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## Taxonomic variability of phosphorus stress in Sargasso Sea phytoplankton

**Abstract**—Low inorganic phosphorus (SRP) concentrations and high inorganic nitrogen to phosphorus ratios suggest that phytoplankton production in the northwestern Sargasso Sea may be controlled to some extent by the availability of phosphorus. Phosphorus stress in marine phytoplankton was qualitatively assessed by using a single-cell enzyme-linked fluorescent (ELF) assay for the enzyme alkaline phosphatase, which is induced at low SRP concentrations. During the highly stratified summer period, ~30% of the observed autotrophic eukaryotes in the surface waters were ELF-labeled, whereas in the well-mixed fall period, nearly 70% of the observed autotrophic eukaryotes in the surface waters were ELF-labeled. During the summer, autotrophic flagellates displayed significantly higher ELF-labeling than did both diatoms and dinoflagellates, and this labeling did not vary with depth, whereas in the fall, autotrophic flagellates and diatoms displayed statistically similar and decreasing percentages of ELF-labeled cells as a function of depth. This assay allowed for rapid assessment of the in situ physiological condition of individual autotrophic phytoplankton in the Sargasso Sea. By using this assay, we were able to identify taxonomic and potential seasonal variability of phosphorus stress within the autotrophic phytoplankton community.

For decades, biologists and geochemists have debated which nutrient, nitrogen or phosphorus, limits marine primary production (e.g., Codispoti 1989). In the 1960s and 1970s, the open-ocean new production (Dugdale and Goe-

ring 1967) and export production (Eppley and Peterson 1979) paradigms were developed based upon a nitrogen-limited ocean, a view that found support in prominent publications (e.g., Hecky and Kilham 1988). In the past two decades this view has changed and it is now widely accepted that marine primary production can be limited by inorganic phosphorus (SRP), iron, and silica, as well as nitrogen (e.g., Martin and Fitzwater 1988; Boyd et al. 1999). Part of this change in thought is due to a greater appreciation of nitrogen-fixing organisms that by definition cannot be nitrogen-limited.

A number of studies in the Sargasso Sea have presented evidence supporting the hypothesis that this region may currently be SRP-limited. Early geochemical studies (Fanning 1992; Michaels et al. 1996) noted dissolved inorganic N:P ratios that were substantially greater than the canonical Redfield (1958) ratio, and that have recently been confirmed by high-sensitivity nutrient analytical methods (Wu et al. 2000; Cavender-Bares et al. 2001). The biological interpretation of nutrient limitation associated with these high N:P ratios is not straightforward, because there is little physiological information on the N:P ratio at which phytoplankton transition from nitrogen to phosphorus limitation. Examination of available data suggests that this ratio may range from ~20 to 50 (reviewed by Geider and LaRoche 2002).

The enzyme alkaline phosphatase (AP), which is induced by SRP limitation in many phytoplankton species (Cembella

et al. 1984), has been used as a physiological indicator of phosphorus stress in marine phytoplankton. This enzyme hydrolyzes phosphate groups from molecules within the dissolved organic phosphorus (DOP) pool, which is often many-fold greater than the SRP pool (e.g., Ammerman et al. 2003). At the Bermuda Atlantic Time-series Study (BATS) site, chlorophyll-normalized bulk AP activity peaked during the late spring–early summer ~1 month after the seasonal phytoplankton biomass maximum (Ammerman et al. 2003). These elevated AP ratios suggested increased phosphorus stress in the phytoplankton community, but the AP measurements included heterotrophic AP activity (to an unquantified extent), resulting in an overestimate of the autotrophic component when normalized to chlorophyll. A similarly elevated chlorophyll-normalized bulk AP activity was found at the northern extreme of the Sargasso Sea during summer (Guildford and Hecky 2000). The results from these bulk AP assays have found support from a study employing an enzyme-linked fluorecent (ELF) single-cell AP assay in this ocean region (Dyhrman et al. 2002). A significant observation from the latter study was that not all phytoplankton groups in the same water mass had the same AP activity, an observation previously made for freshwater systems (e.g., Rengefors et al. 2003). We collected samples of phytoplankton populations at several depths during summer and fall, and, by using the single-cell ELF-97 assay, tested the hypothesis that phosphorus stress in Sargasso Sea phytoplankton differed both among phytoplankton groups and between seasons.

**Methods**—Although previously tested with cyanobacteria and dinoflagellate and cryptophyte species (Gonzales-Gil et al. 1998; Dyhrman and Palenik 1999; Dyhrman et al. 2002; Nedoma et al. 2003), this assay was tested with several additional eukaryotic phytoplankton species isolated from the Sargasso Sea. Cultures of *Chaetoceros* sp. (Bacillariophyceae, Culture Collection of Marine Phytoplankton [CCMP] 199), *Helicotheca thamesis* (Bacillariophyceae, CCMP 826), *Akashiwo sanguinea* (Dinophyceae, CCMP 1837), and *Tetraselmis* sp. (Chlorophyceae) were grown in f/2 medium (*Chaetoceros* sp., *H. thamesis*, and *Tetraselmis* sp.) or L1 medium (*A. sanguinea*) at 22°C ( $\pm 0.5^\circ\text{C}$ ) and 120  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  on a 14:10 light:dark cycle. Cultures were maintained in nutrient-replete growth by frequent dilutions with fresh culture media. Inoculating cells into fresh media where SRP had been omitted induced phosphorus stress.

Field samples were obtained from Hydrostation S (32°10'N, 64°30'W) and the BATS site (31°40'N, 64°10'W), both of which are located in the northwestern quadrant of the oligotrophic Sargasso Sea, during the cruises listed in

Table 1. During BATS cruises, samples were collected from two separate casts taken on consecutive days so that the total number of separate summer casts was four (two at the BATS site and two at Hydrostation S) and the total number of separate fall casts was five (four at the BATS site and one at Hydrostation S). Bulk water samples were collected at 1-, 60-, 100-, and 160-m (BATS site) or 1-, 50-, 100-, and 150-m (Hydrostation S) depths on each cast in 12-liter Teflon-coated Ocean Test Equipment bottles with Teflon-coated stainless steel rings mounted on a 24-position SeaBird SBE-32 rosette. From each collection depth, duplicate sample filters (see below) were made and these duplicates were averaged to represent a single value for each depth on a specific cast. Statistical analyses (analysis of variance and Student's *t*-test, StatView Statistical Software) were conducted on these averaged data ( $n = 4$  or  $n = 5$ ), and, therefore, the error estimates presented reflect both environmental and methodological variability. Collection of nutrient and hydrographic data followed standard BATS protocols.

To assess phosphorus-stress, the standard ELF staining procedure (e.g., Gonzales-Gil et al. 1998) was used but with the following slight modifications. Samples (several milliliters for cultures and ~0.25 to 1 liter for field samples) were gently filtered (50 mm Hg) onto Irgalan Black–stained 0.4- $\mu\text{m}$  polycarbonate filters and placed in a clean petri dish for cell membrane permeabilization with 70% ethanol. Small cyanobacteria, likely *Synechococcus* (based upon cell size), present in the field samples were not found to be ELF-labeled, and it was not clear if 70% ethanol adequately permeabilized cyanobacterial cell membranes. A 10% dimethylsulfoxide (DMSO) solution in ethanol was tested to increase cell membrane permeability, but cyanobacteria remained unlabeled. More importantly, the use of the 10% DMSO in ethanol solution resulted in clearer images of ELF-labeled eukaryotic phytoplankton cells both in culture and in the field and therefore was used for the data reported herein.

After permeabilization with 10% DMSO in ethanol for 30 min, filters were carefully transferred back to the filter tower, vacuum rinsed with 0.2- $\mu\text{m}$ -filtered Sargasso Sea water and placed in a clean petri dish for ELF-labeling. The concentrated ELF substrate was diluted 20-fold with the provided buffer (Molecular Probes ELF-97 Endogenous Phosphatase Detection Kit) and filtered through a 0.2- $\mu\text{m}$  syringe filter to remove any fluorescent ELF particles. The filter was saturated with the diluted ELF substrate (0.4 ml) and incubated in the dark for 45 min. After incubation, filters were carefully transferred back to the filter tower and vacuum rinsed with 0.2- $\mu\text{m}$ -filtered seawater, and slides were prepared with the ELF mounting medium. This protocol was rigorously

Table 1. Sample summary, mixed layer depths (MLD), and depth of the phosphocline for the summer (Jun–Jul 2002) and fall (Oct–Nov 2002) sampling periods.

Sample information	Summer	Fall
Cruises	BATS 166, Hydrostation 968/969	BATS 169/170, Hydrostation 974
Total No. casts	4	5
MLD (m)	15	45
Phosphocline depth (m)	160–200	100–120

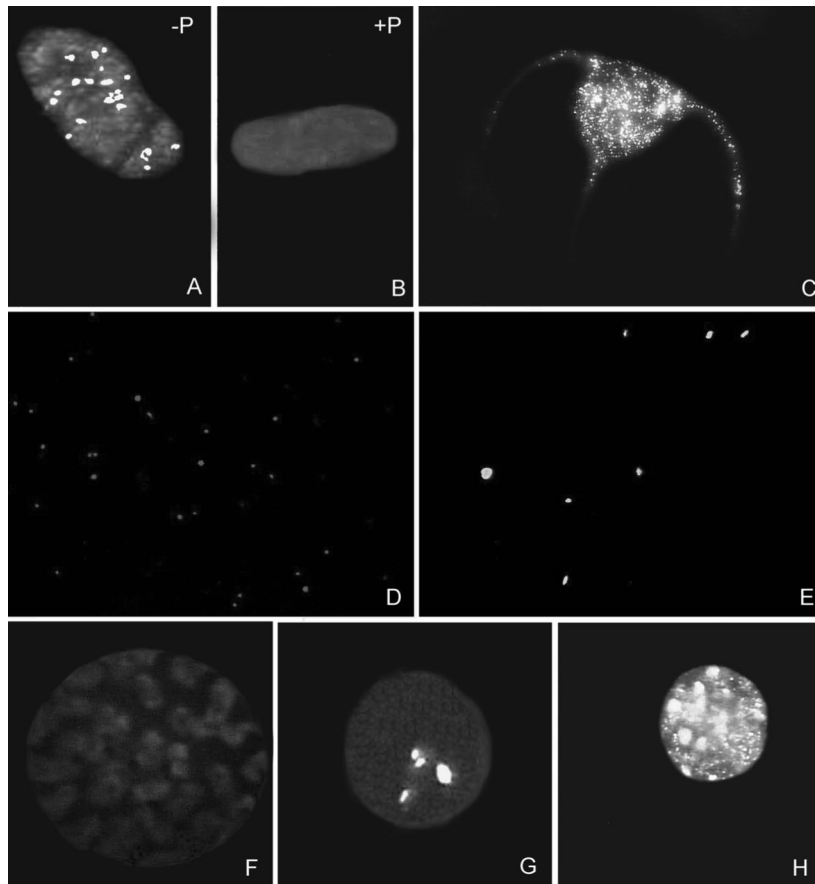


Fig. 1. Fluorescent micrographs of phytoplankton assayed using the ELF-97 substrate. Unless noted, all images were taken under the Olympus UV filter cube (U-MWU), and positive ELF-labeling shows up as lighter colors. (A) Positive ELF-97 staining in a P-depleted *Tetraselmis* sp. laboratory culture; (B) absence of ELF-97 labeling in a P-sufficient *Tetraselmis* sp. laboratory culture; (C) positive ELF-97 staining in the dinoflagellate *Ceratium* sp. collected at 5 m during BATS cruise 169; (D) field of cyanobacteria highlighted under the Olympus CY3 filter set (green excitation light); (E) same field as in panel C, except with UV excitation, highlighting the presence of positively labeled eukaryotic cells and the absence of any labeling, in the cyanobacterial cells; (F–H) three images of centric diatoms showing (F) no, (G) little, and (H) very high levels of ELF-97 staining.

tested by using phosphorus-depleted and phosphorus-sufficient cultures to ensure that the fluorescent labeling of cells was correctly attributable to the induction of AP. For purposes of this manuscript, only one set of phosphorus-depleted and phosphorus-sufficient images are presented (Fig. 1).

All microscopy and visualization was conducted on an Olympus AX-70 Research Microscope at either  $\times 600$  or  $\times 1,000$  magnification, depending upon the size of the particle being examined. The ELF substrate was visualized by using an Olympus ultraviolet (UV) Excitation (U-MWU) filter cube set with an excitation waveband of 330–385 nm, a dichromatic beam splitter at 400 nm, and a barrier filter at 420 nm. Phytoplankton cells were grouped into either prokaryotes (i.e., cyanobacteria) or eukaryotes, with the eukaryotes further divided into diatoms, dinoflagellates, and other autotrophic flagellates. This grouping was achieved in the following manner. Given the preponderance of cyano-

bacterial cells in the samples, 15 random microscope fields were examined under the Olympus CY3 Green Excitation filter cube set (U-MWIG; excitation 520–550 nm, dichromatic beam splitter at 565 nm, and a barrier filter at 580 nm; Fig. 1D) to excite the phycoerythrin pigments within the cyanobacteria and the total number of cells in the field were counted. Each field was then examined under the UV filter set to determine if any of the cyanobacteria contained the insoluble fluorescent ELF product. Quantification of autotrophic eukaryotes was performed in two ways. Random microscope fields were examined under the Olympus Wide Blue Excitation (excitation filter at 420–480 nm, dichromatic beam splitter at 500 nm, and a barrier filter at 515 nm) and UV filter cube sets to quantify the number of autotrophic eukaryotes (as defined by residual chlorophyll *a* fluorescence) and those autotrophic eukaryotes labeled with the ELF product, respectively. The larger diatoms, dinoflagellates, and autotrophic flagellates were quantified for each

whole filter, as opposed to random fields, by using the same two filter sets as for the smaller eukaryotes. Examining the sample filters under transmitted light allowed classification of autotrophic cells into diatom, dinoflagellate, or other flagellate groups. The numbers of cells counted at each depth provides a semiquantitative estimate of phytoplankton group abundance at that depth, because the actual cell number per volume seawater was not counted and it is possible that cells are lost during the ELF washing procedure.

**Results and discussion**—Samples collected from the Sargasso Sea during the summer and fall (Table 1) provided evidence of AP activity (as detected by ELF-labeling) in autotrophic phytoplankton. Although ELF cannot distinguish between inducible and constitutive AP activity, with the exception of the cultured marine dinoflagellate *Alexandrium tamerense* (Gonzales-Gil et al. 1998), ELF-labeling patterns to date have only detected inducible AP activity. With this caveat in mind, the presence of ELF-labeling in natural phytoplankton populations could be interpreted as an indication of phosphorus stress. Phytoplankton collected from the field displayed ELF-labeling patterns similar to those observed in cells from phosphorus-stressed cultures, supporting prior hypotheses of potential phosphorus stress in Atlantic subtropical gyres (e.g., Ammerman et al. 2003; Vidal et al. 2003). Staining was commonly observed in diatoms, dinoflagellates, and autotrophic flagellates, but very rarely observed in coccoid cyanobacteria such as *Synechococcus* (Fig. 1). Of the thousands of small coccoid (1- to 2- $\mu\text{m}$ ) cyanobacterial cells that were enumerated, only a tiny fraction ( $\sim 0.2\%$ ) were ELF-labeled, despite the testing of different cell permeabilization protocols. Variability in the level of ELF fluorescence was quite large from cell to cell (compare Fig. 1F–H), likely because of differences in the extent of cellular AP protein expression or specific activity of individual AP enzyme complexes.

The number of cells counted within each group decreased with depth, with autotrophic flagellates most abundant at all depths sampled, followed by diatoms and then dinoflagellates (Fig. 2). The percentage of autotrophic cells that were ELF-labeled differed between seasons, depths, and autotrophic group. In the surface waters, the percentage of total cells that were ELF-labeled was significantly ( $p < 0.05$ , paired  $t$ -test) lower in summer (mean  $\pm$  SE;  $28.6 \pm 12.5\%$ ) than in fall ( $69.4 \pm 5.9\%$ ), whereas at deeper depths, the percentage of total autotrophic cells labeled was higher in summer than in fall (Fig. 2B,E), and depth-dependent differences were mostly nonsignificant. The significant difference between the percentage of autotrophic cells labeled at 160 m in summer and fall was driven entirely by the disparity in the number of cells counted in each autotrophic group. In the summer at 160 m, autotrophic flagellates dominated the total cells counted, whereas in the fall, equal numbers of diatoms and autotrophic flagellates were counted. For this reason, this seasonal difference at 160 m is not interpreted further.

For diatom and autotrophic flagellate groups, significant seasonal, depth, and seasonal  $\times$  depth interaction differences were found (Table 2). For both cell groups, the mean seasonal differences were the strongest source of variability ( $p$

$< 0.001$ ), but in opposite directions. The autotrophic flagellates displayed higher levels of ELF-labeling in summer than in fall, whereas diatoms displayed higher levels of ELF-labeling in fall than in summer. Moreover, during summer, diatoms displayed significantly ( $p < 0.05$ , paired  $t$ -test) lower levels of ELF-labeling than autotrophic flagellates at all depths, whereas in the fall, no statistical difference was found in ELF-labeling between diatoms and flagellates regardless of the depth. Autotrophic flagellates only displayed significant differences in percent ELF-labeling between the surface and 160-m samples, whereas nearly all depth comparisons were significant for the diatoms (Table 2). This appears to be due in part to the lower cast-to-cast (i.e., environmental) variability in labeling of diatoms than of flagellates (Fig. 2). Dinoflagellates were found at all depths during the summer, but only in surface waters in the fall, which prevents meaningful statistical comparisons for this autotrophic group. Examination of the ELF-labeling data presented herein suggests that there may be important species-specific responses to phosphorus stress embedded within a bulk phytoplankton community response.

Several potential explanations exist for the differences in ELF-labeling between the various phytoplankton taxonomic groups, the very low levels of ELF-labeling in diatom and dinoflagellate groups during the stratified summer period, and the absence of ELF-labeling in cyanobacteria. A number of marine phytoplankton species, in particular flagellates, have some form of mixotrophic metabolism (reviewed by Antia et al. 1991), and therefore expressed AP levels might be a function of nutritional mode. Many flagellate species are osmotrophic (i.e., can utilize dissolved organic compounds) and therefore ELF-labeling would be expected to be high when SRP levels in the Sargasso Sea are reduced during seasonal stratification and these species are utilizing the abundant DOP pool. During periods of destratification, when mixed layers deepen and phosphoclines shoal, ELF-labeling in these flagellates would potentially decrease. This is exactly what was observed when the mixed layer deepened from 15 to 45 m and detectable ( $\sim 20 \text{ nmol L}^{-1}$ ) SRP concentrations shoaled to 120 m during the course of this study (Fig. 2; Table 1). Some flagellates, and in particular large dinoflagellates, may have a phagotrophic nutritional mode (i.e., they ingest whole prey cells) and therefore would not necessarily be expected to express AP activity and be ELF-labeled. Consequently, seasonal changes in nutritional mode of the resident phytoplankton, as well as phosphorus availability, could explain seasonal changes in ELF-labeling of flagellated species. These relationships between nutritional modes (or simply changes between nutritional modes) in marine phytoplankton and nutrient cycling in oligotrophic ocean gyres are not well understood at this time and are a potential area for future research.

Diatoms also may possess osmotrophic, but not phagotrophic, metabolisms (e.g., Antia et al. 1991; Stoecker 1999), so changes between mixotrophic nutritional modes are not a likely explanation for the significant increase in the percentage of ELF-labeled diatoms from summer to fall. One possible mechanism that could explain this seasonal increase in ELF-labeling is that these diatoms are vertically migrating to “mine” SRP from deeper depths (e.g., Taylor et al. 1988;

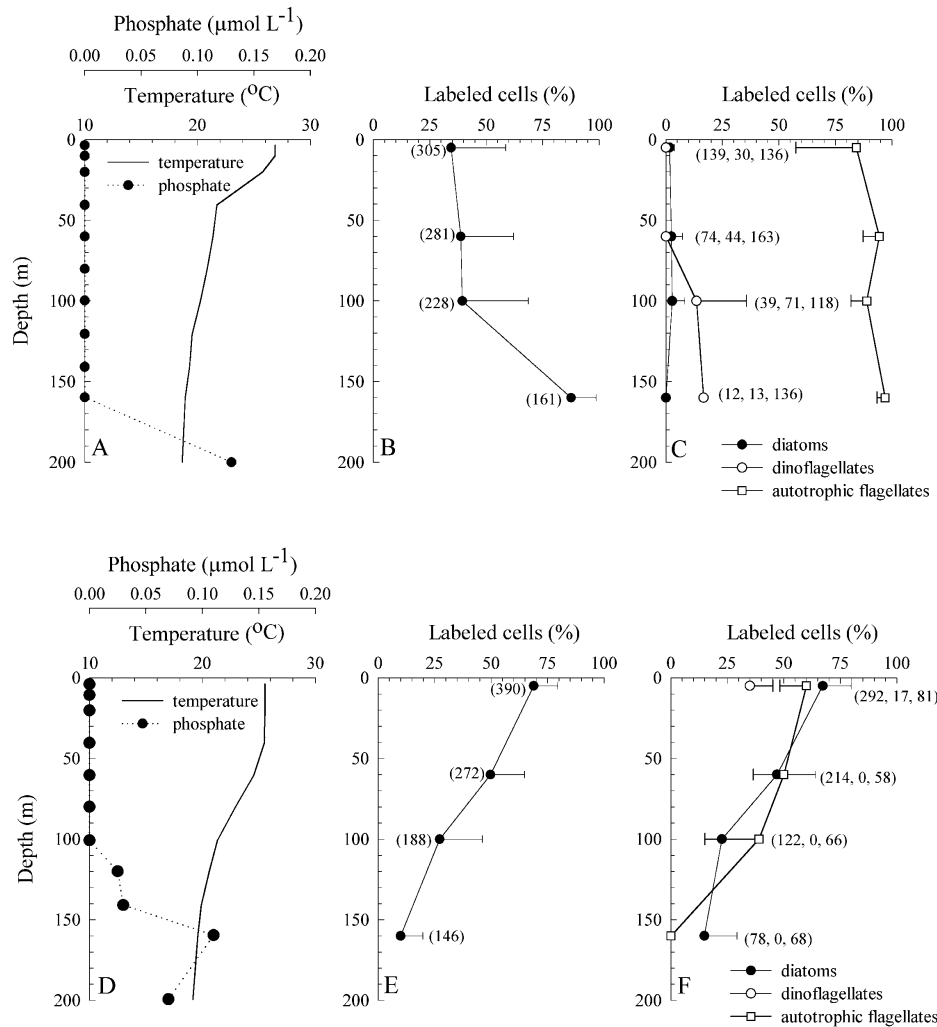


Fig. 2. Depth-dependent variability in water-column chemical and physical properties, and ELF-97 cell labeling. (A, D) Average temperature and phosphorus concentration profiles for each season; (B, E) the fraction ( $\pm$ SD) of total eukaryotic cells counted that were labeled with ELF-97; and (C, F) the taxonomic breakdown of cellular labeling between diatoms, dinoflagellates, and other autotrophic flagellates during the (A–C) summer and (D–F) fall seasons. The numbers in parentheses next to data points represent the total number of autotrophic cells counted (B, E) and the number in each phytoplankton group (C, F; in the order as given in the legend) used in the calculation of the percentages presented. Note, because of the standardized sampling depths, samples from similar depths are pooled and no error bars in the depth (y-axis) are given for clarity in the figure. In addition, x-axis error bars are only presented in one direction to improve clarity between profiles for each phytoplankton group.

James et al. 1992). In the Sargasso Sea, vertical migrators have been found predominantly during the summer (e.g., Villareal and Lipschultz 1995), when the lowest levels of ELF-labeling were observed in diatoms. At any depth and any point in time, “populations” of vertical migrators could be composed of cells that are either descending to or ascending from the phosphocline. Given that several days are required to repress AP activity after reexposure to SRP (e.g., Dyhrman and Palenik 1999), a timescale equal to or longer than estimated vertical migration timescales (Villareal and Lipschultz 1995), factors that impact the match or mismatch of vertical migration and enzyme regulation timescales may

impact the depth-dependent pattern in ELF-labeling. For example, if we assume there are no seasonal changes in vertical migration rates, low ELF-labeling during the highly stratified summer condition might reflect a stable-state scenario between descending and ascending cells that is driven entirely by physiological rate processes and a vertical migration timescale longer than the AP repression timescale. In contrast, the deeper mixed layer (i.e., a more physically active upper ocean) in the fall might facilitate trapping of near-surface vertical migrators in the surface water (if mixing rates exceed physiological descent rates), whereas the shoaling phosphocline would allow vertical migrators to replenish

Table 2. Analysis of variance of seasonal and depth-dependent variability in percentage ELF-labeling for autotrophic flagellates and diatoms. Post hoc test were Fisher's least square differences tested with an alpha value of 0.05.

Source of variability	Mean square	DF*	<i>f</i> -value	<i>p</i> -value	Power
Flagellates					
Depth	791.13	3	3.19	0.039	0.67
Season	31,247.10	1	125.94	<0.001	1.00
Depth × season	1,637.61	3	6.60	0.002	0.96
Residual	248.11	28			
1 m vs. 55 m	0.990				
1 m vs. 100 m	0.243				
1 m vs. 155 m	0.005				
55 m vs. 100 m	0.238				
55 m vs. 155 m	0.004				
100 m vs. 155 m	0.068				
Fall vs. summer	<0.001				
Diatoms					
Depth	1,309.65	3	9.00	0.002	0.99
Season	11,644.33	1	80.02	<0.001	1.00
Depth × season	1,228.99	3	8.45	0.004	0.99
Residual	145.52	28			
1 m vs. 55 m	0.066				
1 m vs. 100 m	0.002				
1 m vs. 155 m	<0.001				
55 m vs. 100 m	0.026				
55 m vs. 155 m	0.002				
100 m vs. 155 m	0.336				
Fall vs. Summer	<0.001				

\* degrees of freedom

SRP reserves closer to the surface, therefore shortening the overall vertical migration distance and therefore shortening the timescale relative to the AP repression timescale. This set of physical conditions could conceivably result in the decrease in percent of ELF-labeled diatoms with depth observed during the fall. Interactions, including match or mismatch in temporal scales, between physiological and physical processes in the oligotrophic gyres remain to be fully understood but are very important in our ability to understand currently observed phenomena as well as to predict, based on models, the functioning of and future changes to these ecosystems.

A related explanation for the observed differences in ELF-labeling between diatoms and dinoflagellates compared to other flagellates relates to the ability of diatoms and dinoflagellates to form very large internal nutrient pools (e.g., Villareal and Lipschultz 1995). The expression of phosphorus-stress indicators, such as inducible AP activity, is linked to the balance between the SRP availability and cellular phosphorus demand (i.e., product of cell quota and growth rate). The formation of large internal SRP pools would tend to buffer large changes in external SRP concentrations, which when coupled with slower overall growth rates (much slower than the competing autotrophic flagellates) in these larger diatoms and dinoflagellates, would tend to reduce phosphorus stress and therefore could limit AP expression and ELF-labeling. The exact opposite would be true for the smaller, faster-growing autotrophic flagellates.

Unlike eukaryotic autotrophs, marine cyanobacteria, like-ly *Synechococcus*, were not ELF-labeled by any of the pro-

ocols that were employed in this study. Rengefors and colleagues (2003) have made similar observations in freshwater coccoid cyanobacteria; however, this lack of labeling is not specific to all marine cyanobacteria because the ELF assay has been successful at detecting phosphorus stress in the filamentous cyanobacteria *Trichodesmium* collected from the Sargasso Sea (Dyhrman et al. 2002). It is possible that the small, coccoid cyanobacterial cells lack an AP that is detected by ELF, are not phosphorus stressed, or the assay conditions were not optimized (e.g., Nedoma et al. 2003). Sargasso Sea cyanobacteria are known to possess both high-affinity phosphate transport systems (e.g., Scanlan and Wilson 1999) and DOP degradation pathways (e.g., phosphonate degradation [Palenik et al. 2003]). We cannot quantitatively assess these three possible explanations in this study, and this would be a fruitful area of future research.

A full understanding of the mechanisms of phosphorus cycling in the ocean, and many other nutrient cycles, requires a working knowledge of in situ physiological traits of the organisms that are at the center of these complex nutrient cycles. Moreover, how these physiological traits impact the coupling of biological communities to physical perturbations is key to building a predictive capability of the ocean's response to future changes. The relatively simple physiological diagnostic marker employed in this study allows for the assessment of a cell's physiological state in situ as opposed to in vitro assessment after incubations. We have now successfully employed this assay to generate initial evidence for taxonomic and temporal variability of phosphorus stress in

the oligotrophic ocean gyres that is yet to be fully appreciated.

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## Synchronized hatch and its ecological significance in rainbow smelt *Osmerus mordax* in St. Mary's Bay, Newfoundland

**Abstract**—Early life history stages in most marine animals are subject to high mortality through predation, starvation, and dispersal. Accordingly, the potential exists for the selection of behavioral mechanisms that reduce mortality. We examined the ecological significance of synchronization in hatch and the initiation of larval drift in rainbow smelt, *Osmerus mordax*, populations in St. Mary's Bay, Newfoundland. Larval abundances from six 24-h ring net surveys (2-h intervals) in Colinet and Salmonier Rivers during 2002/2003 suggest synchronized hatch following dusk (~2200 h). Monitoring of egg hatching in situ confirmed synchrony was at hatch and not emergence. Larval abundance showed no relationship with temperature or flow rates, and the consistency in hatch pattern suggested a light/dark cue. In experimental manipulations in which eggs were exposed to light and dark conditions for 2-h periods, hatch percentages were up to five times higher ( $p < 0.005$ ) in dark treatments. We hypothesized that the linkage of hatch to low light levels represents a mechanism to avoid elevated larval predation in daylight conditions. Egg predation determined from predator gut content analysis suggested that extreme predation risk from small (<20 cm) salmonids peaked during the day, prior to dusk, and was lowest during night (2200–0400 h). Microcosm experiments demonstrated that newly hatched larvae exposed to predators in dark conditions did not change in number, but mortality averaged 60% in light conditions. Our results suggest that predation pressure during the early life history of aquatic organisms might play a strong role in the optimization of aquatic life histories.

The early life history of many aquatic organisms is characterized by high mortality rates resulting from predation, starvation, and advection from suitable areas (Rumrill 1990; Pepin 1991; Houde 2002). Survival through this period might be determined by the proportion of eggs or larvae that experience favorable conditions (Frank and Leggett 1983; Cushing 1990). Survival and subsequent recruitment could therefore be enhanced if developmental stages could be cued to the timing and location of favorable conditions.

Synchrony and active manipulation of “developmental events” has been observed in various taxa, including fishes (e.g., Frank and Leggett 1983), marine invertebrates (e.g.,

Barry 1989), insects (Dingle 1985), and plants (e.g., Gill 1981; Christensen 1985). These behaviors might dampen the effect of environmental variance and dramatically affect recruitment success and life history evolution (Leggett 1985). However, few attempts have been made to examine how hatch timing contributes to spatial and temporal distribution of aquatic species and the subsequent consequences for survival.

Rainbow smelt, *Osmerus mordax*, display increased nighttime abundance of drifting larvae (Johnston and Cheverie 1988) and appear to synchronize their larval drift from river spawning sites to estuarine habitats further downstream. Throughout eastern North America, smelt time their reproduction to follow the spring thaw, and spawning is characterized by synchronized nightly migrations upstream of the maximum tidal incursion where small (~1 mm) adhesive, demersal eggs are released (McKenzie 1964). Eggs develop in freshwater streams or rivers, and hatch occurs at 10–20 d at 14–16°C. Larvae are then immediately transported downstream to the estuary. Smelt therefore represent a model species for the examination of synchronization and embryological control of hatch. Whether this synchrony in larval drift represents hatch or posthatch emergence from the substrate, how it is cued, and its adaptive significance have not been addressed. We hypothesized that nighttime drift of smelt larvae results from hatch synchrony cued by decreasing light conditions, which is a proxy for decreased predation risk and a “safe site” (Frank and Leggett 1982a; Bradbury et al. 2000) for hatching larvae. Thus, the objective of this study was to document the process and mechanism of synchronous larval drift in estuarine smelt populations and examine the hypothesis that this behavior represents a response to reduce high predation risk during the early larval period. Specifically, can behavior associated with hatching eggs and larval drift influence subsequent survival?

**Methods**—Smelt spawning locations were identified through interviews with local residents and subsequent snorkeling surveys during the spring of 2001 in Salmonier and Colinet Rivers, St. Mary's Bay, southeast Newfoundland,