Table 2

Amplified genes from Trichodesmium *and* Katagnymene spiralis

	PhoA	*PstS1*	*PstS2*
T. erythraeum	XXX	XXX	XXX
T. tenue	XXX	XXX	X
K. spiralis	XXX	X	X
T. thiebautii		X	

XXX—entire gene
X—gene fragment

Kategnymene are approximately 98% identical to the corresponding genes in *T. erythraeum.* Given the morphological distinction of the *Trichodesmium* species, this high degree of similarity was surprising. However, coverage of the entire gene in each species may reveal regions within the genes that are less highly conserved. This hypothesis is consistent with the results for the only other functional gene to be sequenced from multiple *Trichodesmium* species, *hetR,* which shows a lower degree of similarity, 91%–95% (7).

Despite extensive efforts to amplify all the genes by varying annealing temperature, primers, and concentrations of magnesium, enzyme, and template, we were unable to amplify *pstS2* or *phoA* from *T. thiebautii.* This is particularly interesting because *T. thiebautii* has been shown to have alkaline phosphatase activity (2), and because of the degree of similarity of *phoA* among the other species. Additionally, *T. thiebautii*'s evolutionary relationship, inferred from *Trichodesmium* ITS sequences (6), indicates *T. tenue* and *K. spiralis* are more closely related to each other than to *T. thiebautii.* It may be that *phoA* in *T. thiebautii* is very different compared to the other species or that a different gene and gene product are used in the hydrolysis of dissolved organic phosphorus for this species.

Future work is needed to determine whether *pstS2* and *phoA* are present in *T. thiebautii.* Although other species and isolates of *Trichodesmium* should be analyzed, our initial identification of putative P-regulated genes in these *Trichodesmium* species has improved our knowledge of phosphorus scavenging mechanisms in this important genus.

This work was funded by NSF grant OCE-0220945, and by NSF Research Experience for Undergraduates grant OCE-0097498.

Literature Cited

1. **Capone, D., J. Zehr, H. Paerl, B. Bergman, and E. Carpenter. 1997.** *Science* **276:** 1221–1229.
2. **Dyhrman, S., E. Webb, and D. Anderson. 2002.** *Limnol. Oceanogr.* **47:** 1832–1836.
3. **Sanudo-Wilhelmy, S. A., C. Gobler, D. Hutchins, M. Yang, K. Lwiza, J. Burns, D. Capone, J. Raven, and E. Carpenter. 2001.** *Nature* **411:** 66–69.
4. **Scanlan, D., and N. West. 2002.** *FEMS Microbiol. Ecol.* **40:** 1–12.
5. **Scanlan, D., and W. H. Wilson. 1999.** *Hydrobiologia* **401:** 149–175.
6. **Orcutt, K. M., U. Rasmussen, E. A. Webb, J. B. Waterbury, K. Gundersen, and B. Bergman. 2002.** *Appl. Environ. Microbiol.* **68:** 2236–2245.
7. **Janson, S., B. Bergman, E. Carpenter, S. Giovannoni, and K. Vergin. 1999.** *FEMS Microbiol. Ecol.* **30:** 57–65.

Reference: *Biol. Bull.* **205:** 231–232. (October 2003)

Molecular Quantification of Toxic *Alexandrium fundyense* in the Gulf of Maine

*Madeline Galac[1], Deana Erdner[2], Donald M. Anderson[2], and Sonya Dyhrman[2,]**
[1] State University of New York at Stony Brook, Stony Brook, NY
[2] Woods Hole Oceanographic Institution, Woods Hole, MA

The toxic dinoflagellate *Alexandrium fundyense* is widespread in the northeastern part of North America, including the Gulf of Maine, and is responsible for seasonal harmful algal blooms in these regions. Even at low cell densities, *A. fundyense* produces toxin that can accumulate in shellfish and cause paralytic shellfish poisoning (PSP). PSP can be debilitating or lethal to humans and other shellfish consumers and is a public health concern (1). Accurate measurements of *A. fundyense* distributions, at a low cell density, are critical to continued PSP monitoring and mitigation efforts.

Traditional assessments of *A. fundyense* cell number rely on microscopic counts of species abundance. However, it is difficult to visually distinguish *A. fundyense* from other dinoflagellates and other species of *Alexandrium,* as there are often only subtle morphological differences between taxa. Oligonucleotide probe-based methods (2) help to distinguish between *Alexandrium* and other genera and are commonly employed in the Gulf of Maine to map cell distribution. Although these approaches are useful, we developed and applied an assay that does not require microscopy. Various studies have used quantitative PCR (qPCR) to assay cell numbers of dinoflagellates such as *Pfiesteria piscicida* (3). In this study we mapped *A. fundyense* distribution using a qPCR assay that appears to be specific and sensitive.

Primers were designed to amplify a 174 base pair region of the large ribosomal subunit (LSU) gene (4). This gene was chosen because it has been sequenced from many species of *Alexandrium,* is commonly used for phylogenic analyses (5), and is present in high copy number.

Building on previous work (4), the specificity of the LSU primers was tested against DNA extracted from phytoplankton cultures with Qiagen's DNEasy Kit, according to the manufacturer's instructions, using standard hot start amplification conditions

* Corresponding author: sdyhrman@whoi.edu

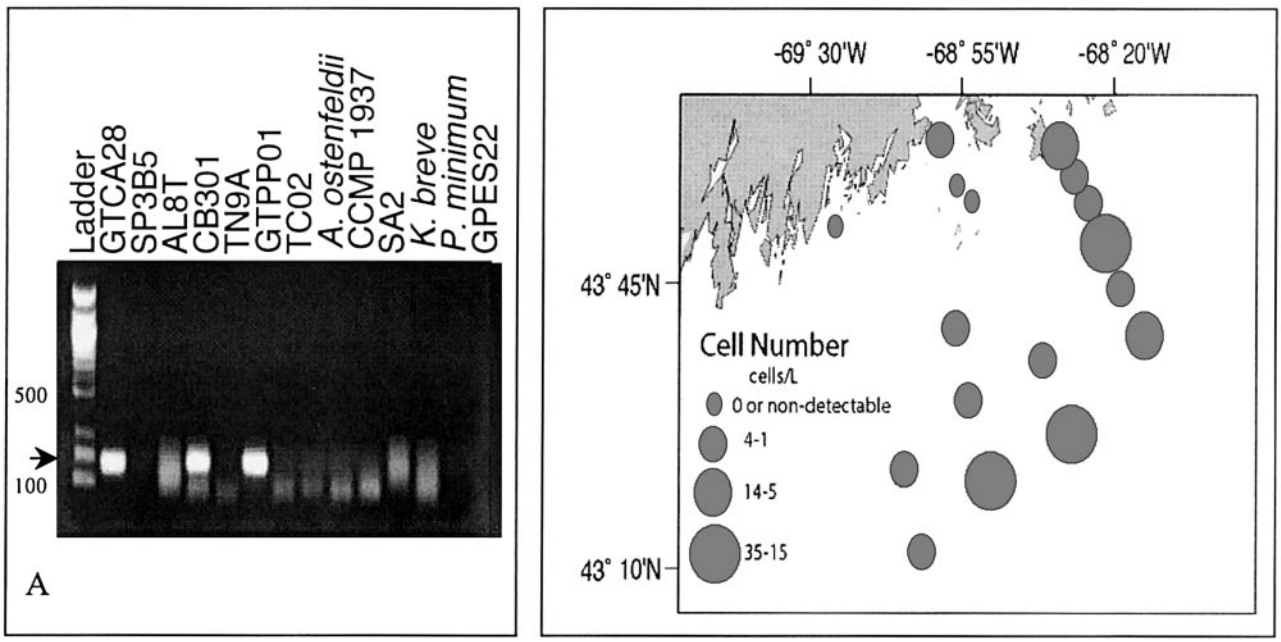

Figure 1. *Specificity of large ribosomal subunit (LSU) gene primers and a map of* Alexandrium fundyense *distribution. (A) The arrow indicates a 174-bp fragment of the LSU gene, as shown by the 100-bp ladder. Amplification occurred only in strains of* A. fundyense *that are present in the Gulf of Maine (GTCA28, CB301, GTPP01). Amplification did not occur in* Alexandrium *strains from other parts of the world (SP3B5, AL8T, TN9A); nor did amplification occur in other* Alexandrium *species such as* A. ostenfeldii *and* A. andersoni *that could occur with* A. fundyense *in the Gulf of Maine (*A. ostenfeldii, *TC02). Other dinoflagellate species also did not amplify (CCMP1937, SA2,* Karenia breve, Prorocentrum minimum, *GPES22). (B) A map of* A. fundyense *distribution in surface water of the Gulf of Maine during a cruise from 29 May to 6 June 2003.*

and a 60 °C annealing temperature. Under these conditions amplification occurred only with *A. fundyense* isolated from the Gulf of Maine (Fig. 1A).

Field samples were collected from surface water along transects in the Gulf of Maine from 29 May through 6 June 2003. At each station, 4 l of surface water was collected, prescreened through a 64-μm sieve, and collected on a 15-μm filter. The samples were each extracted with Qiagen DNEasy kit.

Using qPCR, the number of cells in a field sample was determined. In this study, qPCR was performed using Stratagene Brilliant SYBR Green QPCR Master Mix, and a fluorescence threshold was set by the analytical software for the BioRad iCycler. The PCR cycle during which this threshold was crossed for each sample designated the C_T. Sample C_T can be compared to the C_T of standards with a known cell count to specify the number of cells present in the sample (4).

To ensure that different strains of *A. fundyense* have the same copy number of the LSU gene, standard curves were built from two Gulf of Maine strains in culture (GTCA28 and CB301). Both strains resulted in similar standard curves ($y = -2.5281x + 30.664$, $R^2 = 0.9514$ for the first strain and $y = -2.0291x + 27.61$, $R^2 = 1$ for the second strain). A dilution series of a known number of cells was also analyzed with a field sample matrix. No significant PCR inhibition of the target was detected from extraneous DNA in the field samples.

The number of cells detected in the Gulf of Maine ranged from 1 to 35 cells per liter (Fig. 1B). This range is similar to the range of cell numbers calculated from other methods (live counts done during the cruise and oligonucleotide probe counts [Anderson, unpubl. data]), which we did not expect to be exactly the same since the samples were taken from different Niskein bottles and processed differently. Of the stations tested, station 86 and offshore stations 104 and 105 had the highest concentrations of cells. To confirm amplification of *A. fundyense* from the field samples, PCR products generated from samples 89 and 128 were sequenced at the Marine Biological Laboratory, Woods Hole, Massachusetts, using the facility's protocols and were shown to be *A. fundyense*.

In summary, qPCR appears to be a specific and sensitive approach to monitoring the abundance of *A. fundyense*. This method shows great promise for mapping the *A. fundyense* populations in the Gulf of Maine.

This research was supported by an EPA Star Award through the ECOHAB Program (R-83041501-0) and a NSF-REU site grant (OCE-0097498) to the Boston University Marine Program.

Literature Cited

1. **Anderson, D. M. 1997.** *Limnol. Oceanogr.* **42:** 1009–1022.
2. **Anderson, D. M., D. M. Kulis, B. A. Keafer, and E. Berdalet. 1999.** *J. Phycol.* **35:** 870–883.
3. **Bowers, H. A., T. Tengs, H. B. Glasgow, J. M. Burkholder, P. A. Rublee, and D. W. Oldach. 2000.** *Appl. Environ. Microbiol.* **66:** 4641–4648.
4. **La Du, J., D. Erdner, S. Dyhrman, and D. Anderson. 2002.** *Biol. Bull.* **203:** 244–245.
5. **Scholin, C. A., M. Herzog, M. Sogin, and D. M. Anderson. 1994.** *J. Phycol.* **30:** 999–1011.