a 488 nm laser. For enumeration of small sized phytoplankton, 500 µl subsamples were processed as described in Sherr et al. (2005). Populations of coccoid cyanobacteria (*Synechococcus* or SYN) and of photosynthetic eukaryotes (PEUK) were distinguished by differences in fluorescence in orange (cyanobacteria) and in red (eukaryotic phytoplankton) wavelengths. We have previously determined that 75% to 85% of the red-fluorescing cells in the PEUK region of our cytograms are < 5 µm in size (Sherr et al. 2005). *Prochlorococcus* appears on the red-fluorescence axis of the flow cytograms to the left of the PEUK region, which was confirmed by a few observations of *Prochlorococcus* in cytograms of samples collected at a basin station in September 2001.

For heterotrophic bacteria, 250 µl subsamples were diluted with 250 µl of DiW, and stained with SYBR Green I and potassium citrate for 15 min, following the protocol of Marie et al. (1997). Bacterial counts were made during a three minute sample run at low flow rate. Regions were established in cytograms of side scatter and green fluorescence to define bacterial cells with high nucleic acid content (HNA) and low nucleic content (LNA). The cytogram for each sample was individually inspected, and HNA and LNA regions manually moved to conform to the appropriate areas of the bacterial dot-plot. Mean cell-specific SYBR fluorescence was obtained for total bacteria and for HNA and LNA cells, along with abundance of cells within each group. Logical gating in

Becton-Dickinson Cell Quest software was used to exclude coccoid cyanobacteria, based on orange fluorescence, from the abundance counts of heterotrophic bacteria.

Each subsample was spiked immediately before processing with a known amount of either 3.0 μm (for phytoplankton) or 1.0 μm (for bacteria) Polysciences Fluoresbrite yellow-green beads from respective stock solutions of beads that had been precalibrated with Becton-Dickinson True-Count beads. The number of beads enumerated in each sample run was used to accurately determine the sample volume processed and thus the abundances of SYN, PEUK, and bacteria.

Notes:

The Thesaurus does not contain the noted fields (N1-N6). The data for N1, N2, N3, N5 and N6 are provided as cells per ml (rather than the thesaurus recommended units of cells per cubic meter). Note N4, the "FL_ratio_HNAtoLNA" is the ratio of fluorescence of High Nucleic Acid particles to Low Nucleic Acid particles in that sample.

References cited:

Corwith, H.L., Wheeler, P.A., 2002. El Nino related variations in nutrient and chlorophyll distributions off Oregon. *Progress in Oceanography* 54, 361-380. Fleischbein, J., Hill, J., Huyer, A., Smith, R.L., Wheeler P.A., 1999. Hydrographic data from the GLOBEC long-term observation program off Oregon, 1997 and 1998. Data Report 172, Ref. 99-1. OSU.

Gordon, L. I., Jennings, J. C., Ross, A. A., Krest, J. M., 1995. A suggested protocol for continuous flow automated analysis of seawater nutrients (phosphate, nitrate, nitrite and silicic acid) in the WOCE Hydrographic Program and the Joint Global Ocean Fluxes Study, WOCE Operations Manual, WOCE Report No. 68/91. Revision 1995.

Marie, D., Partensky, F., Jacquet, S., Vaulot, D., 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Applied and Environmental Microbiology* 63, 186-193.

Parsons, T. R., Maita, Y., Lalli, C.M. 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*, 173 pp., Pergamon Press, Oxford, UK.

Sherr, E.B., Sherr, B.F., Wheeler, P.A. 2005. Distribution of coccoid cyanobacteria and small eukaryotic phytoplankton in the upwelling ecosystem off the Oregon coast during 2001 and 2002. *Deep-Sea Research II* 52:317-330

Wetz, M.S., J. Hill, H. Corwith, and P. A. Wheeler. 2004. Nutrient and Extracted Chlorophyll Data from the GLOBEC Long-Term Observation Program, 1997-2004 Data Report 193

[Figure 1](#). Station locations for the five GLOBEC NEP-LTOP sampling lines off Oregon and northern California: NH - Newport Hydroline off Newport, Oregon; HH - Heceta Head line off Heceta Head, Oregon; FM - Five Mile Point line 20 km south of Coos Bay, Oregon; RR - Rogue River line off the mouth of the Rogue River at Gold Beach, Oregon; and CC - Crescent City line off of Crescent City California just south of the Oregon/California border indicated on the map. Depth contours of 50 m, 200 m, and 2000 m are shown.

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Data Files

File
fcm.csv (Comma Separated Values (.csv), 207.63 KB) MD5:f5e601aca73162f544858f55d9e46d84
Primary data file for dataset ID 2457

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Parameters

Parameter	Description	Units
year	Year	dimensionless
cruiseid	Cruise identifier (e.g. W0302A = R/V Wecoma cruise 0302A).	dimensionless
transect	Transect identifier.	dimensionless
station	Station number.	dimensionless
station_std	Standard station name.	dimensionless
depth_w	Bottom depth.	unknown
month_gmt	Month.	dimensionless
day_gmt	Day of month.	dimensionless
time_gmt	Time (GMT); 24 hr clock (HHMM).	dimensionless
lat	Latitude.	decimal degrees
lon	Longitude.	decimal degrees
depth	Sample collection depth.	meters
bact_total_abund	Total abundance of bacteria. Parameter originally named 'bact_total_abund_cellsperml (N1)'. 	cells per mL
bact_HNA_abund	Abundance of HNA (high-nucleic acid content) bacteria. Parameter originally named 'bact_HNA_abund_cellsperml (N2)'. 	cells per mL
bact_LNA_abund	Abundance of LNA (low nucleic acid content) bacteria. Parameter originally named 'bact_LNA_abund_cellsperml (N3)'. 	cells per mL
FL_ratio_HNAtoLNA	Ratio of fluorescence of High Nucleic Acid particles to Low Nucleic Acid particles in that sample. Also referred to as 'N4'. 	dimensionless (ratio)
coccus_s	Abundance of coccoid cyanobacteria (Synechococcus). Original parameter name 'Synecho_abund_perml (N5)'. 	cells per mL
ph_euk_abund	Abundance of photosynthetic eucaryotes. Parameter originally named 'PEUK_abund_perml (N6)'. 	cells per mL
temp	Temperature	degrees C
sal	Salinity	psu
PO4	PO4.	umoles/L
SiO2	SiO2.	umoles/L
NO3_NO2	Nitrate and Nitrite.	umoles/L
chl_a	chlorophyll	ug/L

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Instruments

Dataset-specific Instrument Name	Niskin Bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m.
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

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Deployments

W0103B

Website	https://www.bco-dmo.org/deployment/57603
Platform	R/V Wecoma
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/mar01cr.pdf
Start Date	2001-03-20
End Date	2001-03-24

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p> <p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. Duplicate subsamples of seawater for nutrient analyses were collected in acid-washed Nalgene™ 60 ml HDPE bottles and immediately frozen at -20 °C for nutrient analysis. For determination of chlorophyll-a concentration, subsamples of whole seawater were vacuum filtered onto 25 mm Whatman GF/F filters, and the filters stored frozen in glass Vacutainer™ tubes (-20 °C) until processed on shore. Nutrient and chlorophyll-a: Analysis of nutrient and chlorophyll-a concentrations during the GLOBEC cruises were carried out by the NEP-LTOP service group, results are available as a data report (Wetz et al. 2004). Analyses for concentration of nitrate plus nitrite, phosphate and silicic acid (silicate) were performed using a hybrid Technicon AutoAnalyzerII(tm) and Alpkem RFA300(tm) system following protocols modified from Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). Flow cytometric analysis of cell abundances: In the laboratory, samples were thawed and kept on ice in a dark container until run on a Becton-Dickinson FACSCaliber flow cytometer with a 488 nm laser. For enumeration of small sized phytoplankton, 500 µl subsamples were processed as described in Sherr et al. (2005). Populations of coccoid cyanobacteria (Synechococcus or SYN) and of photosynthetic eukaryotes (PEUK) were distinguished by differences in fluorescence in orange (cyanobacteria) and in red (eukaryotic phytoplankton) wavelengths. We have previously determined that 75% to 85% of the red-fluorescing cells in the PEUK region of our cytograms are < 5 µm in size (Sherr et al. 2005). Prochlorococcus appears on the red-fluorescence axis of the flow cytograms to the left of the PEUK region, which was confirmed by a few observations of Prochlorococcus in cytograms of samples collected at a basin station in September 2001. 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Each subsample was spiked immediately before processing with a known amount of either 3.0 µm (for phytoplankton) or 1.0 µm (for bacteria) Polysciences Fluoresbrite yellow-green beads from respective stock solutions of beads that had been precalibrated with Becton-Dickinson True-Count beads. The number of beads enumerated in each sample run was used to accurately determine the sample volume processed and thus the abundances of SYN, PEUK, and bacteria.</p>
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Website	https://www.bco-dmo.org/deployment/57604
Platform	R/V Wecoma
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/jul01cr.pdf
Start Date	2001-07-06
End Date	2001-07-09

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p> <p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. 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Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). 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W0109A

Website	https://www.bco-dmo.org/deployment/57605
Platform	R/V Wecoma
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/sep01cr.pdf
Start Date	2001-09-04
End Date	2001-09-10

Description	<p>Methods & Sampling</p> <p>At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p>
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W0111B

Website	https://www.bco-dmo.org/deployment/57606
Platform	R/V Wecoma
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/nov01cr.pdf
Start Date	2001-11-27
End Date	2001-11-29

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p> <p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. 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Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). Flow cytometric analysis of cell abundances: In the laboratory, samples were thawed and kept on ice in a dark container until run on a Becton-Dickinson FACSCaliber flow cytometer with a 488 nm laser. For enumeration of small sized phytoplankton, 500 µl subsamples were processed as described in Sherr et al. (2005). Populations of coccoid cyanobacteria (Synechococcus or SYN) and of photosynthetic eukaryotes (PEUK) were distinguished by differences in fluorescence in orange (cyanobacteria) and in red (eukaryotic phytoplankton) wavelengths. We have previously determined that 75% to 85% of the red-fluorescing cells in the PEUK region of our cytograms are < 5 µm in size (Sherr et al. 2005). Prochlorococcus appears on the red-fluorescence axis of the flow cytograms to the left of the PEUK region, which was confirmed by a few observations of Prochlorococcus in cytograms of samples collected at a basin station in September 2001. For heterotrophic bacteria, 250 µl subsamples were diluted with 250 µl of DiW, and stained with SYBR Green I and potassium citrate for 15 min, following the protocol of Marie et al. (1997). Bacterial counts were made during a three minute sample run at low flow rate. Regions were established in cytograms of side scatter and green fluorescence to define bacterial cells with high nucleic acid content (HNA) and low nucleic content (LNA). The cytogram for each sample was individually inspected, and HNA and LNA regions manually moved to conform to the appropriate areas of the bacterial dot-plot. Mean cell-specific SYBR fluorescence was obtained for total bacteria and for HNA and LNA cells, along with abundance of cells within each group. Logical gating in Becton-Dickinson Cell Quest software was used to exclude coccoid cyanobacteria, based on orange fluorescence, from the abundance counts of heterotrophic bacteria. Each subsample was spiked immediately before processing with a known amount of either 3.0 µm (for phytoplankton) or 1.0 µm (for bacteria) Polysciences Fluoresbrite yellow-green beads from respective stock solutions of beads that had been precalibrated with Becton-Dickinson True-Count beads. The number of beads enumerated in each sample run was used to accurately determine the sample volume processed and thus the abundances of SYN, PEUK, and bacteria.</p>
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W0202A

Website	https://www.bco-dmo.org/deployment/57607
Platform	R/V Wecoma
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/feb02cr.pdf
Start Date	2002-02-19
End Date	2002-02-21

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p> <p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. Duplicate subsamples of seawater for nutrient analyses were collected in acid-washed Nalgene™ 60 ml HDPE bottles and immediately frozen at -20 °C for nutrient analysis. For determination of chlorophyll-a concentration, subsamples of whole seawater were vacuum filtered onto 25 mm Whatman GF/F filters, and the filters stored frozen in glass Vacutainer™ tubes (-20 °C) until processed on shore. Nutrient and chlorophyll-a: Analysis of nutrient and chlorophyll-a concentrations during the GLOBEC cruises were carried out by the NEP-LTOP service group, results are available as a data report (Wetz et al. 2004). Analyses for concentration of nitrate plus nitrite, phosphate and silicic acid (silicate) were performed using a hybrid Technicon AutoAnalyzerII(tm) and Alpkem RFA300(tm) system following protocols modified from Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). 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For heterotrophic bacteria, 250 µl subsamples were diluted with 250 µl of DiW, and stained with SYBR Green I and potassium citrate for 15 min, following the protocol of Marie et al. (1997). Bacterial counts were made during a three minute sample run at low flow rate. Regions were established in cytograms of side scatter and green fluorescence to define bacterial cells with high nucleic acid content (HNA) and low nucleic content (LNA). The cytogram for each sample was individually inspected, and HNA and LNA regions manually moved to conform to the appropriate areas of the bacterial dot-plot. Mean cell-specific SYBR fluorescence was obtained for total bacteria and for HNA and LNA cells, along with abundance of cells within each group. Logical gating in Becton-Dickinson Cell Quest software was used to exclude coccoid cyanobacteria, based on orange fluorescence, from the abundance counts of heterotrophic bacteria. Each subsample was spiked immediately before processing with a known amount of either 3.0 µm (for phytoplankton) or 1.0 µm (for bacteria) Polysciences Fluoresbrite yellow-green beads from respective stock solutions of beads that had been precalibrated with Becton-Dickinson True-Count beads. The number of beads enumerated in each sample run was used to accurately determine the sample volume processed and thus the abundances of SYN, PEUK, and bacteria.</p>
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W0204A

Website	https://www.bco-dmo.org/deployment/57608
Platform	R/V Wecoma
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/apr02cr.pdf
Start Date	2002-04-04
End Date	2002-04-10

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p>
	<p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. Duplicate subsamples of seawater for nutrient analyses were collected in acid-washed Nalgene™ 60 ml HDPE bottles and immediately frozen at -20 °C for nutrient analysis. For determination of chlorophyll-a concentration, subsamples of whole seawater were vacuum filtered onto 25 mm Whatman GF/F filters, and the filters stored frozen in glass Vacutainer™ tubes (-20 °C) until processed on shore. Nutrient and chlorophyll-a: Analysis of nutrient and chlorophyll-a concentrations during the GLOBEC cruises were carried out by the NEP-LTOP service group, results are available as a data report (Wetz et al. 2004). Analyses for concentration of nitrate plus nitrite, phosphate and silicic acid (silicate) were performed using a hybrid Technicon AutoAnalyzerII(tm) and Alpkem RFA300(tm) system following protocols modified from Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). Flow cytometric analysis of cell abundances: In the laboratory, samples were thawed and kept on ice in a dark container until run on a Becton-Dickinson FACSCaliber flow cytometer with a 488 nm laser. For enumeration of small sized phytoplankton, 500 µl subsamples were processed as described in Sherr et al. (2005). Populations of coccoid cyanobacteria (Synechococcus or SYN) and of photosynthetic eukaryotes (PEUK) were distinguished by differences in fluorescence in orange (cyanobacteria) and in red (eukaryotic phytoplankton) wavelengths. We have previously determined that 75% to 85% of the red-fluorescing cells in the PEUK region of our cytograms are < 5 µm in size (Sherr et al. 2005). Prochlorococcus appears on the red-fluorescence axis of the flow cytograms to the left of the PEUK region, which was confirmed by a few observations of Prochlorococcus in cytograms of samples collected at a basin station in September 2001. For heterotrophic bacteria, 250 µl subsamples were diluted with 250 µl of DiW, and stained with SYBR Green I and potassium citrate for 15 min, following the protocol of Marie et al. (1997). Bacterial counts were made during a three minute sample run at low flow rate. Regions were established in cytograms of side scatter and green fluorescence to define bacterial cells with high nucleic acid content (HNA) and low nucleic content (LNA). The cytogram for each sample was individually inspected, and HNA and LNA regions manually moved to conform to the appropriate areas of the bacterial dot-plot. Mean cell-specific SYBR fluorescence was obtained for total bacteria and for HNA and LNA cells, along with abundance of cells within each group. Logical gating in Becton-Dickinson Cell Quest software was used to exclude coccoid cyanobacteria, based on orange fluorescence, from the abundance counts of heterotrophic bacteria. Each subsample was spiked immediately before processing with a known amount of either 3.0 µm (for phytoplankton) or 1.0 µm (for bacteria) Polysciences Fluoresbrite yellow-green beads from respective stock solutions of beads that had been precalibrated with Becton-Dickinson True-Count beads. The number of beads enumerated in each sample run was used to accurately determine the sample volume processed and thus the abundances of SYN, PEUK, and bacteria.</p>

W0207A

Website	https://www.bco-dmo.org/deployment/57610
Platform	R/V Wecoma
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/jul02cr.pdf
Start Date	2002-07-09
End Date	2002-07-15

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p> <p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. Duplicate subsamples of seawater for nutrient analyses were collected in acid-washed Nalgene™ 60 ml HDPE bottles and immediately frozen at -20 °C for nutrient analysis. For determination of chlorophyll-a concentration, subsamples of whole seawater were vacuum filtered onto 25 mm Whatman GF/F filters, and the filters stored frozen in glass Vacutainer™ tubes (-20 °C) until processed on shore. Nutrient and chlorophyll-a: Analysis of nutrient and chlorophyll-a concentrations during the GLOBEC cruises were carried out by the NEP-LTOP service group, results are available as a data report (Wetz et al. 2004). Analyses for concentration of nitrate plus nitrite, phosphate and silicic acid (silicate) were performed using a hybrid Technicon AutoAnalyzerII(tm) and Alpkem RFA300(tm) system following protocols modified from Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). Flow cytometric analysis of cell abundances: In the laboratory, samples were thawed and kept on ice in a dark container until run on a Becton-Dickinson FACSCaliber flow cytometer with a 488 nm laser. For enumeration of small sized phytoplankton, 500 µl subsamples were processed as described in Sherr et al. (2005). Populations of coccoid cyanobacteria (Synechococcus or SYN) and of photosynthetic eukaryotes (PEUK) were distinguished by differences in fluorescence in orange (cyanobacteria) and in red (eukaryotic phytoplankton) wavelengths. We have previously determined that 75% to 85% of the red-fluorescing cells in the PEUK region of our cytograms are < 5 µm in size (Sherr et al. 2005). Prochlorococcus appears on the red-fluorescence axis of the flow cytograms to the left of the PEUK region, which was confirmed by a few observations of Prochlorococcus in cytograms of samples collected at a basin station in September 2001. For heterotrophic bacteria, 250 µl subsamples were diluted with 250 µl of DiW, and stained with SYBR Green I and potassium citrate for 15 min, following the protocol of Marie et al. (1997). Bacterial counts were made during a three minute sample run at low flow rate. Regions were established in cytograms of side scatter and green fluorescence to define bacterial cells with high nucleic acid content (HNA) and low nucleic content (LNA). The cytogram for each sample was individually inspected, and HNA and LNA regions manually moved to conform to the appropriate areas of the bacterial dot-plot. Mean cell-specific SYBR fluorescence was obtained for total bacteria and for HNA and LNA cells, along with abundance of cells within each group. Logical gating in Becton-Dickinson Cell Quest software was used to exclude coccoid cyanobacteria, based on orange fluorescence, from the abundance counts of heterotrophic bacteria. Each subsample was spiked immediately before processing with a known amount of either 3.0 µm (for phytoplankton) or 1.0 µm (for bacteria) Polysciences Fluoresbrite yellow-green beads from respective stock solutions of beads that had been precalibrated with Becton-Dickinson True-Count beads. The number of beads enumerated in each sample run was used to accurately determine the sample volume processed and thus the abundances of SYN, PEUK, and bacteria.</p>
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AT7-21

Website	https://www.bco-dmo.org/deployment/57490
Platform	R/V Atlantis
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/sep02cr.pdf
Start Date	2002-09-27
End Date	2002-10-03
Description	<p>funded by NSF OCE-0000733 UNOLS schedule link The original data from this cruise are available from the NSF R2R data catalog.</p> <p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p> <p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. Duplicate subsamples of seawater for nutrient analyses were collected in acid-washed Nalgene™ 60 ml HDPE bottles and immediately frozen at -20 °C for nutrient analysis. For determination of chlorophyll-a concentration, subsamples of whole seawater were vacuum filtered onto 25 mm Whatman GF/F filters, and the filters stored frozen in glass Vacutainer™ tubes (-20 °C) until processed on shore. Nutrient and chlorophyll-a: Analysis of nutrient and chlorophyll-a concentrations during the GLOBEC cruises were carried out by the NEP-LTOP service group, results are available as a data report (Wetz et al. 2004). Analyses for concentration of nitrate plus nitrite, phosphate and silicic acid (silicate) were performed using a hybrid Technicon AutoAnalyzerII(tm) and Alpkem RFA300(tm) system following protocols modified from Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). Flow cytometric analysis of cell abundances: In the laboratory, samples were thawed and kept on ice in a dark container until run on a Becton-Dickinson FACSCaliber flow cytometer with a 488 nm laser. For enumeration of small sized phytoplankton, 500 µl subsamples were processed as described in Sherr et al. (2005). Populations of coccoid cyanobacteria (Synechococcus or SYN) and of photosynthetic eukaryotes (PEUK) were distinguished by differences in fluorescence in orange (cyanobacteria) and in red (eukaryotic phytoplankton) wavelengths. We have previously determined that 75% to 85% of the red-fluorescing cells in the PEUK region of our cytograms are < 5 µm in size (Sherr et al. 2005). Prochlorococcus appears on the red-fluorescence axis of the flow cytograms to the left of the PEUK region, which was confirmed by a few observations of Prochlorococcus in cytograms of samples collected at a basin station in September 2001. For heterotrophic bacteria, 250 µl subsamples were diluted with 250 µl of DiW, and stained with SYBR Green I and potassium citrate for 15 min, following the protocol of Marie et al. (1997). Bacterial counts were made during a three minute sample run at low flow rate. Regions were established in cytograms of side scatter and green fluorescence to define bacterial cells with high nucleic acid content (HNA) and low nucleic content (LNA). The cytogram for each sample was individually inspected, and HNA and LNA regions manually moved to conform to the appropriate areas of the bacterial dot-plot. Mean cell-specific SYBR fluorescence was obtained</p>

for total bacteria and for HNA and LNA cells, along with abundance of cells within each group. Logical gating in Becton-Dickinson Cell Quest software was used to exclude coccoid cyanobacteria, based on orange fluorescence, from the abundance counts of heterotrophic bacteria. Each subsample was spiked immediately before processing with a known amount of either 3.0 μm (for phytoplankton) or 1.0 μm (for bacteria) Polysciences Fluoresbrite yellow-green beads from respective stock solutions of beads that had been precalibrated with Becton-Dickinson True-Count beads. The number of beads enumerated in each sample run was used to accurately determine the sample volume processed and thus the abundances of SYN, PEUK, and bacteria.

W0212A

Website	https://www.bco-dmo.org/deployment/57611
Platform	R/V Wecoma
Report	http://globec.who.edu/nep/reports/ccs_cruises/dec02cr.pdf
Start Date	2002-12-03
End Date	2002-12-05

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p> <p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. Duplicate subsamples of seawater for nutrient analyses were collected in acid-washed Nalgene™ 60 ml HDPE bottles and immediately frozen at -20 °C for nutrient analysis. For determination of chlorophyll-a concentration, subsamples of whole seawater were vacuum filtered onto 25 mm Whatman GF/F filters, and the filters stored frozen in glass Vacutainer™ tubes (-20 °C) until processed on shore. Nutrient and chlorophyll-a: Analysis of nutrient and chlorophyll-a concentrations during the GLOBEC cruises were carried out by the NEP-LTOP service group, results are available as a data report (Wetz et al. 2004). Analyses for concentration of nitrate plus nitrite, phosphate and silicic acid (silicate) were performed using a hybrid Technicon AutoAnalyzerII(tm) and Alpkem RFA300(tm) system following protocols modified from Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). 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Website	https://www.bco-dmo.org/deployment/57612
Platform	R/V Wecoma
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/feb03cr.pdf
Start Date	2003-02-14
End Date	2003-02-16

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p> <p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. Duplicate subsamples of seawater for nutrient analyses were collected in acid-washed Nalgene™ 60 ml HDPE bottles and immediately frozen at -20 °C for nutrient analysis. For determination of chlorophyll-a concentration, subsamples of whole seawater were vacuum filtered onto 25 mm Whatman GF/F filters, and the filters stored frozen in glass Vacutainer™ tubes (-20 °C) until processed on shore. Nutrient and chlorophyll-a: Analysis of nutrient and chlorophyll-a concentrations during the GLOBEC cruises were carried out by the NEP-LTOP service group, results are available as a data report (Wetz et al. 2004). Analyses for concentration of nitrate plus nitrite, phosphate and silicic acid (silicate) were performed using a hybrid Technicon AutoAnalyzerII(tm) and Alpkem RFA300(tm) system following protocols modified from Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). Flow cytometric analysis of cell abundances: In the laboratory, samples were thawed and kept on ice in a dark container until run on a Becton-Dickinson FACSCaliber flow cytometer with a 488 nm laser. For enumeration of small sized phytoplankton, 500 µl subsamples were processed as described in Sherr et al. (2005). Populations of coccoid cyanobacteria (Synechococcus or SYN) and of photosynthetic eukaryotes (PEUK) were distinguished by differences in fluorescence in orange (cyanobacteria) and in red (eukaryotic phytoplankton) wavelengths. We have previously determined that 75% to 85% of the red-fluorescing cells in the PEUK region of our cytograms are < 5 µm in size (Sherr et al. 2005). Prochlorococcus appears on the red-fluorescence axis of the flow cytograms to the left of the PEUK region, which was confirmed by a few observations of Prochlorococcus in cytograms of samples collected at a basin station in September 2001. 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W0304A

Website	https://www.bco-dmo.org/deployment/57613
Platform	R/V Wecoma
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/apr03cr.pdf
Start Date	2003-04-01
End Date	2003-04-06

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p> <p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. Duplicate subsamples of seawater for nutrient analyses were collected in acid-washed Nalgene™ 60 ml HDPE bottles and immediately frozen at -20 °C for nutrient analysis. For determination of chlorophyll-a concentration, subsamples of whole seawater were vacuum filtered onto 25 mm Whatman GF/F filters, and the filters stored frozen in glass Vacutainer™ tubes (-20 °C) until processed on shore. Nutrient and chlorophyll-a: Analysis of nutrient and chlorophyll-a concentrations during the GLOBEC cruises were carried out by the NEP-LTOP service group, results are available as a data report (Wetz et al. 2004). Analyses for concentration of nitrate plus nitrite, phosphate and silicic acid (silicate) were performed using a hybrid Technicon AutoAnalyzerII(tm) and Alpkem RFA300(tm) system following protocols modified from Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). Flow cytometric analysis of cell abundances: In the laboratory, samples were thawed and kept on ice in a dark container until run on a Becton-Dickinson FACSCaliber flow cytometer with a 488 nm laser. For enumeration of small sized phytoplankton, 500 µl subsamples were processed as described in Sherr et al. (2005). Populations of coccoid cyanobacteria (Synechococcus or SYN) and of photosynthetic eukaryotes (PEUK) were distinguished by differences in fluorescence in orange (cyanobacteria) and in red (eukaryotic phytoplankton) wavelengths. We have previously determined that 75% to 85% of the red-fluorescing cells in the PEUK region of our cytograms are < 5 µm in size (Sherr et al. 2005). Prochlorococcus appears on the red-fluorescence axis of the flow cytograms to the left of the PEUK region, which was confirmed by a few observations of Prochlorococcus in cytograms of samples collected at a basin station in September 2001. For heterotrophic bacteria, 250 µl subsamples were diluted with 250 µl of DiW, and stained with SYBR Green I and potassium citrate for 15 min, following the protocol of Marie et al. (1997). Bacterial counts were made during a three minute sample run at low flow rate. Regions were established in cytograms of side scatter and green fluorescence to define bacterial cells with high nucleic acid content (HNA) and low nucleic content (LNA). The cytogram for each sample was individually inspected, and HNA and LNA regions manually moved to conform to the appropriate areas of the bacterial dot-plot. Mean cell-specific SYBR fluorescence was obtained for total bacteria and for HNA and LNA cells, along with abundance of cells within each group. Logical gating in Becton-Dickinson Cell Quest software was used to exclude coccoid cyanobacteria, based on orange fluorescence, from the abundance counts of heterotrophic bacteria. Each subsample was spiked immediately before processing with a known amount of either 3.0 µm (for phytoplankton) or 1.0 µm (for bacteria) Polysciences Fluoresbrite yellow-green beads from respective stock solutions of beads that had been precalibrated with Becton-Dickinson True-Count beads. The number of beads enumerated in each sample run was used to accurately determine the sample volume processed and thus the abundances of SYN, PEUK, and bacteria.</p>
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NH0307A

Website	https://www.bco-dmo.org/deployment/57560
Platform	R/V New Horizon
Report	http://globec.who.edu/nep/reports/ccs_cruises/jul03cr.pdf
Start Date	2003-07-02
End Date	2003-07-08

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p> <p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. 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Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). 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W0309B

Website	https://www.bco-dmo.org/deployment/57617
Platform	R/V Wecoma
Report	http://globec.whoi.edu/nep/reports/ccs_cruises/sep03cr.pdf
Start Date	2003-09-26
End Date	2003-10-01

Description	<p>Methods & Sampling At each station sampled, water was collected, using 5-liter Niskin bottles mounted on a CTD rosette, for analysis of bacteria and phytoplankton stocks via flow cytometry from 4 to 6 depths in the upper 100 to 150 m. Samples were also collected from the same Niskin bottles as part of the LTOP service program for determination of concentrations of inorganic nutrients and chlorophyll-a. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.</p>
	<p>Processing Description For flow cytometry samples, 3 ml aliquots were pipetted into 4 ml cryovials and preserved with 0.2% (w/v) final concentration of freshly made paraformaldehyde. The samples were gently mixed and let sit in the dark at room temperature for 10 minutes before quick-freezing and storage in liquid nitrogen. On return of samples to the lab, the cryovials were stored at -80 °C until flow cytometric analysis was performed. Duplicate subsamples of seawater for nutrient analyses were collected in acid-washed Nalgene™ 60 ml HDPE bottles and immediately frozen at -20 °C for nutrient analysis. For determination of chlorophyll-a concentration, subsamples of whole seawater were vacuum filtered onto 25 mm Whatman GF/F filters, and the filters stored frozen in glass Vacutainer™ tubes (-20 °C) until processed on shore. Nutrient and chlorophyll-a: Analysis of nutrient and chlorophyll-a concentrations during the GLOBEC cruises were carried out by the NEP-LTOP service group, results are available as a data report (Wetz et al. 2004). Analyses for concentration of nitrate plus nitrite, phosphate and silicic acid (silicate) were performed using a hybrid Technicon AutoAnalyzerII(tm) and Alpkem RFA300(tm) system following protocols modified from Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low-nutrient surface seawater. Deionized water (DiW) was used as a blank, and triplicate DiW blanks were run at the beginning and end of each run, to correct for any baseline shifts. In this protocol, the coefficients of variation for duplicates at low nutrient concentrations are typically < 1% (Fleischbein et al., 1999), while at high nutrient concentrations, coefficients of variation are 2-3 % for nitrate and silicate (Corwith and Wheeler 2002). Chlorophyll was extracted for > 12 h in the dark at -20 °C (Parsons et al., 1984) using either 90% acetone or 95% methanol as the solvent. Fluorescence was measured with a Turner Designs(TM) 10-AU fluorometer that had been calibrated with purified chlorophyll-a (Sigma Co.). Flow cytometric analysis of cell abundances: In the laboratory, samples were thawed and kept on ice in a dark container until run on a Becton-Dickinson FACSCaliber flow cytometer with a 488 nm laser. For enumeration of small sized phytoplankton, 500 µl subsamples were processed as described in Sherr et al. (2005). Populations of coccoid cyanobacteria (Synechococcus or SYN) and of photosynthetic eukaryotes (PEUK) were distinguished by differences in fluorescence in orange (cyanobacteria) and in red (eukaryotic phytoplankton) wavelengths. We have previously determined that 75% to 85% of the red-fluorescing cells in the PEUK region of our cytograms are < 5 µm in size (Sherr et al. 2005). Prochlorococcus appears on the red-fluorescence axis of the flow cytograms to the left of the PEUK region, which was confirmed by a few observations of Prochlorococcus in cytograms of samples collected at a basin station in September 2001. For heterotrophic bacteria, 250 µl subsamples were diluted with 250 µl of DiW, and stained with SYBR Green I and potassium citrate for 15 min, following the protocol of Marie et al. (1997). Bacterial counts were made during a three minute sample run at low flow rate. Regions were established in cytograms of side scatter and green fluorescence to define bacterial cells with high nucleic acid content (HNA) and low nucleic content (LNA). The cytogram for each sample was individually inspected, and HNA and LNA regions manually moved to conform to the appropriate areas of the bacterial dot-plot. Mean cell-specific SYBR fluorescence was obtained for total bacteria and for HNA and LNA cells, along with abundance of cells within each group. Logical gating in Becton-Dickinson Cell Quest software was used to exclude coccoid cyanobacteria, based on orange fluorescence, from the abundance counts of heterotrophic bacteria. Each subsample was spiked immediately before processing with a known amount of either 3.0 µm (for phytoplankton) or 1.0 µm (for bacteria) Polysciences Fluoresbrite yellow-green beads from respective stock solutions of beads that had been precalibrated with Becton-Dickinson True-Count beads. The number of beads enumerated in each sample run was used to accurately determine the sample volume processed and thus the abundances of SYN, PEUK, and bacteria.</p>

Project Information

U.S. GLOBEC Northeast Pacific (NEP)

Website: <http://nepglobec.bco-dmo.org>

Coverage: Northeast Pacific Ocean, Gulf of Alaska

Program in a Nutshell

Goal: To understand the effects of climate variability and climate change on the distribution, abundance and production of marine animals (including commercially important living marine resources) in the eastern North Pacific. To embody this understanding in diagnostic and prognostic ecosystem models, capable of capturing the ecosystem response to major climatic fluctuations.

Approach: To study the effects of past and present climate variability on the population ecology and population dynamics of marine biota and living marine resources, and to use this information as a proxy for how the ecosystems of the eastern North Pacific may respond to future global climate change. The strong temporal variability in the physical and biological signals of the NEP will be used to examine the biophysical mechanisms through which zooplankton and salmon populations respond to physical forcing and biological interactions in the coastal regions of the two gyres. Annual and interannual variability will be studied directly through **long-term observations** and detailed **process studies**; variability at longer time scales will be examined through **retrospective analysis** of directly measured and proxy data. Coupled **biophysical models** of the ecosystems of these regions will be developed and tested using the process studies and data collected from the long-term observation programs, then further tested and improved by hindcasting selected retrospective data series.

Program Information

U.S. GLOBAL ocean ECosystems dynamics (U.S. GLOBEC)

Website: <http://www.usglobec.org/>

Coverage: Global

U.S. GLOBEC (GLOBAL ocean ECosystems dynamics) is a research program organized by oceanographers and fisheries scientists to address the question of how global climate change may affect the abundance and production of animals in the sea.

The U.S. GLOBEC Program currently had major research efforts underway in the Georges Bank / Northwest Atlantic Region, and the Northeast Pacific (with components in the California Current and in the Coastal Gulf of Alaska). U.S. GLOBEC was a major contributor to International GLOBEC efforts in the Southern Ocean and Western Antarctic Peninsula (WAP).

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0000733
National Oceanic and Atmospheric Administration (NOAA)	NA67RJ0151 (NEP)
National Oceanic and Atmospheric Administration (NOAA)	NA86OP0589 (NEP)

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