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Molecular Approaches to Understanding Population Dynamics of the Toxic Dinoflagellate *Alexandrium fundyense*

Jane La Du[1], *Deana Erdner, Sonya Dyhrman, and Don Anderson (Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543)*

Toxic dinoflagellates of the genus *Alexandrium* are the primary organisms responsible for seasonal harmful algal blooms (HABs) in coastal New England waters. Members of the genus *Alexandrium* can produce powerful neurotoxins that accumulate in filter-feeding shellfish, causing debilitating or lethal paralytic shellfish poisoning (PSP) in humans and other higher trophic consumers (1).

To better understand the formation and persistence of HABs, it is important to have accurate measures of population dynamics, specifically growth rate and abundance of toxic cells. Recently, molecular assays have been used to quantify the abundance of the HAB organism *Pfiesteria piscicida* (2), and similar assays may be useful for efficient analysis of field samples and for prediction of potential *A. fundyense* HABs. Analogous molecular tools could be used to assess growth rate of *A. fundyense* populations.

This project employed gene sequencing and specific primer design targeting two *Alexandrium fundyense* (CA28) gene fragments: one encoding for ribulose-1,5-bisphosphate carboxylase (RubisCO, *rbcL*); the other encoding for large subunit ribosomal RNA (LSU). This research also explored the use of quantitative polymerase chain reaction (QPCR) to assess growth rate through *rbcL* gene expression and to quantify cell density through LSU gene abundance. QPCR is highly sensitive, enabling users to monitor increases in PCR product formation during amplification (3). By using an appropriate standard, the starting quantity of the specific mRNA or DNA target in a sample can be calculated.

[1] Oregon State University, Corvallis, OR.

The *rbcL* gene was chosen because dinoflagellates, unlike other photosynthetic eukaryotes, express only a Form II RubisCO (4). RubisCO is the key enzyme for fixing CO_2 into organic cellular components during the Calvin-Benson cycle. Measurement of *rbcL* gene expression may be a useful marker for determining changes in cellular growth rate. The LSU gene has a high copy number and is species-specific, making it a good candidate for quantifying CA28 cell density through QPCR.

Degenerate *rbcL* primers, based on three published dinoflagellate *rbcL* sequences (GenBank as of June 2002) were used to PCR amplify a weak 320 base pair product (5). This product was sequenced, and the resulting sequences were aligned and used to design specific *rbcL* primers (forward primer 5′-CACTTTG-CAGCTGAGTCTTCCA-3′, reverse primer 5′-CAACGCATC-CACGGTTTTTGTG-3′) that target an 81 base pair fragment of the *rbcL* gene.

The LSU forward primer (5′-GGAATGCAAAGTGGGTGG-3′) was designed from published *Alexandrium* strain sequences (6). A previously designed oligonucleotide (NA1) was used as a reverse primer. The NA1 oligonucleotide is specific to toxic North American ribotypes of the *Alexandrium fundyense/tamarense/catenella* species complex (7). These primers targeted a 174 base pair sequence.

Gradient PCR with an annealing temperature range of 50–70 °C was used to determine the highest annealing temperature possible for optimal product formation and primer specificity. Optimal annealing temperatures for the specific rbcL and LSU primers were 63.5 °C and 58 °C respectively.

The specific *rbcL* primers produced robust amplification of the *rbcL* gene fragment as compared to the degenerate primers (Fig.

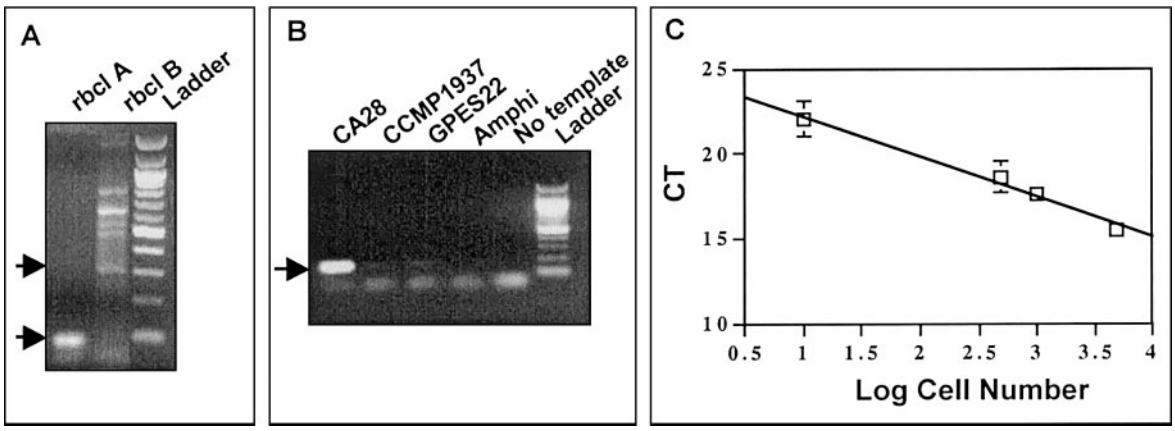

Figure 1. *PCR amplification comparisons of designed rbcL and LSU primers, and QPCR standard curve of LSU amplification from known cell densities. (A) Arrows indicate new rbcL primers (A)* vs. *old primers (B). (B) Specificity and amplifiction robustness of new CA28 LSU primers. (C) QPCR standard curve comparing* C_t vs. *cell number from LSU amplification.*

1A). The LSU primer pair also produced strong amplification and was highly specific to *Alexandrium* when tested against members of three other dinoflagellate genera, *Gymnodinium* sp. (CCMP1937), *Lingulodinium polyedra* (GPES22), and *Amphidinium carterae* (Amphi) (Fig. 1B).

The LSU primers were tested on *A. fundyense* clonal culture DNA, extracted from a range of known cell quantities. QPCR was performed using SYBR green fluorescent dye with amplification conditions established previously from gradient PCR. Successful amplification resulted in a standard curve (Fig. 1C) that compares the PCR cycle number that crosses a designated fluorescence threshold (C_T) to cell density ($R^2 = 0.98$).

The specific *rbcL* primers designed and tested during this project may be used for further research on *rbcL* expression to determine if there is a correlation between growth rate and *rbcL* message abundance. This could be a valuable tool for rapid analysis of *Alexandrium* growth rate in field populations.

The LSU primers described here may be useful for analysis of *Alexandrium* cell densities within mixed field samples, augmenting our abilities to monitor *Alexandrium fundyense* population density in the field. Future work is needed to optimize conditions for DNA extraction from mixed samples containing known *A. fundyense* cell densities. If successful, *A. fundyense* cell numbers in field samples could be quantified by QPCR determination of LSU abundance through comparison to a standard curve.

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Pelagic Larval Duration of the Caribbean Wrasse, *Thalassoma bifasciatum*

Jason Philibotte (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543)

Most coral reef fishes have a pelagic larval life stage that varies in length from a few days to several months (1). It has been suggested that reef fish developed a larval life stage for one or more of the following reasons: increased food availability, reduced threats of predation over reefs, or improved dispersal (2). The pelagic stage can also be instrumental in determining reef diversity, biogeographic range, and population dynamics of coral reef fish (1, 3, 4). The optimal method for determining pelagic larval duration (PLD) in reef fish is the otolith aging technique. Microincrement formation (daily growth rings) can help determine the primary factors that influence the recruitment patterns (spawning patterns, lunar cycles, oceanographic processes, etc.) of larval fish. In this study, PLD of the bluehead wrasse, *Thalassoma bifasciatum,* from Glover's Atoll, Belize, was determined using the otolith aging technique. These results, along with results from previous studies conducted throughout the Caribbean, were used to determine variability in the mean PLD and the overall range of PLD for *T. bifasciatum*. Sampling seasons within studies were used as separate data sets to calculate the variability in the mean PLD.

Fifty-eight specimens of *T. bifasciatum* were captured with a dip net from reefs around Glover's Atoll. Samples were collected on December 12 to 15, 1994. The fish were measured to the nearest 1 mm and preserved in 95% ethanol. The sagittae and the lapilli, the two largest of the three pairs of otoliths, were used in this study. The sagittae were removed from the semicircular canal at the base of the cranium, and the lapilli were removed from the lateral wall above the sagittae. Otoliths were cleaned in xylene for 24 h and then placed on a slide in immersion oil. Otoliths were examined using a compound microscope with magnifications between 100× and 1000×. Victor (1) validated the presence of daily growth rings and discovered a settlement mark on the otoliths of *T. bifasciatum*. The settlement mark is a series of closely set rings that forms when the larva makes the transition from the plankton to the benthos. PLD was determined by counting the number of increments from the focus to the settlement mark and adding 2 days, in order to account for the time delay in otolith formation after fertilization.

This study found PLD of *T. bifasciatum* to range from 38 to 54 days, with a mean of 45 days. The spawning dates of the larvae collected ranged from October 6 to 27, 1994. The larvae subsequently settled out of the plankton from November 21, 1994, to December 15, 1994, a period of 25 days.

The mean and range of PLD varies considerably among and within species (3, 4). Larvae with longer PLD will potentially have higher levels of dispersal (1) and more variance around the mean PLD (2) due to longer exposure to oceanographic processes. For example, Pomacentrids have a short and consistent PLD: Wellington and Robertson (5) showed that 12 species from Panama had PLDs ranging from 17 to 37 days; the largest range within a species was 14 days. Comparatively, Labrids have a longer PLD with greater variability. For 100 species of Labrids in the Pacific and Caribbean, Victor (6) determined PLDs ranging from 15 to 121 days; the largest range within species was 56 days. Of these 100 species, 20 had a mean PLD greater than *T. bifasciatum* (49.3