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Oregon State University (OSU, Corvallis, Oregon, U.S.A.) was the site, from May 23–24, 2005, of an international workshop on Oceanic Ecodynamyics COmparison in the Subarctic Pacific (OECOS) organized by Drs. Charles B. Miller and Tsutomu Ikeda, and sponsored by PICES (with assistance from the OSU Research Office and the OSU College of Oceanic and Atmospheric Sciences). Japanese and North American scientists discussed the fundamental questions and observational details of proposed comparative studies of ecological processes in the upper waters of the oceanic subarctic Pacific.

I. Scientific Issues Posed by OECOS

The physical setting of the subarctic Pacific was well described by Favorite *et al.* (1976). North of the latitude where the 33 pss salinity isocline turns vertically and surfaces, about 43°N, the water column is characterized by a 1 pss halocline at about 100 m depth, a density gradient which supports substantial internal waves. It is also a barrier to vertical mixing throughout the year that prevents (1) full replenishment of surface nutrients to deep concentrations and (2) complete removal of euphotic zone biota during winter mixing. From spring through autumn, there is also a seasonal thermocline, usually at about 35 m, which divides the euphotic zone into two distinct habitats. The region is a current gyre with slow, disperse flow along the southern side, slow flow east of the dateline, and stronger flow with rapid central cores (the Alaska 'stream') along the northern side. Flow is northward along the British Columbia–Alaska coast (the Alaska Current) and southward past Kamchatka, the Okhotsk Sea entrance and northern Japan (the Oyashio). There is also evidence, although not particularly clear evidence, that this gyre divides into two south of the central Aleutians, resulting in partially closed western and eastern subarctic gyres.

Oceanic sectors of the seas are defined as being far from the influence of land. The cores of both western and eastern gyres (whether or not they are indeed separate) definitely qualify as oceanic. In the Gulf of Alaska, at sufficient distance from shore, the oceanic character includes HNLC conditions, that is, high nitrate (HN) and low chlorophyll (LC), throughout the year. Despite the presence of high concentrations of major nutrients (*e.g.*, nitrate always greater than 6 μM), phytoplankton never bloom. The bulk of phytoplankton are always nanoplankton, with cells smaller than about 5 μm, and chlorophyll levels rarely exceeding 0.5 μg l^{-1}.

It has been established that the HNLC character of subarctic Pacific waters far from land is attributable to limited availability of iron in the euphotic zone (suggested by Martin and Fitzwater, (1988)). Several mesoscale iron-addition experiments, the Japanese SEEDS project (Tsuda *et al.*, 2005b) and the Canadian–Japanese SERIES experiment (Boyd and Harrison, 2006), have shown that adding soluble iron to the upper mixed layer induces strong increases in standing stocks of microplanktonic (>5 μm) diatoms, algae that without iron addition, are present in very low abundance. A partial explanation for the low chlorophyll condition (Miller *et al.*, 1991) is that microheterotroph (protozoan) grazers are capable of rapid increase, supported by eating the consistently small phytoplankton. With iron limitation firmly established, it remains to be explained fully the processes and variations of the lower trophic levels under normal circumstances without iron addition.

Despite the continuously low chlorophyll concentrations in oceanic sectors, there is substantial seasonality of phytoplankton production rates. In the course of the spring transition, during thermal stratification, rates more than double (Welschmeyer *et al.*, 1993; Harrison, 2002). This surely is attributable to the reduced extent of vertical mixing and consequent increase in cellular light exposure. Nitrate levels are reduced in this period. This is the time that the production *vs.* grazing balance of the HNLC regime must be the most challenged by the increasing growth potential of the phytoplankton. In the east, there are oscillations in chlorophyll levels between about 0.15 and 0.6 μg l^{-1}, which imply shifting in trophic relations among phytoplankton, protozoa, and possibly higher levels, including copepods. It is in this period that a suite of copepod species (three species of

Neocalanus, and *Eucalanus bungii*), which mostly reside at depth in diapause stages for the rest of the year, run through active recruitment to older stages, grow, and prepare for a return to diapause after accumulation of large lipid stores. At least in the eastern gyre these copepods do not eat much phytoplankton directly, as shown by the absence of chlorophyll in their guts (Dagg, 1993a,b), but they grow (Miller, 1993a,b) and thus must be supported by eating food without green pigments, presumably protozoa. Thus, a food chain of at least three or four steps is implied just to get to copepod level.

For the eastern sector, the dynamics of phytoplankton oscillations have been a subject of speculation (Strom *et al.*, 2000), but there has been, as yet, little direct research. One aim of OECOS is to understand the control of these oscillations. A section is devoted to this, below.

In the western sector, atmospheric dust transport extends farther to sea, allowing spring blooms dominated by diatoms to extend well out into the western gyre, although it appears that the core of the gyre, centered at approximately 51°N, 160°E, is persistently HNLC. After the bloom passes, apparently due to the onset of iron limitation, HNLC conditions are established for the remainder of the year since major nutrients are not exhausted (with the extremely rare exception of silicate; Wong and Matear, 1999). The spring bloom creates an opportunity to compare aspects of subarctic ecology with (east) and without (west) iron limitation, at least during the spring transition. The spring bloom in the Oyashio region should be fully characterized from before its onset to its termination. Nutrient drawdown, floristics, fate of primary organic matter, and the feeding and growth responses of the copepod complex should be characterized in detail. The dominant spring copepods are the same species with the same prolonged diapause phases as those that inhabit the eastern subarctic subgyre, so the comparison of growth under field conditions should be very informative. It is expected that the availability of large phytoplankton will shorten the food chain, provide much more food, and allow much more rapid growth of copepods.

OECOS participants propose that much can be learned from parallel studies and comparisons of processes in eastern and western subarctic sectors by taking advantage of both differences (bloom *vs*. continuous HNLC conditions) and similarities (identical copepod communities) between them.

II. Participant Contributions to the OECOS Workshop

Those who participated in the May 2005 OECOS Workshop were selected as scientists whose interests and access to techniques allow them to address specific issues in respect to subarctic Pacific ecodynamics. Their names and interests are given on pages iii and iv, and a picture of the group (save for Moira Galbraith representing Dr. David Mackas) is shown below (Fig. 1). Every participant provided an essay on aspects of subarctic Pacific oceanography that he or she is interested in investigating. Those essays are presented here, with short introductions by the Workshop organizers, Drs. Charles B. Miller and Tsutomu Ikeda, to provide context.

Fig. 1 OECOS/PICES Workshop participants: (top row from left) Timothy Cowles, Tsutomu Ikeda, Peter Strutton, Charles Miller, Ken Furuya, Sei-ichi Saitoh, Harold Batchelder; (middle row) Jay Cullen, Zanna Chase, Michael Dagg, Nicholas Welschmeyer, Karen Selph; (bottom row) Kenshi Kuma, Toru Kobari, Atsushi Yamaguchi, Takashi Ota, Suzanne Strom, Deana Erdner.

A. ASPECTS OF PHYTOPLANKTON ECOLOGY IN THE SUBARCTIC PACIFIC

Automated and semi-automated analyses of phytoplankton floristics should be particularly suitable for studies of phytoplankton in the subarctic Pacific. In high-nitrate low-chlorophyll (HNLC) regions, substantial fractions of Synechococcus *and nano-eukaryotes in the phytoplankton will make abundance analyses by flowcytometry particularly valuable. The analyses are quantitively precise and suitable for very high data frequencies. Quantitative video-microscopy can also be used to good advantage for slightly larger classes of cells: haptophytes, prymnesiophytes, coccoid chlorophytes, very small diatoms and other nanophytoplankton. In addition, particularly with epifluorescence arrangements, it can evaluate heterotrophic flagellate abundance, a key aspect of the ecosystem. Dr. Karen Selph was invited to the Workshop to discuss the potential of these methods for OECOS.*

Microbial community compositions

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As part of the effort to characterize the plankton community dynamics in the subarctic Pacific Ocean during OECOS, I propose to use a combination of flow cytometry and epifluorescence microscopy for abundance and biomass estimation of the microbial community.

Three complementary methods of estimating microbial abundances will be used. First, shipboard flow cytometry on freshly collected, live phytoplankton will allow immediate enumeration of two fluorescently-distinct groups: *Synechococcus* and photosynthetic eukaryotes. Using the cellular scatter signal collected by the flow cytometer, a rough estimate of cell size can also be obtained. In addition, should rarer cytometrically-distinct populations appear, this on-board method will allow more sample volume to be processed to get a statistically significant number of counts for the rare populations. This instrument has been successfully employed during two other field projects to date: in the Antarctic waters of the Drake Passage (Fig. 2) and in the equatorial Pacific.

Another aliquot of sample will be preserved and frozen (LN_2) for enumeration of non-pigmented bacteria on a land-based flow cytometer. Given that one of the hypotheses for the lower limit of the phytoplankton abundance cycle is that microheterotrophs switch from consuming autotrophs to grazing on heterotrophs, enumeration of the non-pigmented bacteria will be important. These samples will also provide a backup for phytoplankton abundance estimates. However, it is expected that the samples processed live will give somewhat higher numbers for phytoplankton relative to those preserved and frozen, as preservation tends to destroy some delicate cells.

Finally, samples will also be taken for digital imaging by epifluorescence microscopy. This will allow enumeration and biovolume estimation of both the pigmented and non-pigmented members of the microbial community. In addition, if no other investigator is collecting samples for the abundance of ciliates, I will take acid Lugol's samples for later analysis on land using an inverted microscope. That would round out the community abundance picture.

Methods

Ship-board flow cytometry will be performed using a Beckman-Coulter EPICS XL, coupled with a syringe pump for quantitative sample delivery (a system similar to Selph *et al.* (2001)). Samples (50 ml) will be collected and run live within 2 h to estimate the abundance of phytoplankton. Phytoplankton are distinguished on the basis of chlorophyll (red fluorescence, 680

nm), phycoerythrin (orange fluorescence, 575 nm) and by forward and 90° side-scatter signatures. Calibration beads (1 and 6 µm) are added to each sample as an internal standard for fluorescence. In order to discern any diel or other time-varying patterns, this sampling will be repeated at a frequency of up to six profiles of 10 depths each per 24-h period. Raw data (listmode files) will be processed using the software FlowJo (Treestar, Inc.).

Land-based flow cytometry will be done on a Beckman-Coulter EPICS Altra flow cytometer equipped with multiple lasers and a syringe pump delivery system. This instrument, which is part of a facility under my direction at the University of Hawaii (www.soest.hawaii.edu/sfcf), is optimized for the detection of picoplankton. Samples will be taken and preserved (2 ml aliquots) on board ship, and then frozen. Back on land, the samples will be thawed, stained with Hoechst 33342 (1 µg ml^{-1}, v/v, final concentration), and incubated at room temperature in the dark for 1 h (Monger and Landry, 1993). Aliquots (100 µl) will be analyzed for populations of phytoplankton and non-pigmented bacteria, distinguished on the basis of chlorophyll (red fluorescence, 680 nm), phycoerythrin (orange fluorescence, 575 nm), DNA (blue fluorescence, 450 nm), and by forward and 90° side-scatter signatures. Calibration beads (0.5 and 1.0 µm yellow-green beads and 0.5 µm UV beads) will be added to each sample as an internal standard for fluorescence. Raw data will be processed as described for the live samples.

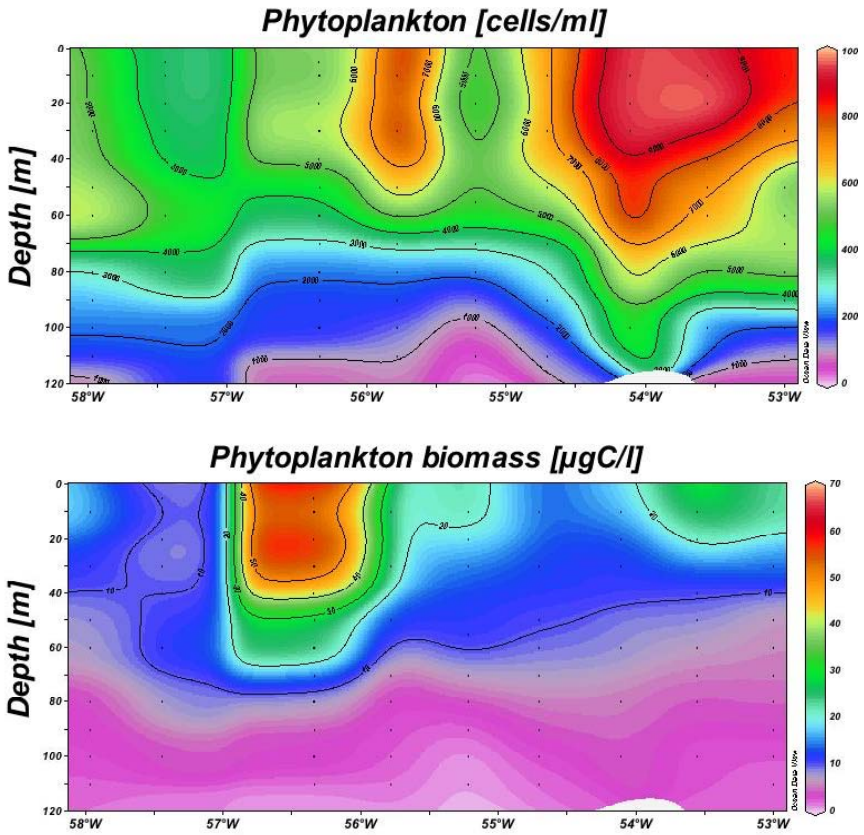

Fig. 2 Example of flow cytometry data on live samples from the Drake Passage using the Beckman-Coulter EPICS XL (Selph and Apprill, unpub. data). Top panel: Total phytoplankton abundance (cells ml^{-1}). Bottom panel: Phytoplankton biomass (µg C l^{-1}) derived from abundance data which was converted to biomass using the forward-scatter measurements from flow cytometry and a biovolume:biomass conversion factor. Contour plots were generated using Ocean Data View (www.awi-bremerhaven.de/GEO/ODV, R. Schlitzer).

Also on board ship, samples will be taken for image analysis by epifluorescence microscopy to characterize the microbial community composition. Cells in the nanoplankton size range (2–20 μm) will be enumerated from 50-ml samples preserved with paraformaldehyde (0.4% final concentration, v/v) and stained with 40 μl of 0.033% (w/v) proflavin, a protein stain. The samples will be concentrated on 1-μm, 25-mm black polycarbonate membrane filters and stained with DAPI (50 μg ml^{-1}, final concentration) for 0.5 min in the final stage of filtration. Cells in the microplankton range (20–200 μm) will be counted from 250- or 500-ml samples preserved and cleared with sequential additions of alkaline Lugol's fixative (0.1%, final concentration), 1% borate-buffered formaldehyde and 0.009% sodium isothiosulfate (protocol modified from Sherr and Sherr (1993)). These samples will be stained with 200 μl of proflavin, filtered onto 8-μm, 25-mm black polycarbonate membrane filters, and stained with DAPI (as above). Filters from both preparations will be mounted on glass slides with a drop of immersion oil and viewed on shipboard with a video image analysis system before freezing at –80°C. The video system consists of a Olympus BX51 epifluorescence microscope on an anti-vibration platform, a 100 W power supply, and a Microfire digital camera. At least 30 random images per slide will be digitized on shipboard and downloaded to a PC computer with Media Cybernetics Image Pro Plus software. Nanoplankton and micro-plankton will be viewed and imaged at 640X and 400X, respectively.

Logistics

These operations would require two people on board ship: one person to run the flow cytometer and the other to make and image the epifluorescence microscope slides. All major equipment (flow cytometers and epifluorescence microscope-imaging system) is available from my lab at the University of Hawaii.

The work Dr. Selph proposes would be an excellent characterization of short-term ecosystem variation, going far beyond just the chlorophyll data available in earlier studies. The flow cytometry data can be available in real-time to help us track where we are in the cycle as observations go forward. It is essential to effective 'reactive' planning during the cruises that most aspects of our results be progressively evident as we generate them, rather than emerging at some meeting a year or more after the observations at sea.

An intriguing aspect of work in the central Gulf of Alaska is that the specific composition of the algal flora is different on every visit one makes, as shown by Beatrice Booth's work in SUPER (Subarctic Pacific Ecosystem Research). Phytoplankton are always predominantly picoplankton and nanoplankton (mostly less than 7–8 μm), but the dominant cells can be coccoid chlorophytes, any of the divisions of small flagellates, or even small diatoms. We cannot fully understand what is happening without knowing what sorts of algae are operating around us; we cannot just measure chlorophyll. We are likely to see transitions in phytoplankton composition, although we may not be able to explain such transitions when they occur. However, they should be documented. In this regard, Dr. Nicholas Welschmeyer proposes in the next essay to do 'CHEMTAX' high-performance liquid chromatography (HPLC) work on a twice daily basis aboard ship, with regularly posted results. That would provide another dimension to the picture.

It will be essential that growth rates of phytoplankton be estimated by up-to-date, reliable techniques that take full account of the impact of microheterotrophs in incubation bottles. This is important, in part, because the trophic link from pico- and nanophytoplankton to protozoan grazers initiates transfer to larger organisms: copepods, euphausiids, fish, squid, and mammals. Nicholas Welschmeyer has developed a dilution technique using carbon-14 which seems ideally suited to high-nitrate low-chlorophyll (HNLC) situations and thus to the core problems to be studied by OECOS. At the Workshop he discussed these methods (and some other aspects of an approach to subarctic Pacific ecology).

Subarctic Pacific lower trophic interactions: Production-based grazing rates and grazing-corrected production rates

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Background

The eastern subarctic Pacific is characterized year-round by low surface chlorophyll concentrations, typically falling in the range 0.15–0.6 μg Chl l^{-1}. The rare, large phytoplankton cells (diatoms) have been shown to be iron-limited. However, the dominant small phytoplankton classes grow at moderate to high rates and are actively grazed by microzooplankton such that the bulk chlorophyll crop remains relatively constant (Miller *et al.*, 1991; Strom and Welschmeyer, 1991). Under conditions of experimental *in situ* iron fertilization, the large diatom component will bloom (Harrison *et al.*, 1999), however, natural iron fertilization events in the eastern subarctic Pacific are not known. A primary goal of the collaborative OECOS project in the Gulf of Alaska is to define the conditions that control lower and upper limits of the phytoplankton community abundance under normal, iron-stressed conditions. In particular, we seek to understand what sets the *lower* limit of roughly 0.15 μg Chl l^{-1} characteristic of the subarctic Pacific; effectively asking, "What prevents chlorophyll from being grazed away?"

Since the range between upper and lower limits of chlorophyll concentration is relatively narrow, it could be argued that we are simply observing natural variability about a mean low value as is the case during the winter low of many planktonic environments. However, as elaborated by Strom *et al.* (2000), the spring–summer subarctic algal biomass variation appears to be in a cycle, mirrored by inverse variation of regenerated ammonium concentration. This implies grazing and nutrient cycling. Strom *et al.* (2000) show that, while one explanation for the non-zero lower limit implies a minimum food threshold for the onset of grazing activity, the collection of laboratory feeding experiments shows no evidence for feeding thresholds in the micrograzers tested. In order to resolve the issue in the field, it is important to determine algal growth rates and microzooplankton grazing rates as accurately as possible at both upper and lower limits of Chl standing crop, *while the cycle is in operation!*

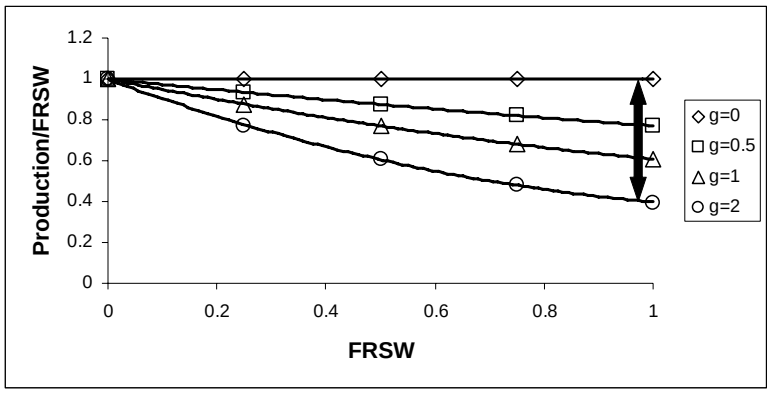
Our proposed contribution to this effort is to deploy a series of analyses focusing on microzooplankton grazing rates, algal growth/production rates and compositional changes in the phytoplankton community. All the methods proposed here are necessarily biased by analytical technique (radioisotopes, pigments, active fluorescence, oxygen, and total inorganic carbon (TCO_2)), and they will need corroboration by more organismic methods (microscopy, FlowCam, and flow cytometry) that will be contributed by collaborators.

^{14}C experiments

We propose to deploy a new variant of the dilution experiment, which provides sensitive, carbon-based estimates of community microzooplankton grazing. The technique is based on measurement of the photosynthetic rate (^{14}C uptake) determined in a conventional dilution series. The method measures grazing rate on the basis of the time-integrated effect of dilution on primary productivity, rather than on the net change in prey (measured usually as Chl) concentration, as in conventional dilution protocol. One benefit of the ^{14}C method is particularly important: the technique yields bulk community grazing rates without relying on Chl as an accurate tag for algal biomass. It is well known that variable C/Chl ratios and variable grazer digestive efficiencies can wreak havoc on final grazing rate calculations when the experiments are based on net Chl changes. Necessarily, when grazer degradation of Chl is not 100% efficient, the calculated rates must be regarded as *conservative* estimates of the true grazing rate (Waterhouse and Welschmeyer, 1995). Unfortunately, the absolute value of micrograzer Chl digestive efficiency is not known, so it cannot be controlled for in natural samples. These problems can be remedied by analyzing conventional dilution experiments on a prey-specific basis (say, cytometrically enumerated *Synechococcus* sp., or fluorescently tagged prey analogs). The bulk community grazing pressure would then have to be inferred from activity on specific prey items. The ^{14}C-uptake-based dilution experiment is proposed here as a method to obtain the *bulk* community grazing rate.

Briefly, the dilution-normalized ^{14}C-uptake rate is plotted against dilution (Fig. 3), yielding a curve which is best-fit to predict 1) the dilution-corrected production rate in the absence of grazing (y-intercept) relative to 2) the production rate determined under ambient grazer/prey concentrations (dilution = 1.0). The resulting grazer-influenced productivity ratio, G_r, is combined in Eq. 1 (Fig. 3, top) to yield a numerical solution for g (d^{-1}), the community grazing rate; the analytical relationship is summarized in Figure 3, bottom. Application of this technique yields very high community grazing rates in Monterey Bay, the California Current, and in the oligotrophic Central North Pacific (Fig 4, upper right; $g > 2.0\ d^{-1}$). The data provide clear verification that the well-acknowledged, but under-appreciated, grazer-mediated remineralization of ^{14}C (as respiratory $^{14}CO_2$ and dissolved organic ^{14}C ($DO^{14}C$) sloppy feeding/excretion) is real, and substantial, in every ^{14}C incubation. We are currently 'correcting' the derived grazing rates with the remineralization function of Laws (1984, 2000) which takes into account grazer growth/assimilation efficiencies. Even under those corrections we find, contrary to a recent note by Dolan and McKeon (2004), that Chl-based dilution experiments are likely to yield significant *underestimates* of grazing rates (Fig. 4, lower).

$$dP/dt = \mu\hat{P} = \mu P_0[(\exp(\mu\text{-}g)t - 1)/(\mu\text{-}g)t]$$



G_r = Grazer-free production / Production under full grazing

$Gr = (1\text{-}g/\mu)[\exp(\mu t)\text{-}1]/[\exp(\mu\text{-}g)t\text{-}1]$

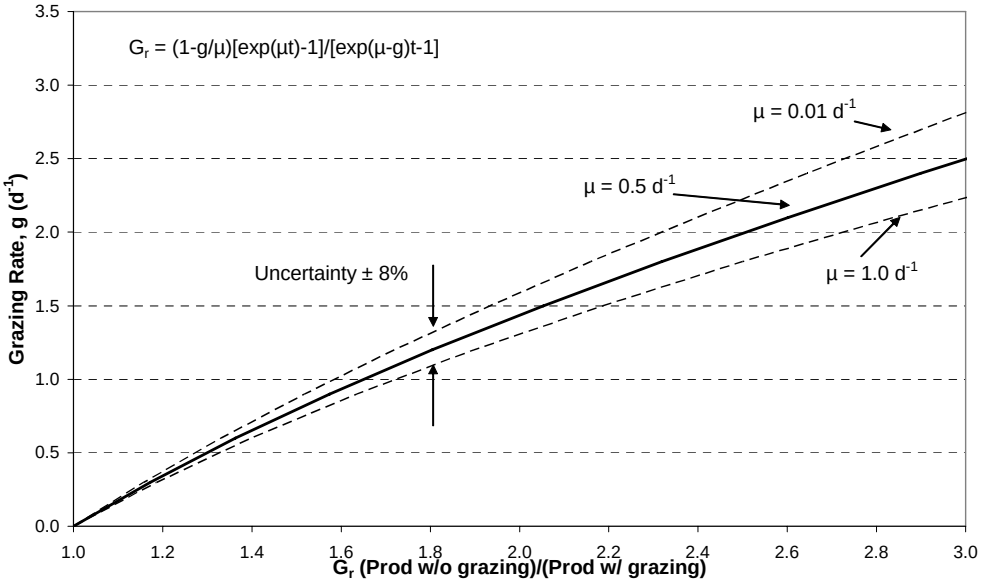
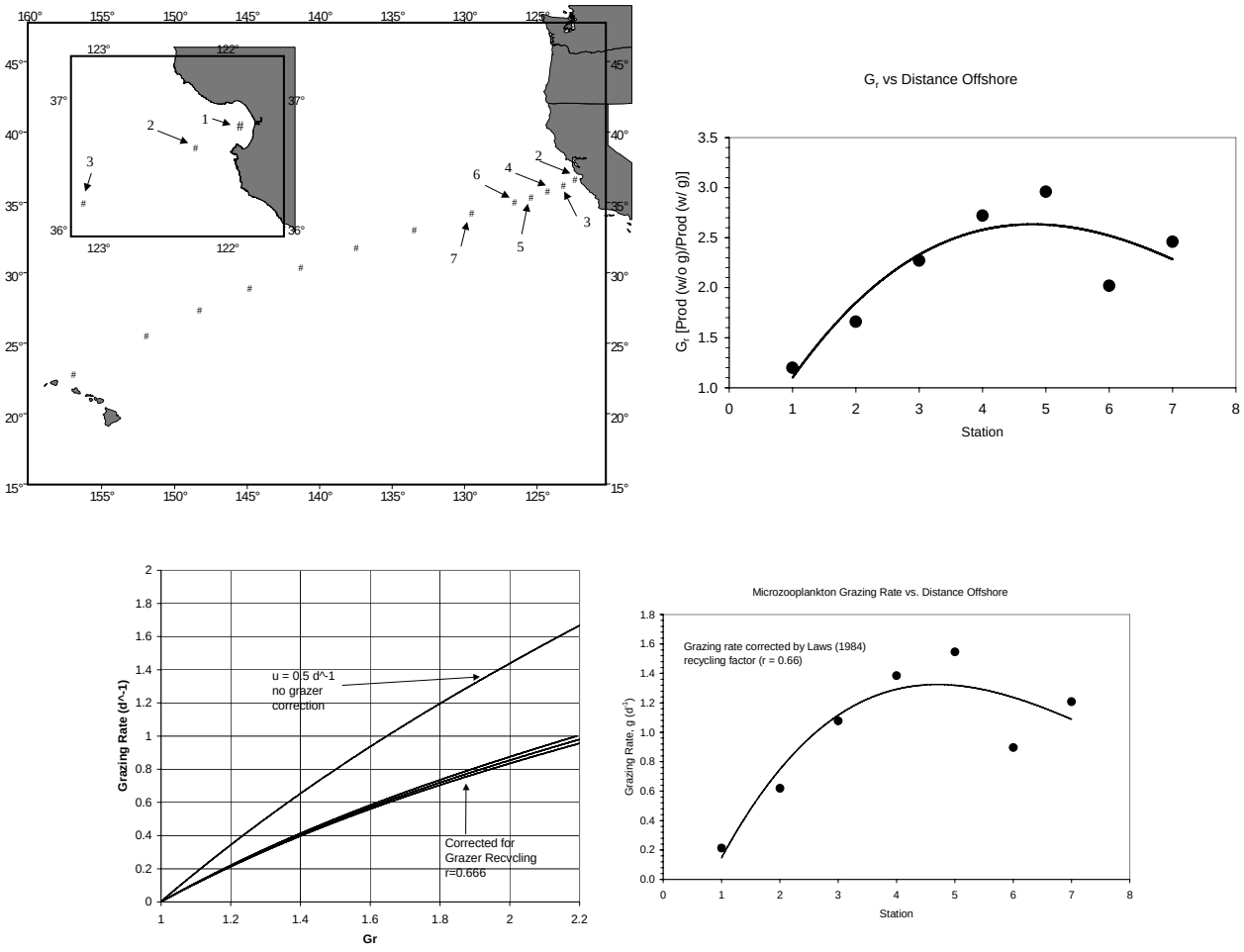


Fig. 3 Top: Production-based estimates of microzooplankton grazing rates. Principle: algal specific growth is density independent, but primary production (dP/dt = uP) is not. Modeled calculations of primary production is at a constant algal growth rate of 0.5 d^{-1}, but under four community grazing rates, g, all are subjected to dilution treatments. Bottom: The shift in production on the right side of Figure 3 (top) is defined as G_r, and is shown analytically in the bottom figure. Experimental protocol: measure G_r empirically, then calculate g (d^{-1}), the community grazing rate.

The production-based dilution method makes use of ^{14}C estimates of production for one simple reason – *sensitivity*. However, it can also be analyzed with conventional light–dark gross production measurements based on oxygen. In that case, the method is free of radiotracer remineralization biases. Table 1 gives results from Elkhorn Slough, California, where Chl levels are high enough to provide high-precision potentiometric end-point O_2 determinations (Furuya, 1995) with an adequate signal. Production-based dilution grazing experiments at times were more than twice as high as side-by-side, conventional, Chl-based dilution grazing



^{14}C Recycling Correction: Laws 1984 J. theor. Biol. 110:425-434

Correction Factor = (1-r)(1-β) where

r = 1 – growth efficiency and,

β = (1-μ/g)[exp(μt)-1]/[exp(μt)-exp(gt)]

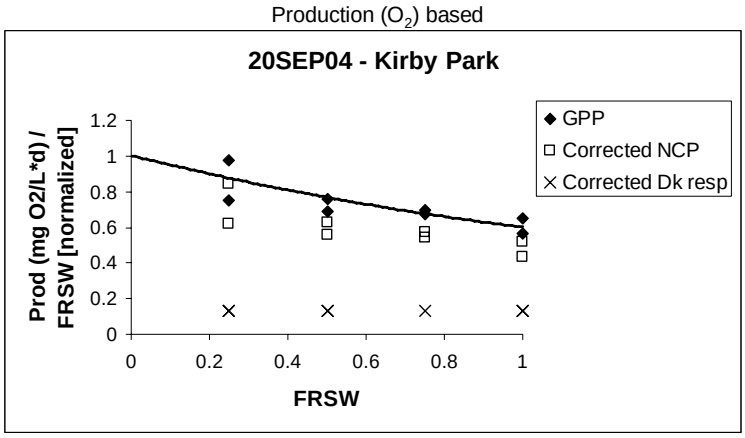
Fig. 4 Upper left: Station locations near Monterey Bay (inset) and offshore California where production and grazing were measured by ^{14}C dilution experiments. Upper right: Uncorrected values of G_r suggest very high grazing rates (see Figure 3, bottom). Lower: Recycling correction still yields rates of grazing substantially higher than those based on Chl-based dilution.

results (Table 1; Fig. 5). Can we use an oxygen-based technique in the subarctic Pacific? In most cases, the answer will be no (not enough sensitivity). However, when the subarctic chlorophyll cycle begins to approach the upper limit, ~0.5 μg Chl a l^{-1}, we calculate a reasonable chance (with adequate replication) that a 48-h incubation could provide field calibration of the necessary 'remineralization' correction for the ^{14}C version of the experiment. We propose to deploy the ^{14}C version of the experiment as the routine (daily) measure of community microzooplankton grazing rates (augmented by oxygen measurements when signal level allows). Additionally, we propose to provide high precision coulometric determinations of TCO_2 (UIC Inc. CM5012 Coulometer) to be paired up with O_2 measurements, yielding appropriate photosynthetic

Table 1 Elkhorn Slough Field Experiments. Production (O_2)-based grazing *vs*. Chl-based grazing.

Date	Location	Oxygen-based g (d^{-1})	Chl-based g (d^{-1})
24-Aug-04	MB	0.867	0.4092
2-Sep-04	KP	1.037	0.4689
20-Sep-04	KP	0.996	0.7172
1-Oct-04	KP	0.637	0.2066

MB = Monterey Bay
KP = Kirby Park, Elkhorn Slough



G_r = Grazer-free prod / Prod under full grazing = 1/0.608 = 1.646

g = 0.996 d^{-1}

Fig. 5 Primary production in Elkhorn Slough (at Kirby Park) measured by oxygen production in a dilution series. FRSW is fraction of raw seawater (estuary water); GPP is gross primary production; NCP is net carbon production corrected by subtracting dark O_2 consumption. All production values were normalized to dilution (FRSW).

quotients and (we expect) a corroboration of ^{14}C carbon-based community rates.

We propose to be responsible for routine measurements of primary productivity by ^{14}C technique in the OECOS field work. The measurements collected over the Subarctic Pacific Ecosystem Research (SUPER) program yielded significantly higher productivity than gathered on the Canadian Weathership program (Welschmeyer *et al.*, 1993). The more recent Canadian Joint Global Ocean Flux Study (JGOFS) measurements show similar, or even slightly higher values, than derived in SUPER (Harrison *et al.*, 1999). We will apply the same procedures, including *in situ* water column incubations, to maintain historical continuity. However, since we now know that more than 75% of water column integrated productivity takes place within the mixed layer (Welschmeyer *et al.*, 1991), and that the manipulated iron fertilization experiments were essentially mixed-layer phenomena, it seems logical to simplify, and focus much of the laborious ^{14}C experimental efforts on mixed-layer sampling, utilizing shipboard incubators simulating *in situ* conditions. We have numerous rotating plankton wheels that hold all bottle sizes, from 125 ml to 2.7 l, to be used for all variants of the ^{14}C dilution experiments proposed here: 1) ^{14}C-based grazing rates (discussed above), 2) algal

growth rates determined by ^{14}C labeling of pigments (Welschmeyer *et al.*, 1991), and 3) release of $DO^{14}C$, in absolute rate and as a function of grazing.

The last point regarding $DO^{14}C$ release requires some comment. Karl *et al.* (1998) made it clear that large fractions of total net primary production were accounted for in the $DO^{14}C$ analyses made at the Hawaii Ocean Times-series (HOTS) project. That measurement was ignored in SUPER and in the more recent Canadian experiments. However, we have determined in Monterey Bay that more than half the total released $DO^{14}C$ is due to microzooplankton grazing activity (sloppy feeding/excretion), not algal physiological release *per se* (Chevalier, 2001). That result is based, again, on results from ^{14}C dilution experiments. We will use the magnesium-induced co-precipitation dissolved organic carbon (MAGIC DOC) adsorption procedure (Karl, 1998) in OECOS dilution experiments, searching for variability in the grazer-induced release of $DO^{14}C$ for two purposes: 1) to produce the most accurate measurements of total primary production that we can (particulate and dissolved) and 2) to identify possible links between the fresh release of labile DOC and potential increases in taxon-specific components of the phytoplankton community.

The dilution-based ^{14}C experiments will also be used to compute true net primary productivity, corrected for grazing-induced remineralization. Basically, if we again derive microzooplankton grazing rates similar to (or higher!) than measured during SUPER (0.3–0.5 d^{-1}), it is reasonably safe to assume that all historical estimates of primary production in the subarctic Pacific, based on 24-h ^{14}C incubations, are low because the grazer-remineralized $^{14}CO_2$ and $DO^{14}C$ were never accounted for; that error would be *ca*. 30–40%. Manipulations of the ^{14}C-dilution experiment above can be used to produce 'grazer-corrected' estimates of the true net primary production rate.

Active fluorescence

In all of the proposed variants of the dilution experiment (ours and those of other OECOS investigators), we will eventually ask what kind of subjective nutrient addition (if any) should be made to our otherwise trace-metal clean treatment bottles (Landry *et al.*, 1995). (Who will keep our hands metal clean?) We propose that at the end of each experiment, aliquots of all dilution samples be analyzed for dark-adapted estimates of photochemical efficiency, F_v/F_m, utilizing Walz Pulse Amplitude Modulated fluorescence instrumentation (Water PAM-FT) to check for uniformity in algal physiological condition across the dilution series. Recall that a primary assumption of dilution protocol is that the algal-specific growth rate is density independent. That same assumption holds for the ^{14}C-dilutions described here.

We also propose to set up in-line continuous monitoring of surface F_v/F_m utilizing paired, flow-through Water PAM-FT units, one with red excitation and one with blue excitation. Phytoplankton F_v/F_m response to iron limitation is well documented (Behrenfield *et al.*, 1996), and surface, flow-through monitoring (especially at night) should be adequate to detect changes over the course of several weeks. Moreover, contrary to popular belief, blue-excitation, active-fluorescence instrumentation (such as the Chelsea Fast Repetitive Rate (FRR)) does not illicit uniform F_v/F_m response for all phytoplankton groups, particularly picophytoplankton with phycobilins. Figure 6 shows a recent transect through Monterey Bay, with paired red and blue PAM units, each recording twice per minute. Data from a Chelsea FRR fluorometer on the same in-line flow system were omitted for clarity; and all three instruments showed the same gross trends in F_v/F_m (note large variations which are inherent in daytime determinations subject to strong irradiance fluctuation). However, the later samples (>14:00 h) were collected offshore (right side of plot) and they showed small, but consistent decreases in F_v/F_m for blue excitation relative to red excitation. Coincidentally, high-performance liquid chromatography (HPLC) pigment calibration extractions along the transect showed that cyanophyte contributions increased from 5% to 20% of the total algal community as determined from CHEMTAX (zeaxanthin) analysis (Mackey *et al.*, 1996). We propose that similar fluctuations in the relative contributions of *Synechococcus* and continuous eukoryotes could be monitored in

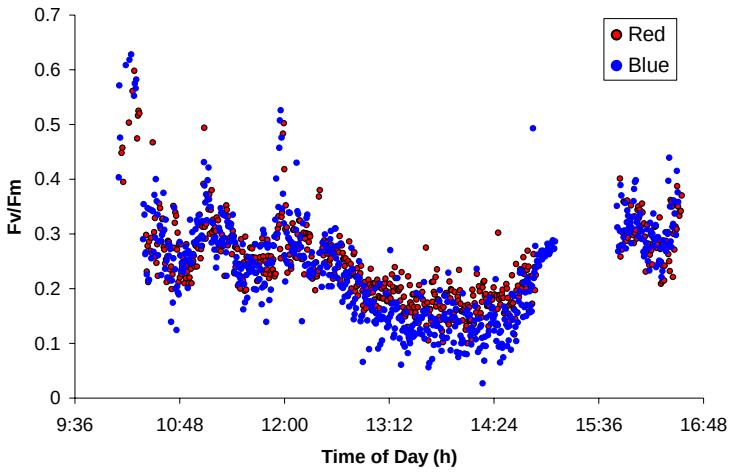


Fig. 6 Monterey Bay, April 5, 2005; paired, Water PAM-FT instruments. Early sampling (~10:00 h local time) commenced just outside Moss Landing Harbor; at 13:00 h we were roughly 15 miles (25 km) offshore. Note that F_v/F_m determined from blue excitation active fluorescence is lower than that determined from red excitation in the offshore sites. Relative abundance of cyanophytes increased offshore.

unattended mode during the full period of each OECOS cruise.

The Water PAM fluorometers described above are new and are now sensitive enough for offshore oligotrophic work. However, they are available in flow-through mode only; not for *in situ* deployment. We propose to collect data from a CTD-mounted Chelsea FRR instrument for determination of depth-dependent changes in active fluorescence to check for depth-related variability in physiological condition, especially at the base of the mixed layer. Data from the instrument could also be used to make real time fluorescence-based estimates of instantaneous production (Kolber and Falkowski, 1993). However, we consider vertical profiling of physiological fluorescence conditions (F_v/F_m and absorbance cross-section) to be more significant to the project than real-time instantaneous production calculations *per se*, given that ^{14}C productivity will still serve as ground truth.

Pigments

Finally, we propose a CHEMTAX HPLC analysis of mixed-layer phytoplankton community structure during the full course of each cruise. If the collaborative OECOS efforts *do* show that microzooplankton grazing rates indeed become reduced at the lower limits of observed chlorophyll cycling, we will, of course, ask, "why?" Possibly there is a discernible, repeated shift in relative proportions of algal groups at the high and low ends of standing crop variation, leading to prey-selective threshold response (not yet found in the lab). We have applied Simon Wright's (Australian Antarctic Division, Tasmania) CHEMTAX analysis to gross distinctions in phytoplankton structure in the Monterey Bay area with reasonable success. Figure 7a shows examples of CHEMTAX analysis of algal community distinctions in Elkhorn Slough where the diatom/dinoflagellate-dominated lower portion of the slough is easily separated from the cryptophyte-dominated upper section. Figure 7b shows stations separated by only a few hundred meters during the low-tide ebb of the Elkhorn Slough plume into Monterey Bay proper.

The natural history of pigments in the subarctic Pacific is fairly simple; key diagnostic pigments include peridinin (dinoflagellates), 19-butanoyloxyfucoxanthin (pelagophytes), fucoxanthin (diatoms), 19-hexanoyloxyfucoxanthin (prymnesiophytes), prasinoxanthin (prasinophytes), zeaxanthin (cyanophytes), and Chl *b* (prasinophytes/ chlorophytes). We propose to take one or two reasonably foolproof HPLC systems to sea to provide daily postings of noon and midnight mixed-layer CHEMTAX composition for the duration of the cruise. These HPLC systems

(30 samples each per day by autosampler could be used by any OECOS group to analyze their specific experiments if so desired.)

Summary of our proposed contributions:

1. Determine microzooplankton community grazing rates *via* ^{14}C dilution experiments.
2. Produce ^{14}C estimates of primary production (particulate and dissolved) by:
   a. Depth-integrated *in situ* incubations;
   b. Simulated *in situ*, deck-based incubations (mixed layer);
   c. Photosynthetron-style P *vs*. I curves (??).
3. Produce ^{14}C-based estimates of algal growth rates (pigment labeling) for the mixed layer only for:
   a. Bulk community grazing rates, based on ^{14}C-labeling of Chl *a* ;
   b. Group-specific grazing rates, based on ^{14}C-labeling of carotenoids.

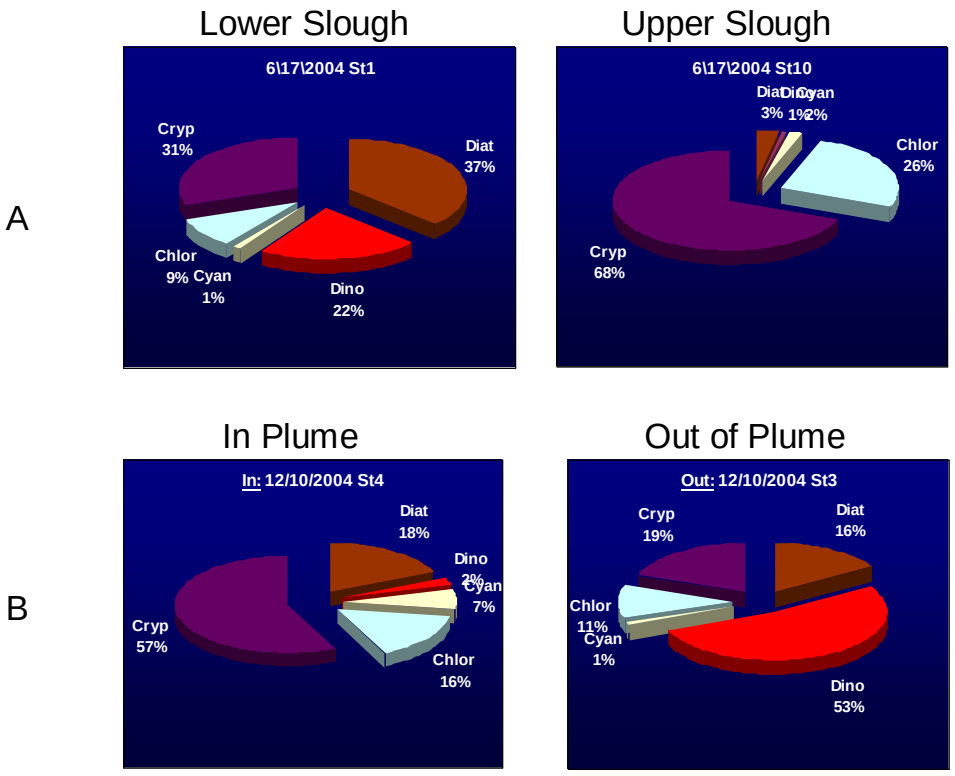


Fig. 7 Examples of CHEMTAX pigment analysis in Elkhorn Slough/Monterey Bay. (a) Lower Slough (Station 1) *vs*. Upper Slough (Station 10), separated by 5 km. Diatom/dinoflagellate dominance in lower slough; cryptophyte dominance in upper slough. (b) Extreme low tides carry the Elkhorn Slough plume into Monterey Bay. Stations inside and outside of the plume were only separated by several hundred meters. The 'cryptophyte' signal of Elkhorn Slough can be clearly seen in Monterey Bay proper.

4. Make active fluorescence measurements using:
   a. Continuous surface flow-through F_v/F_m (Water PAM red and blue instruments);
   b. Depth profiles (Chelsea FRR, or a successful competitor at the time of proposal);
   c. F_v/F_m in each dilution series.
5. Analyze algal pigments using:
   a. Group-specific (HPLC) methods;
   b. Simple fluorometric Chl methods (all CTDs, size fractionation and wherever else needed).

Evaluations of phytoplankton abundance, floristics, and production rates also must be done in the western subarctic Pacific. Two approaches were presented at the OECOS Workshop. An array of ship-based techniques was suggested by Dr. Ken Furuya, and satellite-based estimates of phytoplankton standing stocks and production were evaluated by Dr. Sei-ichi Saitoh. In the essay below, Dr. Furuya suggests that both floristic analyses (microscopy, CHEMTAX and flow cytometry) and evaluation of phytoplankton physiological status be accomplished by recently developed techniques.

Phytoplankton bloom dynamics and their physiological status in the western subarctic Pacific

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Compared to the eastern side, the western subarctic Pacific is characterized by high variability in surface chlorophyll *a* concentration that is associated with diatom blooming (Shiomoto *et al.*, 1998). Phytoplankton bloom dynamics are central to OECOS in the western subarctic Pacific, and we propose diagnosis of the physiological status of phytoplankton as a component of OECOS based on the following background.

Photosynthetic characteristics of phytoplankton during summer

Geographical variability in the relationship between photosynthesis and irradiance was examined in the subarctic North Pacific Ocean in July–August 1997 and 1999. Photosynthesis *versus* irradiance (P–E) curves were obtained for seawater that was collected by trace metal-clean techniques. Most regions sampled during this study were characterized by high nutrients (> 8 μM nitrate) and low chlorophyll (0.2–0.6 μg l^{-1}), except the western gyre in 1997, where chlorophyll concentrations of 1.3–3.0 μg l^{-1} were recorded. Ambient iron concentration at the surface was consistently subnanomolar during both years (<0.22 nM). Surface chlorophyll *a* was statistically higher in the western gyre in 1997, while no difference was observed in 1999. Phytoplankton class composition, as revealed by CHEMTAX analysis of bio-marker pigments, was different between these regions in 1997 (Fig. 8). Maximum photosynthetic rates ($P^B m$) varied between 1.79 and 5.24 mgC (mg chl)$^{-1}$ h^{-1} with no obvious geographical difference between the western and eastern gyres. In contrast, there were noticeable east–west gradients in the initial slope of the P–E curves (α^B) for surface populations in 1997: Station P (50°N, 145°W), located in the eastern gyre had the lowest α^B (0.088 ± 0.041 mgC (mg chl)$^{-1}$ h^{-1} (μmol m^{-2} s^{-1})$^{-1}$, n = 3), and it increased westward. In July 1999, no significant east–west gradient was seen in α^B, which ranged between 0.012 and 0.029 mgC (mg chl)$^{-1}$ h^{-1} (μmol m^{-2} s^{-1})$^{-1}$, while sporadically high α^B was observed in the western gyre area. Bottle incubations conducted in 1999 with iron enrichment of 1–2 nM resulted in a significant enhancement of both α^B and maximum quantum yield (oceanic quantum yields in the subarctic Pacific are atypically low compared to Pacific coastal sites and the North Atlantic, Fig. 9). This signifies the role of iron in controlling the productivity potential of phytoplankton. These observations indicate that east–west gradients exist in algal photosynthetic activity, but that the gradients are not stable. What is responsible for the difference?

Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study

Limitations on natural iron fertilization and nitrogen concentrations that saturate uptake capacity at the surface lead to an hypothesis that iron availability controls bloom dynamics. This was validated by large-scale iron fertilization experiments (Tsuda *et al.*, 2003, 2005b). Interestingly, the second experiment conducted in 2004 resulted in a different response of

phytoplankton populations. While the first experiment yielded a distinct growth of diatoms, the second one resulted in a much lower increase in chlorophyll *a* and less dominance of diatoms. What is responsible for the difference? This is not fully explained, yet, but it indicates the importance of algal conditions before the experiments.

Diagnosis of physiological status

These observations demonstrate that it is crucial to examine the composition and physiological status of phytoplankton in order to analyze bloom dynamics and their environmental control. A 50-day long observation would be beneficial to allow us to monitor the whole span from pre-blooming through the bloom-to-bloom termination. We propose to conduct time-series observations to investigate bloom dynamics with emphases on 1) factors initiating blooming and 2) the physiological conditioning of phytoplankton before the bloom. Algal physiological status is diagnosed by active fluorescence to monitor light acclimation and iron stress, and by flow cytometry. Since growth responses to environmental changes differ according to algal group, group-specific properties are requisite for insights to population dynamics. Thus, we will apply a variety of techniques for taxonomic analysis of the algal community. While there are limited numbers of measurements that successfully indicate physiologic status, flow-cytometric techniques have been proposed for esterase activity, peroxidase activity, nitrate reductase induction, photosynthetic capacity, carbon-incorporation rate and membrane integrity. Many of these should be useful in OECOS studies.

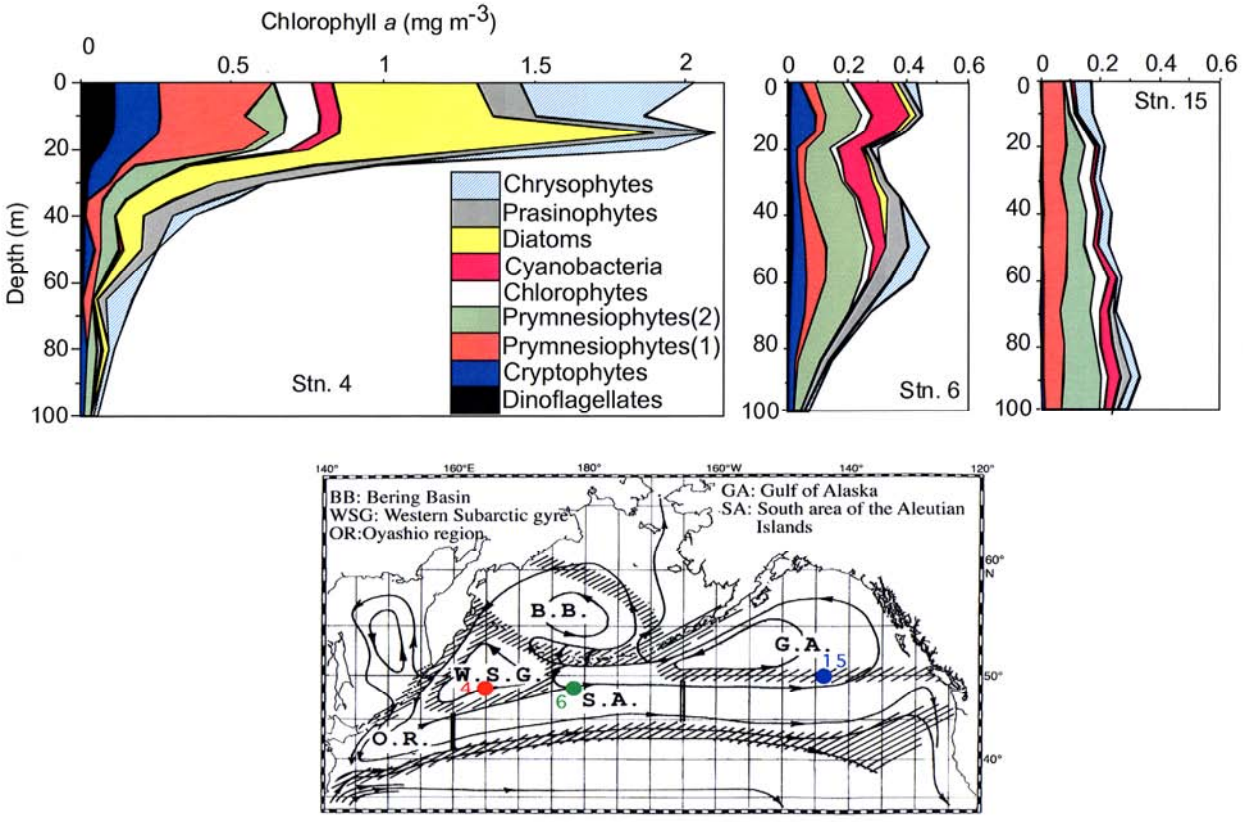

Fig. 8 Example of phytoplankton marker-pigment analysis (CHEMTAX): Comparison of a low-level bloom in the western subarctic gyre with typical high-nitrate low-chlorophyll (HNLC) communities farther to the east (July–August 1997).

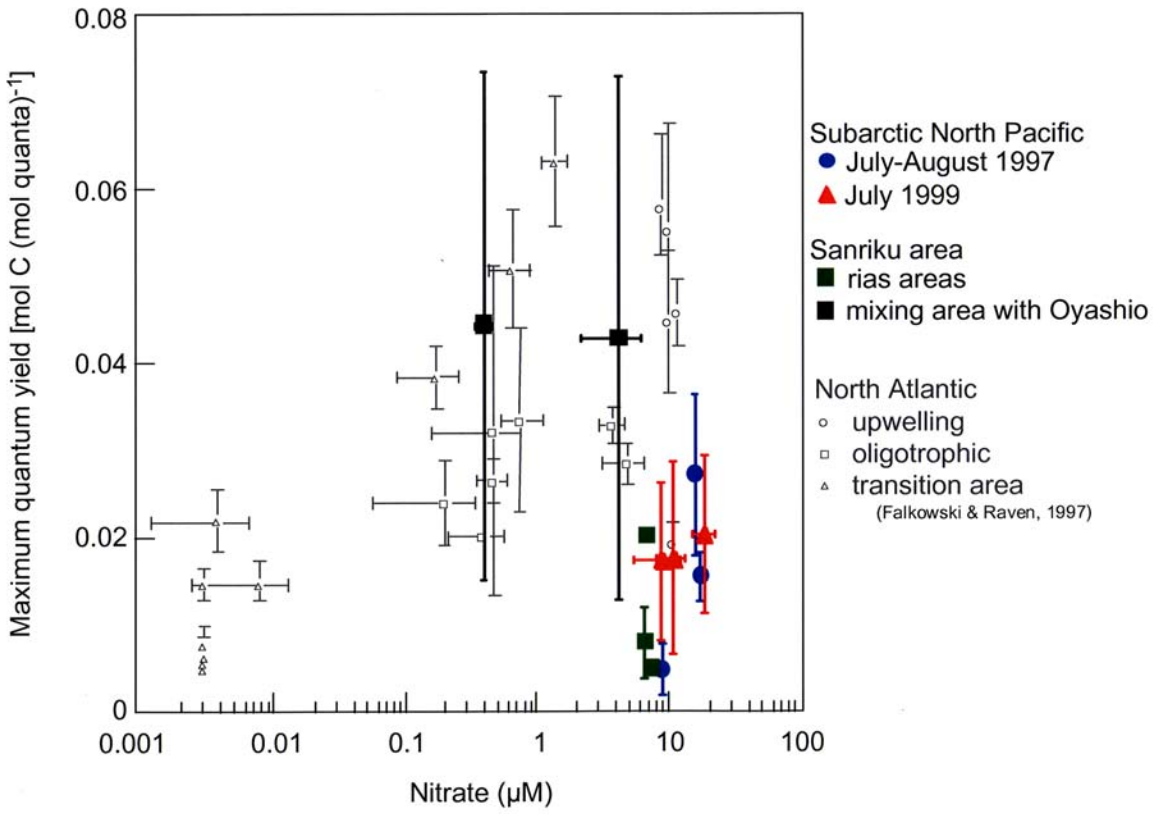

Fig. 9 Quantum yields of photosynthesis (carbon fixed relative to absorbed light) in the oceanic subarctic Pacific are typically very low for the available nitrogenous nutrients compared to coastal areas (as Sanriku area–rias areas are ■ points at <5 μM nitrate) and sites in the North Atlantic.

Our proposed contributions are:

1) Bulk measurements for:
   a. continuous measurement of chlorophyll and primary production at a fixed depth by natural fluorescence (Yoshikawa and Furuya, 2004);
   b. Pulse Amplitude Modulation (PAM) analysis of electron transport from PS-II to PS-I.

2) Group-specific properties, including:
   a. phytoplankton composition as revealed by microscopy for nano- and microplankton, flow cytometry for pico- and nanophytoplankton and CHEMTAX analysis of biomarker pigments for class-specific composition;
   b. Flow cytometric diagnosis of algal physiological status.

We will need to have close collaboration with other groups, in particular, with those working in bio-optics, trace metal chemistry, and nutrient dynamics.

Temporal changes in these properties will be investigated during the 50-day observation from pre-blooming to its termination, because such observations can cover the time scales of episodic events, such as temporal stabilization of the water column, iron supply events, and continuously sunny days. Since we need to differentiate biological and chemical processes from physical ones, areas with strong advection or high spatial variability in oceanic conditions should be avoided. Selection of the study site is an issue of critical importance.

Satellite applications are more difficult in the subarctic Pacific than in many other ocean areas because of the extremely high frequency of cloudiness (>85%). This is particularly hampering to analyses based on ocean color, although, as Dr. Sei-ichi Saitoh's essay here demonstrates, the situation is not hopeless. Applications of microwave satellite techniques, such as scatterometry (wind proxies), sea surface altimetry and sea surface temperature (SST) could be of considerable value to OECOS, placing its time-series observations in a spatially expansive evaluation of physical conditions.

Temporal and spatial variability of phytoplankton biomass and productivity in the northwestern Pacific

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Introduction

Biogeochemical processes in the ocean play an important role in environmental changes. These processes are affected by the biological pump; hence, monitoring the variability of the distribution of chlorophyll-*a* (chl-*a*) and primary production is a way of monitoring oceanic biogeochemical cycles. The subarctic North Pacific represents one of the world's most biologically productive regions.

Ocean-color remote sensing is a useful new tool for continuously monitoring the temporal and spatial variability of chl-*a* concentration. Past studies (Banse and English, 1994, 1999) have discussed phytoplankton seasonality in the western and eastern Pacific Ocean using Coastal Zone Color Scanner (CZCS) data sets. Goes *et al.* (2001) discussed the impact of the recent El Niño event of 1997/1998 on the biological production of the North Pacific using satellite and ship data. Sugimoto *et al.* (2001) reported the features of El Niño events and their biological impacts in the western North Pacific. A recent comparative study on primary production in the Western Subarctic Gyre (WSG) and Alaskan Gyre (AG) focused on different effects of iron (Harrison *et al.*, 1999).

Results from Sasaoka *et al.* (2002)

Ocean-color imagery clearly showed seasonal and interannual variability in the spatial abundance and distribution of chl-*a* in the study area. Chl-*a* concentrations were generally low (0.24–0.537 mg chl m^{-3}) for most of the year, except for a few peaks (1.0–2.0 mg chl m^{-3}) observed in the spring and fall bloom seasons (May, June, September, and October). Chl-*a* concentrations (>10 mg chl m^{-3}) were consistently high along the Kuril Islands and in the coastal waters around the Kamchatka Peninsula, and in 1998 they were clearly higher than in 1999.

The WSG was characterized by positive sea-surface temperature anomalies (SSTA) during the summer to fall of 1998. These anomalies appear to be a high-latitude response to the 1997/1998 ENSO event. High concentrations of chl-*a* appeared in the WSG from September to November, only, in 1998. We suggest that the high chl-*a* around the WSG from summer to fall in 1998 was facilitated by (1) negative SSTA in winter, despite the negative wind anomaly, which provided a larger amount of nutrients to the sea surface, (2) a positive wind anomaly from April to June, which might cause light limitation of the phytoplankton growth, due to the deepening of the surface-mixed layer in summer, and (3) positive SSTA, even with the slightly positive wind anomaly, which was accompanied by water column stabilization and hence release from light limitation of the phytoplankton. Warmer SST also might have enhanced the phytoplankton growth. During summer to fall in 1999, the phytoplankton biomass between 42°N and 43°N along 165°E was greater than in other years. The enhanced chl-*a* concentration coincided with a distinct frontal temperature gradient located between 40°N and 45°N in September 1998 and 1999.

Questions raised by Goes *et al.* (2004)

- How important are winds in regulating biological production in the subarctic Pacific Ocean?
- To what extent are winds responsible for the large west–east gradients in biological production in the subarctic Pacific Ocean?
- How does their variable strength over seasonal and interannual scales impact biological production in the western and eastern parts of the subarctic Pacific?

Approach

Monthly fields of primary production are calculated using the vertically generalized production model (VGPM) of Behrenfeld and Falkowski (1997a,b) and Kameda and Ishizaka (2005). Those models are validated using *in situ* measurement in this region.

We will develop more accurate in-water algorithms for this region using the MODerate-resolution Imaging Spectroradiometer (MODIS) data sets. Near-real-time satellite data will be provided for ship operation from the MODIS receiving and analysis station at Hokkaido University that has just started to receive MODIS data in the middle of March 2005.

One of the main issues to be addressed by OECOS, at least in the eastern sector, is what drives the short-term variability of the phytoplankton standing stock. Despite the consistently low stock levels, chlorophyll concentrations < 0.5 μg l^{-1}, there are oscillations amounting approximately to a factor of three: 0.15 to 0.45 μg l^{-1}. These occur over periods of a week or more, up to 20 days (P.W. Boyd, unpublished fluorometer data from a mooring at Ocean Station P (OSP)), with very strong autocorrelation of successive measures in high-frequency time series (Fig. 10). Similar variation, with similar periodicities, was observed by Bishop et al. *(2002) with 'carbon explorers', free vehicles that obtain transmissometery profiles two to five times daily in the vicinity of OSP. Upper water column light transmissivity can be converted to particulate organic carbon content with reasonable accuracy (Bishop, 1999), and shows strong pulses in surface layers at 10- to 20-day intervals between April 10 and the end of May in 2001.*

The opposite phasing of chlorophyll and ammonia variations (Fig. 10C) is the best demonstration that they are driven by biological interactions. Metabolic breakdown of phytoplankton (and other food-chain components) during chlorophyll decrease regenerates ammonia. Ammonia is taken up during chlorophyll increase. These are, of course, net changes resulting from modest differences between larger daily rates of plant growth and plant consumption. Diel variations, up during daylight, down at night, are well demonstrated by the transmissometry data of Bishop et al. *(2002).*

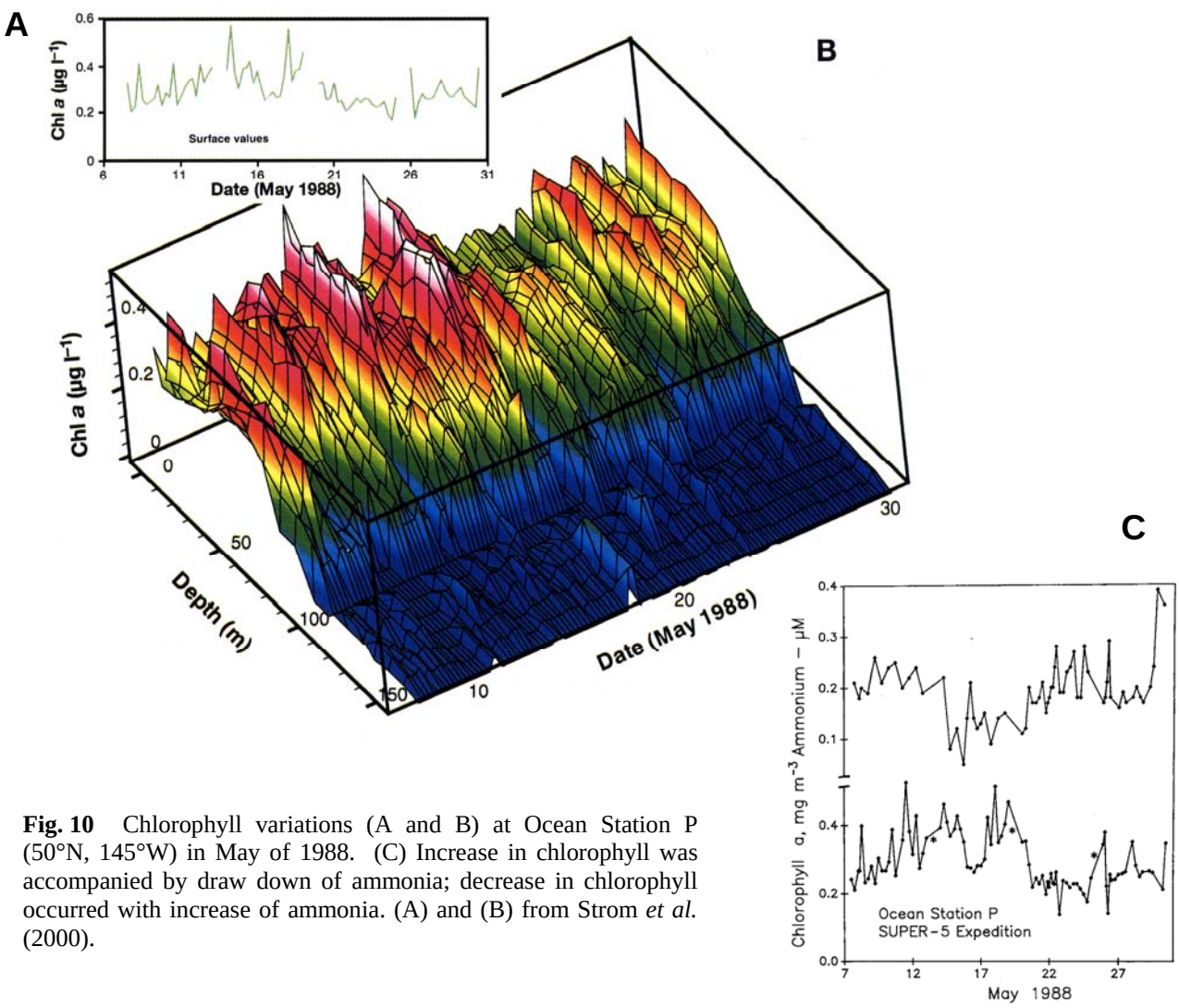

Fig. 10 Chlorophyll variations (A and B) at Ocean Station P (50°N, 145°W) in May of 1988. (C) Increase in chlorophyll was accompanied by draw down of ammonia; decrease in chlorophyll occurred with increase of ammonia. (A) and (B) from Strom *et al.* (2000).

These oscillations beg for detailed explanation, and Strom et al. *(2000) have considered some of the possibilities. There are others, and we will announce those recognized so far as we proceed through this report. Among explanations for limitation of phytoplankton increase at the peaks of the cycle is iron limitation. At some sufficiently low concentration, even the smallest phytoplankton would not have enough surface relative to cell mass to acquire an iron quota. At that point, growth would slow and iron stress should become evident in the physiology of the cells. Work along these lines is the specialty of Dr. Deana Erdner, an OECOS participant who contributed the next essay. She also suggests application of fast repetition rate fluorometry (FRRf) to obtain F_v/F_m as a measure of photosynthetic capability of electron transport systems. That is very likely to be useful to OECOS, and it is also suggested by several other contributors to this report.*

The use of molecular indicators of phytoplankton iron limitation

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My general area of interest and expertise is the physiological ecology of phytoplankton, primarily eukaryotic phytoplankton but including cyanobacteria and bacteria. More specifically, my work has focused on the application and integration of molecular tools with traditional ecological techniques for understanding the factors that affect the distribution and growth of marine microbes in the environment, particularly nutrient stress.

One aspect of my research to date has been the development of molecular markers for iron limitation in eukaryotic phytoplankton. I used the presence and abundance of two proteins, ferredoxin and flavodoxin, to quantify the severity of iron stress. Ferredoxin (Fd) and flavodoxin (Flv) are both redox proteins that catalyze the same step in photosynthetic electron transfer. Ferredoxin contains an Fe-S center, whereas Flv uses flavin mononucleotide as a cofactor to effect electron transfer. My work and the work of others (LaRoche *et al.*, 1995; Doucette *et al.*, 1996; Erdner *et al.*, 1999; McKay *et al.*, 1999) demonstrated that when phytoplankton cells are iron replete, they utilize Fd for electron transfer. However, when cells become iron stressed, they replace the Fd with the functionally similar Flv, thereby decreasing their iron requirements (the amount of Fd in an iron-replete *Thalassiosira weissflogii* cell can account for about 40% of the total cellular iron quota). It has been demonstrated that some organisms produce Flv constitutively while others do not produce it at all (Erdner *et al.*, 1999), but these seem to be the exception. The presence of Flv has been used to demonstrate iron limitation in diatoms in the subarctic Pacific (LaRoche *et al.*, 1996). The relative abundance of the two proteins (the Flv index: [Flv]/[Fd + Flv]) was used as an index of the severity of iron limitation during the IronExII experiment in the equatorial Pacific (Erdner and Anderson, 1999), and in the Southern Ocean (Maucher and DiTullio, 2003).

For the proposed OECOS project, I am interested in monitoring changes in the iron nutritional status of the phytoplankton community on short time scales. Field studies to date have used samples collected several days apart, providing rather coarse resolution. The physiological response of the phytoplankton occurs much more rapidly; diatoms are capable of switching to Fd synthesis, accompanied by complete degradation of Flv protein, within 24 hours after iron resupply (Erdner, 1997; McKay *et al.*, 1999). The response of other physiological processes is even faster, with photosynthetic efficiency (as F_v/F_m) responding on the scale of hours. This has been observed both in the laboratory (Erdner, 1997; McKay *et al.*, 1999) and in the field (Erdner and Anderson, 1999), where cells show near-maximal F_v/F_m but still express only Flv. It appears that Fd may be the 'first thing to go', and the last to return, when cells become iron-stressed.

Because the limit cycles observed in the eastern subarctic Pacific have periods as short as 6–10 days, it would be desirable to monitor the iron status of the phytoplankton on a daily basis. The amplitude of the cycle is likely to be influenced by both phytoplankton growth (and therefore limitation) and grazing. Protistan grazing has been demonstrated to regenerate iron that can be used by phytoplankton (Barbeau *et al.*, 1996), thus grazing itself can play a role in relieving the iron limitation of the phytoplankton community. We can envision a scenario in which, at the 'top' of the limit cycle, phytoplankton biomass has reached its maximum, due to limitation by iron, which slows their growth and allows grazing to exceed growth. As grazing continues, biomass decreases, but at the same time the grazing regenerates iron for the phytoplankton. This allows the phytoplankton to grow faster, until the point where growth exceeds grazing (the 'bottom' of the limit cycle). The physiological status of the phytoplankton will be quite dynamic throughout this cycle, and measurements such as F_v/F_m and the Flv index could reveal the changes that are occurring.

To date, field measurements of Fd and Flv have relied on detection of the proteins, using either antibodies (LaRoche *et al.*, 1996) or high-performance liquid chromatography (HPLC) with spectrophotometric detection (Erdner and Anderson, 1999; Maucher and DiTullio, 2003). The antibodies are very specific, with the only available antisera being reactive to diatoms alone. HPLC methods detect proteins from all taxa, but require large sample volumes (100s of liters) in order to extract sufficient protein for analysis. This limits the sampling resolution, but more importantly, hinders the ability to look at responses in different parts of the phytoplankton community. For the OECOS work, it will be possible to use at least the HPLC methods for analysis. A desirable modification would be the use of size fractionation to gain information on differential responses to iron amongst *Synechococcus*, picoeukaryotes, nanoplankton, *etc*. This could be achieved through the use of sequential filtration using both nitex and glass fiber filters (142 mm diameter).

Greater sensitivity and resolution could be achieved through methods targeting genes encoding Fd and Flv. Very little work has been done in this area, primarily due to the lack of gene sequences for Flv. However, with the advent of full genome sequences for a number of marine phytoplankton (*Synechococcus, Prochlorococcus, Trichodesmium, Crocosphaera, Thalassiosira pseudonana, Phaeodactylum tricornutum* and *Emiliania huxleyi*), as well as expressed sequence tag libraries for diatoms (*Pseudo-nitzschia* multiseries) and dinoflagellates (*Alexandrium tamarense, Alexandrium ostenfeldii* and *Karenia brevis*), sequences for both Fd and Flv are now available for a variety of taxa. These sequences provide information with which the homologous sequences can be isolated from other species. If the genes are regulated in the same fashion as the proteins, then measurement of gene expression could provide a sensitive and specific way of determining Fd and Flv levels, and the assay could be tailored to provide data on different taxonomic or functional groups. This approach obviously needs to be verified and developed for use in natural populations. The work to achieve this has just begun in our laboratory, and will be the topic of future proposed research. It is possible that such an approach could be available for use in a few years.

B. IRON CONCENTRATION AND CHEMICAL SPECIATION

If iron is sometimes limiting and sometimes not, it will be essential to OECOS, on both sides of the Pacific, to have accurate estimates of iron concentration and chemical speciation. Recent work (reviewed in Shaked et al. *(2005).* Limnol. Oceanogr. ***50****: 872–882) suggests some of the complexity of the uptake process and its interaction with organic iron-binding siderophores in seawater. OECOS will need sophisticated chemists to correctly characterize variations in iron availability to support phytoplankton growth within this array of complex chemistry. Drs. Zanna Chase, Jay Cullen and Kenshi Kuma will contribute this sophistication to OECOS. Their essays follow.*

Iron measurements during OECOS

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One of the hypotheses to be explored during OECOS is that iron limitation sets the upper limit of the observed phytoplankton variations. Measurements of total dissolved iron, iron speciation, and physiological indicators of iron stress are needed. We describe sampling and analysis of iron, as well as ideas about additional measurements that would be relevant to the question of the influence of iron in this system.

Sampling considerations

The key to any successful field campaign involving iron measurements is the clean collection of samples. Surface waters can be cleanly collected by pumping through tubing attached to a towed body deployed outside the ship's wake. To avoid contamination from the ship, *this approach necessitates that the ship always be moving through the water.* This would be the most convenient way of collecting samples, as the clean sample stream could be directed straight into the analytical instruments, which greatly reduces the chance for contamination. Alternatively, samples could be collected manually by Zodiac trip.

We also need at least one profile per day with a dedicated CTD-rosette deployed on conducting, plastic-coated cable. Sample bottles are typically Go-Flos. We recently saw such a system (jointly owned by Dr. Christopher Measures, University of Hawaii, and William Landing, University of Florida) deployed during an iron intercomparison cruise (Sampling and Analysis of Iron – SAFe) in the Pacific (Fig. 11). It collected a full profile to 1000 m depth in about 1 hour. Samples were clean. This approach is much faster than hanging Go-Flos off Kevlar line. Dr. Jay Cullen was funded in April 2005 to build such a rosette system. It is likely that this rosette will be available for the OECOS study.

A dedicated, clean analytical and sampling van would be ideal for sampling from the Go-Flos and for the iron analyses. Again, Dr. Measures has an ideal system that could serve as a model (Fig. 12). Dr. Cullen may also soon have access to such a facility.

Iron speciation measurements

Total dissolved Fe

By this we mean iron in a 0.2 μm-filtered sample treated in such a way as to release all iron from organic ligands, and to dissolve all colloids. Typically this is achieved by acidification to pH < 2 or by UV treatment. Many at-sea techniques exist. If we sample by continuous

surface pumping we would probably use a flow injection analysis technique, such that samples can be directed straight into the analytical system, with minimal handling. Dr. Chase has experience with colorimetric and chemiluminescence techniques, both of which performed well during the recent SAFe intercomparison exercise. The electro-chemical techniques used to measure iron speciation (see below) can also be used at sea to measure total dissolved iron.

Fe(II)

It is more soluble than Fe(III), more readily available to phytoplankton, and it may exhibit diurnal cycles associated with photoreduction. Fe(II) may also be elevated in response to aerosol or rain events. We would probably use some version of the spectrophotometric or chemi-luminescent (*Bowie et al.*, 2002) methods already published. For these measurements, it is very important to run the sample as soon as possible after collection. This analysis may not be critical, but would be interesting.

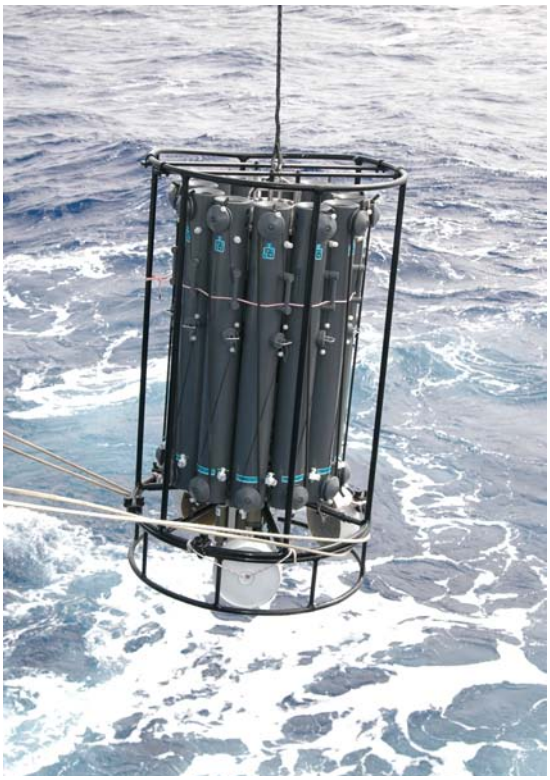

Fig. 11 The trace metal clean rosette of Go-Flos deployed during SAFe in the North Pacific.

Fig. 12 Collecting filtered water from the Go-Flos in the University of Hawaii clean van. The 12 bottles are racked on either side of the van and filtration is accomplished through clean-air pressure

Organically complexed Fe

The concentrations of ligand-bound iron (FeL) and total inorganic iron (Fe′) will be determined by competitive ligand equilibration/adsorptive cathodic stripping voltammetry (Gledhill and Van den Berg, 1994; Rue and Bruland, 1995), using the competing ligand 2-(2-Thiazolylazo)-p-cresol. (Croot and Johansson, 2000). Dr. Cullen has extensive experience in the analysis of trace metals in seawater and marine particles. The measured concentrations of Fe_T, Fe′, and FeL will then allow us to calculate the concentration and conditional stability constants of total excess dissolved iron binding ligand (L_T) in the seawater.

Colloidal Fe

It could be determined by ultrafiltration (0.02 µm), but this is labor intensive and would need to be justified scientifically.

Particulate Fe

This would tell us how much iron is associated with particles. If some assumptions are made about the lithogenic contribution, it is possible to estimate biological ‘metal quotas’ using these samples. Alternatively, samples can be washed to remove extracellular metals (Tovar-Sanchez *et al.*,

2003). Samples would be collected from the Go-Flos onto cleaned filters and stored for future analysis by Inductively coupled Plasma-Mass Spectrometry (ICP-MS) (Cullen and Sherrell, 1999).

Archived samples

These would be collected and acidified such that multi-element ICP-MS techniques could be used to measure a suite of other bio-active metals (Zn, Co, Cu, Mn, and Cd). As ship-based methods exist for many of these metals, it would also be possible to measure them at sea, but this would involve trade-offs in time and space allocations, so this idea should be discussed.

Monitoring phytoplankton iron stress

Fast repetition rate fluorometry (FRRf) provides a very sensitive measure of phytoplankton photosynthetic competency. During all of the iron addition experiments, the FRRf was the first instrument to pick up a response to iron addition.

A series of quick, 'bioassay' incubations could be performed to assess iron stress in the photosynthetic community. Briefly, samples (500 ml?) would be collected from the clean sampler and incubated in the presence and absence of added iron for 12–24 hours (or possibly less time – this is to be determined). They would then be assayed for photosynthetic competency with FRRf. The ratios of the responses in the with Fe and without Fe treatments would provide an indications of iron stress. This method should be much more sensitive than looking for a growth response (*e.g*., chlorophyll) and multiple assays per day could be run.

Quantifying iron inputs

This would also be a wonderful opportunity to study aerosol iron inputs. The eastern North Pacific does experience episodic iron enrichment from Asian dust storms (Bishop *et al*., 2002). A ship stationed at a single point for 50–60 days would enable us to provide unique measurements of the aerosol input to this area. Coupling these measurements to the in-water measurements of iron and iron speciation would tell us much about the delivery of iron to the surface of the ocean. Thus we should consider including daily collection of aerosol samples, and collection of rain when possible. Dr. Chase has had some experience with aerosol collection on land, and could consult with experts on how this is done at sea.

Underway sampling with a towed body will likely be possible during OECOS time-series work, but only when other over-side or net towing activities allow it. Thus, there might be several underway iron samplings each day. We could steam off in a suitable direction from a defined time-series station, which would be a drifter location or geographic position. This would be the time for air sampling as well, since air sampling is very difficult, at least in light winds when sitting still in a ship's diesel cloud. Air sampling takes a tower on the bow to reduce the amount of sea spray included in samples (still substantial). The ship must move upwind to ensure that the sampler will be upwind of the stacks. Clean rain on a ship is very difficult to obtain, but perhaps we can find a way (sampling balloons?).

The Go-Flo rosette system will need a dedicated winch. We will need counts for both east and west OECOS vessels of the number of dedicated winches required. We do not want to be changing out cables to different instruments. So far, we see needing at least:

- *CTD and nutrient/chlorophyll rosette*
- *Iron rosette*
- *Multiple plankton net*
- *Any underway profiling system (Timothy Cowles' work in the east)*

More sampling equipment may be required, but attention to minimizing this number and preparation to provide specialty winches is in order.

The measurement of iron, nutrients and other chemical components in the northwestern North Pacific Ocean

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For the OECOS project, I am interested in monitoring iron (total, dissolved, and other forms), nutrients, and other chemical components in the surface waters (0–300 m depth) throughout the vertical water mixing during late winter (1), the spring phytoplankton bloom after water column stabilization (2), and the end of spring bloom (3) when:

- Vertical water mixing during winter transports iron and nutrients to the surface layer.
- Upward transport of iron and other nutrients during water mixing induces the spring phytoplankton bloom after water column stabilization.
- Change of iron, nutrients and phytoplankton species occurs during the spring phytoplankton bloom.

Sampling methods

Seawater samples need to be collected in the surface waters (0–300 m depth) on a daily basis in the northwestern North Pacific Ocean by use of acid-cleaned, Teflon-coated, 10-liter Niskin X sampling bottles (General Oceanics) attached to a CTD-RMS, or on Kevlar line. Sample filtration for analyses of labile dissolved Fe (<0.22 μm) concentrations, Fe(III) hydroxide solubility, and humic-type fluorescence intensity is carried out by connecting an acid-cleaned 0.22-μm pore size Durapore membrane filter (Cartridge type-Millipak 100, Millipore) to a sampling bottle spigot and then filtering with gravity filtration. Unfiltered samples are collected for total dissolvable Fe, Chl *a*, and nutrient concentrations. The unfiltered and filtered seawater (100 ml in precleaned 125-ml, low density polyethylene (LDPE)) used for dissolvable (unfiltered) and labile dissolved (<0.22 μm) Fe concentration

analyses is buffered at pH 3.2 with 10 M formic acid–2.4 M ammonium formate buffer solution (0.5 ml per 100-ml sample solution) in a class 100 clean-air bench on board as soon as the samples are collected. The buffered samples (pH 3.2) are kept at room temperature for 1–2 months until Fe analysis is conducted in the laboratory (Takata *et al.*, 2004).

Analytical methods

Iron concentration

The Fe concentration in each buffered sample is determined by an automated Fe analyzer (Kimoto Electric Co. Ltd.) using a combination of chelating resin concentration and luminol-hydrogen peroxide chemiluminescence (CL) detection in a closed flowthrough system (Obata *et al.*, 1993). Briefly, Fe in a buffered sample solution is selectively collected on 8-quinolinol immobilized chelating resin and then eluted with dilute 0.3 N HCl. The eluent is mixed with luminol solution, 0.6 N aqueous ammonia and 0.7 M H_2O_2 solution successively, and then the mixture is introduced into the CL cell. Finally, the Fe concentration is determined from the CL intensity.

Fe(III) hydroxide solubility and humic-type fluorescence intensity

The frozen 0.22-µm-filtered samples are thawed and warmed to room temperature (18°C) in a clean room (dark condition) overnight before the start of the experiments for Fe(III) solubility and humic-type fluorescence intensity. The Fe(III) solubility (20°C) in the defrosted filtered seawater sample at each depth is determined by a simple filtration (0.025-µm Millipore cellulosic membrane filter) involving γ-activity measurement of ^{59}Fe(III) after adding radioactive ^{59}Fe(III) into the seawater sample, as reported in previous studies (Kuma *et al.*, 1996; Nakabayashi *et al.*, 2001; Tani *et al.*, 2003; Takata *et al.*, 2004). Just after the frozen samples (10 ml in a polypropylene tube) are thawed and warmed overnight to room temperature in the dark, the humic-type fluorescence intensity is measured in a 1-cm quartz cell with a Hitachi F-2000 fluorescence spectrophotometer at 320-nm excitation and 420-nm emission, using 10-nm bandwidths (Hayase and Shinozuka, 1995).

Chl a *and nutrient concentrations*

The concentrations of size-fractionated Chl *a* (0.2, 2, and 10 µm) at each depth (5- to 150-m depth) are determined by the fluorometric method using N, N-dimethylformamide extraction (Suzuki and Ishimaru, 1990). Major nutrient concentrations are determined using a Technicon autoanalyzer. Hydrographic data (salinity, temperature and depth) are obtained using a CTD.

Dr. Kuma proposes to take on basic major nutrient (NO_3, NH_4, $Si(OH)_4$, PO_4) determinations for the Western OECOS Group. The Eastern Group has not dealt with this issue, but views high-frequency nutrient, temperature, and salinity profiles as quite readily generated through a service function. This will require suitable contract arrangements for provision of this data, and chemists with appropriate auto-analyzer equipment aboard. Apart from nutrient drawdown over the course of the time series, we do not associate any cutting-edge questions with the acquisition of these essential background data.

C. PHYSICAL OCEANOGRAPHY, FINE-SCALE DISTRIBUTION PATTERNS AND AUTONOMOUS DRIFTERS

In regard to the physical setting, apart from frequent CTD/rosette profiling of the water column, it is anticipated that both western and eastern OECOS time series could benefit from (1) high-frequency data telemetered to a ship from drifters defining the station locations and (2) frequent characterization of the vertical fine structure of physical and biological features of the water column. Most importantly, the drifter would provide continuity to chlorophyll and surface physical data during any intervals that shipboard work is shut down by stormy weather. High-resolution bio-optical profiles would identify vertically restricted layers of very high concentration that are seen in many ocean areas, and would possibly explain how zooplankton find enough food in systems seen at low resolution as trophically very dilute, while spectral absorbance profiles would give us phytoplankton data equivalent to a limited CHEMTAX analysis, but with centimeter-scale resolution. It is not certain what we will learn from that, but certainly it will be something. Drifters are addressed in an essay by Dr. Peter Strutton and bio-optical profiling in another by Dr. Timothy Cowles. The bio-optical profiling will also contribute much of the small-scale data on mixing processes that we will need to interpret distributional patterns in OECOS work.

The use of drifters in Lagrangian experiments: Positives, negatives and what can really be measured

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Drifter design and general comments

Figure 13 illustrates the design that was used by the Monterey Bay Aquarium Research Institute (MBARI) team during the Southern Ocean Iron Experiment (SOFeX). I use it as an example because I have personal experience with it. The drifter was relatively easy to deploy off the stern of the vessel, through the A-frame, but it was difficult to recover – we lost two holey sock drogues in the screws. The drifter also experienced significant slippage, perhaps because of high winds and a relatively large surface expression. Adding a second holey sock drogue (*i.e.*, to increase subsurface drag) did not do much to reduce slippage. The 'disposable' optics-only drifters (Mark Abbott, Oregon State University) that we deployed had a very small surface expression and anecdotal evidence suggests they did not slip as much (they stayed with the N patch, but that patch became very large, so maybe that was not such a difficult feat).

The size of the surface float of the MBARI drifters was largely determined by the packet radio antenna (communications with the ship), GPS antenna, CO_2 air intake, and above-water radiometer. The logger/controller canister was not watertight enough to be mounted underwater; it was about 30 cm in diameter, so it increased the size of the surface expression. The float itself was almost 1 m in diameter.

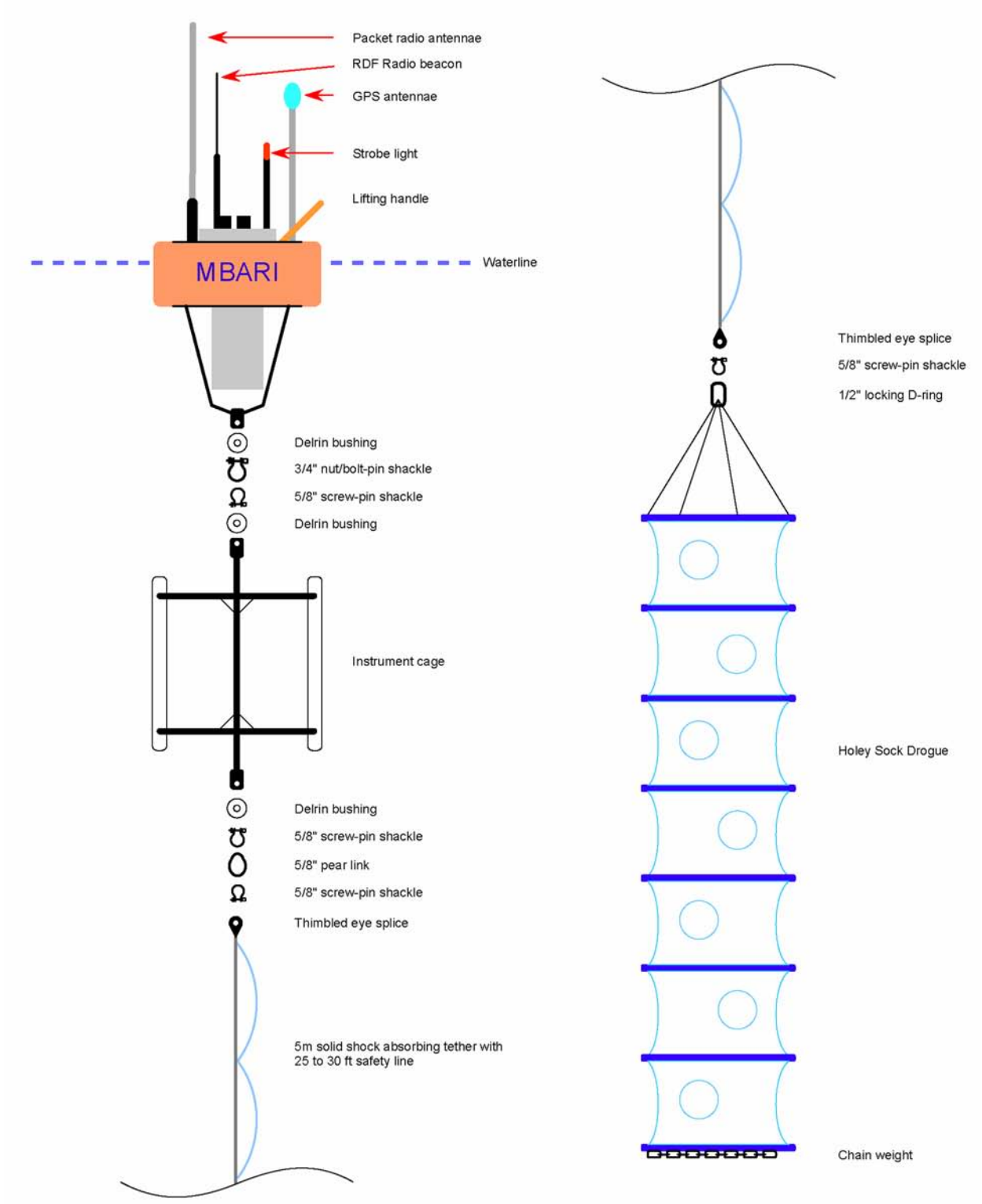

Fig. 13 Schematic of the MBARI drifter that was used during the Southern Ocean Iron Experiment SOFeX.

Fig. 14 The drifter, deployed. The small red float nearby is at the end of a tag line.

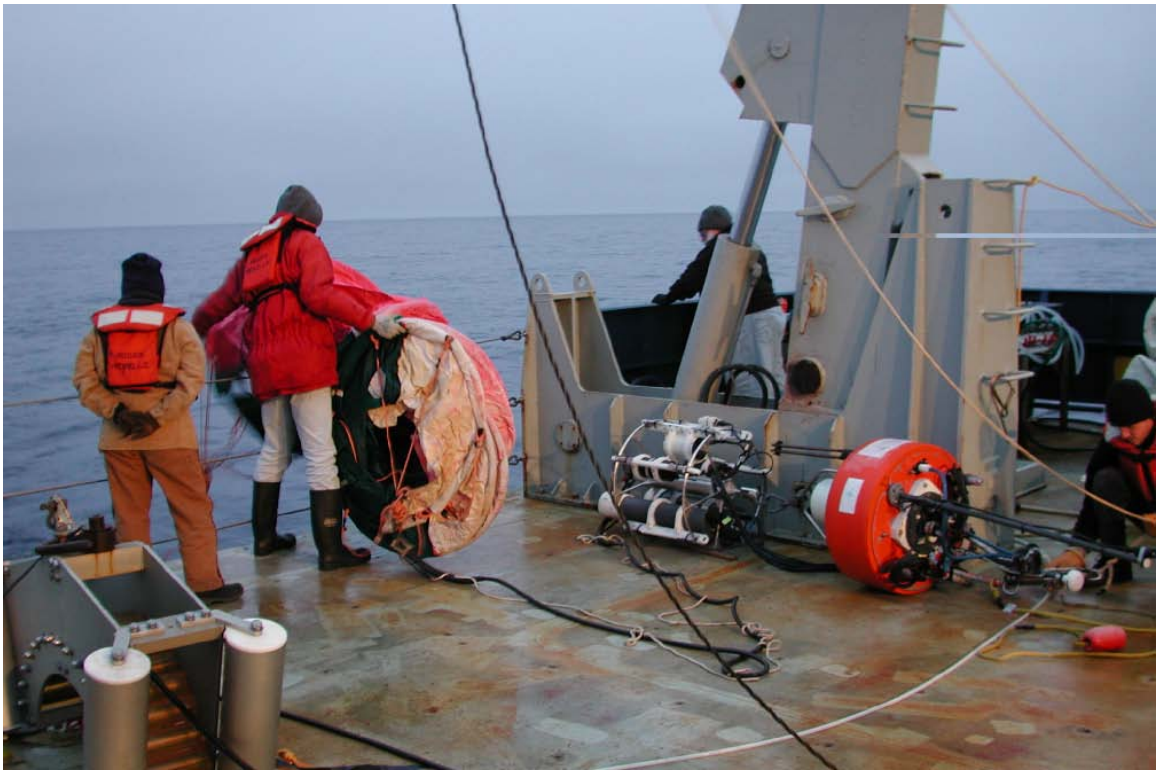

Fig. 15 Deployment of the MBARI drifter off the stern of the R/V *Revelle*.

Instruments

The MBARI SOFeX drifters were deployed (Fig. 14) with the following instruments (see the cage mounted beneath the float in Fig. 15):

- MBARI In Situ Ultraviolet Spectrometer (ISUS) optical nitrate sensor. At the time, these were made at MBARI, but they are now commercially available from Satlantic Inc. for about $30,000 (or maybe a little less).
- MBARI CO_2 system, built by Gernot Friederich's lab. These are not commercially available, but it might be possible to buy one from MBARI for less than $20,000.
- HOBILabs hydroscat instruments are intended to measure fluorescence and scattering. I have recently had success in the Labrador Sea with WETLabs fluorometers and VSF (volume scattering function) instruments. They are relatively small, easy to operate, and collect excellent data (http://bioloc.oce.orst.edu/strutton/research_labsea.htm).
- SeaBird MicroCAT 37-SM CT package (P optional, but not necessary for drifters).
- SeaBird SBE43 O_2 sensor.
- Satlantic OCR radiometer (7 wavelengths of upwelling radiance, Lu). A single wavelength irradiance (Ed490) sensor was mounted at the top of one of the antennas.
- GPS and packet radio communications were mounted on the surface float.

Which of these instruments would be important for OECOS?

The answer to this question should probably be guided by the parameters we wish to measure when the ship is unable to sample due to weather.

CO_2 was essential for the SOFeX drifter, since one of the parameters we were trying to quantify was drawdown of CO_2 as a result of Fe fertilization (Fig. 16). My feeling is that we are likely to observe less significant (less important?) fluctuations in CO_2 during OECOS. This is a point for discussion.

Nitrate fluctuations were also observed during SOFeX, and were helpful for determining budgets. In OECOS we are likely to observe progressive drawdown of nitrate through the course of the spring transition. Probably this need not be represented by really high-frequency and gapless data, and, since the sensors are expensive, nitrate should likely be left to shipboard analysis.

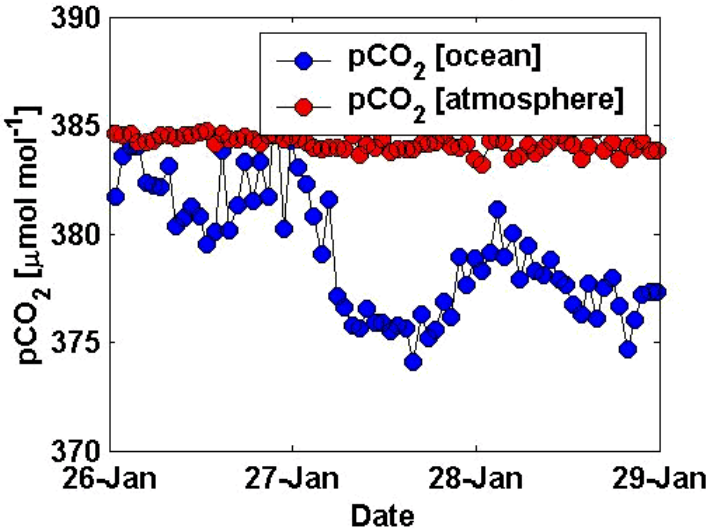

Fig. 16 CO_2 drawdown, measured by the drifter, after Fe fertilization of the SOFeX south patch. The magnitude of the drawdown observed from underway ship-based measurements was of the same magnitude.

Chlorophyll is essential, can be easily determined *via* fluorescence (even with the considerable daily variability in many parts of the ocean), and the sensors are cheap (less than $5,000).

Particulate Organic Carbon (POC) can be determined by backscatter or beam attenuation. Again, these sensors are cheap (less than $5,000). Making the connection to POC requires calibration samples to be taken, but this will likely be done anyway.

Temperature, salinity and O_2 sensors are all relatively inexpensive and should be standard equipment for any drifter. T and S help to indicate any slippage of the drifter into different water masses, and O_2 helps determine chemical budgets.

Upwelling radiance measurements might be added, but they are somewhat redundant if we already have chlorophyll measurements. Standard radiometers have wavelengths at roughly the SeaWiFS wavelengths (412, 443, 490, 510, 555, 670 and 683 nm). Band ratio algorithms (usually 443 nm/555 nm) can be used to get chlorophyll, but they do not work any better than fluorescence. The 683-nm channel can be used to derive a measure of photosynthetic efficiency (Abbott and Letelier, 1998).

Irradiance sensors (above-water) can complement the in-water radiance measurements, but the ship will measure irradiance underway.

Hardware, electronics and communications

For the MBARI drifters, all instruments were connected to a central controller (Chavez *et al.*, 1997). The advantage of this is that subsets of data can be transmitted to the ship when packet radio connections are made. If the drifter is never recovered, then at least the data are saved. It also permits monitoring of the data, so if an instrument goes bad, the drifter can be recovered and the problem can be fixed. In reality, recovery might not happen except for major failures.

The disadvantage of this centralized controller system is that it requires significant hardware and software support. A simpler option might be to have each instrument, or groups of instruments, internally record data, which can be downloaded after recovery.

For locating the drifter, a minimum requirement (possibly sufficient for slow current regimes) would be an ARGOS transmitter. It can transmit positions several times per day *via* satellite to an e-mail account (on the ship). ARGOS works well at most latitudes. During SOFeX we found that the GPS unit that was reporting to the controller often failed, and at least one drifter package was nearly lost because of this. It is likely this was a symptom of working at high latitudes (poor satellite coverage). A strobe light really helps for finding the drifter in fog or at night.

OECOS projects would certainly want radio transfer of drifter data to the ships at short intervals so that characterization of the habitat is continuously available. Some of the variables that Dr. Strutton lists, as measured in earlier drifter work, can also be measured for the mixed layer aboard ship during rough weather, using water from clean intake lines: nutrients (even iron), CO_2, chlorophyll, and salinity. A drifter, then, might be asked to get data from several levels, even quite deep ones. As Dr. Cowles's essay points out below, we do not have very good understanding of shear in flow among layers in the open subarctic, so that the effect of a long drogue line with multiple instrument packages cannot be determined. It will be important that drifters not eat the entire OECOS budget, and that the drifter projects have about the same general cost as most other component projects.

Dr. Strutton says that nitrate data are expensive. In the subarctic Pacific nitrate changes slowly over the seasonal time scale, whereas ammonia concentration variation is rapid (time scales of days with changes in both directions) and much more interesting. We need to work toward the development of a drifter-mountable ammonia analyzer.

The interaction between plankton distribution patterns and vertical and horizontal physical processes in the eastern subarctic North Pacific

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The onset of surface warming in early spring initiates a 2-layer system above the permanent halocline (Denman and Gargett, 1988), with *Neocalanus plumchrus* and *N. flemingeri* concentrated above a shallow pycnocline (~40 m), and *N. cristatus* and *Eucalanus bungii* concentrated between that shallow pycnocline and the seasonal pycnocline (~100–150 m) (Mackas *et al.*, 1993; confirmed by Goldblatt *et al.*, 1999).

Work from the Subarctic Pacific Ecosystem Research (SUPER) program at Ocean Station P (OSP) indicated that wind forcing sustains moderate to high turbulence in the shallow surface mixed layer, with little penetration of that energy below the shallow (temporary) pycnocline. This barrier to mixing creates two distinct physical environments within the upper 100 m, and appears to support distinct ecological relationships in these respective depth ranges (*e.g.*, Mackas *et al.*, 1993). Finally, it appears that these two upper 'ecological' intervals are effectively isolated from mixing with the waters below the permanent pycnocline (maintained by a halocline, ~32–33 pss). Denman and Gargett (1988) argued that the strong stratification created by the permanent pycnocline (100–150 m) prevented realistic estimates of vertical flux of nutrients (or other passive tracers) through the pycnocline.

The ecological interactions within and between these distinct intervals clearly contribute to the unique temporal patterns in phytoplankton, protist, and mesozooplankton abundance seen in this region of the subarctic Pacific (*e.g.*, Miller *et al.*, 1991; Strom *et al.*, 2000). Among the many compelling questions raised by these patterns, I am particularly interested in understanding the link between the vertical scales of physical processes and the corresponding vertical patterns in biological distributions.

Eastward flow dominates the OSP region (*e.g.*, Warren and Owens, 1988) throughout the 4000-m water column. General upper ocean conditions have been described by many authors – see Whitney and Freeland (1999) for a recent summary of the region. Considerable data exist on the surface flow (~10 cm s^{-1}) near OSP (*e.g.*, Niiler and Paduan, 1995; Bograd *et al.*, 1999, based on drifters), and the vertical structure of the velocity field at OSP was documented in late August 1977 during the Mixed Layer Experiment (MILE; Davis *et al.*, 1981), and again in 1987 by the Ocean Storms experiment (several papers in *Journal of Physical Oceanography* 1995, **25**(11)), but there is a shortage of data with good vertical resolution of the velocity structure. Davis *et al.* (1981) observed shears within the upper 50 m of 0.01–0.05 s^{-1} over vertical intervals of 3 m. D'Asaro *et al.* (1995) report roughly equivalent shear values for the base of the seasonal pycnocline, but those values represent storm conditions. The microstructure profiles discussed by Denman and Gargett (1988) indicated significant vertical shear at the base of the permanent pycnocline (based on instrument tilt), but that shear was not sufficient to induce local small-scale vertical mixing. Davis *et al.* (1981) estimated that conditions favorable to small-scale mixing were infrequent in August 1977.

These apparent vertical gradients in shear resemble those we have observed off the coast of Oregon using a free-fall vertical profiling system (Cowles, 2003) that resolves vertical hydrographic and bio-optical structure on scales of 2–4 cm (see Fig. 17). We find that steep gradients in density often have steep gradients in vertical shear, confined within a 1–2 m depth interval near the density gradient. Local maxima (or extremely steep gradients) in phytoplankton abundance often occur near these shear gradients, creating a vertical distribution pattern that could be described as

'vertically patchy' for grazers. These steep gradients in phytoplankton persist for many hours, creating significant vertical heterogeneity in food resources for micrograzers and localized zones with high growth potential for those micrograzers.

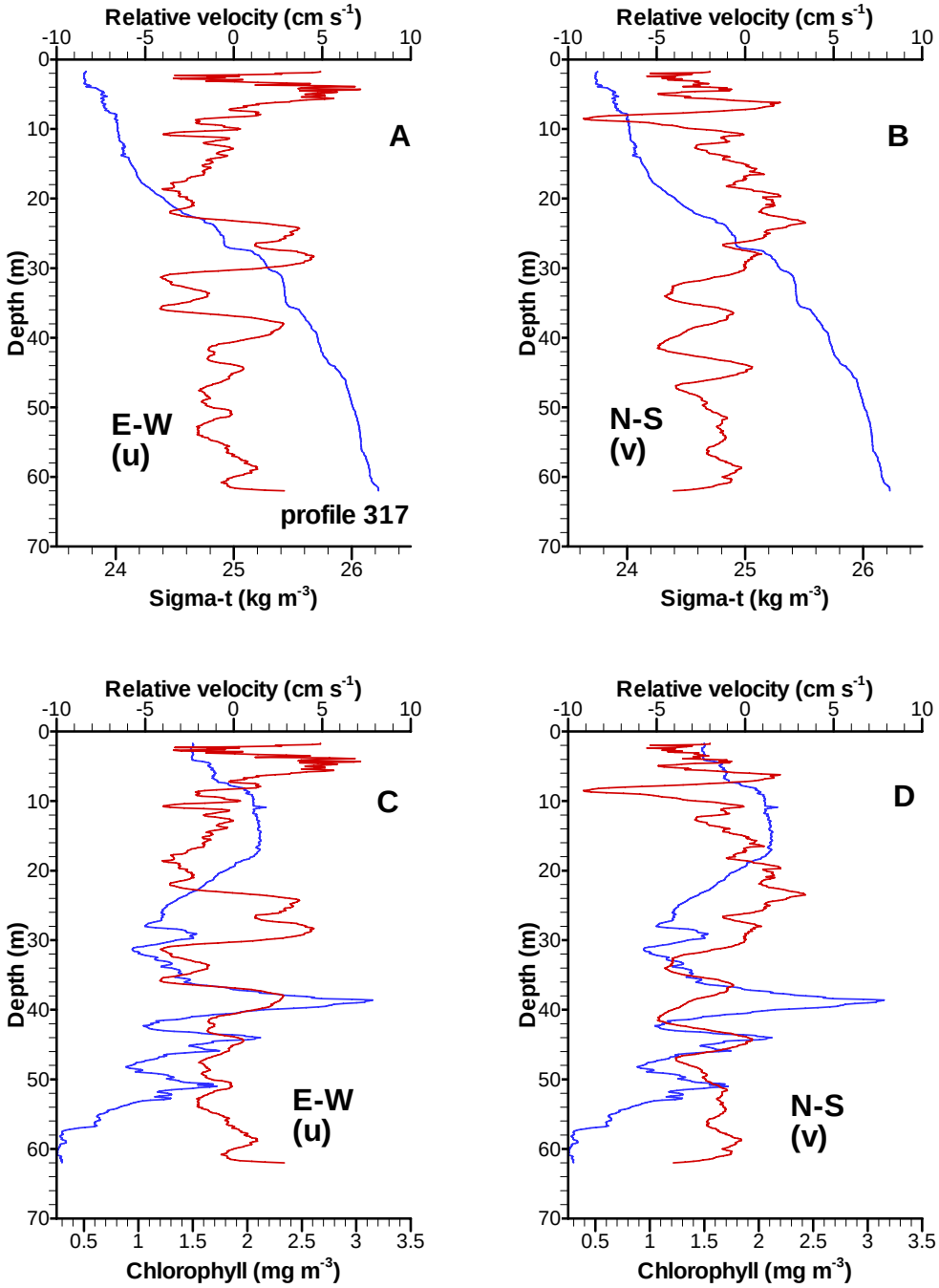

Fig. 17 Vertical profile 317, September 20, 1998, over the Oregon continental shelf. (A) Sigma-t (blue) and the u-component of horizontal velocity (red), relative to the free-fall profiler. (B) Sigma-t (blue) and the v-component (red), (C) Chlorophyll (blue) and the u-component (red), (D) Chlorophyll (blue) and the v-component (red). Note the alignment of the density steps and chlorophyll gradients with the changes in relative horizontal velocity.

Previous work at OSP suggests that the vertical patterns in microzooplankton abundance largely define the distribution and grazing patterns of *N. plumchrus* and *N. flemingeri* (Mackas *et al*., 1993; Dagg, 1993a; Gifford, 1993; Strom *et al.*, 1993). It is reasonable to hypothesize that the vertical pattern of microzooplankton abundance is strongly correlated to the pattern of phytoplankton abundance. The vertical structure of the phytoplankton pattern, and its relationship to finescale physical processes, has not yet been resolved on small enough vertical scales to address this interplay between physics and biology in the subarctic Pacific (*e.g*., Boyd and Harrison, 1999).

My research interests within OECOS are motivated by the opportunity to address several questions that arise from these earlier observations.

- Do phytoplankton develop in (or get spread into) vertical layers in response to the shear at the base of the shallow pycnocline? (This is relevant to a 'patchy' resource hypothesis.)
- Is the structure of the spring mixed layer controlled by vertical processes? To what extent are horizontal processes influencing the ecological interactions above the seasonal pycnocline?
- Given that considerable horizontal mesoscale variability (passage of eddies – see Niiler and Paduan (1995); D'Asaro *et al.* (1995); Bograd *et al.* (1999)) has been observed in this region, to what extent is variability in horizontal advection influencing the vertical distribution patterns we observe?
- Do the vertical gradients in plankton distribution (phytoplankton *and* zooplankton) match the vertical gradients in physical structure, including small-scale mixing? It was suggested by Mackas *et al.* (1993) that the distinct differences in turbulence between the ecological intervals of the upper 100 m layer also influenced the distribution of the larger copepods.

My research objectives in OECOS are to:

- characterize the finescale (~1 m) vertical gradients in phytoplankton and zooplankton abundance (in response to the 'patchy food' hypothesis);
- characterize the horizontal variability in physical properties and processes (temperature, salinity, and velocity);
- simultaneously assess phytoplankton distributions (with bio-optics) and zooplankton distributions (with bio-acoustics) (also in response to the 'patchy food' hypothesis).

Research approaches

These objectives (and research questions) can be approached by using high-resolution profiles to address the vertical scales of variability, and mesoscale mapping surveys to address the horizontal scales of variability.

Vertical processes and patterns

My research group uses a free-profiling system that descends at ~0.20 m s^{-1} and resolves T, S, horizontal velocity, shear, chlorophyll fluorescence, spectral absorption and attenuation, and bio-acoustic backscatter (for small zooplankton). T and S are resolved to 1 cm, while the bio-optical variables are resolved to 3–5 cm. Bio-acoustic backscatter is resolved in 1 m depth bins. Current velocity (horizontal) and vertical shear are resolved in 0.5 m depth bins. The profiler can also carry a small rosette system to collect discrete water samples during a profile, with depths of sample collection selected by the user watching the real-time output of the CTD system.

Horizontal processes and patterns

Regional/mesoscale variability in hydrography, phytoplankton, and acoustic proxies for zooplankton can be documented with mapping surveys using a towed, undulating SeaSoar vehicle (CTD and bio-optics) and a towed bio-acoustics array. In conjunction with shipboard ADCP, such surveys can reveal the upper 200 m structure across a 150 km × 150 km region with a 5-day survey (non-stop) of 8 parallel lines, or across a 100 km × 100 km region with a 3-day survey of 6 lines, each assuming approximately 20-km spacing

between each section (the local Rossby radius). This type of survey would provide 1 m vertical resolution of bio-acoustical backscattering – and would be a valuable complement to the stratified zooplankton tows that would be done by David Mackas and Moira Galbraith.

In order for the Eastern OECOS Group to incorporate a ~100 × 100 km SeaSoar grid in their work, it would take an additional ship (if 6-hourly station work is to be sustained at the time-series site). Could SeaSoar work be accomplished by a separate, shorter visit of an intermediate-size vessel, e.g.*, R/V* Wecoma*? That would probably take a justification based mostly on physics and separate from the main goals of OECOS. Could it be part of a Canadian project, perhaps aboard the CCGS* John P. Tully *in an annual Line P transect? Perhaps an autonomous vehicle could carry out such a survey and return to the main ship. These devices are pretty close to ready for service.*

How much of what we need to know about shear can come from a shipboard acoustic Doppler current profiler (ADCP)? How well can an ADCP be held in vertical 'register' with the water-column profiler results?

Operation of the profiler will be weather-dependent, so we will not have profiles during storms ('wind events'), but only during the intervals between them. Some stratification may develop and have significant association with biological distributions as these intervals progress. A reasonable guess is that thin layers are much more likely to be in the second layer, between the seasonal and permanent pycnoclines. There is likely to be enough energy to keep the upper water column mixed with high frequency down to about 35 meters.

Fine-scale profiles take time and require replication. It will be useful, even at this early stage, to figure out a time budget and schedule for over-side work aboard the RV OECOS.

D. MICROZOOPLANKTON

A key aspect of the food web in high-nitrate low-chlorophyll (HNLC) regions, and in fact all oligotrophic oceans, is the complexity among autotrophs and heterotrophs at very small sizes. Much of the production is from cyanobacteria and picoeukaryotes, and most of the rest from nanophytoplankton. Grazing on the smallest primary producers begins with protozoans of only a few microns, and ciliates and heterotrophic dinoflagellates account for most of the rest of the first food chain transfer. All of these small grazers are preyed upon by others of the same size or only slightly larger, so there is considerable trophic complexity before organic matter is transferred to mesozooplankton. It is certain that most nutrition for mesozooplankton, particularly the copepods dominant in the spring transition, is not phytoplankton but microplankton, primarily protists. Thus, to understand the control of phytoplankton stocks and production rates to evaluate food available to mesozooplankton, it is necessary to examine protistan ecology. Two experts in this area, Drs. Suzanne Strom and Takashi Ota, discuss appropriate approaches in the following essays.

Microzooplankton processes in oceanic waters of the eastern subarctic Pacific: Project OECOS

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Microzooplankton (protozoan phagotrophs <200 µm) were first identified as significant consumers of phytoplankton in the eastern subarctic Pacific during research into the "Major Grazer Hypothesis" in 1984 (Miller, 1993b). Low rates of phytoplankton removal by other processes (copepod grazing, sinking, and advection), coupled with growing recognition throughout the oceanographic community of the importance of microzooplankton (*e.g.*, Azam *et al.*, 1983), suggested that micrograzers must be responsible for the bulk of the herbivory in this high-nitrate, low-chlorophyll region. Subsequent research during the Subarctic Pacific Ecosystem Research (SUPER) and Canadian Joint Global Ocean Flux Study (JGOFS) programs has borne out this prediction. While one can quibble over the details (and the microzooplankton researchers certainly have), rates of microzooplankton grazing on phytoplankton, as measured by the seawater dilution technique, generally vary between about 50 and 150% of phytoplankton intrinsic growth rates in this ecosystem. Microzooplankton grazing in concert with iron limitation of phytoplankton growth rates is thought to be responsible for the high-nitrate, low-chlorophyll condition (*i.e.*, the lack of phytoplankton blooms with attendant macronutrient depletion) in the oceanic waters of the eastern subarctic Pacific (Miller *et al.*, 1991; Boyd *et al.*, 2004). We have recently found that this situation extends inshore as far as the outer continental shelf in the northern Gulf of Alaska (Strom *et al*., 2006).

Features of this previous research relevant to OECOS include:

- Every study so far (Strom and Welschmeyer, 1991; Landry *et al.*, 1993b; Rivkin *et al.*, 1999; Liu *et al*., 2002) shows **grazing : growth** rate ratios ranging between about 0.5 and 1.5, with a few exceptionally low grazing rates in each data set. Does this range represent 'snapshots' of different portions of the limit cycles experienced by the phytoplankton and (perhaps) their consumers?
- Experiments conducted in winter (Boyd *et al*., 1995b) gave rates similar to the lower end of the spring–summer range, and showed near equivalency between growth and grazing.
- All rates measured to date, with the possible exception of those during the Subarctic Pacific

Iron Experiment for Ecosystem Dynamics Study (SERIES) (Boyd *et al.*, 2004; specifics of SERIES grazing experiments are in press) have to be considered iron-contaminated.

While the growth (*i.e.*, cell division) rates of the ambient phytoplankton community probably do not respond measurably to Fe contamination or addition in 24 hours, it is unclear how physiological shifts might affect phytoplankton chlorophyll content, grazing pressures, and the **grazing : growth** relationship. This needs to be investigated during OECOS by comparing 'clean' with 'typical' (of the past) incubations, and using physiological (*e.g.*, F_v/F_m) and taxon-specific measures of phytoplankton processes.

I envision two major areas of investigation for the microzooplankton component of the OECOS eastern program. Below, I outline some of the compelling questions in each area, then describe the methods available to address these questions. The mix of approaches that we use will depend on the refinement of our hypotheses, and on methodological feasibility.

Major Question I: ***How do microzooplankton interact with their prey to maintain the observed upper and lower limits of phytoplankton biomass in the eastern subarctic Pacific?***

Historically, phytoplankton chlorophyll concentrations (Miller *et al.*, 1991) and carbon biomass levels (Booth *et al.*, 1993) have been observed to remain relatively constant year-round in this ecosystem. However, within these narrow bounds, chlorophyll levels can vary on time scales suggestive of the 'limit cycles' produced by modeled predator–prey and limiting-nutrient interactions (Strom *et al.*, 2000). This observation, especially if characteristic of the ecosystem, leads to a series of questions about the workings of the lower trophic levels.

1. *Will we observe such cycles when we are again on station?*

We will have to obtain measurements daily for the component organisms and nutrients, and for processes hypothesized to be important in driving these cycles. Finer temporal spacing of measurements could be nested in this daily sampling as personpower allows. The key experimental design criterion will be the ability to make consistent, internally comparable measurements over time.

2. *What keeps phytoplankton stocks from going any lower than they do (ca. 0.1 µg chl/l)?*

Candidate explanations put forward by Strom *et al.* (2000) include grazing thresholds and prey switching (*e.g.*, among phytoplankton, to bacteria, or to other heterotrophic protists). We can examine the first of these in two possible ways – first, by using a sequence of highly dilute treatments within seawater dilution experiments to look for a 'leveling off' of net growth rates, as would be expected if grazing ceases (reaches a threshold) at some low phytoplankton concentration. Such an effect was hinted at in Sargasso Sea research (Lessard and Murrell, 1998), although this involved a somewhat different treatment of the data. With the exception of the Rivkin *et al.* (1999) study, which presents methological issues, the subarctic Pacific dilution experiment data summarized above only employed treatments at 25% full-strength seawater and above, with a limited number of total dilution levels, so they do not allow evaluation of the grazing threshold hypothesis. We can also explore the use of variation in food vacuole contents as a measure of feeding intensity (Dolan and Simek, 1998). This will require some preliminary laboratory work.

Prey switching can be examined by looking for temporal changes in the relative grazing intensity on different prey types. Phytoplankton can be categorized by size class or taxon (*e.g.*, using flow cytometry (Liu *et al.*, 2002) or high-performance liquid chromatography (Strom and Welschmeyer, 1991)). Clearance rates on bacteria can be compared day-to-day by adding fluorescently labelled bacteria cells (Sherr *et al.*, 1987) in tracer amounts to incubation experiments. Assessing grazing by protists on heterotrophic protists is trickier. We can try to examine this using size-fractionation (or 'trophic cascade') experiments (Calbet and Landry, 1999), but the amount of microscopy required to enumerate, say, heterotrophic flagellates in multiple treatments per

experiment with any sort of time resolution (daily?) is daunting.

3. *What is the role of microzooplankton grazing in dictating iron availability (type and amount), and how does this influence the upper and lower biomass levels and growth rates of the phytoplankton?*

Perhaps some of the iron speciation measurements proposed by Chase and Cullen (this report) can be related to time series in microzooplankton abundance and activity. Another idea is to use a backwards approach: use a commercially available siderophore such as desferrioxamine (Wells, 1999) to soak up all (?) bioavailable iron, then see how much difference this makes in some fast-responding aspect of phytoplankton physiology such as F_v/F_m. Presumably, if the phytoplankton are already strongly iron-limited, say at the peak in the cycle, the change in their physiological state would be slight, while the change would be larger if there were considerable available iron in the ecosystem. Perhaps the cyclic changes will be obvious in a temporal series of F_v/F_m and flavodoxin/ferridoxin without any fancy manipulations.

Major Question II: ***How do microzooplankton couple phytoplankton and copepod trophic levels?***

As already summarized by several previous essay writers, the interzonal migrators *Neocalanus* spp. (and likely the other dominant spring copepods *Eucalanus bungii* and *Metridia pacifica*) consume little phytoplankton production directly (Frost *et al.*, 1983; Dagg, 1993a). Rather, their diet consists largely of microzooplankton, with perhaps a substantial detrital component in some cases (Gifford and Dagg, 1991; Dagg, 1993b; Gifford, 1993). The larger microzooplankton (ciliates and heterotrophic dinoflagellates >30 µm) appear to be particularly important for *Neocalanus*, as indicated by recent experiments in the coastal Gulf of Alaska (Liu *et al*., 2005).

1. *To what extents do phytoplankton and microzooplankton size and species composition change as phytoplankton stock varies? Does this affect trophic coupling to copepods?*

I am intrigued by the observation (Miller, 1993a) that individual *Neocalanus flemingeri* C5 biomass was considerably higher in 1988 than in 1987. Looking back at data for the lower trophic levels during those cruises, total (average) chlorophyll, autotrophic and heterotrophic carbon biomass were similar for the two spring periods (Booth *et al.*, 1993), while primary productivity actually averaged somewhat lower in spring 1988 than in 1987 (Welschmeyer *et al*., 1993). Examining the size composition of the phyto- and microzooplankton (Fig. 18) indicates that larger auto- and heterotrophs were relatively more abundant in 1988. The taxonomic composition (Fig. 19) shows that the spring of 1988 hosted a 'bloom' of the diatom *Nitzschia cylindroformis*, which may, in turn, have supported the elevated 1988 biomass of larger ciliates. There may have been fewer trophic levels between the bulk of the phytoplankton and large copepods in 1988 compared with 1987. Analysis of size and taxonomic composition should indicate whether the trophic structure leading from phytoplankton to copepods changes as the lower trophic levels progress through limit cycles. We can also use size fractionation experiments (*e.g*., Capriulo and Carpenter, 1980; Calbet and Landry, 1999) to investigate the number of trophic levels between various size classes of microzooplankton consumers and specific phytoplankton size classes, and taxa (*e.g*., *Synechococcus* spp. as enumerated with flow cytometry). We have done these experiments with some success during U.S. Global Ocean Ecosystem Dynamics (GLOBEC)-sponsored research in the coastal Gulf of Alaska. Evaluations of size, taxonomic composition, and trophic relationships within the microplankton will also be an important point of comparison between eastern and western subarctic Pacific ecosystems (*cf.* Taniguchi, 1999).

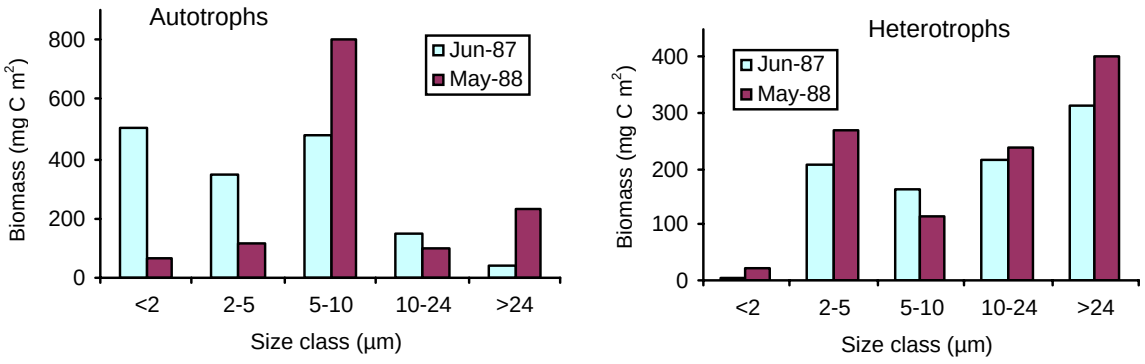

Fig. 18 Size composition of auto- and microheterotrophs during spring 1987 and 1988 (data replotted from Booth *et al.*, 1993).

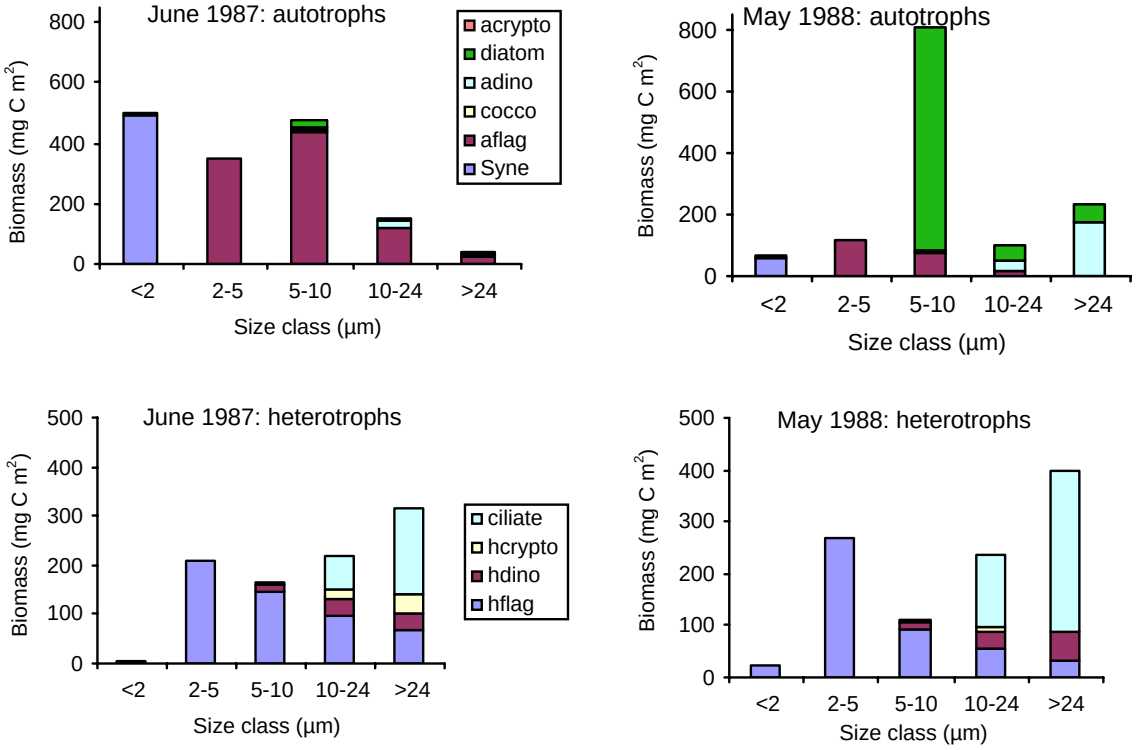

Fig. 19 Taxonomic composition of auto- and microheterotroph communities during spring 1987 and 1988 (data replotted from Booth *et al.*, 1993); acrypto = autotrophic cryptophyte, adino = autotrophic dinoflagellate, cocco = coccolithophorid, aflag = autotrophic flagellate, Syne = *Synecoccus*, hcrypto = heterotrophic cryptophyte, hdino = heterotrophic dinoflagellate, hflag = heterotrophic flagellate.

2. *Are there significant 'top-down' effects of* Neocalanus *on phyto- and microzooplankton?*

In other words, does the grand pageant of interzonal migration, the spring surface layer phasing of growth and development of the different species, cascade down to the lower trophic levels?

This could be an important link between the two geographic components of our program. Landry *et al.* (1993a) explored the issue in two mesocosm experiments during SUPER, and found some increased cropping of diatoms and enhancement of bacterial production in the presence of 0.75 C5 *N. plumchrus* per liter. Lower copepodid densities (but still several-fold higher than ambient) had little impact on lower trophic levels. Additional evidence suggesting little top-down influence was the relatively low net production of ciliates in the absence of copepods – leaving little apparent ciliate production to be cropped by larger consumers (Landry *et al.*, 1993a). We observed similarly low net production of microzooplankton during incubations of seawater (with no meso- or macrozooplankton) in the coastal Gulf of Alaska during GLOBEC studies.

On the other hand, microzooplankton biomass in the eastern subarctic Pacific appears to be relatively constant year-round, much like the observed pattern for chlorophyll. Overall microzooplankton biomass levels at Ocean Station P in February 1994 and March 1993 (Boyd *et al*., 1995a) were nearly identical to those observed during spring and summer in 1987 and 1988 (Booth *et al.*, 1993). This directly contradicts the 'best fit' (to empirical chlorophyll and nitrate data) nutrients – phytoplankton – zooplankton – detritus (NPZD) model of Frost (1993), which yields a roughly 4-fold range in microzooplankton biomass over the annual cycle. The seasonal excursions predicted by Fasham (1995) and Denman and Peña (1999) in their NPZD models are even larger. If the large seasonal cycle in primary production moves up into the microzooplankton community but does not appear as accumulated biomass, where does it go? Is it just 'burned up' by passing through multiple microzooplankton trophic levels? Do subarctic microzooplankton have extremely low gross growth efficiencies? Given the paucity of data available so far, it will be extremely interesting just to observe the degree to which microzooplankton biomass increases in concert with spring primary production during the OECOS time series.

In thinking about these issues I worry that we are missing an important planktonic group in our analyses – organisms between about 50–300 µm that occur at abundances of about 10–100 per liter. These would not be seen in 'countable' quantities in a settled 10–50 ml sample (as is usual for counting microzooplankton), and would tend to be undersampled and overlooked in net tow samples aimed at larger zooplankton. You can see a list of such organisms at the bottom of Booth *et al.*'s (1993) Table 9; they include large ciliates, sarcodines, copepod nauplii, and could include larvaceans. I can imagine sampling for, and enumerating, these – others (*e.g*., Gowing and Garrison, 1992) have used reverse concentration mechanisms to sample for the sarcodines – but how would we know what these organisms were doing in the environment (*i.e*., feeding rates, prey types)? They might be quite important as prey for *Neocalanus* spp.

A challenge will be dealing experimentally with trophic coupling between the large copepods and the microbial community.

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*Dr. Strom suggests (and see Michael Dagg's essay in this report) that short-term cycling could reflect a top-down effect of mesozooplankton feeding on microheterotrophs (MH), although she then found no particular evidence for it in available data. Testing this will take sophisticated evaluation of microherbivore stocks and feeding upon them, with particular attention to the 50–300 µm class of animals that she correctly notes gets too little attention. There is also a whole level of zooplankton in the 300–1000 µm class (*Microcalanus, Oithona *copepodites, and a considerable list of other forms) that can add steps to the food web. These are very difficult to deal with technically, but perhaps they must be considered and their roles defined.*

Functional role of microzooplankton in the pelagic marine ecosystem during phytoplankton blooms in the western subarctic Pacific

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It has become obvious that microzooplanktonic assemblages perform multiple functions in marine ecosystems. Namely, they are not only primary consumers of pico- and nano-sized producers but are also significant regenerators of nutrients. In addition, they are important food sources for metazoan zooplankton and fish larvae. Which function is more important than the others depends on the food web structure of the ecosystem.

How do microzooplankton function during the spring bloom in the Oyashio region?

We have already learned several differences in ecological features between the western and eastern subarctic Pacific. For example, strong spring blooms are regular annual events dominated by larger diatoms in the western subarctic Pacific (Kasai *et al.*, 1997), while no pronounced spring phytoplankton bloom occurs in the eastern North Pacific (Miller, 1993a; Strom *et al.*, 2000). In the west, microzooplankton biomass is highest during or slightly after the diatom blooms, while there are no clear seasonal trends in the eastern subarctic Pacific. Shinada *et al.* (2001) demonstrated that the microbial food chain largely channels carbon flow throughout the season, with the exception that the grazing food chain (phytoplankton eaten by copepods) is functional along with the microbial food chain only in the spring phytoplankton bloom period in the Oyashio region. On the other hand, the grazing food chain is never significant in the eastern sector, and microzooplankton grazing is principally responsible for sustaining high-nitrate low-chlorophyll (HNLC) conditions year-round (Strom *et al.*, 2000). From these comparisons, it is clear that the planktonic food chain structure in the Oyashio region differs from that in the eastern subarctic Pacific due to seasonal activation of the grazing food chain. This implies that carbon transfer efficiencies from primary producers to mesozooplankton may be higher in the Oyashio region as compared to the eastern subarctic Pacific. These conclusions, however, will need further verification because they were derived from monthly experiments.

During the OECOS program, we propose to perform dilution experiments in conjunction with application of a size-fractionation method at least every 2–3 days to depict temporal changes in microzooplankton grazing rates and growth rates of phytoplankton. In addition, we must conduct grazing experiments with copepods.

How can we know the *in situ* metabolic activity of microzooplankton?

The magnitude of microzooplankton activity can be evaluated from their productivity, standing stocks, and individual growth rates. Cell cycle methods (McDuff and Chisholm, 1982) or the frequency of dividing cells (FDC) method are known to be among the best tools for estimating *in situ* growth rates of protists. These methods have been successfully applied for several types of planktonic organisms, *e.g.*, dinoflagellates (Weiler and Chisholm, 1976), *Prochlorococcus* (Vaulot *et al.*, 1995) and *Synechococcus* (Campbell and Carpenter, 1986). These methods were also applied to heterotrophic organisims such as tintinnid ciliates (Coats and Heinbokel, 1982) and oligotrichous ciliates (Ota, unpublished data; Growth rates of 16 natural populations of 12 species were successfully estimated in subarctic waters.). It has been well demonstrated that the growth rate of ciliates with plentiful food is a negative function of body size and a positive function of temperature. Therefore, it is interesting to compare the *in situ* growth rates determined by the cell cycle method ($\mu_{in\ situ}$) with apparent growth rates estimated from incubation experiments, or maximum potential growth rates estimated from cell size and ambient temperature (*e.g.*, Müller and Geller, 1993). Those

comparisons will give information regarding the growth limitations affecting natural populations. In the OECOS program, therefore, we propose to conduct time-series sampling over 24 h at least three times (early, mid-, end stages) throughout the bloom period to define *in situ* growth rates of dominant species of microzooplankton.

Another expectation during the spring bloom in the western subarctic Pacific

The western (at least east of 165°E) and eastern subarctic Pacific gyres are known to be HNLC regions, although the former area has greater aeolian dust flux, a major source of iron in the oceanic ecosystem. In the western gyre we investigated the response of microzooplankton to the change in phytoplankton growth rate, species composition, and biomass during the phytoplankton bloom induced by an *in situ* iron fertilization study (SEEDS, Subarctic Pacific Iron Experiment and Ecosystem Dynamics Study). The results indicated the important role of heterotrophic dinoflagellate grazing in the food-web dynamics and biogeochemical cycle of both the natural and iron-enriched situations (Saito *et al.*, 2005). Briefly, the iron-enrichment experiment (SEEDS) induced a dramatic diatom bloom dominated by a chain-forming diatom from Day 9, after the addition, to the end of the experiment (Day 13). In this period, there was also a massive occurrence of a heterotrophic dinoflagellate, *Gyrodinium* sp. Mathematical simulations show *Gyrodinium* sp. would have almost grazed down the diatoms within 10–11 days after the bloom initiation and would have respired most of the carbon fixed by diatoms in the sea-surface. This *Gyrodinium* sp. was originally a minor component of the microzooplankton assemblage, and was the only microheterotroph that could feed on the large, chain-forming diatoms that increased after the iron-enrichment. Such emergences of minor species as principal players in SEEDS were not predicted prior to the experiment, due to our limited understanding of the species present and their physiological characteristics. Although those results were derived from anthropogenic perturbations, similar events may occur during the natural 'perturbations' of the bloom period. In order to understand the mechanisms of variations (or stabilities) of ecosystem and biogeochemical processes, we need further studies of food-web components and their functions, not only for dominant species but also for rare species. Especially, it should be worthwhile to focus on the dynamics and physiology of heterotrophic dinoflagellates, protozoans that can function as significant grazers during the spring bloom in the western subarctic Pacific.

Evaluation of protistan growth rates from proportions of cells in different cell-cycle stages has been extremely laborious work. Recurring application at high frequency in OECOS time-series studies will pose severe demands for data production. Possibly this could be relieved by some of the video-microscopy recording techniques proposed above by Drs. Selph and Furuya. Species present in sufficient abundance could be stained, concentrated and presented to the recording system so as to make large numbers of cell-phase evaluations possible in near-real time. Even for samples to be finally evaluated ashore, developmental work involving at least partial automation will be needed to raise the data volume to the levels needed for OECOS. Nevertheless, this is a promising idea that can be evaluated thoroughly when Dr. Ota's study of oligotrich growth rates becomes available.

High-frequency dilution experiments also challenge our ability to generate data at high rates. Probably each OECOS program should have a cooperative scheme for dilution experiments, so that they can provide data on production and grazing at the same time for all interested participants. Perhaps ^{14}C-uptake dilution studies (see Dr. Welschmeyer's essay earlier in this report) can be designed to produce all possible outputs from a single incubation series. It is possible that the 2-point experiments proposed recently by Michael Landry (ASLO meeting, 2005, Santiago de Compostela) can be evaluated quickly enough to provide times series of phytoplankton growth and loss to micrograzers. Maybe dilution experiments can be evaluated by flow cytometry to break out separate rates (μ and g) for different classes of phytoplankton.

E. MESOZOOPLANKTON

The OECOS idea, a comparative study between eastern and western subarctic ecosystems, arose in a discussion between Drs. Tsutomu Ikeda and Charles Miller about data requirements for evaluating development of the copepods termed (by Vinogradov (1968)) interzonal migrators (IZM), species that strongly dominate the net-caught zooplankton during the spring transition all across the subarctic Pacific. These species have 1- or 2-year life cycles, in all cases with prolonged resting stages at depth. Essays provided for the OECOS meeting cover many aspects of the life history, vertical distribution, and ecological roles of these species. One principal goal of an OECOS program will be to obtain growth evaluations for IZM copepods in order to compare a strongly food-limited habitat expected in the eastern gyre with a more food-replete habitat during a spring bloom in the west. We need to evaluate development rates and growth using similar methods, and we need to compare feeding rates and food selection. We know that these copepods feed on large phytoplankton when it is abundant in coastal zones, and that they have much faster development than those in continuously high-nitrate low-chlorophyll (HNLC) conditions at Ocean Station P. We expect to see this difference in our comparison and to evaluate it quantitatively.

The meeting included six students of mesozooplankton: Michael Dagg, Moira Galbraith (Monday only, representing herself and David Mackas), Tsutomu Ikeda, Toru Kobari, Charles Miller, and Atsushi Yamaguchi. All of their essays are presented serially here, without further comment.

Vertical zonation of mesozooplankton, and its variability in response to food availability, density stratification, and turbulence

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In the spring season (roughly March to May or June) the upper 100 m of the subarctic Pacific is occupied by, and feeds, a very high biomass of large 'interzonal migrant' copepods. Research at Station P during the weathership era (Frost, 1983; Fulton, 1983; Miller *et al.*, 1984b, 1988, and others) documented the major seasonal features of life history and deep dormancy. Subsequent research during the Subarctic Pacific Ecosystem Research (SUPER), Joint Global Ocean Flux Study (JGOFS) and Surface Ocean Low Atmosphere Study/Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study (SOLAS/SERIES) programs (Landry and Lehner-Fournier, 1988; Dagg, 1993a; Gifford, 1993; Goldblatt *et al.*, 1999, and others) showed that the copepods do not directly regulate the bulk of phytoplankton production, but instead get most of their diet from protist microzooplankton, and from the relatively sparse and temporally intermittent population of large phytoplankton.

The focus for our previous work during SUPER (Mackas *et al.*, 1993), and one focus for our proposed OECOS work, is on the layered vertical distribution of these copepods within the upper 100–150 m. This vertical zonation (which was confirmed in Canadian JGOFS studies, Goldblatt *et al.*, 1999) is persistent throughout the day–night cycle and strongly affects where rates of feeding activity (and mortality of microplankton) are highest. *Neocalanus plumchrus* and *N. flemingeri* occupy the surface mixed layer; *N. cristatus* and *E. bungii* are usually, but not always, concentrated below the mixed layer. Centers of vertical distributions of all four species become deeper when wind is strong and upper-layer density stratification is weak, suggesting that turbulence intensity may be one of the environmental cues affecting their depth preferences. Subsequent

observations during Canadian JGOFS (Goldblatt *et al*., 1999) and SOLAS/SERIES (Tsuda *et al.*, in press) support the general pattern of vertical partitioning of the upper 100 m, but Tsuda *et al.* added the important new observation that *N. cristatus* moved upward into the surface mixed layer during (and within) a localized summer season diatom bloom induced by iron enrichment.

The field sampling and lab identifications required to describe upper layer vertical distributions are laborious but relatively straightforward: stratified oblique tows within the upper 250 m, using a multiple net sampler such as MOCNESS or BIONESS, followed by microscopic identification and counting to species and developmental stage. Sampling intervals should be approximately every second day, with some added day–night comparisons.

Our second research interest within OECOS is seasonal developmental timing of the copepods, and the cause(s) for relative brevity of the surface layer biomass maximum and the later stages of the annual *N. plumchrus* cohort. In the 1990s, we began to realize that the timing of the biomass maximum and of the onset of seasonal dormancy for *N. plumchrus* varies on decadal time scales by nearly 2 months, and that differences in timing are very well-correlated with interannual and latitudinal variability in surface warming and stratification (Mackas *et al.*, 1998; Bertram *et al*., 2001; Batten *et al.*, 2003; Mackas and Batten, 2005). Evidence to date is that control of this timing takes place in the upper layer during the spring growing season, and not as a consequence of changing date of emergence from dormancy and spawning. In the open Pacific, the duration of the deep spawning by *N. plumchrus* is very long (Miller and Clemons, 1988) and, in particular, is much longer than the biomass peak produced by the main pulse of C4 and C5 pre-dormant copepodites. In contrast to the strong correlation with mixed-layer temperature anomalies, there is no correlation of interannual timing variability with temperature anomalies at the dormancy depth (Mackas *et al.*, 1998). As yet, we do not know how a very broad cohort of eggs becomes a much narrower cohort of late copepodites. Candidates include:

- a progressive temperature-driven acceleration of development (likely to occur, but probably not large enough to account for the observed range of variation),
- stalling of development, and accumulation of hatched juveniles at some relatively early life stage until food and temperature environments allow further progression (Saito and Tsuda (2000) saw hints of this at the late naupliar stage in laboratory rearing experiments),
- and/or temperature-correlated variations in survivorship that preferentially eliminate a large subset of the annual cohort of nauplii and early-stage copepodites.

This question can be addressed using the same samples and numerical data that Charles Miller will be using to generate ‘Heinle graphs’ and growth rate estimates (later in this report).

Marine ecosystem characteristics and seasonal abundance of dominant calanoid copepods in the Oyashio region

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Interannual variations in developmental timing of dominant calanoid copepods have been evaluated at Site H, in the Oyashio region, based on time-series sampling conducted in 1996–1997 and 2002–2004. The major results of this study are that:

1. Both temperature and chlorophyll *a* varied interannualy, but no effects on reproduction timing or generation length were seen for copepods with annual life cycles;

2. Abundances of *Neocalanus plumchrus* and *Eucalanus bungii* were correlated with the magnitude of the spring phytoplankton bloom. This suggests that food supply is an important factor affecting their population sizes;

3. Salp blooms in 2003 affected the recruitment of *N. plumchrus* and *E. bungii* but not recruitment of other copepods, suggesting possible food competition between young copepodids of the two species and salps;

4. Interannual variations in the recruitment season of *Metridia pacifica* may be interpreted by the shorter generation length, lack of a diapause phase, and close coupling of feeding and spawning of this species as compared with the other copepods.

For details, see Figure 20.

All previous sampling programs were based typically on only one sampling per month. This sampling interval is too coarse to analyze the rapid development sequence of important copepods during the season of the phytoplankton bloom. High-frequency sampling in the OECOS program will reveal several aspects currently not known in the Oyashio ecosystem:

1. Accurate growth rate estimates of four diapausing copepods (three *Neocalanus* species and *E. bungii*);

2. Grazing impacts of *Neocalanus* spp. and *E. bungii* on the spring phytoplankton bloom;

3. Spatial (vertical) and temporal niche separation, if any, of several zooplankton species;

4. Egg production monitoring for some zooplankton (*e.g.*, *M. pacifica*, *E. bungii*) through the phytoplankton bloom.

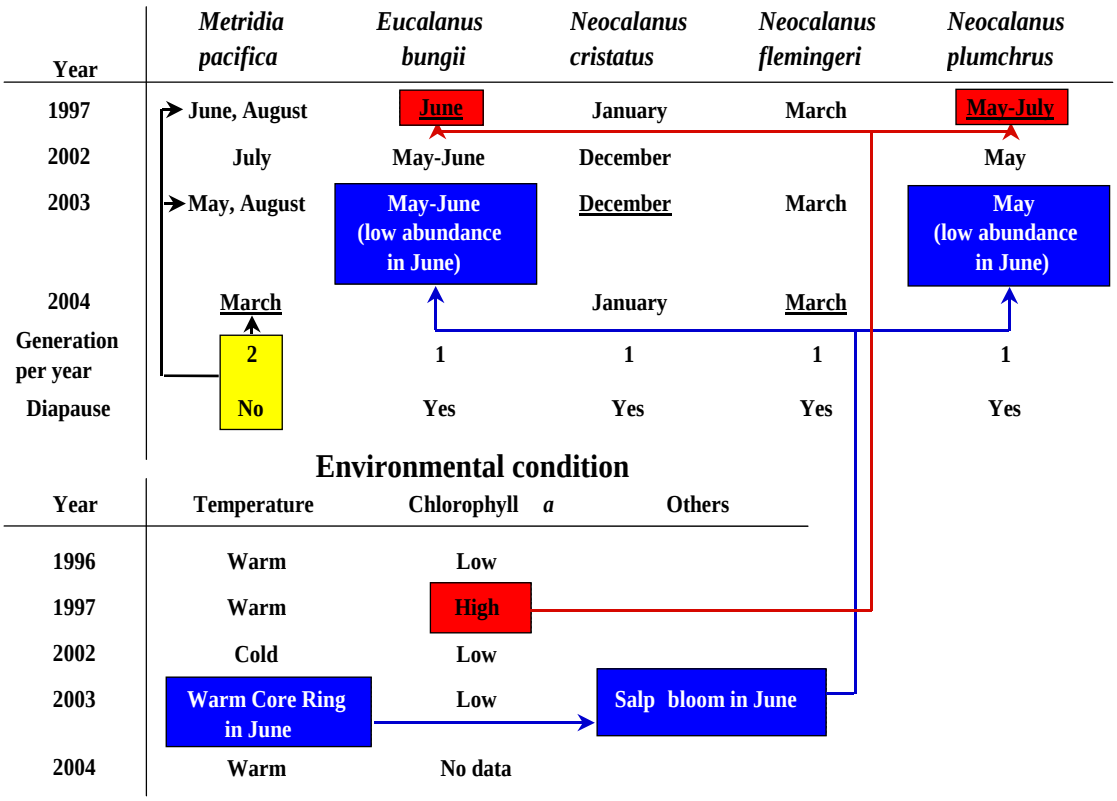

Fig. 20 Coupled tables showing the timing of recruitment of early copepodid stages for each copepod species. The left column is year, number of generations per year and presence of diapause phase in their life cycle. The underlined months indicate the year when the maximum abundance was seen. The lower table shows environmental conditions in each year: temperature, chlorophyll *a*, and other factors. The presence of a warm core ring in June 2003 induced a large Salpida bloom, which apparently resulted in low abundance of *Eucalanus bungii* and *Neocalanus plumchrus*. From chlorophyll *a*, a spring bloom of large magnitude in 1997 parallels high abundance of *E. bungii* and *N. plumchrus* in that year. Among the five copepod species studied, only *Metridia pacifica* had multiple generations per year, and no diapause phase in their life cycle. *Metridia pacifica* showed greater variations in timing of recruitment of early copepodid stages. Thus, the habitat-responsive reproductive strategy of this species allows it to initiate spawning more flexibly than the other species.

OECOS: Proposed mesozooplankton research in the Oyashio region, western subarctic Pacific

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Background

Life cycles of large- and medium-sized, grazing copepods (*Neocalanus cristatus, N. plumchrus, N. flemingeri, Eucalanus bungii*, and *Metridia pacifica*) have been studied extensively at Station P in the eastern subarctic Pacific by U.S. and Canadian scientists during the 1980s. In the western subarctic Pacific, Japanese scientists have pursued intensive studies on life cycles of dominant mesozooplankton in the Oyashio region since 1995. The time series of mesozooplankton data at Site H in the Oyashio region were developed by sampling at monthly intervals over one full year using vertical hauls with closing nets at 5 discrete depths between the surface and 2000 m. Whenever possible, some short-term reference stations were set in the central and eastern subarctic Pacific, Bering Sea, Okhotsk Sea, and Japan Sea during the program, and compared with the results at Site H.

As a result, information about life cycles (spawning season, development pattern, generation time, longevity, *etc*.) has accumulated rapidly on a number of zooplankton species in the Oyashio region, including copepods (*N. cristatus, N. plumchrus, N. flemingeri, Eucalanus bungii, M. pacifica, M. okhotensis, Paraeuchaeta elongata, Heterorhabdus tanneri, Pleuromamma scutullata, Gaidius variabilis, Pseudocalanus minutus, P. newmani, Oncaea* spp., and *Triconia* spp.), a euphausiid (*Euphausia pacifica*), amphipods (*Themisto japonica, T. pacifica, Primno abyssalis*, and *Cyphocaris challengeri*), an ostracod (*Discoconchoecia pseudodiscophor*a), a hydromedusa (*Aglantha degitale*), and chaetognaths (*Sagitta elegans* and *Eukrohnia hamata*). Most of these results have been published (see bibliography at the end of this essay). For the large- to medium-sized grazing copepods mentioned above, the differences in the life cycles of *Neocalanus* spp. between Site H and Station P are not appreciable. As exceptions, *E. bungii* had a 1-year life cycle, in contrast to their 2-year life cycle at Station P, and *M. pacifica* completed 2 generations at Site H in contrast to 3 generations at Station P. Some details of these results were presented by Drs. Toru Kobari and Atsushi Yamaguchi at the OECOS Workshop.

A common phenomenon among these copepods was that the body size of specimens at Site H was much larger than that at Station P. This regional difference in body size leads us to examine the possible existence of regional populations of *N. cristatus*, which is the most important mesozooplankton species in terms of biomass in the Oyashio region (see below). Nucleotide sequence data of mitochondrial 16S rRNA and COI genes of *N. cristatus* collected from various regions within the subarctic Pacific and its marginal seas revealed no such distinctive subpopulations for this species. Thus, regional variations in body size of *N. cristatus* can be ascribed to environmental conditions (temperature, food abundance, *etc*.) that vary from one region to the next. In this regard, Site H is colder, yet has a much more abundant food supply (incidence of massive phytoplankton blooms in spring) compared with the conditions at Station P (warmer temperature, and no incidence of phytoplankton blooms). *Euphausia pacifica* exhibits a marked regional variation in life cycle patterns in the subarctic Pacific and its marginal seas, but our current mitochondrial 16S rRNA data also showed no genetic distinctions among specimens from distant locations.

Through the analyses of life cycles of various mesozooplankton mentioned above, the contribution of the population biomass of each species or group to the total mesozooplankton biomass becomes evident. Of the total biomass

(16 g DW m^{-2}, at 0–2000 m, annual mean), the most important species is *N. cristatus* at 31%, followed by *N. plumchrus* (15%), *E. bungii* (13%), *N. flemingeri* (6%) and *M. pacifica* (6%). These large- to medium-sized grazing copepods altogether contribute 40% of the total mesozooplankton biomass at Site H.

OECOS: The new phase of research in the Oyashio region

Low-frequency sampling (*e.g.,* 1 sample per month), mentioned above, was enough to let us gain information about the overall picture of mesozooplankton life cycles for species with a generation length of 1 year or more. However, precise calculation of population production of these mesozooplankton requires high resolution data on their developmental sequences. For example, all large- to medium-sized grazing copepods achieved rapid development from early to late copepodite stages through phytoplankton blooms which lasted *ca*. 3 months (April–June). As seen in our low-frequency sampling, abundance peaks of 3 to 4 copepodite stages occurred simultaneously, in the sample of the same month, making it difficult to estimate development time of each stage. High-frequency sampling (1 sample per day) during OECOS should solve that problem.

Population production calculations for oceanic zooplankton have been regarded as difficult, largely due to problems in estimating mortality rates by repeated samplings on the same populations over the time. This is partly because populations do not remain in place for sampling. Clearly, mortality is a central issue for a zooplankton production study in OECOS, especially for the design of the high-frequency sampling program. On the other hand, a simple 'growth-rate method', ignoring mortality, may be applicable:

Production Rate = individual growth rate (for suitable categories × their biomass).

A thought about high-frequency sampling in the Oyashio region

Two sampling methods may be considered: (1) at fixed locations (possibly two, to examine the effect of small-scale patchiness), and (2) by following buoys with drogues (whether at one location or several locations across the stream axis is open to discussion). For (1), it is assumed that the population of a given mesozooplankton species in the Oyashio region is homogenous upstream from the station, with life cycles synchronized throughout. For (2), the starting position needs to take into account the Russian Exclusive Economic Zone (EEZ) (likely not accessible) and the complex flow field of the Oyashio region. The main stream of the Oyashio flows at a speed of 20–30 cm s^{-1}, which may drift the buoy eastward from the region in a few weeks, toward offshore waters characterized by HNLC conditions.

Bibliography of mesozooplankton studies in the Oyashio region

Below is a suggested reading list, in chronological order, of recent mesozooplankton studies for the Oyashio region.

Ozaki, K. and Ikeda, T. 1997. The effect of temperature on the development of eggs and nauplii of the mesopelagic copepod *Paraeuchaeta elongata*. *Plankton Biol. Ecol.* **44**: 91–95.

Ozaki, K. and Ikeda, T. 1998. Development time and generation length of the mesopelagic copepod *Paraeuchaeta elongata* reared in the laboratory. *Plankton Biol. Ecol.* **45**: 75–78.

Yamaguchi, A., Ikeda, T. and Shiga, N. 1998. Population structure and life cycle of *Pseudocalanus minutus* and *Pseudocalanus newmani* (Copepoda: Calanoida) in Toyama Bay, southern Japan Sea. *Plankton Biol. Ecol.* **45**: 183–193.

Tsuda, A., Saito, H. and Kasai, H. 1999. Life histories of *Neocalanus flemingeri* and *Neocalanus plumchrus* (Calanoida: Copepoda) in the western subarctic Pacific. *Mar. Biol.* **135**: 533–544

Ozaki, K. and Ikeda, T. 1999. Vertical distribution, population structue and life cycle of the mesopelagic copepod *Paraeuchaeta elongata* off Cape Esan, southwest Hokkaido, Japan. *Plankton Biol. Ecol.* **48**: 48–53.

Lee, H.W., Ban, S., Ando, Y., Ota, T. and Ikeda, T. 1999. Deleterious effect of diatom diets on egg production and hatching success in the marine copepod *Pseudocalanus newmani*. *Plankton Biol. Ecol.* **46**: 104–112.

Nishiuchi, K. 1999. Life cycle and inter-annual variation in Chaetognatha in the northern North

Pacific Ocean. Ph.D. thesis, 116 pp., Graduate School of Fisheries Sciences, Hokkaido University. (in Japanese)

Kobari, T. and Ikeda, T. 1999. Vertical distribution, population structure and life cycle of *Neocalanus cristatus* (Crustacea: Copepoda) in the Oyashio region, with notes on its regional variations. *Mar. Biol.* **134**: 683–696.

Yamaguchi, A. and Ikeda, T. 2000. Vertical distribution, life cycle and body allometry of two oceanic copepods (*Pleuromamma scutulata* and *Heterorhabdus tanneri*) in the Oyashio region, western North Pacific Ocean. *J. Plankton Res.* **22**: 29–46.

Shinada, A., Ikeda, T., Ban, S. and Tsuda, A. 2000. Seasonal changes in micro-zooplankton grazing on phytoplankton assemblages in the Oyashio region, western subarctic Pacific. *Plankton Biol. Ecol.* **47**: 85–92.

Yamaguchi, A. and Ikeda, T. 2000. Vertical distribution, life cycle, and developmental characteristics of the mesopelagic calanoid copepod *Gaidius variabilis* (Aetideidae) in the Oyashio region, western North Pacific. *Mar. Biol.* **137**: 99–109.

Yamada, Y. and Ikeda, T. 2000. Development, maturation, brood size and generation length of the mesopelagic amphipod *Cyphocaris challengeri* (Gammaridea: Lysianassidae) off southwest Hokkaido, Japan. *Mar. Biol.* **137**: 933–942.

Kobari, T. and Ikeda, T. 2001. Life cycle of *Neocalanus flemingeri* (Crustacea: Copepoda) in the Oyashio region, western subarctic Pacific, with notes on its regional variations. *Mar. Ecol. Prog. Ser.* **209**: 243–255.

Saito, H. and Tsuda, A. 2000. Egg production and early development of the subarctic copepods *Neocalanus cristatus*, *N. plumchrus* and *N. flemingeri*. *Deep-Sea Res* **47**: 2141–2158.

Tsuda, A., Saito, H. and Kasai, H. 2001. Geographical variarion of body size of *Neocalanus cristatus, N. plumchrus* and *N. flemingeri* in the subarctic Pacific and its marginal seas; implication in the origin of large form *N. flemingeri* in Oyashio area. *J. Oceanogr.* **57**: 341–352.

Kobari, T. and Ikeda, T. 2001. Ontogenetic vertical migration and life cycle of *Neocalanus plumchrus* (Crustacea: Copepoda) in the Oyashio region, with notes on regional variations in body sizes. *J. Plankton Res.* **23**: 287–302.

Yamaguchi, A. and Ikeda, T. 2001. Abundance and population structure of three mesopelagic *Paraeuchaeta* species (Copepoda: Calanoida) in the Oyashio region, western subarctic Pacific Ocean with notes on their carcasses and epizoic ciliates. *Plankton Biol. Ecol.* **48**: 104–113.

Lee, H.-W., Ikeda, T. and Ban, S. 2001. Metabolism, body composition (C and N) and estimated net growth efficiency of a calanoid copepod *Pseudocalanus newmani* raised at different temperatures in the laboratory. *Plankton Biol. Ecol.* **48**: 114–120.

Shinada, A., Ikeda, T., Ban, S. and Tsuda, A. 2001. Seasonal dynamics of planktonic food chain in the Oyashio region, western subarctic Pacific. *J. Plankton Res.* **23**: 1237–1247.

Kaeriyama, H. and Ikeda, T. 2002. Vertical distribution and population structure the three dominant pelagic ostracods (*Discoconchoecia pseudodiscophora*, *Orthoconchoecia haddoni*, and *Metaconchoecia skogsbergi*) in the Oyashio region, western North Pacific. *Plankton Biol. Ecol.* **49**: 66–74.

Kaeriyama, H. and Ikeda, T. 2002. Body allometry and developmental characteristics of the three dominant pelagic ostracods (*Discoconchoecia pseudo-discophora*, *Orthoconchoecia haddoni*, and *Metaconchoecia skogsbergi*) in the Oyashio region, western North Pacific. *Plankton Biol. Ecol.* **49**: 97–100.

Yamada, Y., Ikeda, T. and Tsuda, A. 2002. Abundance, growth and life cycle of the mesopelagic amphipod *Primno abyssalis* (Hyperiidea: Phrosinidae) in the Oyashio region, western subarctic Pacific. *Mar. Biol.* **141**: 333–341.

Ikeda, T., Hirakawa, K. and N. Shiga. 2002. Production, metabolism and production/biomass (P/B) ratio of *Metridia pacifica* (Crustacea: Copepoda) in Toyama Bay, southern Japan Sea. *Plankton Biol. Ecol.* **49**: 58–65.

Padmavati, G. and Ikeda, T. 2002. Development of *Metridia pacifica* (Crustacea: Copepoda) reared at different temperatures in the laboratory. *Plankton Biol. Ecol.* **49**: 93–96.

Lee, H.W., Ban, S., Ikeda, T. and Matsuishi, T. 2003. Effect of temperature on development, growth and reproduction in the marine copepod *Pseudocalanus newmani* at satiating food concentration. *J. Plankton Res.* **25**: 261–271.

Yamada, Y. and Ikeda, T. 2003. Metabolism and chemical composition of four pelagic amphipods in the Oyashio region, western subarctic Pacific Ocean. *Mar. Ecol. Prog. Ser.* **253**: 233–241.

Kobari, T., Ikeda, T., Kanno, Y., Shiga, N., Takagi, S. and Azumaya, T. 2003. Interannual variations in abundance and body size in *Neocalanus* copepods in the central North Pacific. *J. Plankton Res.* **25**: 483–494.

Shinada, A., Ban, S. and Ikeda, T. 2003. Seasonal changes in nano/micro-zooplankton herbivory and heterotrophic nano-flagellates bacterivory off Cape Esan, southwestern Hokkaido, Japan. *J. Oceanogr.* **59**: 609–618.

Yamaguchi, A., Ikeda, T., Watanabe, Y. and Ishizaka, J. 2004. Vertical distribution patterns of pelagic copepods as viewed from the “predation pressure” hypothesis. *Zool. Studies* **43**: 475–485.

Padmavati, G., Ikeda, T. and Yamaguchi, A. 2004. Life cycle, population structure and vertical distribution of *Metridia* spp. (Copepoda: Calanoida) in the Oyashio region (NW Pacific Ocean). *Mar. Ecol. Prog. Ser.* **270**: 181–198.

Ikeda, T., Sano, F. and Yamaguchi, A. 2004. Metabolism and body composition of a copepod *Neocalanus cristatus* (Crustacea) from the bathypelagic zone of the Oyashio region, western North Pacific. *Mar. Biol.* **145**: 1181–1190.

Taniguchi, M., Kanehisa, T., Sawabe, T., Christen, R. and Ikeda, T. 2004. Molecular phylogeny of *Neocalanus* copepods in the subarctic Pacific Ocean, with notes on non-geographical genetic variations for *Neocalanus cristatus*. *J. Plankton Res.* **26**: 1249–1255.

Yamada, Y., Ikeda, T. and Tsuda, A. 2004. Comparative life-history study on sympatric hyperiid amphipods (*Themisto pacifica* and *T. japonica*) in the Oyashio region, western North Pacific. *Mar. Biol.* **145**: 515–527.

Nishibe, Y. and Ikeda, T. 2004. Vertical distribution, abundance and community structure of oncaeid copepods in the Oyashio region, western subarctic Pacific. *Mar. Biol.* **145**: 931–941.

Kaeriyama, H. and Ikeda, T. 2004. Metabolism and chemical composition of mesopelagic ostracods in the western North Pacific Ocean. *ICES J. Mar. Sci.* **61**: 535–541.

Ikeda, T., Sano, F. and Yamaguchi, A. 2004. Metabolism and body composition of a copepod (*Neocalanus cristatus*: Crustacea) from the bathypelagic zone of the Oyashio region, western subarctic Pacific. *Mar. Biol.* **145**: 1181–1190.

Shoden, S., Ikeda, T. and Yamaguchi, A. 2005. Vertical distribution, population structure and lifecycle of *Eucalanus bungii* (Copepoda: Calanoida) in the Oyashio region, with notes on its regional variations. *Mar. Biol.* **146**: 497–511.

Nishibe, Y. 2005. The biology of oncaeid copepods (Poecilostomatoida) in the Oyashio region, western subarctic Pacific: its community structure, vertical distribution, life cycle and metabolism. Ph.D. thesis, 92 pp., Graduate School of Fisheries Science, Hokkaido University.

Some background on *Neocalanus* feeding

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In the southeast Bering Sea, a region of high phytoplankton stocks during the spring bloom and low concentration during post-bloom periods, experiments (Dagg and Wyman, 1983) indicated high feeding rates on phytoplankton (based on gut pigments) in the spring and much lower rates in the post-bloom summer period (Table 2). *Neocalanus cristatus* rates were much higher than those of *N. plumchrus* (likely to be a mixture of *N. plumchrus* and *N. flemingeri* which were not separated at that time).

In bottle incubation experiments (Dagg *et al*., 1982; done at copepod concentrations that I now realize were much too high, so rates are underestimates) using a particle counter, and pooling data from *N. cristatus* CV (Stage V) and CIV and *N. plumchrus* CV, ingestion at high food concentrations (> 400 µg C l^{-1}) was variable, but the average was 26.5 µg C cop^{-1} d^{-1}. At lower food concentrations, ingestion was linearly related to food concentration, decreasing as food concentrations decreased.

In lab experiments (Dagg and Walser, 1986) with *N. plumchrus*, CV feeding on *Thalassiosira weissflogii* gut pigment content was high but variable at chlorophyll concentrations > 3 µg l^{-1} and decreased towards zero at lower chlorophyll concentrations. Lowest gut pigment levels were 1–2 ng cop^{-1} and high levels averaged about 26 ng cop^{-1}.

During extensive experimentation in SUPER (Subarctic Pacific Ecosystem Research) (Dagg, 1993a,b; at Ocean Station P (OSP)), two approaches for measuring feeding rates on phytoplankton by all three *Neocalanus* species were used: the gut pigment method, and a bottle incubation method using chlorophyll disappearance during incubation as the index of feeding. Both methods gave similar rates for *N. plumchrus* CV and *N. flemingeri* CV of between about 20 and 80 ng chl cop^{-1} d^{-1}. (I also have some data for CIV and CIII). These are low ingestion rates of phytoplankton, equivalent to only a few µg C cop^{-1} d^{-1}. Adding the ingestion of microzooplankton (measured by Gifford (1993)) approximately doubles the total ingestion rate. Comparison with calculated ingestion rate demands (based on body size–respiration relationships) suggests these copepods are barely able to grow in the eastern subarctic Pacific (although they *do* grow (Miller and Nielsen, 1988; Miller, 1993a)). This suggests we are missing something: for example, alternative sources of food, or patchiness that can lead to small-scale, high-rate feeding events. On the other hand, these rates are quite consistent with expected feeding behavior of these copepods in high-nitrate low-chlorophyll (HNLC), nano-dominated food environments like the eastern subarctic Pacific. These copepods are feeding at rates very much below saturation. Supportive data comes from a comparison of *N. plumchrus* (including

Table 2 *Neocalanus* feeding rates on phytoplankton in spring and summer in the southeast Bering Sea.

Species	Month	Chlor max (µg l^{-1})	Ingestion rate (ng chl cop^{-1} h^{-1})	Clearance rate (ml cop^{-1} h^{-1})
N. plumchrus	May	9–10	15–135	1.4–13.5
N. cristatus	May		0–406	0–40.4
N. plumchrus	June	0.5–0.7	1–34	1.7–55.6
N .cristatus	June		9–71	10.8–116.5

N. flemingeri) body size and growth rate at OSP with rates from the southeast Bering Sea (Vidal and Smith, 1986; Dagg, 1991). These indicate slower growth at OSP where development is slower and final body size is smaller, suggesting much reduced ingestion. There remain inconsistencies, however, between estimates of food required for observed growth, and measured rates of ingestion. That is, the question, "How do they make it?" remains unanswered.

Neocalanus cristatus CV feeding was different from the other two *Neocalanus* species. Gut pigment contents were higher, between about 3 and 33 ng cop^{-1}, with a slight diel cycling between a minimum in the afternoon and a maximum in early morning (0200–0400 h). By comparison, gut pigment levels in *N. plumchrus* and *N. flemingeri* CV were typically between 0.5 and 4.0 ng cop^{-1}. In addition, for *N. cristatus* CV, the two methods for measuring phytoplankton ingestion gave different results, with the incubation method indicating approximately 90 ng cop^{-1} d^{-1} and the gut pigment method indicating approximately 355 ng cop^{-1} d^{-1}. This latter observation, plus other information (depth distribution, microscopic examination of gut contents, in-water body orientation, and behavior), led me to conclude that *N. cristatus* CV feeds on sinking aggregates (Dagg, 1993a).

More recent work in the Global Ocean Ecosystem Dynamics (GLOBEC) program from the Gulf of Alaska shelf (Dagg *et al*., 2005) has shown that fifth copepodites of *N. plumchrus* and *N. flemingeri* do not appear to feed any differently from each other when given the same foods (although I am still analyzing some experiments microscopically). However, because they are separated temporally, they do not always live in identical food environments. Both feed at high rates under high phytoplankton concentrations, but rates are variable. Both clearly prefer large particles (> 20 μm), sometimes consume intermediate sized particles (5–20 μm), and rarely consume any small particles (< 5 μm), whether the food environment is dominated by large particles or small ones (Figs. 21 and 22). Micro-zooplankton in their diets are being analyzed now.

One interesting observation in these cruises was that, although the daily ingestion of both copepods varied widely because of variability in phytoplankton availability, these daily differences did not result in differences in the final body size or lipid store (Fig. 23). These copepods efficiently integrate small and mesoscale variation in their food environment over their 60- to 90-day growth period on the shelf and all end up about the same. (The inner shelf waters of the Gulf of Alaska commonly contain high concentrations of large phytoplankton; the middle shelf and the outer shelf usually have low concentrations of phytoplankton dominated by small cells).

As in other studies, *N. cristatus* CV on the shelf in the Gulf of Alaska is different from the other two *Neocalanus* species (Liu *et al.*, 2005). In common however, is the preference for large particles, although this is even more pronounced with *N. cristatus*. Consumption of large (30–70 μm) microzooplankton by *N. cristatus*, combined with their inability to feed on small particles, resulted in a strong cascade effect in bottle incubations, in which abundance of small particles increased dramatically because *N. cristatus* consumed the predators of those cells. Calculations indicated this effect would also occur in nature under some conditions.

Under non-bloom conditions, 73% of the carbon ingested by *N. cristatus* CV was from heterotrophs and 27% from autotrophs. Under bloom conditions, heterotrophs were less important, contributing only 37% *vs.* 63% by autotrophs (Fig. 24).

Each *N. cristatus* CV consumed approximately two times more C d^{-1} under bloom conditions (average = 21.4 μg C cop^{-1} d^{-1}) than under non-bloom conditions (average = 10.0 μg C cop^{-1} d^{-1}), but neither rate was adequate to meet nutritional demands for growth and metabolism. We have concluded that these rates, derived from bottle incubations, are underestimates of *in situ* rates because (a) consumption of large particles is underestimated; (b) some very large particles are not accounted for at all in bottle experiments; and (c) aggregates are not accounted for in bottle experiments. The feeding behavior of *N. cristatus*, one of the most abundant copepods in the North Pacific Ocean, remains incompletely understood.

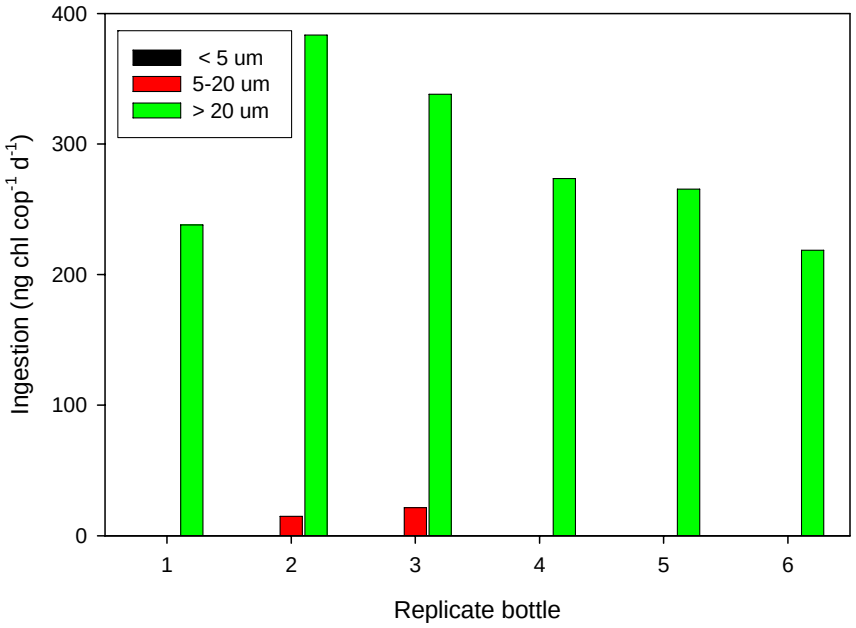

Fig. 21 Results from an experiment with *N. flemingeri* CV with water collected from the inner Gulf of Alaska shelf dominated by large cells (3.75 µg chl l^{-1} comprised of 3.09 µg l^{-1} > 20 µm, 0.36 µg l^{-1} 5–20 µm, and 0.30 µg l^{-1} < 5 µm. Ingestion rate is high, large cells dominate the diet, and no small cells were consumed.

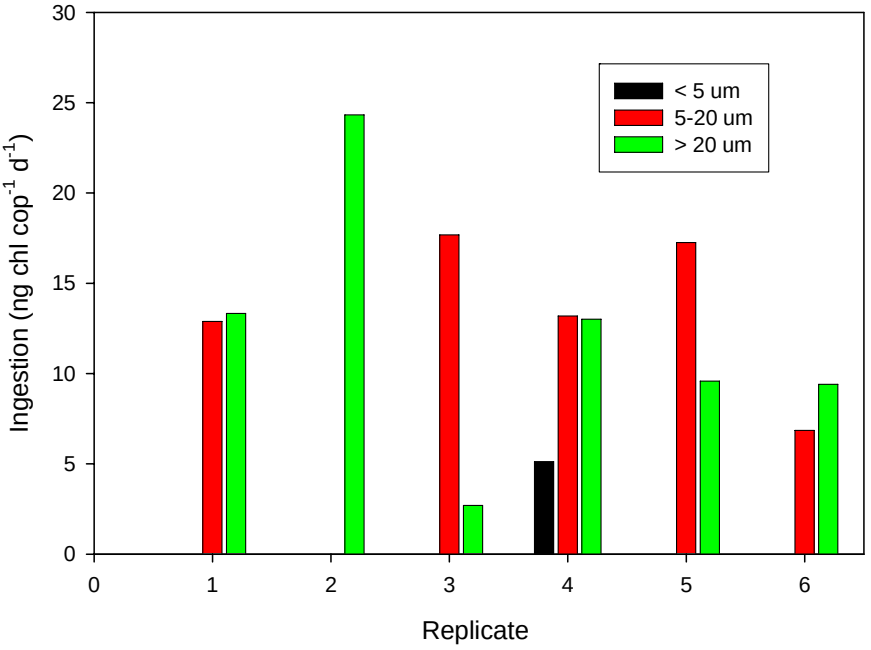

Fig. 22 At low phytoplankton concentrations found on the outer shelf of the Gulf of Alaska and closely analogous to the HNLC oceanic region (total chl = 0.77 µg l^{-1}, with 0.56 µg chl l^{-1} in < 5 µm size category and only 0.05 µg chl l^{-1} in the > 20 µm category), large cells still made up most of the diet (50% of the diet but only 7% of the available chlorophyll). Total phytoplankton ingestion under bloom conditions was approximately 10-fold greater than under non-bloom conditions. (Note the difference in ordinate scales between Figs. 21 and 22).

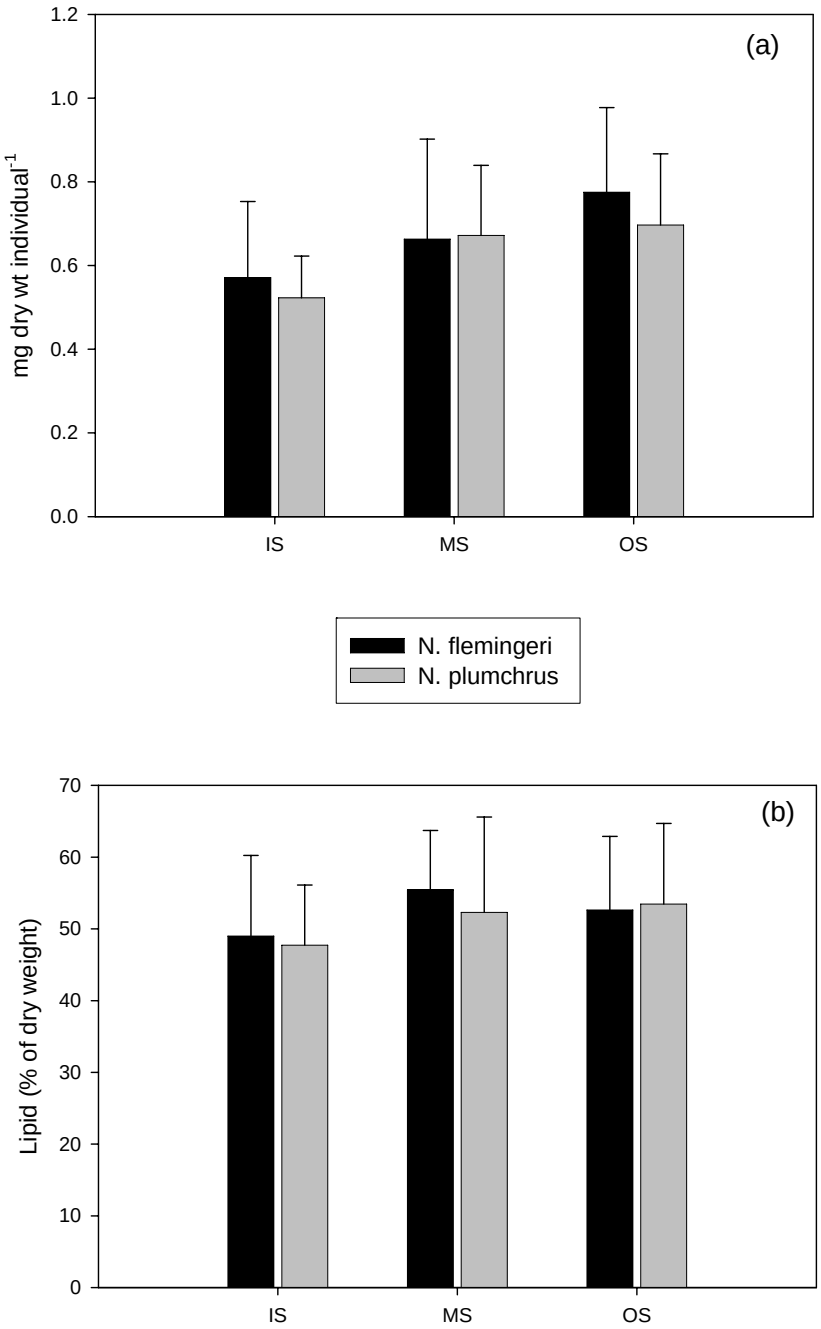

Fig. 23 Body size (upper panel) and % lipid content (lower panel) of *Neocalanus* collected from the inner shelf (IS), mid-shelf (MS), and outer shelf (OS) regions of the Gulf of Alaska showing that, in spite of growing and developing under different food regimes, these copepods attain the same final size.

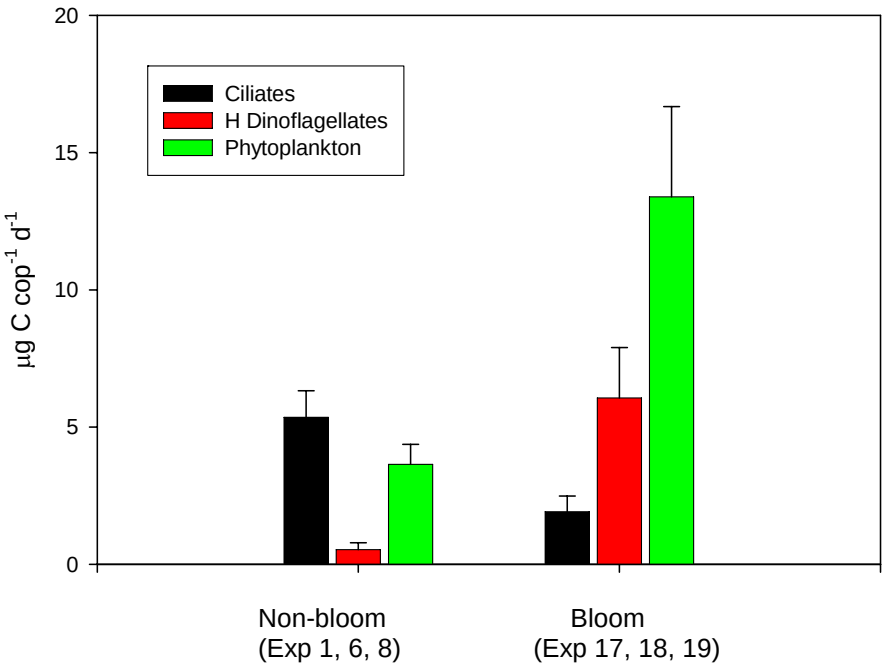

Fig. 24 Sources of nutritional carbon for *N. cristatus* CV, under bloom and non-bloom conditions.

Possible contributions to OECOS

I am interested in (a) the feeding behavior of the above copepods, especially *N. cristatus*, and (b) the mechanisms and pathways by which the feeding activity of *Neocalanus* spp. structure the food web.

Some initial thoughts

- We do not need more bottle experiments – unless in very large (5–10 l) bottles.
- More mesocosm experiments might be useful to elucidate their roles in the system.
- There is a need for methods to examine copepod feeding on large particles, including aggregates.
- We need ways to examine the significance of patchiness-induced feeding events on small T and S scales (microstructure).
- We need to examine gut contents more carefully to get ideas of what they are eating, and apply gut clearance rates to get ingestion rates.

Alternatively we might want to completely ignore feeding and focus on the end result – *i.e.*, growth and development. However, a close comparison of growth and feeding would be useful.

Size and growth of interzonally migrating copepods

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Dominant subarctic copepods

Western and eastern sectors of the subarctic Pacific have the same set of dominant particle-feeding copepods. During the spring transition, the period of thermal stratification, these are: *Neocalanus plumchrus*, *Neocalanus flemingeri*, *Neocalanus cristatus*, *Eucalanus bungii* and *Metridia pacifica*. *Neocalanus* spp. all reproduce at great depth (>500 m), entirely from stored nutriment, producing lipid-rich nauplii (Saito and Tsuda, 2000) that swim (and rise) to upper ocean layers for feeding as copepodites. In the Gulf of Alaska, the first two species listed have their main copepodite development in the near-surface layer, above the initially weak thermocline, with *N. flemingeri* development preceding that of *N. plumchrus* by three to four stages, to nearly a month. Development of *N. cristatus* and *E. bungii* is more spread out in time, with copepodites of the former being present over a longer season, and growth of the latter possibly occurring over more than 1 year. At least in the Gulf of Alaska the *cristatus–bungii* pair lives below the *plumchrus–flemingeri* pair (Mackas *et al.*, 1993; Goldblatt *et al.*, 1999). Possibly the nutrition of the deeper pair is partly fecal matter from the near-surface pair (Dagg, 1993b). OECOS time-series observations in the spring transition period will provide an extended re-evaluation of these relationships in vertical distribution, trophic interaction and development timing in the Gulf of Alaska. More important time-series data from a site in the western subarctic gyre, where there is a very strong spring bloom, will allow us to compare the relationships under very different feeding conditions.

All of the *Neocalanus* species have 1-year life cycles, and they tend to develop as single cohorts with distinct, slow, stage progressions that are evident in sampling with high temporal resolution. This feature means that high resolution time series that are long enough in both the eastern and western sectors should allow comparison of development and growth *in the field* under contrasting nutritional conditions. Several shorter development studies of the sort proposed here have already been done in the eastern sector (Miller and Nielsen, 1988; Miller, 1993a). Changing stage proportions have been used to estimate the development rates of *N. flemingeri* and *N. plumchrus* through a few stages each. Rates for the latter species were slow relative to those observed in much more eutrophic, enclosed coastal waters, particularly the Strait of Georgia, between Vancouver Island and the British Columbia mainland (Evanson *et al.*, 2000; Campbell, 2003). Those coastal studies with resolution of a few weeks were not sufficiently refined to follow cohort development or define stage duration. All stages tended to peak on the same sampling date, a problem identical to that experienced with all sampling to date in the western oceanic sector (*e.g.*, Tsuda *et al.*, 1999; Kobari and Ikeda, 2001a,b; Shoden *et al.*, 2005). Sufficiently prolonged and highly resolved sampling will catch the developmental progressions in both spring bloom (western) and continuously high-nitrate low-chlorophyll (HNLC) (eastern) conditions.

Development rates

The requirement for development rate determinations is daily (better twice daily) samples, say 100–0 m, with nets large enough to catch a few thousand of the dominant copepodite species. These will then be counted by stage, with stage duration estimated by the 'Heinle graph' technique (Fig. 25, Miller, 1993a). This method is subject to some bias because changes in stage proportions due to stage-to-stage differentials in mortality are not separated from changes due to development (modeled by Miller, 1993a; Miller and Tande, 1993). These mortality biases, shown by the models to be modest (~2 days in 13, depending upon stage-to-stage differentials in

mortality rate), cannot be overcome. However, it is almost certain that the western development times will be enough shorter than those in the east, and that the comparison will be clear and approximately quantitative.

There are also measures of stage duration based on incubation techniques. The idea is that a stage is sorted out, incubated (usually with natural food levels), and the molting rate is followed (Kimmerer, 1983; Miller *et al.*, 1984a). Under certain assumptions, the inverse of the fractional molting rate is the stage duration. The key assumption is that the population is in steady state, with all ages-within-stage equally represented. Such methods do not work particularly well in the singe-cohort situation of the subarctic Pacific (Miller and Nielsen, 1988). Suppose that work begins when the cohort of *N. flemingeri* is entering predominantly the third copepodite stage (C3). The stage is going to last up to 2 weeks, so experiments established over the first few days show no molting whatsoever. Eventually, molting rates pick up and rise to a high level when all the remaining C3 are at older ages-within-stage. None of the proportions are particularly well represented by the small samples that are possible to sort. Feeding conditions are impossible to maintain at near-natural levels over days and days. Extended work in refrigerated labs on board is required to obtain nearly worthless results. There are techniques for the screening of samples to remove stages larger and smaller than a mid-range selection, say C2, C3, and C4, which is then checked after an interval for change in mean stage (so-called artificial cohorts: Kimmerer and McKinnon, 1987; Hopcroft *et al.*, 1998; Campbell *et al.*, 2001). This apparently works reasonably well in the tropics for small, very fast developing species, but in the subarctic situation it has all the same problems as single-stage sorting. Screening damages individuals. Earliest stages are likely to be molting very fast, and the next ones not at all, introducing unacceptable biases. Also, food conditions cannot be sustained at normal levels long enough to generate meaningful data. The best route will be to follow cohort development in the water column.

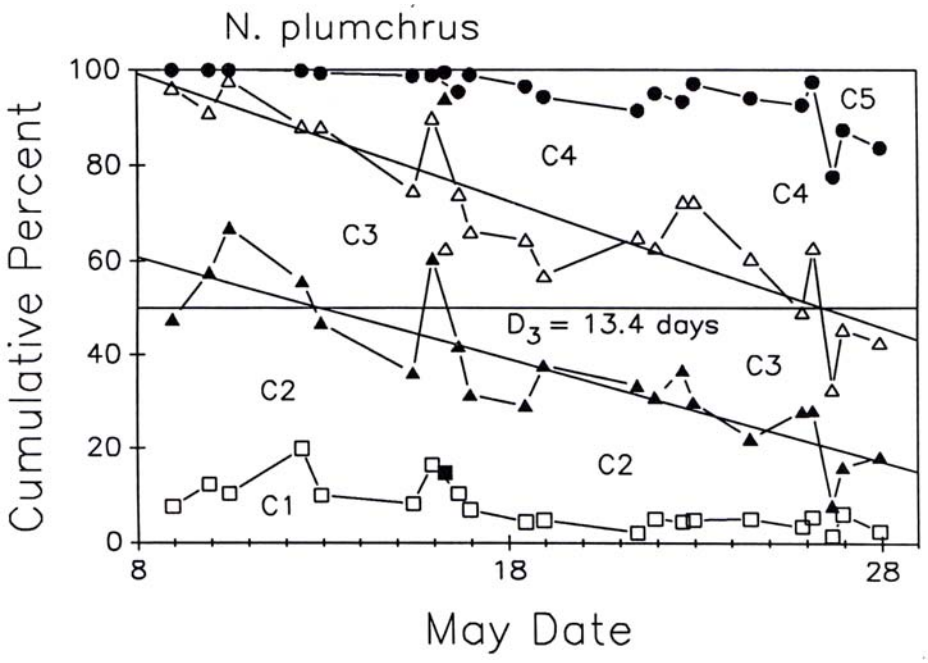

Fig. 25 Proportions of copepodite stages of *N. plumchrus* at Station P in May of 1988. Median duration between median crossings of the C2–C3 transition and the C3–C4 transition is an estimate of typical stage duration for the main annual cohort, 13 days in 1984. These have been termed (by this author) 'Heinle graphs' after Donald Heinle, who used them to evaluate growth in laboratory rearings.

Individual biomass – What we need to measure

Stage duration is a measure of the time aspect (denominator) of a growth rate determination. We also must know how much growth occurs, and this estimate is more difficult to obtain. We need to know the increment in organic content (dry mass or carbon mass) that occurs during the stage: W_{end}–W_{start}. It is generally not possible to look at a specimen to determine its age-within-stage, thus, development-specific weights are not readily attained. Workers often overcome this by measuring mean weights for successive stages, then using the change in those means between stages as a measure of growth. Since stages of large, subarctic copepods are engaged in rather different phases of growth, including massive lipid accumulation to support diapause and all reproduction, it would be informative to do better than that.

At least with live *Neocalanus* it is possible to distinguish individuals about to molt. They have double exoskeletons and thus, possess doubly intense apparent pigmentation. Setae developed for the new exoskeleton within the tissues give the caudal furcae and limbs a distinctive striated appearance. These individuals can be sorted and their mass determined to mark the terminal mass for their stages. Exoskeleton mass can be determined for exuviae (at least approximately) and subtracted from terminal mass. That should be an estimate of the initial mass of the next stage. This should be quite accurate, since very little growth occurs in the last phase of each stage.

Another approach is to measure the variability of individual mass throughout the development of a cohort (at least the part for which a ship is on station). When the oldest cohort members reach, say, C3, all of them will be young-within-stage. Their mass will approximate the initial mass (with associated individual variability). When the oldest C3 are nearing molt and the youngest C3 are newly molted, the full range of mass should be represented, and the bulk should be at about mid-stage mass. This should allow good modeling of individual growth within the stage. If all measures of mass are for individuals for which length is also measured, the effect of body size variation on mass increase over the stage can be accounted for. It will explain (in the statistical sense) much of the individual variability. Copepods do vary dramatically in body length within each stage (*e.g.*, Miller, 1993a; Miller *et al*., 2000).

In order to make these measurements fully meaningful, we need a correct model of copepod growth, particularly the pattern of growth *within* the molt cycle. This has never been demonstrated for copepods, probably because it is so difficult to obtain individual copepods of known age-within-stage. No work comparable to that with crab larvae (Anger, 2001; Steve Sulkin, Shannon Point Marine Laboratory) has been done for copepods. However, there are observations suggesting that the Anger–Sulkin model of the interaction of growth and development in decapod larvae does apply to copepods. This has three parts: the time-course of mass increase through the phases of the molt cycle, and two effects of food rations on growth and molting. Growth follows a pattern (Fig. 26) like that of the zoea I of *Hyas araneus* (a spider crab whose very numerous eggs all hatch in synchrony, then sustain it well enough so that many individuals are available for weighing, *etc*. at each age-within-stage). Growth is very rapid at first with ~70% of the total stage increment laid up in the first half of the molt cycle. Then growth slows as morphogenesis, in preparation for the next molt, takes more of the ingested nutrition. Actually, ingestion and assimilation also slow, with the last fraction of the cycle showing no growth at all.

The molt cycle divides into phases with respect to the effect of withdrawal of food. If food is withdrawn before 30% of the molt-to-molt period passes, growth stops, eventually a very quiescent phase is reached, and molting does not occur. Withdrawal of food shortly after 30% (this is variable among species, of course) stops growth (it must), but it does not stop or delay the next molt, to which the animal is hormonally committed. The next instar just comes out smaller. This time of commitment to the next molt is termed the 'Point of Reserve Saturation' (PRS). Larvae that are starved after PRS, and then have molted, require considerably longer to complete their next stage; there is a recovery period in which nutritional status is restored, and only after which growth and development can resume. There is

also an effect of starvation starting from the beginning of a stage (or before PRS), termed the 'Point of no Return' (PNR). This is the duration of starvation after which even *ad libitum* feeding will not return the animal to health. It will never molt and eventually will die. Dagg (1977) has studied starvation effects in copepods, but not the interaction with the molt cycle, and effects of less than replete diets on zooplankton have been studied very little, apart from those on egg production.

I think that copepodites have exactly this sort of Anger–Sulkin growth pattern. One piece of evidence is that copepodites brought in from the field, sorted to stage, and held in filtered seawater continue to molt for over half of their expected molt-to-molt interval (Fig. 27). This implies that they have a PRS. They can successfully molt because they have, by ~30% of the intermolt interval, accumulated a large fraction of the expected full-nutrition growth increment, exactly as shown for crab zoeae. Crain and Miller (2000) found evidence for this occurring in the field. We showed that copepodites of *Calanus finmarchicus* with postmolt exoskeletal facies (newly molted) accumulated during a starvation event (Campbell *et al.*, 2001) over Georges Bank, northwest Atlantic Ocean. Individuals caught by the food shortage early in a stage, say C_x, did not advance from the postmolt condition, while those past PRS molted to C_{x+1}, raising the fraction of postmolt individuals. Also, those in C_{x-1}, and already past the PRS, proceeded to molt, contributing more to the fraction of postmolt individuals in C_x. Such an increase was demonstrated in several stages.

In any case, to measure growth effectively, especially in comparing food-replete (western) and food-limited (eastern) circumstances in the subarctic Pacific, we need an accurate model of copepod growth rate, one including its oscillation at the period of the molt cycle. Some experimental work on this problem of a correct growth model can proceed before OECOS reaches its field research stage.

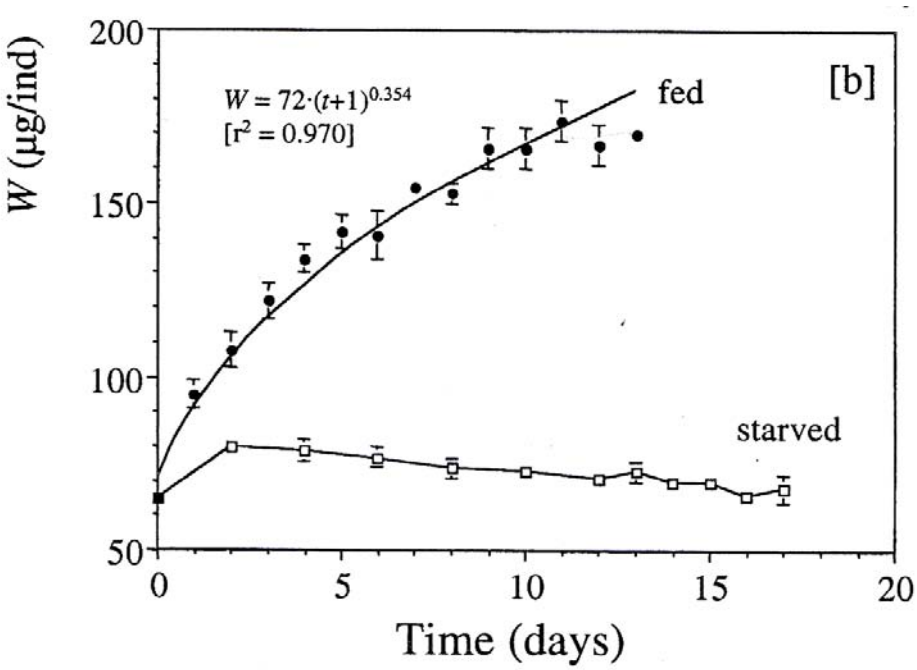

Fig. 26 Time course of weight increase in the planktotrophic zoea I larva of *Hyas araneus* (a spider crab) fed to repletion and starved. Full development takes ~13 days but weight approaches an asymptote after ~9 days, and by 4 days (~30% of the intermolt interval) the animal has achieved over half of the growth for the stage.

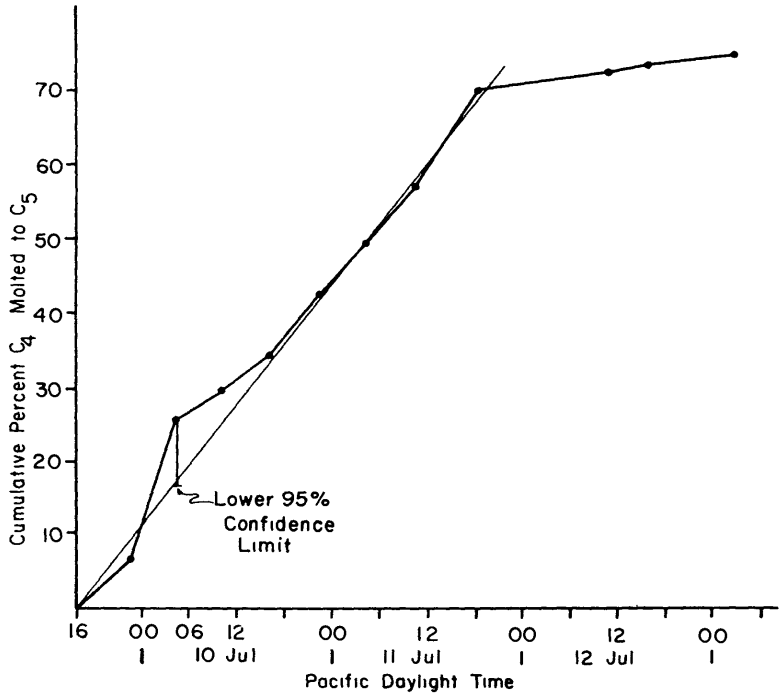

Fig. 27 Cumulative fraction of C4 *Calanus pacificus* sorted from field stock and held without food. About 70% of them molted, having reached PRS before collection and the onset of starvation (Miller *et al*., 1984a).

Individual biomass – Instrumentation

In order to get growth data from the field sufficiently resolved to model individual growth, it is probably necessary to have individual measures of copepod mass. Either dry weight or carbon content of one, newly molted C1 of the species of interest should be measurable. These measures should also be made *at sea*, if at all possible, so that (1) no preservation artifacts are introduced (carbon loss to aldehyde solutions, body breakage and lipid/tissue-fluid loss on thawing, *etc.*), (2) coloration patterns will be available to assist with identification, and (3) the data set will arrive ashore finished. Refined determinations of mass are possible at sea, despite the wild accelerations affecting a ship in a seaway (Childress and Mickel, 1980). A modernized version of this comparative electrobalance system might get down to masses of a few μg, a sensitivity that will be required to obtain individual masses.

Another approach is the 'Salonen machine' (Salonen, 1979) for carbon analysis. This has never been fully exploited, except by Salonen himself and a few of his colleagues in Finland. It is 'just' a carbon analyzer. Salonen's device is a small furnace (actually a quartz tube packed with catalyst at 950°C) for combusting minute samples of organic matter in an oxygen stream that then passes into a non-dispersive infrared gas analyzer (NDIR detector). Salonen claimed, and other workers have confirmed (H.-J. Hirche, pers. comm.), sensitivity of 0.01 μg carbon, with an upper limit of 2 mg, approximately the requirement for individual, oil-filled *N. cristatus* C5. Combustion and accuracy are independent of moisture content, so whole animals can be converted into carbon numbers after simple blotting. With suitable auxiliary detectors, nitrogen can be determined also. A commercial version sold for a while by Salonen was finicky in use (Hirche, pers. comm.), but gave reliable results with sufficient care. Hirche has used it to determine carbon content in single eggs of *Calanus.* Particularly with *Neocalanus* spp. and *Eucalanus*, which store large fractions of lipids, this carbon data will be most useful if we can partition carbon content into storage (lipid) and active tissue fractions. It is possible that lipids can

be extracted, and then both lipid and residual fractions can be measured separately. It is also possible that lipid content can be estimated from pictures (Miller *et al.*, 2000), which are also useful for length measurements (we must be able to determine 'condition factors', that is, mass relative to body length and volume). Then the whole animal can be used for the carbon estimation.

Before we approach the fieldwork, we must model age-within-stage variation of growth (of mass) as it interacts with (1) age-within-stage composition change through time (cohort progress) and (2) individual variation to determine how many individual masses must be measured to adequately evaluate growth patterns. If all five major species are evaluated, the at-sea work load (sampling, sorting, videography, and combustion analysis) will be considerable. This will require further detailed planning. Planning should also include detailed consideration of other techniques: colorimetric protein analysis (*e.g.*, Coomassie Blue or bicinchoninic acid binding of protein with spectrophotometry, modified Lowry oxidation, and several other alternatives), an alternate lipid analysis, and much more.

The OECOS time-series study of copepod size and growth within a season for a single year must be conducted in the face of substantial *year-to-year variability* in growth conditions for interzonally migrating copepod species in the subarctic Pacific (*e.g.*, Miller *et al.*, 1992; Miller, 1993a; Mackas *et al.*, 1998; Kobari *et al.*, 2003a). This variation is well documented by the studies cited with respect to prosome lengths. It is not well characterized in terms of biomass and condition factors, but because of the approximately cubic mass to length relationship, the variation is much larger. For example, I found (Miller, 1993a) a huge difference in *N. flemingeri* C5 individual biomass between 1987 and 1988 at Station P (50°N, 145°W). In 1988 randomly selected individuals were 400 μg DW, while lipid-packed individuals in 1987 were only 270 μg DW. At 53°N in 1988 random individuals were up to 800 μg DW. Evidently the mass variation, when a stage (particularly C5) is fully grown and ready for molt or diapause, can amount to a factor of 3 or more.

The role of food availability in establishing this interannual (and latitudinal; see Kobari *et al.*, 2003a) variation should be illuminated by the west *vs*. east comparison proposed for OECOS, which is expected to be between replete and strongly food-limited conditions, respectively. For the comparison to be valid, it is critical that methods be standardized for application on both sides of the Pacific. A great deal of information exchange should be undertaken to make this standardization possible. It will probably be desirable to have North American and Asian technical groups (the people who will make the measurements at sea) work together on a pilot study someplace to allow instrumental standardization for the measurement process. Possibly OECOS can include some sampling apart from our main, extended time series to get at the issue of longer-term variation in growth conditions. The simplest approach would be a few years of mid-summer samples of *N. plumchrus* C5 and *N. flemingeri* females in diapause taken at depth after the main growing season. This could be part of other programs on both sides of the Pacific.

Biomolecular approaches to growth rate determination

In an early general essay on the OECOS idea, I suggested that one or several recently developed methods for growth rate determination, based on levels of nucleic acid or on activity of specific hormone or enzyme groups, might be applicable. After a study of the papers, I doubt that any of these will have sufficient precision to be of much use in quantitative characterization of individual growth. Partly, this is because it will likely be impossible to determine the phase of growth (particularly within the molt cycle) to which an analysis applies. I may well be wrong. However, any of these techniques might be useful in bulk comparisons of growth averaged across many individuals between west (food replete) and east (food limited). Each of them has substantial cost in analytical work.

Techniques proposed in the literature are estimation of RNA:DNA ratio (Wagner *et al.* 1998, 2001), aminoacyl-tRNA synthetase activity (AARS, Yebra and Hernández-León, 2004), ecdysone titers (Johnson 2004) and chitobiase activity (Sastri and Roff, 2000). The assumption

of RNA:DNA ratios is that most RNA in cells is in ribosomes active in protein synthesis, while all DNA is nuclear and stays in fixed ratio to cell number. Thus, if faster growth requires more protein synthesis and more ribosomes, it will be signaled by higher RNA:DNA. Wagner *et al.* (2001) have shown this is correct. However, RNA:DNA also correlates positively to stage of development (Fig. 28A), or to body weight, even though successive stage growth rates are progressively slower. They overcame that limitation by normalizing RNA:DNA to protein content, after which they were able (mostly) to show a difference in ratio between copepodites on replete diets (growth rate set at 100% in Fig. 28B) and copepodites on reduced rations (growth rate divided by that for high food levels). Clearly the required effect is present, but it is buried several layers inside the growth regulation mechanics of copepodites. A meaningful study in the field for *Neocalanus* spp. would require a preliminary rearing study comparable in detail and excellence to that done by Wagner *et al.* (2001). A field time series would require RNA, DNA, and protein measurements on very large numbers of individuals or samples. Moreover, even their results (Fig. 28B) show large scatter, some of it likely from not distinguishing Anger–Sulkin growth phases.

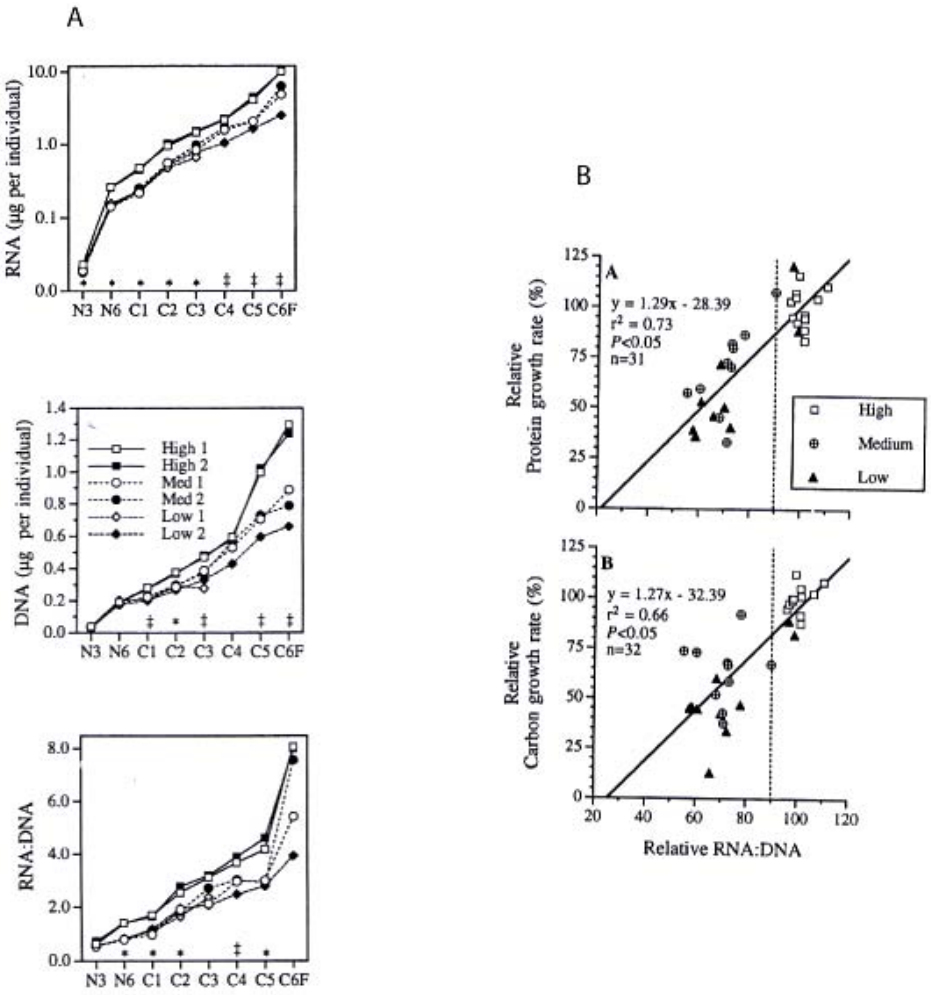

Fig. 28 (A) RNA, DNA, and RNA:DNA for *Calanus finmarchicus* reared in the laboratory at high, medium, and low rations. Both variables and the ratio increase with stage (and size). Note that RNA increases logarithmically with stage; DNA increases linearly. (B) Protein and carbon growth rates, both as a percentage of the rate at high food level (100%) plotted against RNA:DNA divided by protein content. This comparison does show that RNA:DNA responds to growth rate, although in a complex fashion. From Wagner *et al.* (2001).

The AARS technique has an assumption like that of RNA:DNA, that is, faster protein synthesis must require more activity tacking amino acids onto transfer RNA, which is the function of aminoacyl-tRNA synthetases. There are 20 of these enzymes in virtually all animals, all active all of the time, one for each amino acid. Yebra and Hernández-León (2004) measured overall AARS activity in plankter homogenates (15 to 30 *Daphnia* required in the original description) by determining the release rate of pyrophosphate (PPi, *i.e.*, PO_4-PO_4 released as adenosine triphosphate (ATP) is converted to adenosine monophosphate (AMP), the energy charge going to bind lysine, *etc*. to tRNA). PPi is analyzed by its reaction with NADH (nicotinamide adenine dinucleotide), which can be followed with a spectrophotometer. Unfortunately, the correlations demonstrated between growth rate and AARS activity in *Daphnia*, while statistically significant, were too weak to be useful for our studies of individual growth, even if the assay could be miniaturized to determine AARS in one *Neocalanus* early copepodite. Some of the weak correlation may come from the fact that PPi is released by a few other reactions beside AARS activity. Possibly AARS measures would be good enough to show gross regional differences, such as those between the western and eastern subarctic Pacific. Like RNA:DNA, we would need an independent evaluation of AARS to growth relations in our copepods.

Both ecdysone measures and chitobiase activity relate to the molting process. Ecdysone has only been measured by radio-immuno-assay (RIA) and not in single individuals (Johnson, 2004). It requires complex instrumentation and use of radioisotopes. Much like molting-rate studies, it would only show high levels as substantial numbers in a cohort approached molt. Johnson used it to show differences between actively developing individuals and diapause individuals. That is not an issue for OECOS. The best measure of diapause onset is more likely to be development of high levels of molecular chaperone (heat shock) proteins in animals that have not been heat shocked. Chitobiase breaks down the inner layer of the old exoskeleton prior to molting, and at molting it is released into the surrounding water. Sastri and Roff (2000) have proposed measures of its activity in the water as a general measure of secondary production rates. The enzyme breaks down in the water with a time constant around 1 day, so that the activity at any given time depends upon the amount of recent molting (perhaps the surface area of recently shed exoskeleton). This has not, to my knowledge, been applied to a field situation. Results would depend upon stock size as much as stock activity, and separating those sources of variability would depend upon reliable stock measures. I would not propose applying either of these methods at present.

Growth of large interzonal migrating copepods

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Ecological importance

Large interzonally migrating (IZM) zooplankton, such as *Neocalanus* spp. and *Eucalanus bungii*, are predominant in the entire subarctic Pacific and its marginal seas (Mackas and Tsuda, 1999). They account for more than 70% of summer zooplankton biomass in the Oyashio waters (Vinogradov, 1997). Because these species prey on diatoms, flagellates, and ciliates (Tsuda and Sugisaki, 1994; Kobari *et al*., 2003b), they have important roles in carbon uptake from two different food webs, the 'grazing' and microbial food webs. On the other hand, they are major carbon pathways to animals at higher trophic levels, as the food items for various fishes (Odate, 1994; Beamish *et al*., 1999; Yamamura *et al*., 2002), sea birds (Russell *et al*., 1999), and whales (Kawamura, 1982). Therefore, they are key species in the subarctic marine ecosystem. Moreover, their fecal pellets and downward migrations are considered to be a significant biological pump with large carbon flux (Kobari *et al*., 2003b).

Recent studies in the western subarctic Pacific

Over the past 6 years, knowledge of the life histories of IZM copepods has become better integrated (8 published papers) for the western subarctic Pacific (Oyashio). Life cycles of *Neocalanus* spp. in the west are annual (Tsuda *et al*., 1999, 2001; Kobari and Ikeda, 1999, 2000, 2001a,b). Lipid-rich eggs are produced below 500 m depth from autumn to winter, and then rise or migrate upward while hatching and molting through the naupliar stages. *Neocalanus cristatus* appears at the surface first, followed by *N. flemingeri* and then *N. plumchrus*. Throughout the spring phytoplankton bloom, they develop into (Stage 5) C5, except for a small part of the population of *N. flemingeri* that stops at C4. After mid-summer, the young specimens migrate downward to overwinter, the C5 of *N. flemingeri* maturing and mating on the way down, with females as the main resting stage.

For *E. bungii*, two different patterns are proposed for their life histories in the western subarctic Pacific (Shoden *et al*., 2005; Tsuda *et al*., 2005a). In an annual pattern, newly recruited specimens with early birth dates develop into C4 in that year, overwinter at depth, and mature during the spring phytoplankton bloom in the next year. A biennial pattern is more complicated. Newly recruited specimens with late birth dates reach C3 in the current year, overwinter at depth, and develop to C5 by mid-summer in the next year. After a second overwintering, they mature during the spring phytoplankton bloom. During the years with prolonged phytoplankton blooms, they can develop into C6 females and again overwinter. These patterns are flexibly varied with oceanographic conditions, mainly food supply (Shoden *et al*., 2005; Tsuda *et al*., 2005a).

With increasing knowledge of life history in the western subarctic Pacific, east–west differences can be discussed. The western subarctic Pacific is characterized by large seasonal fluctuations of oceanographic conditions, and massive and early phytoplankton blooms (Saito *et al*., 2002). *Eucalanus bungii* is most sensitive to these oceanographic conditions. On the western side, the life span is shortened, and the postembryonic development and reproductive seasons are early. It is considered that life span is shortened by high growth under high food availability, and spawning and development seasons are synchronized with the seasonal peak of primary production (Shoden *et al*., 2005; Tsuda *et al*., 2005a). Unlike *E. bungii*, *Neocalanus* spp. shows similar patterns and timing between the two sites (Tsuda *et al*., 1999; Kobari and Ikeda, 1999, 2001a,b), with an exception for the small part of *N. flemingeri* which has a biennial life history on the western side, (Kobari and Ikeda, 2001a).

Carbon flow through *Neocalanus* spp. has been estimated using time-series data (Kobari *et al.*, 2003b). In the western subarctic Pacific, primary production is very high due to massive phytoplankton blooms. Annual *Neocalanus* production is computed to be 19.3 gC m^{-2} $year^{-1}$ and accounts for 7% of primary production. Removing 4.3 gC m^{-2} $year^{-1}$ from annual production to dormant populations, the remainder is considered to be surface mortality. If predation is the major part of mortality, *Neocalanus* contributes to carbon flow with high transfer efficiency. On the other hand, the ecological importance of dormant populations is also considerable. Although sinking particles and downward migrations both contribute to carbon flux (Fowler and Knauer, 1986), loss during downward migrations is extremely low compared to that of sinking particles. Thus, downward migrations by zooplankton are a very efficient and effective aspect of the biological pump. For *Neocalanus* spp., most of the seasonal downward migration can be considered to be carbon export flux because they end their life history at depth. The estimated export (4.3 gC m^{-2} $year^{-1}$) is similar to the sinking particle flux (4.7 gC m^{-2} $year^{-1}$, Honda, 2000). Considering the large standing stock, the downward migration could be a significant component of global carbon flux.

Important subjects remaining to address

Knowledge of distribution, life cycle, and feeding has been integrated for the large IZM copepods since the 1980s. However, there is little information on growth, production, and interannual variations. All of those should be included in developing ecosystem models for subarctic waters. Although knowledge of long-term changes has been recently integrated (Kobari *et al.*, 2003a; Chiba *et al.*, 2004; Tadokoro *et al.*, 2005), growth processes are still unquantified and should be studied because they are sensitive parameters in population dynamics. Based on the results from the Bering Shelf (Vidal and Smith, 1986), observed growth rates were much different from the estimates of some empirical models, such as the temperature-dependent model of Huntley and Lopez (1992) and the temperature–weight dependent models of Ikeda and Motoda (1978), Hirst and Sheader (1997) and Hirst and Lampitt (1998). Especially strong weight-dependence of growth is observed for late stages. That might result from the weakly developed muscles and stored lipids that apparently are preparation for molt-to-the-adult stage, egg production, and lowered metabolism (Kobari and Ikeda, 1999, 2001a,b; Tsuda *et al.*, 2001). Therefore, it is difficult to estimate growth rate from the empirical models.

Moreover, importance of growth processes (and also mortality) is suggested by the results of the long-term changes in seasonal abundance of *E. bungii* in the western subarctic Pacific (Kobari *et al.*, MS). Adults usually appeared in spring over the study period. However, newly recruited specimens and late stages did not occur until mid-summer in the 1970s and 1990s, while they disappeared by early summer in the 1980s. These findings reveal variations in the progression from spawning to postembryonic development to dormancy. In the Oyashio, phytoplankton blooms terminated early in the 1980s but were long-lasting in the 1990s. These results suggest that growth (and morality) during postembryonic development determines the yearly fluctuations of abundance of this copepod. As spring phytoplankton blooms vary in timing, location, and magnitude in the western subarctic Pacific, the copepod develop-mental responses could be correspondingly varied. This highly variable interaction remains to be documented at the moment because of the short development time at the surface and the complicated hydrography of the Oyashio, which is influenced by Kuroshio warm core rings, strong tidal mixing around the Kuril Islands, and variable wind patterns.

Prospective subjects in the OECOS program

I would like to propose prospective reseach subjects for the OECOS program. A high-frequency sample collection would resolve (1) stage duration time and (2) growth and mortality rates. Better growth and mortality rate estimates for these oceanic copepods could improve our global models, which are mostly constructed from coastal species (Huntly and Lopez, 1992; Hirst and Sheader, 1997; Hirst and Lampitt, 1998; Hirst and Kiørboe, 2002). Details of population dynamics and life history strategies

would be resolved by these findings, as reported for *Calanus finmarchicus* (Hirche *et al.*, 2001; Ohman *et al.*, 2001). By comparing new western area OECOS studies with the eastern OECOS findings, and with the results of SEEDS/SERIES (Tsuda *et al.*, 2005b), controlling factor(s) of growth and mortality could be evaluated. These findings could resolve the responses of life cycle strategies to phytoplankton blooms, especially for *Neocalanus* spp. that have similar seasonal patterns between the east and the west. Moreover, the information on (3) body allometry, (4) chemical or fatty acid composition, (5) feeding or ingestion rate, and (6) daily changes in vertical distribution could suggest what factors initiate and terminate dormancy. Finally, all of these findings can yield estimates of (7) production rate and carbon export by the downward migration of IZM copepods in the western subarctic Pacific.

F. MODELING

Modern study of any complex ocean process involves modeling. Lower trophic level interactions in the subarctic Pacific, from nutrient dynamics to copepod growth (and, of course, beyond that) certainly qualify as complex. Modeling all of the processes, interactions, and cycles that are of interest to OECOS will require substantial and inventive intellectual effort. Dr. Harold Batchelder has undertaken to survey what is required.

Ecosystem and population dynamics modeling

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Introduction and modeling needed to support OECOS investigations

The eastern and western subartic gyres have similar mesozooplankton species composition, but differing seasonal cycles and differing amplitudes of primary production. OECOS proposes to compare the trophodynamics and population dynamics of the lower trophic levels of these two gyres using similar observations over an extended period in the spring, the season when the differences between the two gyres are most dramatic. (See elsewhere in this report for examples of these differences.) In my view, there are three OECOS-related issues in which modeling before and during the program can advance our understanding of the differences between these two gyres: (1) ecosystem modeling that includes iron availability as a prognostic variable, (2) modeling that explores physical–biological coupling and considers potential top-down (mesozooplankton-grazing) or wasp-waist (microzooplankton-grazing) driven trophic cascades, perhaps generating cycles, and (3) individual-based or age–weight structured population dynamics models of the growth of mesozooplankton. It is possible that a single model may be used for the first two of these topics. The third topic (growth of meso-zooplankton) requires models of individual species that include behaviors and details that cannot be readily represented in the more aggregated ecosystem models that would be used for the first two topics.

Ecosystem modeling – Fe considerations

Ecosystem modeling is data intensive. Data are needed for estimating the parameters that govern fluxes of material among model states, and for validation and comparison purposes. Thus, model state variables are usually considered based on functional units (*e.g.*, herbivorous grazers, omnivores, *etc.*), which implies simplification to a manageable number of state variables. Consequently, much of the ecosystem modeling in oceanic domains has occurred for a relatively few locations which have had both long observational data series and intensive field experimental programs, including Hawaiian Ocean Time Series (HOTS), Bermuda Atlantic Time Series (BATS), and Ocean Weathership Stations (OWS) like OWS-Papa in the subarctic Pacific (Batchelder and Miller, 1989; Miller *et al.*, 1991; Frost and Kishi, 1999; Strom *et al.*, 2000). OWS-Papa data sets (observations, annual cycles in wind, mixing, and solar radiation) have been used extensively for box and one-dimensional modeling of planktonic ecosystems (Frost, 1987, 1993; Denman and Peña, 1999, 2002), many with the goal of understanding why phytoplankton blooms do not occur in the high-nitrate low-chlorophyll (HNLC) subarctic Pacific. In the last 18 years, there has been an appreciation of the potential role of iron as a regulator of primary productivity in oceanic HNLC regions, like the Southern Ocean, eastern equatorial Pacific, and subarctic Pacific (Martin *et al.*, 1989; Coale *et al.*, 1996; many others). Iron supply to HNLC regions can occur through

atmospheric processes, such as deposition of terrestrially-derived dust (Mahowald *et al.*, 1999; Fung *et al*., 2000), through oceanic processes *via* upwelling of deep waters with higher Fe concentration, and by interactions with sediments near the coast. Basin- and global-scale ecosystem models have not, until recently, included iron dynamics or the impacts of this micronutrient on biogeochemistry and ecological processes. In the last few years iron cyling has been included in both local one-dimensional simulations and three-dimensional circulations (Leonard *et al.*, 1999; Arrigo *et al.*, 2003; Aumont *et al.*, 2003; Moore *et al.*, 2004; Dutkiewicz *et al.*, 2005). Many of the three-dimensional iron-limited ecosystem models are global and, therefore, have only coarse spatial resolution. OECOS proposes nearly parallel studies of two specific regions of the subarctic Pacific for which the relative importance of Fe supply likely differs. At subarctic latitudes, winds are predominantly from the west and Asian dusts are probably the primary atmospheric source; atmospheric Fe supply is likely to be significantly greater in the Oyashio and western subarctic gyre than in the eastern gyre. Conversely, open ocean upwelling of deep water is likely to be a larger (relative) contributor to Fe surface supply in the eastern gyre than in the western gyre. These differences are amenable to high-resolution (both vertical and horizontal) ecosystem models that consider both atmospheric dust (and Fe) supply and *in situ* Fe dynamics. Bioavailable Fe concentrations and dynamics are dependent upon supply rates (Fung *et al.*, 2000), dissociation and complexation constants (and rates) (Shaked *et al*., 2005), and concentrations of natural organic ligands (humics and fulvics) and other compounds, like siderophores (Barbeau *et al.*, 2003) that form complexes with Fe. Any or all of these can be included in models. To develop ecologically useful models, it will be important to isolate system-controlling aspects of this complexity.

Ecosystem modeling – Trophic cascades and biomass cycling

Modeling of trophic cascade dynamics, imposed perhaps by microzooplankton grazing (removal of primary producer biomass and regeneration to inorganic nutrients (including Fe)), would complement the intensive time-series collections planned as part of OECOS. Colleagues and I (Edwards *et al.*, 2000a,b) examined temporal and spatial responses (one-dimensional and two-dimensional) of a simple nutrient-phytoplankton-zooplankton (NPZ) ecosystem model that was parameterized with either mesozooplankton grazers or microzooplankton grazers. The two parameterizations differed in that the microzooplankton grazers had maximum grazing rates 8 times higher, slightly greater nonlinearity in their Ivlev grazing function, and substantially lower growth efficiency (higher remineralization rate of nutrient) than the macrozooplankton grazers. The base macrozooplankton parameters have been used in previous studies (Franks and Walstad, 1997). The modifications for microzooplankton are based on growth rate estimates and gross growth efficiencies from Strom and Morello (1998) and Straile (1997), respectively. In the absence of vertical diffusion, the models exhibited different behaviors (stable fixed point and oscillatory limit cyles), depending on the maximum phytoplankton growth rate (a function of depth, that is, available irradiance) and parameter choices. The macrozooplankton system, *with* vertical diffusion, became more stable. Conversely, the coupled diffusive-microzooplankton simulation remained unstable, with large oscillations, even at high mixing rates. Nearly full-scale fluctuations (from almost none of the nitrogen to almost all of the nitrogen in the state variable) occur on time scales of tens of days to many months, depending on the parameters, the depth, and magnitude of diffusion. In the two-dimensional model, different parameterizations (macro- *vs.* microzooplankton) led to different spatial scales of variability in an upwelling system (Edwards *et al.*, 2000a). Phytoplankton and zooplankton maxima are quite narrowly banded in the microzooplankton simulation, and near-surface nutrient levels remain high over most of the model domain. In the macrozooplanton simulation, the bands of high phytoplankton near shore and high zooplankton offshore are much broader, and nutrient concentrations offshore are quite low at the surface.

Ecosystem modeling – Mesozooplankton growth and development dynamics

Comparisons of the growth and development rates of the same copepod species in the two subarctic gyres are a key goal of OECOS. Modeling of growth processes should proceed in parallel with the observations and experiments planned. Different, more detailed models are necessary to examine within-cohort growth dynamics. Either individual-based models or age–weight structured multi-class models could be used to explore the responses of the species to variations in temperature, prey type and prey abundance in the two gyres. Individual-based modeling (IBM) is an appropriate framework for identifying the processes controlling growth and development of particular copepods, such as *Neocalanus plumchrus* and *N. flemingeri*, and for examining the impacts, at the population level, of processes that affect individuals. One of these phenomena is the progression of stage distribution during the short period in April–May when these *Neocalanus* spp. are developing and laying up lipid reserves for the extended, deep-dwelling diapause phase of their life cycle. Individual-based models are capable of incorporating the complex processes involved in morphogenesis, lipidogenesis, growth and molting.

An unanswered question in the eastern subarctic gyre is: Why does *N. flemingeri* spawn earlier and more precisely focused in time than *N. plumchrus*? Individual-based modeling (IBM), like that done previously to examine seasonal cycles of development and growth for *Metridia* spp. (Batchelder and Miller, 1989; Batchelder and Williams, 1995), and to examine feeding depths, physiological and behavioral controls of individual copepods and their impact on transport and residence times, and demography, will be valuable in deciphering the dynamics of the large IZM *Neocalanus* of the subarctic Pacific. Individual-based models, coupled with the ecosystem models described in the previous sections, could be used to compare the ecodynamics of *Neocalanus* in the food-deplete eastern and food-replete (at least for a short period in spring) western gyres of the subarctic North Pacific.

III. Reports from Workshop Breakout Groups

The Workshop included almost a full day of discussions in three smaller groups assigned to consider (1) physical and chemical aspects of the ecosystem, (2) phytoplankton and micrograzers, (3) and mesozooplankton. Each group prepared a written report, edited versions of which are provided here.

A. Physical and Chemical Aspects with Emphasis on Iron and Iron Speciation

Participants: Zanna Chase, Timothy Cowles, Jay Cullen, Kenshi Kuma and Peter Strutton

The subarctic Pacific is one of three main high-nutrient, low-chlorophyll (HNLC) ecosystems in the world ocean. We know that adding iron to certain parts of this ecosystem, at certain times of the year, can produce dramatic increases in phytoplankton stocks and growth rates (Boyd and Harrison, 1999; Tsuda *et al.*, 2003). However, important questions remain unanswered regarding the coupling between iron and biology at a range of temporal, spatial and trophic scales; the role of iron speciation; the role of iron in the existence and character of biological limit cycles; and the importance of episodic dust events. An iron chemistry component is critical to the success of OECOS, and also represents a unique opportunity to further our fundamental understanding of iron cycling in an HNLC ecosystem.

Hypotheses

A number of hypotheses related to iron availability were put forward by the group:

1. East–west differences in ecosystem structure are driven by iron. Our assumption is that the western Pacific receives higher iron inputs from Asian dust sources. A corollary of this hypothesis is: *Phytoplankton in the western Pacific have higher Fe:C ratios.*

2. Lack of bioavailable iron sets the upper limit of phytoplankton limit cycles. A corollary of this hypothesis is: *Iron concentration and/or iron speciation shows cyclical behavior in the subarctic Pacific, in synchrony with biological cycles.*

3. Episodic dust inputs modulate the upper limit of the cycles.

4. The lower chlorophyll limit is set by iron limitation of microzooplankton, or more generally, *iron limitation has a direct, physiological impact on higher trophic levels*.

Proposed measurements

A set of iron-related measurements to be included in OECOS, in decreasing order of priority, is listed here together with a brief justification of the measurement and the proposed methodology.

1. **Total dissolved Fe.** This is the standard for iron measurements. It includes filtration through a 0.2 μm filter and acidification to pH < 2. It can be measured at sea using flow injection techniques. Archived samples would be stored for possible future, shore-based analysis of additional metals (with separate funding).

2. **Organically complexed Fe.** This is the dominant form of iron in the ocean. It can be accessed by phytoplankton, but is not as readily available as free iron. The ligands are most likely produced by microorganisms. Quantification and characterization includes ligand concentration and a conditional stability constant by competitive ligand equilibration/ adsorptive cathodic stripping voltammetry (Gledhill and Van Den Berg, 1994; Rue and Bruland, 1995) and is best done at sea.

3. **Particulate Fe.** This includes all iron, cellular and lithogenic iron, caught by a 0.2 µm filter. If filters are washed to remove extracellular metals (Tovar-Sanchez *et al.*, 2003), one can estimate the biological Fe:C quota. It would be interesting to relate this to iron-stress. Samples would be stored for future analysis by Inductively Coupled Plasma Mass Specrometry (ICP-MS) (Cullen and Sherrell, 1999).

4. **Fe(II).** It is more soluble than Fe(III), but with a rapid turnover. Since phytoplankton reduce Fe(III) before taking it up, Fe(II) is more available (Maldonado *et al.*, 2001; Maldonado and Price, 2001; Shaked *et al.*, 2004). Measurement is tricky because of a short half-life. Samples are best run inline using a flow injection, chemiluminescence method (Croot and Laan, 2002). Fe(II) is likely to be the most variable Fe fraction, even on sub-daily time scales, affected by irradiance, O_2, temperature, and pH.

5. **Bioavailable Fe.** There is no single accepted method of chemically determining bioavailable iron. Wells *et al.* (1991) proposed a method based on the reactivity of iron to oxine. The method is fairly simple, and a time series of these measurements could be interesting when combined with other measurements.

6. **Aerosol Fe.** There is a paucity of direct measurements of dust deposition to the open ocean, for obvious reasons. The continuous occupation of an open ocean station, as proposed in OECOS, would be a great opportunity to collect important data from a region known to be influenced by episodic dust events (Bishop *et al.*, 2002). We would consult with William Landing (University of Florida) on how to deploy an aerosol sampler from a ship. Care would need to be taken in positioning the ship during sampling. Samples would be analyzed on shore. As an alternative, surface aluminum could be measured as a proxy for dust input (Measures and Vink, 2000).

Iron and biology

The group will facilitate collection of clean water for biological experiments (see below). The advantage of routinely measuring iron concentrations in (non-radioactive) incubation bottles was also discussed. However, the number of such measurements needs to be kept at a reasonable level, unless a very large number of iron analysts and iron analyzers is brought on board. We estimate that one iron flow injection analysis (FIA) system, being run continuously (*i.e.*, with two analysts), could process *at most* 40 samples per day.

It would be good to include an iron biologist as part of OECOS, meaning someone who does iron-specific experiments on microorganisms, at sea. This person could measure rates of iron uptake and biologically-mediated changes in iron speciation. Expertise and insight are also needed to detect direct effects of iron on higher trophic levels. Finally, the group is interested in working with the modelers to include iron in the biological model.

Sampling

Sampling of the mixed layer, for all species, should be done once a day. In addition, one water column profile (to at least 1000 m) should be taken once every 2 days, for total dissolved Fe only, to serve as quality control. Two to four times during the experiment high-resolution, around-the-clock sampling for Fe(II) should be conducted in order to look for diel variability.

There are two approaches to sampling. The first is a Go-Flo rosette, with clean bottles mounted in an epoxy-coated frame and lowered on Kevlar line. Dr. Cullen has access to a package like this as part of a Canadian collaboration. Advantages of this approach are clean samples from well-defined depths, minimal disturbance to fragile cells, and no limit on sampling depth. Disadvantages include cost, deployment time, possibility of bottle-to-bottle variability, and the challenge of clean sampling and filtration.

The second approach is to use a ship-based pumping system coupled to a submerged 'fish'. Such a system could profile to at least 40 m. Advantages of this approach include rapid deployment, ease of obtaining large volumes of clean water, high vertical resolution, ease of sampling and filtering clean water (which can be done in a clean hood with inline filtering), and low cost. The main disadvantages are the limited depth range and the unknown extent to which pumping may adversely affect fragile organisms.

The best solution is to bring both the pumping and the rosette system. The rosette can be used to collect samples for biological experiments, and for deep profiles, and the pump can be used for continuous underway mapping and high-temporal resolution studies of iron speciation (particularly Fe(II)).

East–west comparison

The Eastern and Western Groups will use the same analytical method (chemiluminescence FIA) and filter size (0.2µm) for measuring dissolved Fe. Both groups will acidify samples to pH 2 and will compare measurements to the Sampling and Analysis of Iron (SAFe) standard seawater.

Iron complexation measurements by cathode stripping voltammetry (CSV) are not currently planned for the Western Group. Drs. Takeda and Obata have expertise in these measurements which could be included if resources allow.

One major difference between the two groups is that the Western Group determines particulate Fe by the difference between a filtered and an unfiltered sample (stored for at least 3 months), whereas the Eastern Group measures particulate Fe by collecting the particles. The Eastern Group will make some comparisons of these two methods.

The Western Group is not planning any Fe(II) measurements. The group decided that size-fractionated dissolved Fe (*e.g.*, colloids) is very labor intensive and is not necessary.

B. PHYTOPLANKTON/MICROZOOPLANKTON STUDIES

Participants: Harold P. Batchelder, Deanna Erdner, Ken Furuya, Takahashi Ota, Sei-ichi Saitoh, Karen Selph, Suzanne Strom and Nicholas Welschmeyer

The group focused on two primary issues:

1. Identifying the most profitable geographic region(s) for an east–west subarctic comparision, and outlining the key questions surrounding this geographic comparison;

2. Identifying the large-scale, unifying questions that might be addressed by a subarctic Pacific plankton research program, with particular attention paid to links between lower trophic levels (phyto- and microzooplankton) and interzonal migrator copepod species.

Identifying geographic region(s) for east–west comparison

Discussion started from the premise that work in the eastern subarctic would occur in the open gyre, the site of previous research identifying chronic iron limitation, biomass limit cycles within the microbial community, decadal changes in the timing of *Neocalanus plumchrus* ontogenetic vertical migration, and other important, yet poorly understood, planktonic processes. The group felt that, although Site H is located in the Oyahio region in waters of oceanic depth, its close proximity to the coast would render the exercise principally a coastal–open ocean comparison, while the exciting questions seemed to center more around the effects of different levels of iron supply on western *versus* eastern oceanic ecosystems. Based on this, the group made the following comparisons between the western and eastern subarctic gyre ecosystems (henceforth WSG and ESG, respectively):

Commonalities

- Animals (particularly the large copepod species);
- Annual primary productivity is about the same.

Differences

- Standing stock of chl is higher in the WSG;
- Diatoms are dominant (sometimes? often?) in the WSG and very low in the ESG;
- *N. cristatus* is dominant in the west *vs. N. plumchrus* in the east; *N. cristatus* prefers larger food particles compared to *N. plumchrus*;
- There is greater copepod standing stock in the west *vs.* the east (in spring only? year-round?);
- There are likely differences in amount, form, and timing of iron delivery.

Identifying the large-scale, unifying questions

The group discussed the potential temporal interactions between the microbial community 'limit cycles' (5- to 10-day oscillations in chlorophyll biomass and, inversely, in ammonium concentration) and the ontogenetic vertical migration (weeks to months at the sea surface) of the dominant copepod species. Potential interactions betweeen the two communities were pointed out, including:

- Top-down effects on the entire microbial community of *Neocalanus* grazing on (large) microzooplankton;
- Daily and seasonal changes in iron availability (from regeneration by micrograzers, as well as external inputs) and implications for copepod production.

Our historical understanding of the role of *Neocalanus* in the open subarctic (particularly the ESG) ecosystem has shifted – the following pendulum model (Fig. 29) was proposed.

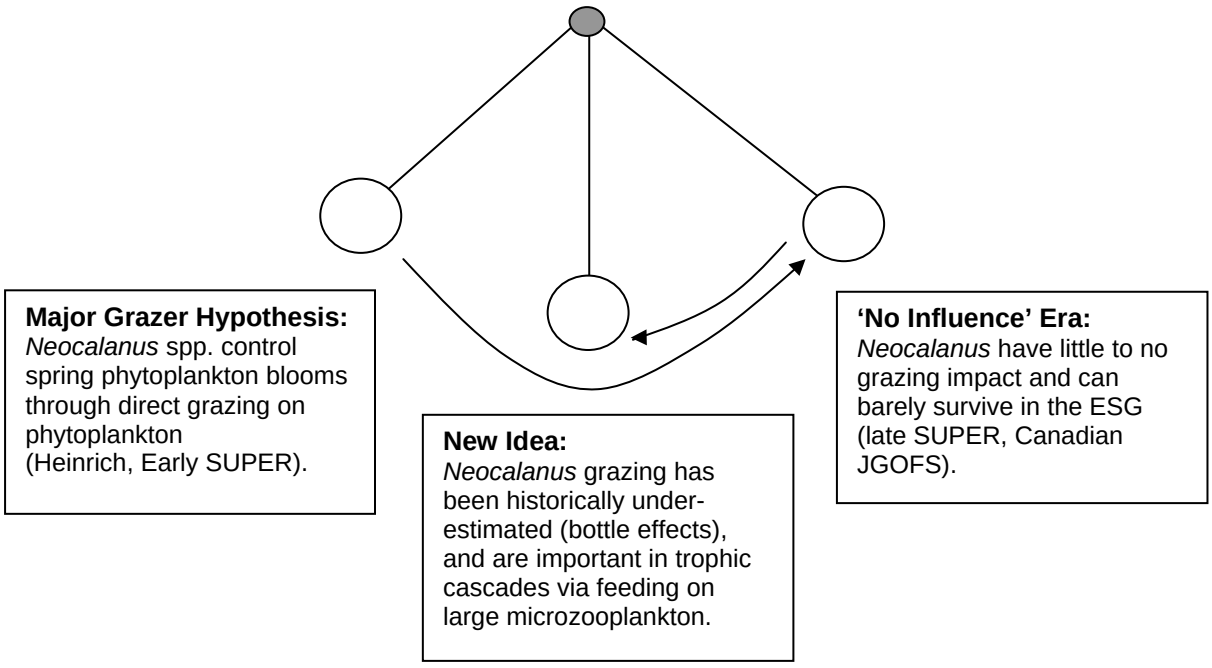

Fig. 29 The pendulum of viewpoints on the balance mechanism of oceanic HNLC ecosystems.

Questions

- Do we fully understand the lack of a spring bloom in the ESG?
- Does the large influx of spring copepods change the dynamics of the lower trophic levels? (The mental experiment of placing a net at 400 m in late winter to trap all the upmigrating copepods was proposed – would this matter for spring lower trophic level dynamics in surface waters?)
- How much of the primary production ends up in copepods?
- How much, and over what time scales, does the composition of the phytoplankton community change?
- Do east–west differences in the frequency of iron inputs affect the cycling within the microbial community?
- Does the dominance of diatoms in the WSG affect *Neocalanus* feeding and trophic cascade?

Needs as we move toward a proposal

- Better documentation of limit cycles from existing data in the ESG;
- Estimates of the amount and efficiency of primary production transfer to copepods (which could be estimated using inverse methods, *e.g.*, based on measured copepod production and assumed growth efficiencies);
- Estimates of the potential copepod community grazing impact on microzooplankton.

Hypotheses

Paradigm I

Iron supply controls mesozooplankton biomass and species composition in the subarctic Pacific.

- Hypothesis A: Variation in iron inputs leads to the dominance of diatoms in the WSG as compared to the ESG.

- Hypothesis B: The large diatom component in the WSG provides a more direct trophic linkage between autotrophic production and mesozooplankton consumers.
- Hypothesis C: Phytoplankton productivity and growth rates vary over different time scales in the west *vs*. the east.
- Hypotheis D: Mean diatom production per day is greater in the west than in the east.

Paradigm II

Differing mesozooplankton biomass and species composition in the east *vs*. west exert differing effects (*via* trophic cascade) on the microbial community.

C. MESOZOOPLANKTON STUDIES

Participants: Michael Dagg, Tsutomu Ikeda, Toru Kobari, Charles B. Miller and Atsushi Yamaguchi

The primary questions regarding mesozooplankton approachable through the OECOS, comparative, spring time series concern the array of interzonally migrating (IZM) copepods (*Neocalanus plumchrus, N. flemingeri* and *Eucalanus bungii*; there is also a significant oceanic stock of *Metridia pacifica*) that is found all across the subarctic Pacific, species common to both the Oyashio region and the Gulf of Alaska. The term 'interzonal migration' refers to the fact that the first four species listed above all have long diapause phases from late spring to late winter, which are spent at depths below 500 meters. Except for *E. bungii*, they all spawn at great depth, producing eggs strictly from stored nutriment. The eggs and nauplii are oil-rich and rise toward the surface. The season at which all of these copepods actively develop in the euphotic zone is spring. In the west they grow through copepodite stages and through a nutriment storage phase during significant, to intense, phytoplankton blooms. In the Gulf of Alaska this growth and storage occurs despite the absence of blooms, but during a time when phytoplankton production rates rise sharply, to produce a 'spring transition'.

If the western OECOS study site is appropriately chosen, western IZM will have copious stocks of large phytoplankton available as food during a substantial spring bloom, and microzooplankton will also be available as a dietary component. In contrast, a site such as Station P (50°N, 145°W) in the east, will not have significant amounts of large phytoplankton, and past work has shown that (1) microzooplankton are nearly their entire diet and (2) development and certainly growth are very slow relative to stocks in enclosed coastal waters with spring blooms, specifically in the Strait of Georgia, British Columbia.

Oceanic sectors of the subarctic Pacific support much greater stocks of mesozooplankton in spring compared to oligotrophic areas in subtropical and tropical waters. These stocks build up through the spring transition, which cannot occur (especially in the east) without having significant effects on the food web of nano- and microplankton that provides them the nutrition for growth. Preliminary experiments and logic both suggest that consumption of microzooplankton by IZM (and other larger plankton) initiates a trophic cascade, which releases nanophytoplankton from grazing pressure. Variation in this effect is likely part of the dynamics of the week-to-week variability of phytoplankton in the region. Such cascades are amenable to observational and experimental evaluation.

Hypothesis I

IZM species common to both ends of the subarctic belt will differ in size-at-stage, feeding rates and growth. Eastern stocks should have less food, grow and develop more slowly, and reach smaller final sizes. Western stocks should grow faster and larger thanks to greater food availability.

Goal

We propose to evaluate the responses of growth and development to the difference in spring transition processes between eastern and western sides of the subarctic Pacific. This should amount to a comparison between food-limited and food-replete habitats, respectively.

Approaches advocated at the workshop (others may be finally adopted)

We propose to compare sizes of IZM copepods between western and eastern sectors of the subarctic Pacific. Much of this can be done before the OECOS time-series study, and a literature review (Kobari, unpublished) has already been prepared. Samples can be exchanged for evaluation of prosome lengths and body weights. While OECOS is planned for very intensive time series in just one spring on each side of the region,

it will be necessary to take account of the interannual variability in size of IZM copepods. That variation is known to be significant (Miller *et al.*, 1992), amounting to a 10% range variation in mean prosome length, but much less than the differences across the range.

We will compare development rates, actually stage durations, between west and east, and develop Heinle graphs for all IZM species on both sides of the ocean. Stage proportions in two or more mixed-layer samples per day for ~50 days will be evaluated. To some extent we will employ adaptive analysis, counting only as many samples as necessary to obtain statistically robust stage durations.

Sizes and weights of stages will be compared using frozen specimens: the Western Group will videograph, then freeze, groups of single individuals in multiwell plates. In the laboratory specimens will be weighed, freeze-dried, weighed again, lipids extracted and weighed again. The Eastern Group is more concerned with coming off the ship with data, rather than frozen specimens. They propose to videograph specimens, blot them, and determine carbon and nitrogen contents with on-board carbon, hydrogen, nitrogen (CHN) equipment. Sufficient comparative data can be obtained by some CHN analyses of western specimens and by some weighing of eastern specimens. It is expected that biomass will be a well behaved function of C and N content.

Laboratory rearing will be done to characterize fully-fed growth and development. Since Growth = *f* (nutrition, temperature), we must do all that is possible (whatever the methodological limitations) to evaluate rations of IZM copepods in both eastern and western regions during OECOS by examining:

- gut pigment content, with the expectation that IZM copepods in the west will contain much more phytoplankton pigment than in the east;
- gut contents *via* microscopy and scanning electron microscopy (SEM);
- incubation studies of removals for phytoplankton and microzooplankton. These can be done as 'concentration' experiments, a series of containers with progressively more copepods. Recent work (Leising *et al.*, 2005) has shown that selection and rejection of specific phytoplankton can be demonstrated in this way, as well as showing trophic cascade effects (increases of particular particles as a result of release from microzooplankton grazing pressure by copepod predation);
- the possibly that there may be layers ('thin layers') of high phytoplankton or microzooplankton concentrations that are important to IZM copepod nutrition. We can examine their importance to ingestion by incubations with concentrated natural particles.

Hypothesis II

The feeding IZM (and other mesozooplankton) serve to modulate the upper water column production cycle via a trophic cascade – important in the east in spring, but not important, at least during the spring, in the west. We know that chlorophyll levels in the oceanic Gulf of Alaska vary between ~0.15 and ~0.6 μg l^{-1}, and progressions between those limits are gradual on time scales of days. Thus, phytoplankton must alternately increase and decrease. Since they are small and eaten primarily by microzooplankton, periods of increase are likely also periods of low microzooplankton stocks, while periods of phytoplankton decrease are likely also periods of mesozooplankton increase. Thus, feeding of IZM copepods (or other mesozooplankton) on microzooplankton could release the nano-phytoplankton from grazing pressure, leading to increasing chlorophyll. Nutrients at the onset of such an event, particularly ammonia, would be high from microheterotroph grazing in the prior phase, supporting the plant growth. Exactly why the IZM copepods would ever release the microzooplankton from feeding pressure is unclear. Turbulence from storms could disrupt distributions or search processes. On the other hand, turbulence events could improve hunting success (as suggested by a large, relatively recent body of literature).

Goals

- To make observations during the time-series sampling of abundance cycles of microheterotrophs and their phase relation to phytoplankton variations;
- To make comparisons of copepod diets between periods of higher and lower phytoplankton abundance;
- To make experimental evaluations of trophic cascade effects.

Approaches

- Phytoplankton and microheterotroph stocks will be evaluated in time-series work 2 to 4 times per day, perhaps more often.
- Diet evaluation is considered under Hypothesis I, above. Comparison of results between different phases of the chlorophyll variation could characterize the role of mesozooplankton in possible trophic cascades.
- 'Concentration' experiments (a series from 0 to many copepods per container) will focus mostly on *Neocalanus* spp. which are dominant in the upper and lower euphotic layers. These would involve big containers (order 10 liters) with recurring measures of chlorophyll, counts of microzooplankton and particle evaluation by a method like Flo-Cam.

Other mesozooplankton issues

What are the ecological significances of the small copepod species (*Oithona, Microcalanus, Pseudocalanus,* and others) that are often extremely numerous? No particular approaches were discussed; we simply raised the issue.

Results from the SUPER studies of IZM copepods showed that they can be strongly partitioned vertically, with *N. plumchrus* and *N. flemingeri* in the layer above the seasonal thermocline (~35 m) and *N. cristatus* and *E. bungii* just beneath it. We need to establish if this is a consistent pattern during the main period of copepodite development and biomass increase, and to know if this partition occurs on both sides of the Pacific, in food-limited and food-replete habitats. It was proposed that densities of these copepods are high enough that good samples can be obtained with a vertically hauled, multiple plankton sampler. Sampling rate might be 6 depth ranges, night *vs*. day, twice a week, which, over ~7 weeks would equal 168 samples. That is very many, suggesting 'adaptive analysis', that is, counting until the result is effectively certain, but not necessarily counting all samples collected. Workers would be David Mackas and Moira Galbraith in the east, and Atsushi Yamaguchi and colleagues in the west.

IV. Issues Arising During the Workshop

A. Phytoplankton Stock Variations in HNLC Systems and Trophic Cascades in the Nano and Micro Regimes

A key aim of OECOS work in the Gulf of Alaska will be a more complete understanding of short-term variation in phytoplankton stocks (see Fig. 10), which Strom *et al.* (2000) considered unexplained. A first requirement is to characterize these variations better than has been done to date. Strom *et al.* based their argument on a single 4-week time series of chlorophyll estimates at 6-hourly intervals, which seemed to show that roughly 8- to 10-day cycles, with amplitude between about 0.2 and 0.6 mg chl-*a* m^{-3}, were likely typical. That is not enough information to say that such variation is typical or to plan a very long, very expensive (in money and human effort) cruise. There were some shorter time series of chlorophyll taken on SUPER cruises in the 1980s, and those will be examined. Dr. Philip Boyd working at the Institute of Ocean Sciences, Canada, deployed some moorings at Station P in the Gulf of Alaska in the mid-1990s and has begun to provide us with long time series of fluorometer data taken from the moorings. The first data set we have seen shows comparable amplitude of variation with very strong autocorrelation (clear progressions, not chaotic changes), but the periods are mostly longer, on the order of 3 weeks and more. Both amplitude and period vary, and we need to see all of Dr. Boyd's data to discover the patterns. These data belong to him and are not published, so we do not show here even the part that we have seen.

In any case, the variations in phytoplankton standing stock need to be explained. Some of the variation at fixed (Eulerian) sites may be transferred past them by advection. However, even if that is true, the variation must be mediated by physical–biological interactions and trophic interactions. The need for revealing that ecology remains. If spatial variation and advective shifting of patterns are important, their description will provide clues to processes. Strom *et al.* (2000) suggested that iron limitation may occur at phytoplankton abundance peaks, which has been considered thoroughly in this worshop.

Several of the essays in this report have invoked a possible hypothesis explaining phytoplankton stock cycling: it could result from a trophic cascade because of (1) diet selection and (2) size-limitations on particles eaten by the dominant large copepods, that is, they mostly do not eat the smallest phytoplankton. These copepods are numerically abundant, even in the most oligotrophic, strongly HNLC regions of the subarctic Pacific. At least in spring, their food selection and limited range of food sizes could drive a cascade at lower tropic levels. Let us pick up the cycle at the (still hypothetical!) peak for microheterotrophs (MH). The copepods graze down the ciliate and hetertrophic dinoflagellate stocks, eventually taking them to levels where copepod feeding is inefficient and slows. At this same point, pico- and nanophytoplankton get a respite from grazing, since switching by the copepods to feed upon them is, at most, weak. Therefore, the phytoplankton increase and ammonia is consumed. As phytoplankton increase, the microzooplankton get better nutrition, increase and eventually reduce the phytoplankton, recycling nitrogen as ammonia. After an interval on short rations, the copepods again, have more microzooplankton to eat, and the cycle is back where we first looked at it. Notice that this does not involve iron. Iron does not need to limit pico- and nanophytoplankton for this cycle to operate; it only needs to stay low enough to inhibit maximal growth rates of micro-phytoplankton.

How could this be tested? Perhaps a time series of feeding rates of copepods on MH could be constructed. Some water would be collected, and then mixed with small (bacterial-scale) fluorescent food for ciliates. A short time would be allowed to pass to get this signal into MH. In that interval a mesozooplankton sample would be taken, and an approximately natural level of copepods from the

tow would be added to the experiment. They would be allowed time to eat but not to defecate; then they would be killed and evaluated for fluorescent gut contents using epifluorescent microscopy to count fluorescent masses in the gut right through the transparent body walls (at least this step works very well with *Neocalanus* spp.). When MH are high, ingestion should be high; when MH are low, ingestion should be low. This is just a functional response evaluation, but if it tracks the fortnightly cycle, possibly offset from it such that high rates persist as MH decline, it would be consonant with the hypothesis. Suggestions are needed.

B. Differences Between East and West in Site Selection for OECOS Time Series

OECOS Workshop participants likely to contribute to a Gulf of Alaska time-series study were in close agreement that the most interesting potential in that region is well out to sea, well into the region that has been consistently HNLC in character since recurring studies began in the 1950s. Since most of the detailed work has been done at Station P (50°N, 145°W), that is a candidate site for the OECOS eastern station. A location somewhat farther north, perhaps 52°N, would put the work closer to the top of the subarctic dome, the shallowest point along the subsurface isopleths of salinity and nutrients.

The selection of an OECOS western station presents more problems. In order to be at a site that has strong spring blooms every year, it would be necessary to be in the main flow of the Oyashio quite close to Hokkaido, perhaps at the frequently sampled Station H. This has the additional attraction of being close to facilities in Hakodate, and thus would require minimal transit time to the station. However, Station H, and areas close around it, are subject to large, advectively driven changes in conditions. Warm core rings from the Kuroshio can wash across the area, which would completely change the character of the planktonic ecosystem. Alternatively, the area is also subject to injections of very cold water emerging from the Sea of Okhotsk. While some of the standard subarctic mesozooplankton would be present in this water, they would very likely be on completely different schedules from populations present at other times. Thus, there would be a risk of chaotic data.

At stations progressively farther to the northeast, say at 50°N, 158°E, there would be a chance that no bloom would be encountered, and that fully HNLC conditions would persist throughout the entire developmental season of IZM copepods. Thus, the comparisons of growth and development between food-replete (western) and food-limited (eastern) conditions could be lost. In addition, stations well to the northeast involve very long transits, up to 10 days from Tokyo, for example, which would reduce the time on station dramatically. These issues were not resolved at the workshop. It is clear they must be left to discussions among OECOS participants in Asia. They know the territory and the aspects of scientific politics that would also be involved.

C. Timing of OECOS Expeditions

It is likely that differences between North American and Asian OECOS participants, in the requirements for obtaining funding and ship time, could force OECOS work in the west and east to be done in different years. At the meeting, Japanese oceanographers felt that the best timing for OECOS western work would be an expedition in April–May 2007, and had reason to think ship time could be obtained for that year. In the United States, oceanographers are facing serious shortfalls in ship funding, and major ship lay-ups are planned for 2006. When those ships are brought

back on-line in 2007, there will be long backlogs of work already funded and ahead of OECOS in the queue for a large vessel. Moreover, the only likely scenario for funding also makes the earliest opportunity for work in the spring of 2008. That scenario is a proposal that was submitted in early 2006 and would be rejected the following summer (nothing here is funded at first request). A second proposal with serious improvements is not possible before the deadlines of early 2007. That proposal would not be reviewed by the spring of 2007, making the spring of 2008 the almost certain earliest opportunity. Therefore, the Eastern Group will try for an expedition in 2008.

After some discussion, it was decided that working in different years is probably not a significant drawback for the comparison of eastern and western OECOS data sets. Year-to-year variations of weather and ocean conditions affect the two regions differently, and the advective transfer of water (and plankton stocks, *etc.*) along the west-wind drift takes several years. Moreover, work in separate years will have some advantages.

- It is possible that we can arrange some exchange of scientists between Eastern and Western Groups for participation in both cruises. It is obvious that participation of North American scientists on a Japanese cruise would pose language problems, particularly for communication with the ship's crew. That is less likely a problem for Japanese scientists participating in the eastern expedition, since they all have reasonably strong skills in English. Careful consideration of cultural differences in scientific operations at sea should be included in evaluating this possibility.

- Difficulties with methods encountered by the Western OECOS Group in the first cruise could possibly be worked out for the eastern cruise a year later. It will be better to have failure-proof methods throughout, but contingencies always affect oceanographic work.

D. CHARACTERIZATION OF PHYSICAL OCEANOGRAPHY

Comments from the Japanese participants after the OECOS Workshop show that most of them feel a strong need for physical oceanographers to participate in their work. They will be considering exactly what physics (mixing, advection, microstructure) needs to be evaluated in the western gyre and will be seeking appropriate workers among their colleagues. Work in the oceanic eastern sector will include solid and recurring physical characterization of the time-series site, and many of the OECOS biological workers, particularly Drs. Strutton and Cowles, have extended experience in interpreting physical data. If a SeaSoar spatial characterization of a large area around the time-series station is to be included, that work will surely entail a separate proposal from physical oceanographers not yet included in OECOS discussions.

V. Concluding Remarks

The contents of this report do not include a detailed plan for what will eventually be proposed for time-series oceanographic observations during the spring transition in western and eastern sectors of the subarctic Pacific. In fact, we did not develop such plans at the Workshop; just exchanging views on the character of the scientific questions to be addressed occupied all of the time.

This report is simply a compendium of information exchanged. Even as such, it leaves out much that was included in the talks (and PowerPoint presentations) given at the Workshop. For both Western and Eastern Working Groups, much remains to be achieved regarding choice of observations, station selection, basic modes of station keeping (drifter following *vs*. fixed stations) and logistics. There are even open issues regarding the character of ecological phenomena to be studied and regarding what questions will be addressed. All of this implies that much work lies ahead to make OECOS as useful as possible, and to justify what will be substantial costs.

Charlie Miller and *Tsutomu Ikeda*
OECOS Workshop Organizers, 2005

VI. References[1]

Abbott, M.R. and Letelier, R.M. 1998. Decorrelation scales of chlorophyll as observed from bio-optical drifters in the California Current. *Deep-Sea Res. II* **45**: 1639–1667.

Anger, K. 2001. The Biology of Decapod Crustacean Larvae. *In* Crustacean Issues 14. *Edited by* R. Vonk, A.A. Balkema Publ., Lisse, 420 pp.

Arrigo, K.R., Worthen, D.L. and Robinson, D.H. 2003. A coupled ocean-ecosystem model of the Ross Sea: 2. Iron regulation of phytoplankton taxonomic variability and primary production. *J. Geophys. Res.* **108**: 3231, doi:10.1029/2001 JC000856.

Aumont, O., Maier-Reimer, E., Blain, S. and Monfrey, P. 2003. An ecosystem model of the global ocean including Fe, Si, P colimitations. *Global Biogeochem. Cycles* **17**: 1060, doi:10.1029/2001 GB001745.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. and Thingstad, F. 1983. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* **10**: 257–263.

Banse, K., and English, D.C. 1994. Seasonality of coastal zone color scanner phytoplankton pigment in the offshore oceans. *J. Geophys. Res.* **99**: 7323–7345.

Banse, K. and English, D.C. 1999. Comparing phytoplankton seasonality in the eastern and western subarctic Pacific and the western Bering Sea. *Prog. Oceanogr.* **43**: 235–288.

Barbeau, K., Moffett, J.W., Caron, D.A., Croot, P.L. and Erdner, D.L. 1996. Role of protozoan grazing in relieving iron limitation of phytoplankton. *Nature* **380**: 61–64.

Barbeau, K., Rue, E.L., Trick, C.G., Bruland, K.W. and Butler, A. 2003. Photochemical reactivity of siderophores produced by marine heterotrophic bacteria and cyanobacteria based on characteristic Fe(III) binding groups. *Limnol. Oceanogr.* **48**: 1069–1078.

Batchelder, H.P. and Miller, C.B. 1989. Life history and population dynamics of *Metridia pacifica*: results from simulation modeling. *Ecol. Model.* **48**: 113–136.

Batchelder, H.P. and Williams, R. 1995. Individual-based modeling of the population dynamics of *Metridia lucens* in the North Atlantic. *ICES J. Mar. Sci.* **52**: 469–482.

Batten, S.D., Welch, D.W. and Jonas, T. 2003. Latitudinal differences in the duration of development of *Neocalanus plumchrus* copepodites. *Fish. Oceanogr.* **12**: 201–208.

Beamish, R.J., Leask, K.D., Ivanov, O.A., Balanov, A.A., Orlov, A.M. and Sinclair, B. 1999. The ecology, distribution, and abundance of midwater fishes of the subarctic Pacific gyres. *Prog. Oceanogr.* **43**: 399–442.

Behrenfeld, M.J. and Falkowski, P.G. 1997a. Photosynthetic rates derived from satellite-based chlorophyll concentration. *Limnol. Oceanogr.* **42**: 1–20.

Behrenfeld, M.J. and Falkowski, P.G. 1997b. A consumer guide to primary productivity models. *Limnol. Oceanogr.* **42**: 1479–1491.

Behrenfeld, M.J, Bale, A., Kolber, Z.S., Aiken, J. and Falkowski, P.G. 1996. Confirmation of iron limitation of phytoplankton photosynthesis in the equatorial Pacific Ocean. *Nature* **383**: 508–511.

Bertram, D.F., Mackas, D.L. and McKinnell, S.M. 2001. The seasonal cycle revisited: interannual variation and ecosystem consequences. *Prog. Oceanogr.* **49**: 283–307.

Bishop, J.K.B. 1999. Transissometer measurement of POC. *Deep-Sea Res. I* **46**: 353–369.

Bishop, J.K.B., Davis, R.E. and Sherman, J.T. 2002. Robotic observations of dust storm enhancement of carbon biomass in the North Pacific. *Science* **298**: 817–821.

Bograd, S.J., Thomson, R.E., Rabinovich, A.B. and LeBlond, P.H. 1999. Near-surface circulation of the northeast Pacific Ocean derived from WOCE-SVP satellite-tracked drifters. *Deep-Sea Res. II* **46**: 2371–2403.

Booth, B.C., Lewin, J. and Postel, J.R. 1993. Temporal variation in the structure of autotrophic and heterotrophic communities in the subarctic Pacific. *Prog. Oceanogr.* **32**: 57–99.

Bowie, A.R., Achterberg, E.P., Sedwick, P.N., Ussher, S. and Worsfold, P.J. 2002. Real-time monitoring of picomolar concentrations of iron(II) in marine waters using automated flow injection-chemiluminescence instrumentation. *Environ. Sci. Technol.* **36**: 4600–4607.

Boyd, P. and Harrison, P.J. 1999. Phytoplankton dynamics in the NE subarctic Pacific. *Deep-Sea Res. II* **46**: 2405–2432.

Boyd, P.W. and Harrison, P.J. (*Eds.*) 2006. SERIES (subarctic ecosystem response to iron enrichment study): A Canadian–Japanese Contribution to Our Understanding of the Iron–Ocean–Climate Connection. *Deep-Sea Res. II* **53** (in press)

Boyd, P.W., Strom, S., Whitney, F.A., Doherty, S., Wen, M.E., Harrison, P.J., Wong, C.S. and Varela, D.E. 1995a. The NE subarctic Pacific in winter:

I. Biological standing stocks. *Mar. Ecol. Prog. Ser.* **128**: 11–24.

Boyd, P.W., Whitney, F.A., Harrison, P.J. and Wong, C.S. 1995b. The NE subarctic Pacific in winter: II. Biological rate processes. *Mar. Ecol. Prog. Ser.* **128**: 25–34.

Boyd, P.W., Law, C.S., Wong, C.S., Nojiri, Y., Tsuda, A., Levasseur, M., Takeda, S., Rivkin, R., Harrison, P.J., Strzepek, R., Gower, J., Mckay, R.M., Abraham, E., Arychuk, M., Barwell-Clarke, J., Crawford, W., Crawford, D., Hale, M., Harada, K., Johnson, K., Kiyosawa, H., Kudo, I., Marchetti, A., Miller, W., Needoba, J., Nishioka, J., Ogawa, H., Page, J., Robert, M., Saito, H., Sastri, A. and Sherry, N. 2004. The decline and fate of an iron-induced subarctic phytoplankton bloom. *Nature* **428**: 549–553.

Calbet, A. and Landry, M.R. 1999. Mesozooplankton influences on the microbial food web: direct and indirect trophic interactions in the oligotrophic open ocean. *Limnol. Oceanogr.* **44**: 1370–1380.

Campbell, L. and Carpenter, E.J. 1986. Diel patterns of cell division in marine *Synechococcus* spp. (Cynechococcus): use of the frequency of dividing cells technique to measure growth rate. *Mar. Ecol. Prog. Ser.* **32**: 139–148.

Campbell, R.G., Runge, J.A. and Durbin, E.G. 2000. Evidence for food limitation of *Calanus finmarchicus* production on the southern flank of Georges Bank during April 1997. *Deep-Sea Res. II* **48**: 531–549.

Campbell, R.G., Wagner, M.M., Teegarden, G.J., Boudreau, C.A. and Durbin, E.G. 2001. Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. *Mar. Ecol. Prog. Ser.* **221**: 161–183.

Campbell, R.W. 2003. Overwintering ecology and ecophysiology of *Neocalanus plumchrus*. Ph.D. thesis, School of Earth and Ocean Sciences, Univ. of Victoria, Victoria, Canada.

Capriulo, G.M. and Carpenter, E.J. 1980. Grazing by 35 to 202 µm micro-zooplankton in Long Island Sound. *Mar. Biol.* **56**: 319–326.

Chavez, F.P., Pennington, J.T., Herlien, R., Jannasch, H., Thurmond, G. and Friederich, G.E. 1997. Moorings and drifters for real-time interdisciplinary oceanography. *J. Atmos. Oceanic Technol.* **14**: 1199–1211.

Chevalier, W. 2001. A comparison of rates of dissolved organic carbon production through phytoplankton extracellular release and through zooplankton grazing. M.Sc. thesis, California State Univ., Stanislaus, CA, U.S.A.

Chiba, S., Ono, T., Tadokoro, T., Midorikawa, T. and Saino, T. 2004. Increased stratification and decreased lower trophic level productivity in the Oyashio region of the North Pacific: A 30-year retrospective study. *J. Oceanogr.* **60**: 149–162.

Childress, J.J. and Mickel, T.J. 1980. A motion compensated shipboard precision balance system. *Deep-Sea Res.* **27A**: 965–970.

Coale, K.H., Fitzwater, S.E., Gordon, R.M., Johnson, K.S. and Barber, R.T. 1996. Control of community growth and export production by upwelled iron in the equatorial Pacific Ocean. *Nature* **379**: 621–624.

Coats, D.W. and Heinbokel, J.F. 1982. A study of reproduction and other life cycle phenomena in planktonic protists using an acridine orange fluorescence technique. *Mar. Biol.* **67**: 71–79.

Cowles, T.J. 2003. Planktonic layers: Physical and biological interactions on the small scale. pp. 31–49. *In* Handbook of Scaling Methods in Aquatic Ecology: Measurements, Analysis, Simulation. *Edited by* L. Seuront and P.G. Strutton, CRC Press, Boca Raton, FL, 624 pp.

Crain, J.A. and Miller, C.B. 2000. Effects of starvation on intermolt development in *Calanus finmarchicus* copepodites: comparison between theoretical models and field studies. *Deep-Sea Res. II* **48**: 551–566.

Croot, P.L. and Johansson, M. 2000. Determination of iron speciation by cathodic stripping voltammetry in seawater using the competing ligand 2-(2-Thiazolylazo)-p-cresol (TAC). *Electroanalysis* **12**: 565–576.

Croot, P.L. and Laan, P. 2002. Continuous shipboard determination of Fe(II) in polar waters using flow injection analysis with chemiluminescence detection. *Anal. Chim. Acta* **466**: 261–273.

Cullen, J.T. and Sherrell, R.M. 1999. Techniques for determination of trace metals in small samples of size-fractionated particulate matter: phytoplankton metals off central California. *Mar. Chem.* **67**: 233–247.

Dagg, M.J. 1977. Some effects of patchy food environments on copepods. *Limnol. Oceanogr.* **22**: 99–107.

Dagg, M.J. 1991. *Neocalanus plumchrus* (Marukawa): Life in the nutritionally dilute subarctic Pacific Ocean and the phytoplankton rich Bering Sea. *Bull. Plankton Soc. Japan, Spec. Vol.*, pp. 217–225.

Dagg, M. 1993a. Grazing by the copepod community does not control phytoplankton production in the subarctic Pacific Ocean. *Prog. Oceanog.* **32**: 163–183.

Dagg, M. 1993b. Sinking particles as a possible source of nutrition for the large calanoid copepod *Neocalanus cristatus* in the subarctic Pacific Ocean. *Deep-Sea Res.* **40**: 1431–1445.

Dagg, M.J. and Wyman, K.D. 1983. Natural ingestion rates of the copepods *Neocalanus plumchrus* and

N. cristatus calculated from gut contents. *Mar. Ecol. Prog. Ser.* **13**: 37–46.

Dagg, M.J., Vidal, J., Whitledge, T.E., Iverson, R.L. and Goering, J.J. 1982. The feeding, respiration and excretion of zooplankton in the Bering Sea during a spring bloom. *Deep-Sea Res.* **29**: 45–63.

Dagg, M.J. and Walser, Jr., W.E. 1986. The effect of food concentration on fecal pellet size in marine copepods. *Limnol. Oceanogr.* **31**: 1066–1071.

Dagg, M.J., Liu, H. and Thomas, A.C. 2006. Effects of mesoscale phytoplankton variability on the copepods *Neocalanus flemingeri* and *N. plumchrus* in the coastal Gulf of Alaska. *Deep-Sea Res.* **53:** 321-332.

D'Asaro, E., Eriksen, C.C., Levine, M.A., Niiler, P., Paulson, C.A. and van Meurs, P. 1995. Upper-ocean inertial currents forced by a strong storm. Part I. Data and comparisons with linear theory. *J. Phys. Oceanogr.* **25**: 2909–2936.

Davis, R.E., de Szoeke, R.A. and Niiler, P.P. 1981. Variability in the upper ocean during MILE. Part II: Modeling the mixed layer response. *Deep-Sea Res.* **28**: 1453–1475.

Denman, K. and Gargett, A. 1988. Multiple thermoclines are barriers to vertical exchange in the subarctic Pacific during SUPER, May 1984. *J. Mar. Res.* **46**: 77–103.

Denman, K.L. and Peña, M.A. 1999. A coupled 1-D biological/physical model of the northeast subarctic Pacific Ocean with iron limitation. *Deep-Sea Res. II* **46**: 2877–2908.

Denman, K.L. and Peña, M.A. 2002. The response of two coupled 1-D mixed layer/planktonic ecosystem models to climate change in the NE subarctic Pacific Ocean. *Deep-Sea Res. II* **49**: 5739–5757.

Dolan, J.R. and McKeon, K. 2004. The reliability of grazing rate estimates from dilution experiments: have we over-estimated rates of organic carbon consumption? *Ocean Sci. Discussions* **1**: 21–36.

Dolan, J.R. and Simek, K. 1998. Ingestion and digestion of an autotrophic picoplankter, *Synechococcus*, by a heterotrophic nanoflagellate, *Bodo saltans*. *Limnol. Oceanogr.* **43**: 1740–1746.

Doucette, G.J., Erdner, D.L., Peleato, M.L., Hartman, J.J. and Anderson, D.M. 1996. Quantitative analysis of iron-stress related proteins in *Thalassiosira weissflogii*: Measurement of flavodoxin and ferredoxin using HPLC. *Mar. Ecol. Prog. Ser.* **130**: 269–276.

Dutkiewicz, S., Follows, M.J. and Parekh, P. 2005. Interactions of iron and phosphorus cycles: A three-dimensional model study. *Global Biogeochem. Cycles* **19**: GV1021, doi:10.1029/ 2004GV002342.

Edwards, C.A., Batchelder, H.P. and Powell, T.M. 2000a. Modeling microzooplankton and macro-zooplankton dynamics within a coastal upwelling system. *J. Plankton Res.* **22**: 1619–1648.

Edwards, C.A., Batchelder, H.P. and Powell, T.M. 2000b. The stability of an NPZ model subject to realistic levels of vertical mixing. *J. Mar. Res.* **58**: 37–60.

Erdner, D.L. 1997. Ferredoxin and flavodoxin as biochemical indicators of iron limitation in marine eukaryotic phytoplankton. Ph.D. thesis, MIT/ WHOI Joint Program in Oceanography.

Erdner, D.L. and Anderson, D.M. 1999. Ferredoxin and flavodoxin as biochemical indicators of iron limitation during open-ocean iron enrichment. *Limnol. Oceanogr*. **44**: 1609–1615.

Erdner, D.L., Price, N.M., Doucette, G.J., Peleato, M.L. and Anderson, D.M. 1999. Characterization of ferredoxin and flavodoxin as markers of iron limitation in marine phytoplankton. *Mar. Ecol. Prog. Ser*. **184**: 43–53.

Evanson, M., Bornhold, E.A., Goldblatt, R.H., Harrison, P.J. and Lewis, A.G. 2000. Temporal variation in body composition and lipid storage of the overwintering, subarctic copepod *Neocalanus plumchrus* in the Strait of Georgia, British Columbia (Canada). *Mar. Ecol. Prog. Ser.* **192**: 239–247.

Falkowski, P.G. and Raven, J.A. 1997. Aquatic Photosynthesis. Blackwell, Oxford, 375 pp.

Fasham, M.J.R. 1995. Variations in the seasonal cycle of biological production in subarctic oceans: a model sensitivity analysis. *Deep-Sea Res*. **42**: 1111–1149.

Favorite, F., Dodimead, A.J. and Nasu, K. 1976. Oceanography of the subarctic Pacific region, 1960–1971. *Bull. Intl. North Pacific Fish. Comm.* **3**: 1–187.

Fowler, S.W. and Knauer, G.A. 1986. Role of large particles in the transport of elements and organic compounds through the oceanic water column. *Prog. Oceanogr*. **16**: 147–194.

Franks, P.J.S. and Walstad, L.J. 1997. Phytoplankton patches at fronts: a model of formation and response to wind events. *J. Mar. Res.* **55**: 1–29.

Frost, B.W. 1983. Interannual variation of zooplankton standing stock in the open Gulf of Alaska. pp. 146–157. *In* From Year to Year: Interannual Variability of the Environment and Fisheries of the Gulf of Alaska and the Eastern Bering Sea. *Edited by* W.S Wooster, University of Washington Sea Grant, Seattle.

Frost, B.W. 1987. Grazing control of phytoplankton stock in the open subarctic Pacific Ocean: a model assessing the role of mesozooplankton, particularly the large calanoid copepods, *Neocalanus* spp. *Mar. Ecol. Prog. Ser*. **83**: 291–303.

Frost, B.W. 1993. A modelling study of processes regulating plankton standing stock and production in the open subarctic Pacific Ocean. *Prog. Oceanogr*. **32**: 17–56.

Frost, B.W. and Kishi, M.J. 1999. Ecosystem dynamics in the eastern and western gyres of the Subarctic Pacific—a review of lower trophic level modeling. *Prog. Oceanogr*. **43**: 317–333.

Frost, B.W., Landry, M.R. and Hassett, R.P. 1983. Feeding behavior of large calanoid copepods *Neocalanus cristatus* and *N. plumchrus* from the subarctic Pacific Ocean. *Deep-Sea Res.* **30**: 1–13.

Fulton, J.D. 1983. Seasonal and annual variations of net zooplankton at Ocean Station P, 1956–1980. *Can. Data Rep. Fish. Aquat. Sci*. **374**: 65 pp.

Fung, I.Y., Meyn, S.K., Tegen, I., Doney, S.C., John, J.G. and Bishop, J.K.B. 2000. Iron supply and demand in the upper ocean. *Global Biogeochem. Cycles* **14**: 281–295.

Furuya, K. and Harada, K. 1995. An automated precise Winkler titration for determining dissolved oxygen on board ship. *J. Oceanogr*. **51**: 375–383.

Gifford, D.J. 1993. Protozoa in the diets of *Neocalanus* spp. in the oceanic subarctic Pacific Ocean. *Prog. Oceanogr*. **32**: 223–237.

Gifford, D.J. and Dagg, M.J. 1991. The microzooplankton-mesozooplankton link: consumption of planktonic protozoa by the calanoid copepods *Acartia tonsa* Dana and *Neocalanus plumchrus* Marukawa. *Mar. Microb. Food Webs* **5**: 161–177.

Gledhill, M. and Van den Berg, C.M.G. 1994. Determination of complexation of iron (III) with natural organic complexing ligands in seawater using cathodic stripping voltammetry. *Mar. Chem.* **4**7: 41–54.

Goes, J.I., Gomes, H.R., Limsakul, A., Balch, W.M. and Saino, T. 2001. El Niño related interannual variations in biological production in the North Pacific as evidenced by satellite and ship data. *Prog. Oceanogr*. **49**: 211–225.

Goes, J.I., Sasaoka, K., Gomes, H.R., Saitoh, S. and Saino, T. 2004. A comparison of the seasonality and interannual variability of phytoplankton biomass and production in the western and eastern gyre of the subarctic Pacific using multi-sensor satellite data. *J. Oceanogr*. **60**: 75–92.

Goldblatt, R.H., Mackas, D.L. and Lewis, A.G. 1999. Mesozooplankton characteristics in the NE subarctic Pacific. *Deep-Sea Res. II* **46**: 2619–2644.

Gowing, M.M. and Garrison, D.L. 1992. Abundance and feeding ecology of larger protozooplankton in the ice edge zone of the Weddell and Scotia Seas during the austral winter. *Deep-Sea Res.* **39**: 893–919.

Harrison, P.J. 2002. Station Papa time series: insights into ecosystem dynamics. *J. Oceanogr*. **58**: 259–264.

Harrison, P.J., Boyd, P.W., Varela, D.E., Takeda, S. Shiomoto, A. and Odate, T. 1999. Comparison of factors controlling phytoplankton productivity in the NE and NW subarctic Pacific gyres. *Prog. Oceanogr*. **43**: 205–234.

Hayase, K. and Shinozuka, N. 1995. Vertical distribution of fluorescent organic matter along with AOU and nutrients in the equatorial Central Pacific. *Mar. Chem.* **48**: 283–290.

Hirche, H.J., Brey, T. and Niehoff, B. 2001. A high-frequency time series at ocean weather ship station M (Norwegian Sea): population dynamics of *Calanus finmarchicus*. *Mar. Ecol. Progr. Ser.* **219**: 205–219.

Hirst, A.G. and Kiørboe, T. 2002. Mortality of marine planktonic copepods: global rates and patterns. *Mar. Ecol. Progr. Ser*. **230**: 195–209.

Hirst, A.G. and Lampitt, R.S. 1998. Towards a global model of in situ weight-specific growth in marine planktonic copepods. *Mar. Biol.* **132**: 247–257.

Hirst, A.G. and Sheader, M. 1997. Are in situ weight-specific growth rates body-size independent in marine planktonic copepods? A re-analysis of the global syntheses and a new empirical model. *Mar. Ecol. Prog. Ser*. **154**: 155–165.

Honda, M. 2000. Study on the carbon cycle in the western North Pacific using sediment traps and ^{14}C. Ph.D. thesis, Hokkaido Univ., Hakodate, Japan.

Hopcroft, R.R., Roff, J.C., Webber, M.K. and Witt, J.D.S. 1998. Zooplankton Growth Rates: the influence of size and resources in tropical marine copepodites. *Mar. Biol.* **132**: 67–77.

Huntley, M.E. and Lopez, M.D.G. 1992. Temperature-development production of marine copepods: a global synthesis. *The American Naturalist* **140**: 201–242.

Ikeda, T. and Motoda, S. 1978. Estimated zooplankton production and their ammonia excretion in the Kuroshio and adjacent seas. *Fish. Bull.***76**: 357–367.

Johnson, C.L. 2004. Seasonal variation in the molt status of an oceanic copepod. *Prog. Oceanogr*. **62**: 15–32.

Kameda, T. and Ishizaka, J. 2005. Size-fractionated primary production estimated by a two-phytoplankton community model applicable to ocean color remote sensing. *J. Oceanogr.* **61**: 663–672.

Karl, D.M., Hebel, D.V. and Bjorkman, K. 1998. The role of dissolved organic matter release in the productivity of the oligotrophic North Pacific Ocean. *Limnol. Oceangr*. **43**: 1270–1286.

Kasai, H., Saito, H., Yoshimori, A. and Taguchi, S. 1997. Variability in timing and magnitude of spring bloom in the Oyashio region, the western subarctic Pacific off Hokkaido, Japan. *Fish. Oceanogr.* **6**: 118–129.

Kawamura, A. 1982. Food habits and prey distributions of three rorqual species in the North Pacific Ocean. *Sci. Rep. Whales Res. Inst.* **34**: 59–91.

Kimmerer, W.J. 1983. Direct measurement of the production:biomass ratio of the subtropical calanoid copepod *Acrocalanus inermis*. *J. Plankton Res.* **5**: 1–14.

Kimmerer, W.J. and McKinnon, A.D. 1987. Growth, mortality and secondary production of the copepod *Acartia tranteri* in Westernport Bay, Australia. *Limnol. Oceanogr.* **32**: 14–28

Kobari, T. and Ikeda, T. 1999. Vertical distribution, population structure and life cycle of *Neocalanus cristatus* (Crustacea: Copepoda) in the Oyashio region, with notes on its regional variations. *Mar. Biol.* **134**: 683–696.

Kobari, T. and Ikeda, T. 2000. Life cycle of *Neocalanus* species in the Oyashio region. *Bull. Plankton Soc. Jpn.* **47**: 129–135.

Kobari, T. and Ikeda, T. 2001a. Life cycle of *Neocalanus flemingeri* (Crustacea: Copepoda) in the Oyashio region, western subarctic Pacific, with notes on its regional variations. *Mar. Ecol. Prog. Ser.* **209**: 243–255.

Kobari, T. and Ikeda, T. 2001b. Ontogenetic vertical migration and life cycle of *Neocalanus plumchrus* (Crustacea: Copepoda) in the Oyashio region, with notes on regional variations in body size. *J. Plankton Res.* **23**: 287–302.

Kobari, T., Ikeda, T., Kanno, Y., Shiga, N., Takagi, S. and Azumaya, T. 2003a. Interannual variations in abundance and body sizes in *Neocalanus* copepods in the central North Pacific. *J. Plankton Res.* **25**: 483–494.

Kobari, T., Shinada, A. and Tsuda, A. 2003b. Functional roles of interzonal migrating mesozooplankton in the western subarctic Pacific. *Prog. Oceanogr.* **57**: 279–298.

Kolber, Z. and Falkowski, P.W. 1993. The use of active fluorescence to measure phytoplankton photosynthesis in situ. *Limnol. Oceanogr.* **38**: 1646–1665.

Kuma, K., Nishioka, J. and Matsunaga, K. 1996. Controls on iron(III) hydroxide solubility in seawater: the influence of pH and natural organic chelators. *Limnol. Oceanogr.* **4**: 396–407.

Landry, M.R. and Lehner-Fournier, J.M. 1988. Grazing rates and behavior of *Neocalanus plumchrus*: implications for phytoplankton control in the subarctic Pacific. *Hydrobiologia* **167/168**: 9–19.

Landry, M.R., Gifford, D.J., Kirchman, D.L., Wheeler, P.A. and Monger, B.C. 1993a. Direct and indirect effects of grazing by *Neocalanus plumchrus* on plankton community dynamics in the subarctic Pacific. *Prog. Oceanogr.* **32**: 239–258.

Landry, M.R., Monger, B.C. and Selph, K.E. 1993b. Time-dependency of microzooplankton grazing and phytoplankton growth in the subarctic Pacific. *Prog. Oceanogr.* **32**: 205–222.

Landry, M.R., Kirshtein, J. and Constantinou, J. 1995. A redefined technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial Pacific. *Mar. Ecol. Prog. Ser.* **120**: 53–63.

LaRoche, J., Murray, H., Orellana, M. and Newton, J. 1995. Flavodoxin expression as an indicator of iron limitation in marine diatoms. *J. Phycol.* **31**: 520–530.

LaRoche, J., Boyd, P.W., McKay, R.M.L. and Geider, R.J. 1996. Flavodoxin as an *in situ* marker for iron stress in phytoplankton. *Nature* **382**: 802–805.

Laws, E.A. 1984. Improved estimates of phytoplankton carbon based on ^{14}C-incorporation into chlorophyll a. *J. Theor. Biol.* **110**: 425–434.

Laws, E.A., Landry, M.R., Barber, R.T., Campbell, L., Dickinson, M. and Marra, J. 2000. Carbon cycling in primary production bottle incubations: inferences from grazing experiments and photosynthetic studies using ^{14}C and ^{18}O in the Arabian Sea. *Deep-Sea Res. I* **47**: 1339–1352.

Leising, A.W., Pierson, J.J., Halsband-Lenk, C., Horner, R. and Postel, J. 2005. Copepod grazing during spring blooms: Does *Calanus pacificus* avoid harmful diatoms? *Prog. Oceanogr.* **67**: 384–405.

Leonard, C.L., McClain, C.R., Murtegudde, R., Hofmann, E.E. and Harding, Jr., L.W. 1999. An iron-based ecosystem model of the central equatorial Pacific. *J. Geophys. Res.* **104**: 1325–1341.

Lessard, E.J. and Murrell, M.C. 1998. Microzooplankton herbivory and phytoplankton growth in the northwestern Sargasso Sea. *Aquat. Microb. Ecol.* **16**: 173–188.

Liu, H., Suzuki, K. and Saino, T. 2002. Phytoplankton growth and microzooplankton grazing in the subarctic Pacific Ocean and the Bering Sea during summer 1999. *Deep-Sea Res. I* **49**: 363–375.

Liu, H., Dagg, M.J. and Strom, S. 2005. Grazing by the calanoid copepod *Neocalanus cristatus* on the microbial foodweb in the coastal Gulf of Alaska. *J. Plankton Res.* **27**: 647–662.

Mackas, D.L. and Batten, S. 2005. Effects on zooplankton of a warming ocean: Recent evidence from the North Pacific. p. 18, Symposium on

Climate Variability and Sub-Arctic Marine Ecosystems, Victoria, B.C., Canada, 16–20 May 2005.

Mackas, D.L. and Tsuda, A. 1999. Mesozooplankton in the eastern and western subarctic Pacific: community structure, seasonal life histories, and interannual variability. *Prog. Oceanogr.* **43**: 335–363.

Mackas, D.L., Sefton, H., Miller, C.B. and Raich, A. 1993. Vertical habitat partitioning by large calanoid copepods in the oceanic subarctic Pacific during Spring. *Prog. Oceanogr.* **32**: 259–294.

Mackey M.D., Mackey D.J., Higgins H.W. and Wright, S.W. 1996. CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. *Mar. Ecol. Prog. Ser.* **144**: 265–283.

Mackas, D.L., Goldblatt, R. and Lewis, A.J. 1998. Interdecadal variation in developmental timing of *Neocalanus plumchrus* populations at Ocean Station P in the subarctic North Pacific. *Can. J. Fish. Aquat. Sci.* **55**: 1878–1893.

Mahowald, N., Kohfeld, K., Hansson, M., Balkanski, Y., Harrison, S., Prentice, I., Schultz, M. and Rodhe, H. 1999. Dust sources and deposition during the Last Glacial Maximum and current clime: A comparison of model results with paleodata from ice cores and marine sediments. *J. Geophys. Res.* **104**: 15,895–15,916.

Maldonado, M.T. and Price, N.M. 2001. Reduction and transport of organically bound iron by *Thalassiosira oceanica* (Bacillariophyceae). *J. Phycol.* **37**: 298–310.

Maldonado, M.T., Boyd, P.W., LaRoche, J., Strzepek, R., Waite, A., Bowie, A.R., Croot, P.L., Frew, R.D. and Price, N.M. 2001. Iron uptake and physiological response of phytoplankton during a mesoscale Southern Ocean iron enrichment. *Limnol. Oceanogr.* **46**: 1802–1808.

Martin, J.H. and Fitzwater, S. 1988. Iron deficiency limits phytoplankton growth in the northeast Pacific subarctic. *Nature* **331**: 341–343.

Martin, J.H., Gordon, R.M., Fitzwater, S.E. and Broenkow, W.W. 1989. VERTEX: Phytoplankton/ iron studies in the Gulf of Alaska. *Deep-Sea Res.* **36**: 649–680.

Maucher, J.M. and DiTullio, G.R. 2003. Flavodoxin as a diagnostic indicator of chronic iron limitation in the Ross Sea and New Zealand sector of the Southern Ocean. *Antarctic Res. Ser.* **78**: 209–219.

McDuff, R.E. and Chisholm, S.W. 1982. The calculation of in situ growth rates of phytoplankton populations from fractions of cells undergoing mitosis: a clarification. *Limnol. Oceanogr.* **27**: 783–788.

McKay, R.M.L., LaRoche, J., Yakunin, A.F., Durnford, D.G. and Geider, R.J. 1999. Accumulation of ferredoxin and flavodoxin in a marine diatom in response to Fe. *J. Phycol.* **35**: 510–519.

Measures, C.I. and Vink, S. 2000. On the use of dissolved aluminum in surface waters to estimate dust deposition to the ocean. *Global Biogeochem. Cycles* **14**: 317–327.

Miller, C.B. 1993a. Development of large copepods during spring in the Gulf of Alaska. *Prog. Oceanogr.* **32**: 295–317.

Miller, C.B. 1993b. Pelagic production processes in the Subarctic Pacific. *Prog. Oceanogr.* **32**: 1–15.

Miller, C.B. and Clemons, M. 1988. Revised life history analysis for large grazing copepods in the subarctic Pacific Ocean. *Prog. Oceanogr.* **20**: 293–313.

Miller, C.B. and Nielsen, R.D. 1988. Development and growth of large, calanid copepods in the ocean subarctic Pacific, May 1984. *Prog. Oceanogr.* **20**: 275–292.

Miller, C.B. and Tande, K. 1993. Stage duration estimation for *Calanus* populations, a modelling study. *Mar. Ecol. Progr. Ser.* **102**: 15–34.

Miller, C.B., Huntley, M.E. and Brooks, E.R. 1984a. Post-collection molting rates of planktonic marine copepods: measurement, applications, problems. *Limnol. Oceanogr.* **29**: 1274–1289.

Miller, C.B., Frost, B.W., Batchelder, H.P., Clemons, M.J. and Conway, R.E. 1984b. Life histories of large, grazing copepods in a subarctic ocean gyre: *Neocalanus plumchrus*, *Neocalanus cristatus*, and *Eucalanus bungii* in the Northeast Pacific. *Prog. Oceanogr.* **13**: 201–243.

Miller, C.B., Frost, B.W., Wheeler, P.A., Landry, M.R., Welschmeyer, N. and Powell, T.M. 1991. Ecological dynamics in the subarctic Pacific, a possibly iron-limited ecosystem. *Limnol. Oceanogr.* **36**: 1600–1615.

Miller, C.B., Fulton, J. and Frost, B.W. 1992. Size variation of *Neocalanus plumchrus* and *Neocalanus flemingeri* in a 20-yr sample series from the Gulf of Alaska. *Can. J. Fish. Aquat. Sci.* **49**: 389–399.

Miller, C.B., Crain, J.A. and Morgan, C.A. 2000. Oil storage variability in *Calanus finmarchicus*. *ICES J. Mar. Sci.* **57**: 1786–1799.

Monger, B.C. and Landry, M.R. 1993. Cytometric analysis of marine bacteria with Hoechst 33342. *Appl. Environ. Microbiol.* **59**: 905–911.

Moore, J.K., Doney, S.C. and Lindsay, K. 2004. Upper ocean ecosystem dynamics and iron cycling in a global three-dimensional model. *Global Biogeochem. Cycles* **18**: GB4028, doi:10.1029/ 2004GB002220

Müller, H. and Geller, W. 1993. Maximum growth rates of aquatic ciliated protozoa: the dependence on body size and temperature reconsidered. *Arch. Hydrobiol.* **126**: 315–327.

Nakabayashi, S., Kusakabe, M., Kuma, K. and Kudo, I. 2001. Vertical distributions of iron(III) hydroxide solubility and dissolved iron in the northwestern North Pacific Ocean. *Geophys. Res. Lett.* **28**: 4611–4614.

Niiler, P.P. and Paduan, J.D. 1995. Wind-driven motions in the northeast Pacific as measured by Lagrangian drifters. *J. Phys. Oceanogr*. **25**: 2819–2830.

Obata, H., Karatani, H. and Nakayama, E. 1993. Automated determination of iron in seawater by chelating resin concentration and chemi-luminescence detection. *Anal. Chem*. **65**: 1524–1528.

Odate, K. 1994. Zooplankton biomass and its long-term variation in the western North Pacific Ocean, Tohoku Sea area, Japan. *Bull. Tohoku Nat. Fish. Res. Inst.* **56**: 115–173.

Ohman, M.D. and Hirche, H.J. 2001. Density-dependant mortality in an oceanic copepod population. *Nature* **412**: 638–641.

Rivkin, R.B., Putland, J.N., Anderson, M.R. and Deibel, D. 1999. Microzooplankton bacterivory and herbivory in the NE subarctic Pacific. *Deep-Sea Res. II* **46**: 2579–2618.

Rue, E.L. and Bruland, K.W. 1995. Complexation of iron(III) by natural organic ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. *Mar. Chem*. **50**: 117–138.

Russell, R.W., Harrison, N.M. and Hunt, G.L. 1999. Foraging at a front: hydrography, zooplankton, and avian planktivory in the northern Bering Sea. *Mar. Ecol. Prog. Ser*. **182**: 77–93.

Saito, H. and Tsuda, A. 2000. Egg production and early development of the subarctic copepods *Neocalanus cristatus*, *N. plumchrus* and *N. flemingeri*. *Deep-Sea Res. I* **47**: 2141–2158.

Saito, H., Tsuda, A. and Kasai, H. 2002. Nutrient and plankton dynamics in the Oyashio region of the western subarctic Pacific. *Deep-Sea Res. II* **49**: 5463–5486.

Saito, H., Suzuki, K., Hinuma, A., Ota, T., Fukami, K., Kiyosawa, H., Saino, T. and Tsuda, A. 2005. Responses of microzooplankton to in situ iron fertilization in the western subarctic Pacific (SEEDS). *Prog. Oceanogr*. **64**: 223–236.

Salonen, K. 1979. A versatile method for the rapid and accurate determination of carbon by high temperature combustion. *Limnol. Oceanogr.* **24**: 177–183.

Sasaoka K., Saitoh, S., Asanuma, I., Imai, K., Honda, M., Nojiri, Y. and Saino, T. 2002. Temporal and spatial variability of chlorophyll *a* in the western subarctic Pacific determined from satellite and ship observations from 1997 to 1999. *Deep-Sea Res. II* **49**: 5557–5576.

Sastri, A.R. and Roff, J.C. 2000. Rate of chitobase degradation as a measure of development rate in planktonic Crustacea. *Can. J. Fish. Aquat. Sci*. **57**: 1965–1968.

Selph, K.E., Landry, M.R., Allen, C.B., Calbet, A., Christensen, S. and Bidigare, R.R. 2001. Microbial community composition and growth dynamics in the Antarctic Polar Front and seasonal ice zone during late spring 1997. *Deep-Sea Res. II* **48**: 4059–4080.

Shaked, Y., Kustka, A.B., Morel, F.M.M. and Erel, Y. 2004. Simultaneous determination of iron reduction and uptake by phytoplankton. *Limnol. Oceanogr.: Methods* **2**: 137–145.

Shaked, Y., Kustka, A.B. and Morel, F.M.M. 2005. A general kinetic model for iron acquisition by eukaryotic phytoplankton. *Limnol. Oceanogr*. **50**: 872–882.

Sherr, E.B. and Sherr, B.F. 1993. Preservation and storage of samples for enumeration of heterotrophic protists. pp. 207–212. *In* Handbook of Methods in Aquatic Microbial Ecology. *Edited by* P.F. Kemp, B.F. Sherr, E.B. Sherr and J.J. Cole, CRC Press, Boca Raton, FL, 777 pp.

Sherr, B.F., Sherr, E.B. and Fallon, R.D. 1987. Use of monodispersed, fluorescently labeled bacteria to estimate in situ protozoan bacterivory. *Appl. Environ. Microbiol.* **53**: 958–965.

Shinada, A., Ikeda, T., Ban, S. and Tsuda, A. 2001. Seasonal dynamics of planktonic food chain in the Oyashio region, western subarctic Pacific. *J. Plankton Res.* **23**: 1237–1247.

Shiomoto, A., Ishida, Y., Tamaki, M. and Yamanaka, Y. 1998. Primary production and chlorophyll *a* in the northwestern Pacific Ocean in summer. *J. Geophys. Res.* **103**: 24,651–24,661.

Shoden, S., Ikeda, T. and Yamaguchi, A. 2005. Vertical distribution, population structure and lifecycle of *Eucalanus bungii* (Copepoda: Calanoida) in the Oyashio region, with notes on its regional variations. *Mar. Biol.* **146**: 497–511.

Straile, D. 1997. Gross growth efficiencies of protozoan and metazoan zooplankton and their dependence on food concentration, predator-prey weight ratio, and taxonomic group. *Limnol. Oceanogr.* **42**: 1375–1385.

Strom, S.L. and Morello, T.A. 1998. Comparative growth rates and yields of ciliates and heterotrophic dinoflagellates. *J. Plankton Res.* **20**: 571–584.

Strom, S.L. and Welschmeyer, N.A. 1991. Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. *Limnol. Oceanogr*. **36**: 50–63.

Strom, S., Postel, J.R. and Booth, B. 1993. Abundance, variability, and potential grazing impact of planktonic ciliates in the open subarctic Pacific. *Prog. Oceanogr*. **32**: 185–203.

Strom, S.L., Miller, C.B. and Frost, B.F. 2000. What sets the lower limits to phytoplankton biomass in high nitrate, low chlorophyll ocean regions? *Mar. Ecol. Prog. Ser*. **193**: 19–31.

Strom, S.L., Olson, M.B., Macri, E.L. and Mordy, C.W. 2006. Cross-shelf gradients in phytoplankton community structure, nutrient utilization, and growth rate in the coastal Gulf of Alaska. *Mar. Ecol. Prog. Ser*. (in press)

Sugimoto, T., Kimura, S. and Tadokoro, K. 2001. Impact of El Niño events and climate regime shift on living resources in the western North Pacific. *Prog. Oceanogr*. **49**: 113–127.

Suzuki, R. and Ishimaru, T. 1990. An improved method for the determination of phytoplankton chlorophyll using N,N-dimethylformamide. *J. Oceanogr*. **46**: 190–194.

Tadokoro, K., Chiba, S., Ono, T., Midorikawa, T. and Saino, T. 2005. Interannual variation in *Neocalanus* biomass in the Oyashio waters of the western North Pacific. *Fish. Oceanogr*. **14**: 210–222.

Takata, H., Kuma, K., Iwade, S., Yamajyoh, Y., Yamaguchi A., Takagi, S., Sakaoka, K., Yamashita, Y., Tanoue, E., Midorikawa, T., Kimura, K. and Nishioka, J. 2004. Spatial variability of iron in the surface water of the northwestern North Pacific Ocean. *Mar. Chem.* **86**: 139–157.

Tani, H., Nishioka, J., Kuma, K., Takata, H., Yamashita, Y., Tanoue, E. and Midorikawa, T. 2003. Iron(III) hydroxide solubility and humic-type fluorescent organic matter in the deep water column of the Okhotsk Sea and the northwestern North Pacific Ocean. *Deep-Sea Res. I* **50**: 1063–1078.

Taniguchi, A. 1999. Differences in the structure of the lower trophic levels of pelagic ecosystems in the eastern and western subarctic Pacific. *Prog. Oceanogr*. **43**: 289–315.

Tovar-Sanchez, A., Sañudo-Wilhelmy, S.A., Garcia-Vargas M., Weaver, R.S., Popels, L.C. and Hutchins, D.A. 2003. A trace metal clean reagent to remove surface-bound iron from marine phytoplankton. *Mar. Chem.* **82**: 91–99.

Tsuda, A. (*Ed.*). 2005. Results from the subarctic Pacific iron experiment for ecosystem dynamics study (SEEDS). *Prog. Oceanogr*. **64**: 91–324.

Tsuda, A. and Sugisaki, H. 1994. In situ grazing rate of the copepod population in the western subarctic North Pacific during spring. *Mar. Biol.* **120**: 203–210.

Tsuda, A., Takeda, S., Saito, H., Nishioka, J., Nojiri, Y., Kudo, I., Kiyosawa, H., Shiomoto, A., Imai, K., Ono, T., Shimamoto, A., Tsumune, D., Yoshimura, T., Aono, T., Hinuma, A., Kinugasa, M., Suzuki, K., Sohrin, Y., Noiri, Y., Tani, H., Deguchi, Y., Tsurushima, N., Ogawa, H., Fukami, K., Kuma, K. and Saino, T. 2003. A mesoscale iron enrichment in the western Subarctic Pacific induces a large centric diatom bloom. *Science* **300**: 958–961.

Tsuda, A., Saito, H. and Kasai, H. 1999. Life histories of *Neocalanus flemingeri* and *Neocalanus plumchrus* (Calanoida: Copepoda) in the western subarctic Pacific. *Mar. Biol.* **135**: 533–544.

Tsuda, A., Saito, H. and Kasai, H. 2001. Life history strategies of subarctic copepods *Neocalanus flemingeri* and *N. plumchrus*, especially concerning lipid accumulation patterns. *Plankton Biol. Ecol*. **48**: 52–58.

Tsuda, A., Saito, H. and Kasai, H. 2005a. Life histories of *Eucalanus bungii* and *Neocalanus cristatus* (Copepoda: Calanoida) in the western subarctic Pacific Ocean. *Fish. Oceanogr*. **13**: 10–20.

Tsuda, A., Saito, H., Nishioka, J. and Ono, T. 2005b. Mesozooplankton responses to iron fertilization in the western subarctic Pacific (SEEDS 2001). *Prog. Oceanogr*. **64**: 237–251.

Tsuda, A., Saito, H., Nishioka, J., Ono, T., Noiri, Y. and Kudo, I. 2006. Mesozooplankton response to mesoscale iron enrichment during the diatom bloom and bloom decline period during SERIES (NE Pacific). *Deep-Sea Res. II* **53** (in press)

Vaulot, D., Marie, D., Olson, R.J. and Chisholm, S.W. 1995. Growth of *Prochlorococcus*, a photo-synthetic prokaryote, in the equatorial Pacific Ocean. *Science* **268**: 1480–1482.

Vidal, J. and Smith, S.L. 1986. Biomass, growth, and development of populations of herbivorous zooplankton in the southeastern Bering Sea during spring. *Deep-Sea Res.* **33**: 523–556.

Vinogradov, M.Y. 1968. Vertical distribution of the oceanic zooplankton. Israel Program for Scientific Translation, Jerusalem, 1970. 339 pp.

Vinogradov, M.Y. 1997. Some problems of vertical distribution of meso- and macrozooplankton in the Ocean. *Adv. Mar. Biol.* **32**: 2–82.

Wagner, M.M., Durbin, E.G. and Buckley, L. 1998. RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. *Mar. Ecol. Prog. Ser.* **162**: 173–181.

Wagner, M.M., Campbell, R.G., Boudreau, C.A. and Durbin, E.G. 2001. Nucleic acids and growth of *Calanus finmarchicus* in the laboratory under

different food and temperature conditions. *Mar. Ecol. Prog. Ser.* **221**: 185–197.

Warren, B.A. and Owens, W.B. 1988. Deep currents in the central subarctic Pacific Ocean. *J. Phys. Oceanogr*. **18**: 529–551.

Waterhouse, T.Y. and Welschmeyer, N.A. 1995. Taxon-specific analysis of microzooplankton grazing and phytoplankton growth rates. *Limnol. Oceanogr*. **40**: 827–834.

Weiler, C.S. and Chisholm, S.W. 1976. Phased cell division in natural populations of marine dinoflagellates from shipboard cultures. *J. Exp. Mar. Biol. Ecol.* **25**: 239–247.

Wells, M.L. 1999. Manipulating iron availability in nearshore waters. *Limnol. Oceanogr*. **44**: 1002–1008.

Wells, M.L., Mayer, L.M. and Guillard, R.R.L. 1991. A chemical method for estimating the availability of iron to phytoplankton in seawater. *Mar. Chem.* **33**: 23–40.

Welschmeyer, N.A. 2004. A ^{14}C-based method for determining microzooplankton grazing rates. Ocean Sciences Meeting, Honolulu, HI, Abstract.

Welschmeyer, N.A., Goericke, R., Strom, S. and Peterson, W. 1991. Phytoplankton growth and herbivory in the subarctic Pacific: A chemotaxonomic analysis. *Limnol. Oceanogr*. **36**: 1631–1649.

Welschmeyer, N.A., Strom, S., Goericke, R., DiTullio, G., Belvin, M. and Peterson, W. 1993. Primary production in the subarctic Pacific Ocean: Project SUPER. *Prog. Oceanogr*. **32**: 101–135.

Whitney, F.A. and Freeland, H.J. 1999. Variability in upper-ocean water properties in the NE Pacific Ocean. *Deep-Sea Res. II* **46**: 2351–2370.

Wong, C.S. and Matear, R.J. 1999. Sporadic silicate limitation of phytoplankton productivity in the subarctic NE Pacific. *Deep-Sea Res. II* **46**: 2539–2556.

Yamamura, O., Honda, S. and Hamatsu, T. 2002. Diets of walleye pollock *Theraga chalcogramma* in the Doto area, northern Japan: ontogenetic and seasonal variations. *Mar. Ecol. Prog. Ser*. **238**: 187–198.

Yebra, L. and Hernández-León, S. 2004. Aminoacyl-tRNA synthetases activity as a growth index in zooplankton. *J. Plankton Res.* **26**: 651–356.

Yoshikawa, T. and Furuya, K. 2004. Long-term monitoring of primary production in coastal waters by an improved natural fluorescence method. *Mar. Ecol. Prog. Ser*. **273**: 17–30.

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[1] Refer to Tsutomu Ikeda's bibliography on page 54 which provides a comprehensive list of suggested reading on recent mesozooplankton studies for the Oyashio region in the western subarctic Pacific.