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Intercalibration of classical and molecular techniques for identification of *Alexandrium fundyense* (Dinophyceae) and estimation of cell densities

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Abstract

A workshop with the aim to compare classical and molecular techniques for phytoplankton enumeration took place at Kristineberg Marine Research Station, Sweden, in August 2005. Seventeen different techniques – nine classical microscopic-based and eight molecular methods – were compared. *Alexandrium fundyense* was the target organism in four experiments. Experiment 1 was designed to determine the range of cell densities over which the methods were applicable. Experiment 2 tested the species specificity of the methods by adding *Alexandrium ostenfeldii*, to samples containing *A. fundyense*. Experiments 3 and 4 tested the ability of the methods to detect the target organism within a natural phytoplankton community. Most of the methods could detect

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cells at the lowest concentration tested, 100 cells L^{-1}, but the variance was high for methods using small volumes, such as counting chambers and slides. In general, the precision and reproducibility of the investigated methods increased with increased target cell concentration. Particularly molecular methods were exceptions in that their relative standard deviation did not vary with target cell concentration. Only two of the microscopic methods and three of the molecular methods had a significant linear relationship between their cell count estimates and the *A. fundyense* concentration in experiment 2, where the objective was to discriminate that species from a morphologically similar and genetically closely related species. None of the investigated methods were affected by the addition of a natural plankton community background matrix in experiment 3. The results of this study are discussed in the context of previous intercomparisons and the difficulties in defining the absolute, true target cell concentration.

Keywords: *Alexandrium fundyense*; *Alexandrium ostenfeldii*; Intercalibration; Microscope; Molecular techniques

1. Introduction

Harmful algal blooms (HABs) are a global concern (Hallegraeff, 1995). Monitoring coastal waters for the presence of potentially harmful microalgae is therefore essential to assess the risk of bloom formation. Normally, this type of monitoring involves microscopic examination of plankton samples, and requires considerable taxonomic experience, because the identification is based on morphological characteristics and the species of interest frequently occur only as a minor component of the plankton community. Microscopic-based methods are therefore continuously fine tuned and modified (e.g., Fritz and Triemer, 1985; Klut et al., 1989; Elbrächter, 1994; Yamaguchi et al., 1995). During the last two decades, the desire to develop methods for rapid and specific identification with high sensitivity has motivated phycologists to explore the capability of molecular based techniques for species identification and enumeration (e.g., Anderson, 1995; Lim et al., 1996; Anderson et al., 1999; Haley et al., 1999; Bowers et al., 2000; Bolch, 2001). As a consequence, numerous methods have been employed in this field, each method with its own advantages and disadvantages.

In August 2005, an inter-comparison workshop on new and classical techniques for determination of the numerical abundance of harmful algal bloom (HAB) species was conducted at the Kristineberg Marine Research Station in Sweden. Scientists with experience in selected enumeration and identification techniques were invited to participate at the workshop. The overall objective was to compare cell count results using a variety of quantitative techniques that included both classical and molecular approaches. The target organism was *Alexandrium fundyense* Balech. Each participant was responsible for one or at most two specific enumeration methods, with the proviso that all methods to be compared should be fully developed and ready for operational use. Samples were provided to participants in a ''blind'' fashion, and only the experiment organisers were cognizant of the identity and composition of the samples distributed. Several experiments were conducted over the course of the workshop, each designed to evaluate a particular parameter or issue. The investigated parameters included the limit of detection, the specificity of the method, the accuracy and precision of the method, and the sensitivity to background species.

The specific objective of the first experiment was to determine the limits of detection of each method. The second experiment tested each method's ability to discriminate *A. fundyense* from the closely related species *Alexandrium ostenfeldii* (Paulsen) Balech et Tangen), known to co-occur in many locations. The third and the fourth experiment examined the accuracy of each counting method when the target organism is in the presence of different amounts of other phytoplankton and detritus (i.e., matrix effects).

Here, we present and compare the results of the intercomparison workshop.

2. Material and methods

2.1. The methods

Seventeen different methods for identification and enumeration of microalgae were tested (Table 1). Each participant conducted one or at most two methods. The classical microscopic methods were represented by techniques based on sedimentation (methods 1–3), filtration (methods 4–6), and different types of counting chambers or slides (methods 7–9). The molecular methods were represented by techniques based on polymerase chain reaction (PCR, method 10), whole-cell ribosomal RNA (rRNA) hybridisation (methods 11–14), rRNA sandwich hybridisation (methods 15 and 17), and rRNA hybridisation (method 16).