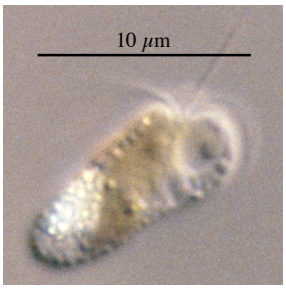
Analysis of growth conditions that promote increased toxin concentrations in dynamic populations of the harmful bloom-forming alga, *Prymnesium parvum* Carter (Haptophyta)

Schonna R. Manning, Ph.D. Candidate
Plant Biology Graduate Program
Section of MCD Biology
The University of Texas at Austin
P.I. Dr. John W. La Claire

The toxin-forming *Prymnesium parvum* is responsible for the deaths of hundreds of thousands of organisms, including amphibians, mollusks, and fishes (Figure 1). This euryhaline alga is currently a seasonal nuisance in Scandinavia, the Mediterranean, the United Kingdom, the Pacific Rim, and the United States. Recurring blooms of *P. parvum* have been found along coastlines, in brackish inlets, as well as in inland fresh water reservoirs. The toxicity of *P. parvum* is attributed to the secondary metabolites prymnesin-1 and -2, which have documented lytic, cytotoxic, neurotoxic and ichthyotoxic properties.

Figure 1. Fish mortality event in a Texas lake attributed to *P. parvum*. This microscopic organism is responsible for killing tons of gill-breathing organisms and typically blooms during the cooler months.



The broad scope of my research is to help resolve the relationships between variables that promote increased toxin concentrations in populations of *P. parvum* and the presence of exotoxins. This objective will be completed in several phases; due to inherent difficulties with the detection of this alga and its toxins, molecular and phytochemical tools are initially necessary for the detection and quantification of *P. parvum* and its associated toxins.

A DNA-based assay was developed that amplifies targeted template by polymerase chain reaction (PCR) for the detection and quantification of *P. parvum* (Figure 2). These PCR methods were designed to amplify four species- and gene-specific products simultaneously in a single reaction tube (multiplex PCR) to increase the number of genetic markers utilized for verification.

Using conventional multiplex PCR, four gene products are amplified simultaneously and the products are visualized in electrophoresis gels as a diagnostic banding pattern (Manning 2006). Multiplex PCR is also applicable to real-time technology using molecular beacons with laser-excited fluorochromes. In real-time, the quantity of DNA is measured by the fluorescence intensity and displayed after each PCR cycle. Collectively, a suite of primers and molecular beacons for multiplex qPCR will allow for the species-specific detection and quantification of *P. parvum* with the confirmation of four species- and gene-specific products (Manning and La Claire 2010, manuscript in review).

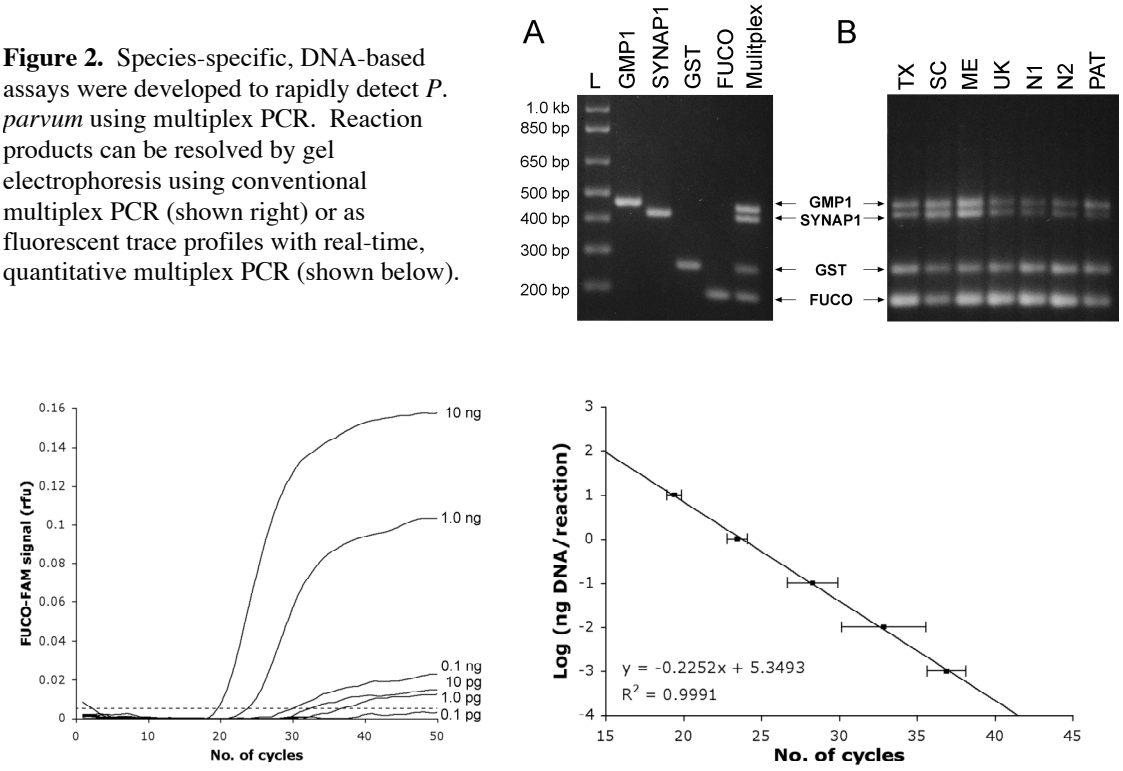


Figure 2. Species-specific, DNA-based assays were developed to rapidly detect *P. parvum* using multiplex PCR. Reaction products can be resolved by gel electrophoresis using conventional multiplex PCR (shown right) or as fluorescent trace profiles with real-time, quantitative multiplex PCR (shown below).

Improved methods are also needed for the rapid isolation and detection of prymnesin-1 ($C_{107}H_{154}Cl_3NO_{45}$; 2263 Da) and prymnesin-2 ($C_{96}H_{136}Cl_3NO_{35;}$ 1969 Da), toxic polyketides produced by *P. parvum* (Figure 3). These secondary metabolites have specific biological actions and unique spectral properties that will be utilized for the quantification of prymnesins and analysis of their respective activities (e.g., metabolic fingerprinting). The development of a small-scale phytochemical extraction protocol will streamline standard methods to a microliter-scale, and the proposed detection methods will be designed for UV-visible spectrophotometry, such that unknown samples can be quantified.

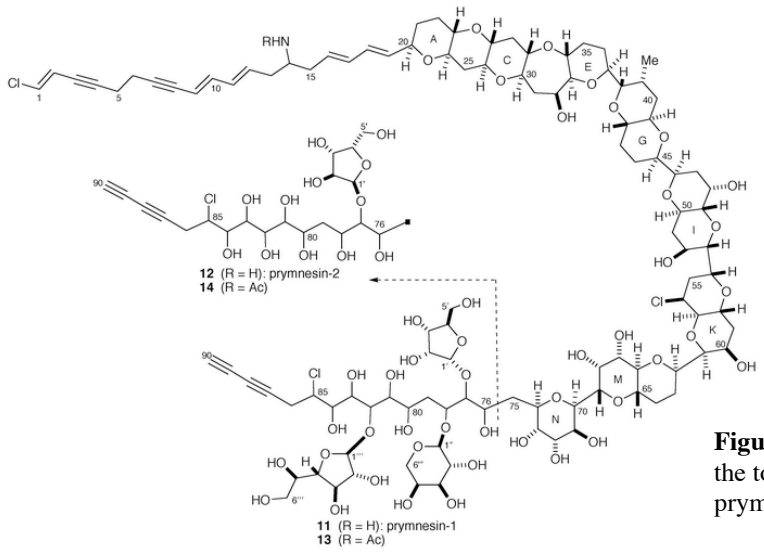


Figure 3. Putative stereochemistry of the toxic polyketides, prymnesin-1 and prymnesin-2

Toxin concentrations will then be evaluated for different cell densities, stages in the growth cycle and different growth conditions such that they can be tested for variations in toxin presence in the cells themselves and in culture supernatants. These types pf experiments will allow the following questions to be addressed:

- Are there variations in toxin production at different points in the growth curve?
- Are there differences in toxin production for *P. parvum* cells in environments with low phosphorus and/or low nitrogen?
- Are there more toxins sequestered in cells than the amount of toxins released into the surrounding environment?
- What are the effects of salinity and temperature on toxin production?

These data will be analyzed statistically by principal components analysis (PCA) to determine which factor(s) contribute(s) most significantly to the synthesis of prymnesins. PCA can also be used to generate predictable models for *P. parvum* blooms and toxicity.

Parallel examinations with *P. parvum* are using microarrays to identify suites of genes that are up-/down-regulated under varying culturing conditions and at different stages in the growth curve). Information obtained from toxin investigations can be corroborated with these microarray analyses to identify genes involved in numerous physiological pathways, including the presently-unknown molecular mechanisms of prymnesin synthesis and toxicity.