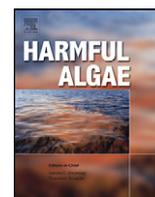


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Incorporating molecular tools into routine HAB monitoring programs: Using qPCR to track invasive *Prymnesium*

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ABSTRACT

Microscopy, a staple of monitoring programs for tracking the occurrence and abundance of harmful algal bloom (HAB) species, is time consuming and often characterized by high uncertainty. Alternate methods that allow rapid and accurate assessment of presence and abundance of HAB species are needed. For many HAB species, such as the toxigenic haptophyte, *Prymnesium parvum*, molecular methods including quantitative real-time PCR (qPCR) have been developed with the suggestion that they should be useful for monitoring programs. However, this suggestion rarely has been put into action. In this study, we modified a recently developed method for detecting *P. parvum* using qPCR and tested its efficacy as an alternative to microscopy for *P. parvum* detection and enumeration in a long-term monitoring program in a recently invaded subtropical US reservoir. Abundance estimates of *P. parvum* were similar for both methods, but we detected *P. parvum* at multiple sites using qPCR where it previously had gone undetected by microscopy. Using qPCR, we substantially reduced processing time, increased detection limit and reduced error in *P. parvum* abundance estimates compared to microscopy. Thus, qPCR is an effective tool for detecting and monitoring *P. parvum*, particularly at pre-bloom densities, and should likewise prove useful in monitoring programs for the other HAB species for which qPCR methods have been developed.

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1. Introduction

Methods for rapidly and accurately processing field samples to assess the presence of harmful algal bloom (HAB) species are vital to any monitoring program attempting to understand HAB species distributions within aquatic systems and networks, their dynamics within those systems, and ultimately preventing or mitigating their harmful affects (HARRNESS, 2005). The capacity to detect the formation of blooms early in their development is particularly important if we hope to subvert their establishment. One such species, *Prymnesium parvum*, has invaded plankton communities in coastal and freshwater systems throughout the world, blooming and killing fish and other organisms (Carter, 1937; Edvardsen & Imai, 2006). Since its discovery as the agent responsible for fish kills in southwestern Texas in the 1980s, *P. parvum* has expanded its range dramatically, blooming and causing fish kills in Oklahoma

and across the southern United States, from California to Florida (Hambright et al., 2010).

Sampling for *P. parvum* typically involves obtaining a water sample, preserving it, and examining small subsamples under the microscope during which *P. parvum* cells are identified and counted. Microscopy can be time and labor intensive, especially if low error and detection limits are important. In many HAB monitoring programs that depend solely on microscopy, accuracy is often sacrificed for increased sample number, leading to conservatively high limits of detection and reduced capability for early detection, invasion status, or bloom initiation. Additionally, some HAB species are relatively small and fragile and can become distorted during preservation (Galluzzi et al., 2008), making their identification and detection even more difficult. Furthermore, enumeration of HAB species is often conducted amidst a diverse plankton assemblage, especially prior to bloom formation, making cells even more difficult to distinguish from other closely related taxa, thereby making highly trained personnel essential (Humbert et al., 2010). Although microscopy is commonly available in most laboratories, the above-mentioned factors render this method inefficient for accurate routine assessments of HABs. Because of these shortcomings, numerous molecular approaches have been developed for assessing HAB species (Humbert et al., 2010; IOC, 2010). Specifically for *P. parvum*,

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methods for identifying and quantifying specific small subunit rRNA genes, such as dot blot hybridization (Simon et al., 2000), flow cytometry (Simon et al., 1997), and solid phase cytometry (Töbe et al., 2006) have been developed. West et al. (2006) also have developed a method based on solid phase cytometry and monoclonal antibodies. While excellent data can be generated using any of these methods, they can be prohibitively expensive, especially in cases involving numerous samples or continuous on-site monitoring.

Quantitative PCR (hereafter qPCR) is another molecular approach that has become a relatively common tool for monitoring HAB species (Humbert et al., 2010; Galluzzi and Penna, 2010; Martins and Vasconcelos, 2011). Owing to factors such as fast processing times and relatively low cost of initial setup and consumables, qPCR provides a relatively inexpensive alternative for HAB species enumeration and monitoring (Galluzzi and Penna, 2010). Multiple qPCR methods have been devised for HAB species, including *Pseudo-nitzschia* (Fitzpatrick et al., 2010), numerous dinoflagellates (Bowers et al., 2000; Galluzzi et al., 2004; Kavanagh et al., 2010), various cyanobacteria (Koskenniemi et al., 2007; Al-Tebrineh et al., 2011), and *P. parvum* (Galluzzi et al., 2008; Manning and La Claire, 2010), but most of these methods have not been incorporated into routine monitoring programs. Since *P. parvum* invaded and bloomed in Lake Texoma, OK–TX in 2004, we have been monitoring its population dynamics using microscopy-based methods (Hambright et al., 2010). Beginning in 2008, we began monitoring *P. parvum* using a modified version of the qPCR method developed by Galluzzi et al. (2008). Here we show that not only can this method be used as a viable alternative to microscopy in a routine monitoring program, but that qPCR also offers a lower limit of detection and higher levels of accuracy than microscopy.

2. Materials and methods

2.1. Study site

Lake Texoma is an impoundment of the Red and Washita Rivers on the border of Oklahoma and Texas, USA. It was constructed in 1944 for flood control, hydropower generation and recreation. *P. parvum* first bloomed in Lake Texoma in the winter of 2003–2004, with large populations restricted to the Red River arm of the lake, and blooms to littoral areas (Hambright et al., 2010). A regular monitoring program has been maintained since 2005 and in January 2008 we began collecting samples for use in molecular assays of *P. parvum* using qPCR at eight littoral sites (L) and five pelagic sites (P), either on the Red River arm (L1, L2, L3, L4, P1, P2), the main body (L5, L6, P3, P4), or the Washita River arm (L7, L8, P5) of the lake (see Hambright et al., 2010 for further site descriptions).

2.2. qPCR primer redesign

Methods for quantifying *P. parvum* via qPCR using primers targeting the ribosomal internal spacer transcribed (ITS2) region were developed by Galluzzi et al. (2008; PymF, 5'-TGTCTGCCGTGGACTTAGTGCT-3' and PymR, 5'-ATGGCACAACGACTTGGTAGG-3'). Galluzzi et al. (2008) did not report any specific amplicons or primer–dimers in their reactions, but we were unable to repeat their methods without formation of primer–dimers (which produced artificially high cell density estimates) using either *P. parvum* monocultures (UTEX strain LB 2797, UTEX Culture Collection, Colorado River, TX, USA; maintained in our lab since 2006), natural lake samples, or culture isolates derived from Lake Texoma. Addition of bovine serum albumin and manipulations of magnesium concentrations (Bustin and Nolan, 2004) failed to alleviate the problem. We deduced that the problem might have been caused by self-complementarity in the GGTTAGG motif located

on the 3' end of PymR (Rychlik, 1993). Therefore, we redesigned the PymR primer by removing 3 bases from the 3' end (creating PymR-3, 5'-ATGGCACAACGACTTGGT-3') thereby removing the potential for the two guanosine doublets to contact and form hydrogen bonds (Hardin et al., 1991). We used Primer BLAST (Rozen and Skaletsky, 2000) to check for target specificity in GenBank, EMBL, DDBJ, and PDB databases and tested the redesigned primer using varying concentrations of lake and culture samples. Our primers only produced matches to the ITS2 rRNA gene of *P. parvum* (GenBank accession numbers: *P. parvum* AM690999.1, *P. parvum* f. *patelliferum* AF289038.1, *P. parvum* FJ907460.1). The use of the PymR-3 primer eliminated the appearance of primer–dimers (as determined by the melt curve analysis with a dissociation protocol at the end of each qPCR run) in all subsequent reactions.

Robustness of the modified protocol was assessed using *P. parvum* strains from diverse geographic locations, including field samples and *P. parvum* cultures derived from Dunkard Creek, West Virginia, PA, USA (UOBS-WANA 576); Lake Granbury (UOBS-Granbury-506); TX, USA; Lake Diversion, TX, USA (UOBS-Diversion 504); Colorado River, TX, USA (UTEX LB 2797); FL, USA (UTEX LB 22837); and Norway (ppar 054, Bjerknes 28, Bognefiorden 28, and NIVA-3/89/3 ES). Our method accurately identified *P. parvum* from all locations and cultures.

2.3. Standard curve

We constructed standard curves using linear plasmid DNA containing the cloned sequence targeted by the primers on the ITS2 region of *P. parvum* strain UTEX LB2797. We purified the plasmid DNA using the UltraClean Standard Mini Plasmid Prep Kit (MoBio Laboratories Inc.). This product was then digested with enzyme Scal (Invitrogen) inside the ampicillin resistance gene and repurified in linear form using an UltraClean 15 DNA Purification Kit (MoBio Laboratories Inc.). We used linear plasmid DNA to avoid overestimation of gene and cell copy numbers that can result from supercoiling of circular plasmid DNA in the qPCR reaction (Hou et al., 2010). We quantified our linear plasmid using a high sensitivity Quant-iT dsDNA assay kit on a Qubit fluorometer (Invitrogen) and calculated plasmid copy concentration based on the average mass of one base pair. We constructed standard curves using 10-fold serial dilutions ranging from 2 to 2×10^6 copies with three analytical replicates. Data from four independent standard curves with freshly made plasmid standards were used for standard curve calibration for lake samples and data from nine independent standard curves were used for standard curve calibration for culture samples. Different curves were used for quantification of the two sample types because different well factors were used in the determination of sample fluorescence for lake and culture samples during the qPCR reaction (persistent and dynamic well factors, respectively). A standard curve based on linear plasmid DNA and no-template negative controls were included in each run and baseline threshold was held constant between runs to ensure the consistency of standards and quantification between runs.

2.4. Microscope vs. qPCR and error comparison

Samples for microscope counts were preserved using Lugol's solution (1% final concentration) and counted within two days from collection (haemocytometer method minimum of six 1 μ L subsamples; detection limit = $\sim 333 \pm 817$ cells mL^{-1} (mean \pm SD)) using a stereomicroscope with DIC Nomarsky illumination at 200 \times magnification. Identification was confirmed at 400 \times magnification. Although this detection limit generates values that individually are not statistically different from zero, our long-term monitoring

program has revealed that such low values, when at multiple sites and times, are biologically significant. Furthermore, this method was used in order to maintain comparability with Texas Parks and Wildlife Department data for *P. parvum* in Lake Texoma during 2004–2006, and to allow timely assessment of *P. parvum* in thirteen lake samples weekly, as standard counts following sedimentation (Lund et al., 1958) would have greatly decreased sample throughput. For qPCR enumeration, we filtered 100–1000 mL (depending on particulate load) of lake water through GF/F glass-fiber filters using gentle vacuum (<0.17 kPa) and then applied methods adapted from Countway and Caron (2006) for processing the material retained on the filter. The filters were folded twice and submerged in 2 mL of lysis buffer (100 mM Tris [pH 8], 40 mM EDTA [pH 8], 100 mM NaCl, 1% sodium dodecyl sulfate), 200 μ L of 0.5 mm zirconia–silica beads, in 15 mL Falcon tubes and stored at -20°C until processing. Frozen filters were thawed in a 70°C water bath for 5 min and lysed by bead beating on a vortexer set to the highest setting for 30 s. Heating and bead beating was repeated two additional times to ensure complete lysis. The crude lysates were then poured into sterile, 2 mL microcentrifuge tubes and stored at -20°C until they were analyzed with qPCR. All samples were analyzed as crude lysates diluted 1:100 in qPCR reactions to remove inhibitory effects of lysis buffer, cellular contents, or other organismal DNA before adding them to qPCR reactions.

For lake samples, we performed qPCR assays in a final volume of 25 μ L containing $1\times$ SYBR Green PCR Mastermix (Applied Biosystems), 100 nM PymF and PymR-3 primers (Invitrogen), and 3 μ L of template from the diluted crude lysates. For each sample, we examined at least three replicate reactions using 96-well plates sealed with optical film B (BioRad) on an iQ5 real-time PCR detection system (BioRad) and analyzed using the associated iQ5 optical system software. Each reaction included an initial denaturation at 95°C for 4 min followed by 40 cycles at 95°C for 15 s and 60°C for 1 min (Galluzzi et al., 2008). Fluorescence within qPCR reactions for lake samples was determined using persistent well factors. Detections were considered significant for samples that produced a detectable signal in at least two replicates and the mean DNA quantity was significantly different from 0 ($P \leq 0.05$).

Because qPCR quantifies the number of copies of a targeted sequence and there are potentially multiple copies of that sequence in a cell, we needed to determine the copy number of the sequence that we targeted for a *P. parvum* cell before we could translate gene quantities into meaningful cell densities. To determine the mean sequence copy number per cell for lake samples, we conducted three independent experiments in which we created 24 independent samples from log-phase growth batch cultures, dilutions of those cultures, and dilutions of steady-state chemostat cultures from a strain of *P. parvum* isolated from Lake Texoma (site L2; UOBS-LebanonPool-249). Cultures were grown in batch and chemostat in modified COMBO medium (Kilham et al., 1998) at N:P = 16:1 (800 μM N: 50 μM P), supplemented with 6 g Instant OceanTM L^{-1} , and maintained on a 12:12 light:dark cycle at 25°C . Sample *P. parvum* abundances ranged from ~ 150 – $15,000$ cells mL^{-1} . We used low-cell densities to avoid inhibition of qPCR reactions due to excessive amounts of template DNA. Culture sample cell densities were determined via flow cytometry (BD-FACSCalibur) and culture samples were filtered and processed for qPCR as noted above for lake samples, except we used 200 nM of PymF and PymR-3 primers to ensure that enough primer was present to bind to all of the available copies of target DNA. Fluorescence in qPCR reactions for culture samples was analyzed using dynamic well factors within separate runs. Copy number was then defined as the slope of simple linear regression of copies of the targeted ITS2 sequence detected by qPCR vs. *P. parvum* abundances detected using flow cytometry (SPSS v19). The copy number regression was forced through the origin, as copy number should

directly correspond to cell number (Motulsky & Christopoulos, 2004). Although data were not normally distributed, data were not transformed to avoid transformational biases that can occur when returning data to its original format and because the primary goal of the analysis was to define the relationship (i.e., the slope) between cell number and gene copies.

To assess the accuracy of the experimentally-determined copy number, we regressed *P. parvum* cell densities for lake samples determined microscopically with cell densities determined by translating lake sample sequence quantities determined via qPCR. Cell densities in lake samples were estimated by dividing the quantities of the targeted sequence in each sample by the number of sequence copies per cell obtained from the regression analysis described above (i.e., the slope). We limited our analysis to samples from January 2008–March 2011 in which *P. parvum* was detected using both methods, $N = 177$. Four samples with standardized residuals $>4\times$ standard deviations were removed. Three of these samples had substantially higher sequence copies than were found in any other sample and would correspond to *P. parvum* abundances much higher than have ever been reported in nature. The fourth sample had extremely low numbers of sequence copies for the number of cells detected microscopically. The regression of cell density from both methods was also forced through the origin, as cell density determined microscopically should directly correspond to cell densities determined via qPCR (Motulsky & Christopoulos, 2004). We also plotted cell density estimates through time from both the microscope and qPCR to further compare the two methods. To estimate and compare the measurement error associated with our qPCR and microscopical methods we determined the coefficient of variation from the analytical replicates and subsamples for each lake sample that produced a significant cell density estimate by either qPCR or microscope count, respectively. These error estimates were plotted against the *P. parvum* cell densities produced from those measurements and we determined the relationship between cell density and error using simple curve fitting (SPSS v19).

3. Results

3.1. Standard curve

The four independent standard curves for quantifying lake samples based on fluorescence assessed with persistent well factors yielded a linear relationship between the threshold cycle (C_t) and the \log_{10} of the starting quantity of linear plasmid ITS2 rDNA sequence (SQ), $C_t = -3.64 \times 64(\text{SQ}) + 34.85$, $r^2 = 0.99$, efficiency = 0.88% (Fig. 1a). The nine independent standard curves for quantifying culture samples from the copy number experiments based on fluorescence assessed with dynamic well factors yielded the relationship $C_t = -3.39(\text{SQ}) + 32.45$, $r^2 = 0.99$, efficiency = 0.97% (Fig. 1b).

3.2. Microscope vs. qPCR and error comparison

The average number of copies of the targeted sequence of ITS2 rDNA per *P. parvum* cell based on samples from the strain isolated from Lake Texoma was 11.7 ± 0.6 (slope \pm SE) (Fig. 2a). Quantities of the targeted sequence obtained through qPCR for the strain isolated from site L2 were also a good fit to cell densities obtained via flow cytometry ($N = 24$, Adj. $r^2 = 0.94$, $P < 0.001$; Fig. 2a). When converted to cell densities using this copy number, gene quantities obtained for lakes samples by qPCR were equivalent to cell density estimates determined microscopically qPCR cell density = 1.1 (microscope cell density; $N = 177$, Adj. $r^2 = 0.85$, $P < 0.001$; Fig. 2b).

Overall, *P. parvum* abundance estimates by both qPCR and microscopy were similar at each of the 13 monitored sites through

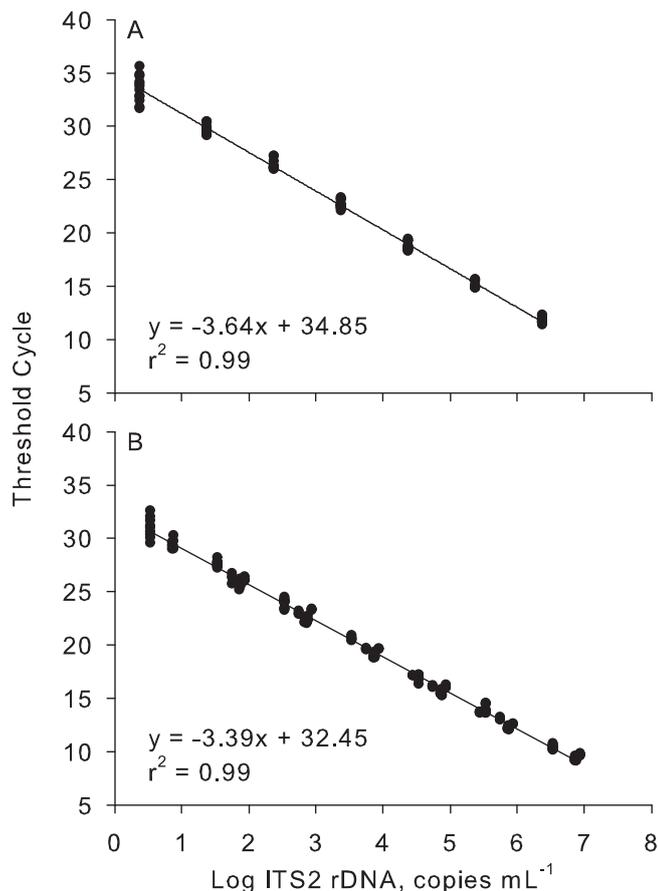


Fig. 1. Composite standard curve calibration of *Prymnesium parvum* qPCR method produced via dilutions of linear plasmid containing the ITS2 rDNA sequence using (a) persistent well factors for lake and (b) dynamic well factors for culture samples.

time (Figs. 3 and 4). The highest abundances by either method were recorded during winter months at sites typically known to experience *P. parvum* blooms (e.g., L2, L3, P1; for detailed site locations see Fig. 1 in Hambright et al., 2010). At other sites with mid-range abundances of *P. parvum* we also saw consistent results between qPCR and the microscope (e.g., L1, L4, L5, P2). The lowest cell density that we detected in the lake was 26 cells mL⁻¹ (P3), increasing our practical detection limit nearly 13-fold over the detection limit of our microscope method employing a haemocytometer (~333 cells mL⁻¹). This increase in limit of detection was most evident in sites with the lowest *P. parvum* abundances, particularly those sites in which blooms have never been observed (e.g., L6, L7, L8, P3, P4, P5).

Coefficient of variation plotted against mean *P. parvum* cell densities revealed similar power curve relationships for both qPCR ($CV = 0.59(\text{dens})^{-0.2}$; Adj. $r^2 = 0.26$, $P < 0.0001$, $N = 271$) and microscopy ($CV = 40.2(\text{dens})^{-0.5}$; Adj. $r^2 = 0.90$, $P < 0.0001$, $N = 187$) with measurement error increasing with decreasing cell density (Fig. 5). However, the coefficients of variation for qPCR-determined *P. parvum* densities were generally much lower than those for the microscope method, rising to no more than 0.7 near the minimum level of detection. By contrast, coefficient of variation for the microscope method rose to 2.5 at its detection limit.

4. Discussion

We modified Galluzzi et al.'s (2008) qPCR method by deleting three bases from the 3' end of the reverse primer and tested whether it could be used as an alternative to microscopy in our

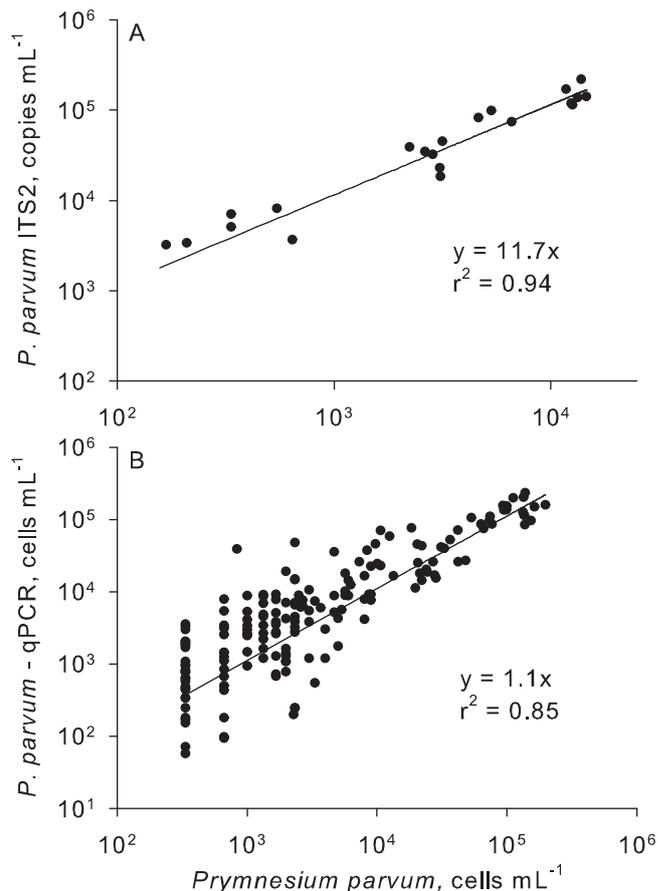


Fig. 2. (a) Linear regression of *P. parvum* abundance (cells mL⁻¹) obtained via flow cytometry and ITS2 rDNA (copies mL⁻¹) obtained via qPCR for culture samples to copy number of the targeted sequence per cell. (b) Linear regression of log *P. parvum* abundance (cells mL⁻¹) obtained via microscope counts and log *P. parvum* abundance (cells mL⁻¹) converted from ITS2 rDNA quantities obtained via qPCR from January 2008–March 2011 that detected *P. parvum* using both methods.

ongoing lake monitoring program. The modified method eliminated the occurrence of primer-dimers, was robust for multiple *P. parvum* strains from North America and Norway and provided similar cell densities in natural samples compared to those obtained by standard microscopical methods. Relative to microscopy, qPCR had a much lower detection limit with substantially lower error and time investment (see also Fitzpatrick et al., 2010). Furthermore, the copy number of the targeted ITS2 rDNA sequence for the strain isolated from Lake Texoma (11.7 ± 0.6) was consistent with values obtained for the two different strains assessed by Galluzzi et al. (2008; Strain KAC 39, Norway: 10.0 ± 2.8 and Strain CCMP 708, Scotland: 15.6 ± 1.6). This consistency suggests that our method is also robust to natural variations that could potentially affect copy number including cell division and sex (sensu a haplo-diploid life cycle; Larsen and Edvardsen, 1998), and genomic variability. Because PrymR-3 lowered detection limit and removed the potential for self-complementarity and primer-dimers during amplification, it may be more suitable for accurately quantifying the presence of *P. parvum* in natural samples than the original PrymR used by Galluzzi et al. (2008).

The high levels of total suspended solids in Lake Texoma (Atkinson et al., 1999) limited the volume of water that we could filter through a GF/F filter, restricting the amount of algae that we were able to concentrate on a filter, and ultimately, our detection limit. For example, if we were to filter 200 mL of lake water, detection of an average of one sequence copy per reaction would translate to 28 cells mL⁻¹, while the same detection after filtering

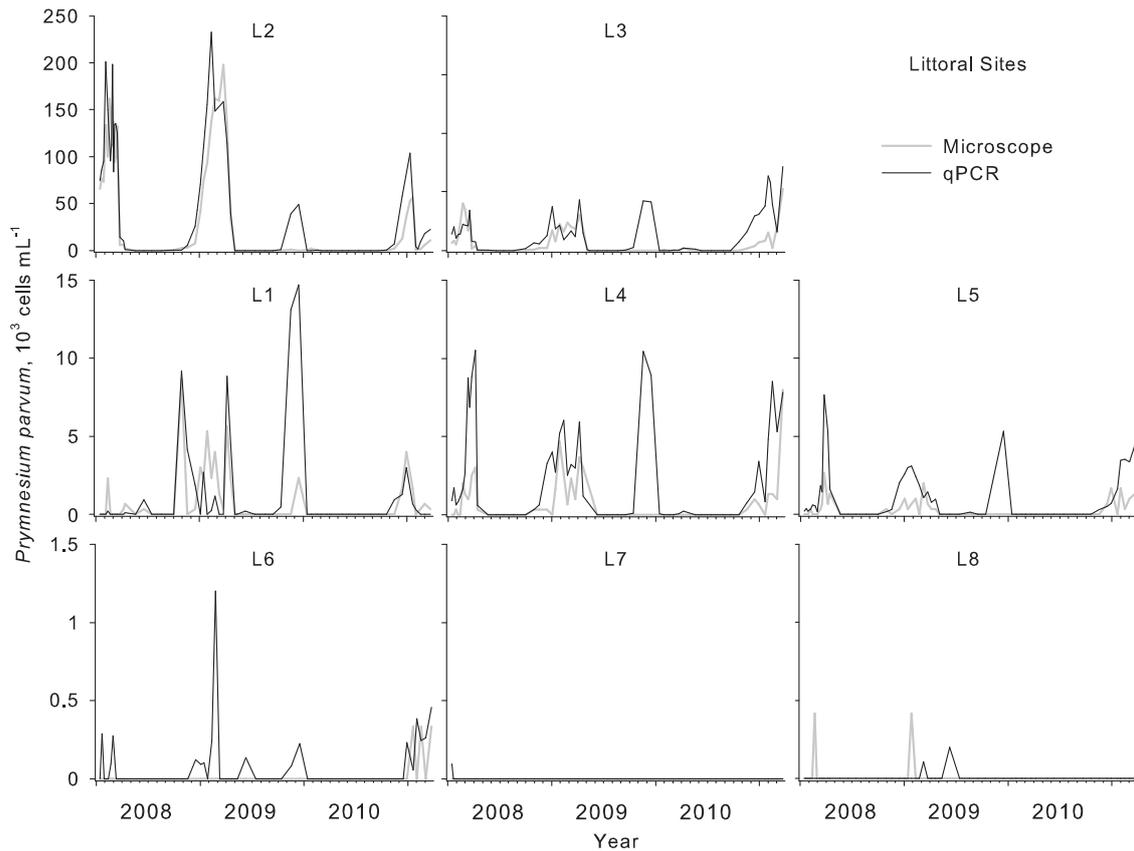


Fig. 3. Comparison of *P. parvum* abundance estimates obtained via microscope (black line) and qPCR (gray line) at littoral stations from January 2008–November 2010 in Lake Texoma. See [Hambright et al. \(2010\)](#), for description of monitoring program and sampling station details.

1 L of lake water would translate to 6 cells mL⁻¹ – a major increase in detection limit. However, our actual detection of 26 cells mL⁻¹ at P3 (800 mL filtered) and other similar findings of low cell densities by qPCR suggests that we have increased our detection limit by at least 13-fold from the detection limit of our haemocytometer and microscope method (26 cells mL⁻¹ vs. 333 cells mL⁻¹). Indeed, a 26 cells mL⁻¹ detection limit is comparable to the minimum limits

of detection that can be obtained using the Ütermohl sedimentation method with extensive subsampling under an inverted microscope ([Lund et al., 1958](#)), which is even more time intensive than our haemocytometer method.

While the patterns of *P. parvum* cell densities based on qPCR were similar to those based on microscope enumeration, we were able to detect *P. parvum* on many occasions in which the detection

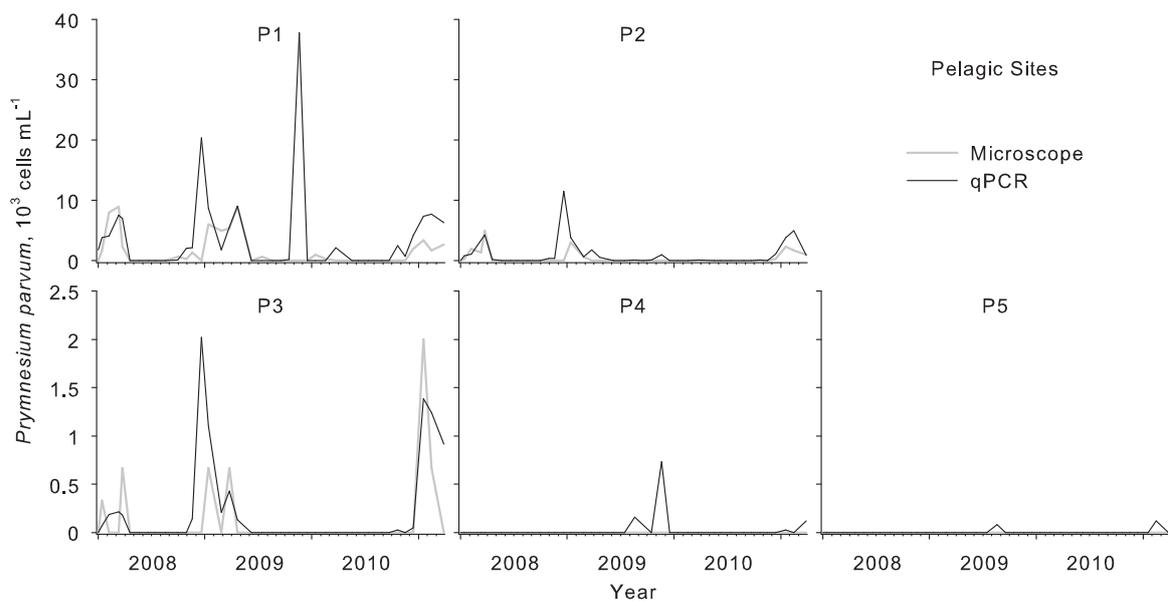


Fig. 4. Comparison of *P. parvum* abundance estimates obtained via microscope (black line) and qPCR (gray line) at pelagic stations from January 2008–November 2010 in Lake Texoma. See [Hambright et al. \(2010\)](#), for description of monitoring program and sampling station details.

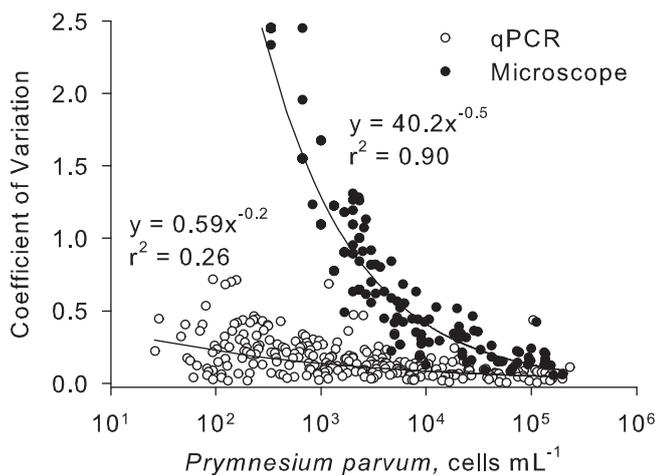


Fig. 5. Error estimates (coefficient of variation) for measurements of *P. parvum* cell density using qPCR (white circles) and microscopy (black circles).

limit of our microscopical methods precluded its detection (e.g., L6, L7, L8, P4, P5). As we approached these low cell densities, measurement error for both methods increased. However, measurement error in the qPCR samples was weakly explained by cell density (26% of the variance) suggesting that something other than cell density is a better predictor of measurement error using qPCR. Measurement error was also substantially lower for qPCR (180% lower at maximum values, i.e., CV = 1.8), which lends credence to values obtained using this method. In late 2009, numerous sites (L1, L2, L3, L4, L5, P1) showed increases in *P. parvum* abundances detected by qPCR that were consistent with bloom formation patterns in previous years, but that went undetected by the microscope even though cell densities were well above its detection limit (up to 50,000 cells mL⁻¹). There are a few instances where *P. parvum* was detected by the microscope, but not by qPCR (e.g., L8 February 2008 and January 2009). As pointed out above, such low values at the microscope detection limit ($\sim 333 \pm 817$ cells mL⁻¹) are not statistically different from zero when considered individually. Given the vast improvement in detection limit using qPCR it is likely that those two instances are in fact due to microscope error (e.g., misidentification of a morphologically similar cell, etc.). The increase in detection limit, the reduction of measurement error, and the detections of *P. parvum* by qPCR that were not detected by microscopy suggest that using qPCR should increase the ability to detect *P. parvum* earlier during an invasion event or during bloom formation and that the enumeration by the microscope-based haemocytometer method can miss early bloom formation even when abundances are above its detection limit.

Since Galluzzi et al. (2008) developed the original qPCR method for assessing *P. parvum* densities using SYBR green, Manning and La Claire (2010) developed a new method incorporating multiplex methods and molecular beacons for qPCR using 4 genomic DNA markers. Although this multiplex method is more technologically advanced it is hindered by a reduced reaction efficiency of the probes with increasing geographic and genetic distance. Hence the multiplex method would likely also be useful in lake monitoring programs, but would need to be optimized to a given system and ecotype. Because we targeted a more conserved genetic marker in the small subunit rDNA, our modified method seems to have avoided this limitation as the gene copy number for the targeted ITS2 region is consistent across 4 different strains and two continents and is able to detect successfully 14 different strains across 2 continents. Thus it appears that our modified Galluzzi et al. method may be more generally applicable in monitoring programs than multiplex methods.

Because we assess *P. parvum* densities at 13 sites in the lake approximately 32 times per year (up to 400 samples annually; Hambright et al., 2010), we have chosen to sacrifice detection limit for quantity of samples by using the haemocytometer-based microscopy method for enumeration. At approximately 15 min per subsample, and at least six subsamples per sample, monitoring *P. parvum* abundances via microscopy is a significant investment of time (~ 600 person-hours annually). Using qPCR, we can quantify *P. parvum* densities in 24 samples with triplicate replication every 3 h. Of these 3 h, only 1 h is required for setting up the qPCR reactions, while during the remaining time the machine is running unattended. Thus, we can count the same number of samples (with lower error and higher resolution, precision, and accuracy) for a given year in 8% of the time necessary with microscopy (~ 50 person-hours).

In conclusion, in this study we show that qPCR can be used successfully to assess and efficiently track *P. parvum* abundances in natural environmental samples. The method is specific, sensitive, and rapid. Since qPCR methods have been and are being developed for many HAB species, it is likely that similar methodology employing qPCR could be expanded in order to help shift the focus from a reactive stance dealing with the harmful effects of *P. parvum* and other HABs after they have bloomed to a more proactive stance aiding in the early detection of blooms or the invasion of new ecosystems. Specifically, detection of *P. parvum* with qPCR could prove useful if combined with routinely collected environmental data to predict *P. parvum* presence in new systems where it has previously gone undetected or in predicting which systems *P. parvum* might invade in the future. This would be particularly salient for newly forming monitoring programs for systems in which *P. parvum* has recently or is currently invading.

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