Harmful Algae 75 (2018) 94–104

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

A novel portable filtration system for sampling and concentration of microorganisms: Demonstration on marine microalgae with subsequent quantification using IC-NASBA

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ARTICLE INFO

Article history:
Received 9 March 2018
Received in revised form 19 March 2018
Accepted 24 March 2018
Available online xxx

Keywords:
Karenia brevis
Tetraselmis suecica
Filtering system
Concentrator
NASBA
Quantification

ABSTRACT

This paper presents a novel portable sample filtration/concentration system, designed for use on samples of microorganisms with very low cell concentrations and large volumes, such as water-borne parasites, pathogens associated with faecal matter, or toxic phytoplankton. The example application used for demonstration was the in-field collection and concentration of microalgae from seawater samples. This type of organism is responsible for Harmful Algal Blooms (HABs), an example of which is commonly referred to as "red tides", which are typically the result of rapid proliferation and high biomass accumulation of harmful microalgal species in the water column or at the sea surface. For instance, *Karenia brevis* red tides are the cause of aquatic organism mortality and persistent blooms may cause widespread die-offs of populations of other organisms including vertebrates. In order to respond to, and adequately manage HABs, monitoring of toxic microalgae is required and large-volume sample concentrators would be a useful tool for *in situ* monitoring of HABs. The filtering system presented in this work enables consistent sample collection and concentration from 1 L to 1 mL in five minutes, allowing for subsequent benchtop sample extraction and analysis using molecular methods such as NASBA and IC-NASBA. The microalga *Tetraselmis suecica* was successfully detected at concentrations ranging from 2×10^5 cells/L to 20 cells/L. *Karenia brevis* was also detected and quantified at concentrations between 10 cells/L and 10^6 cells/L. Further analysis showed that the filter system, which concentrates cells from very large volumes with consequently more reliable sampling, produced samples that were more consistent than the independent non-filtered samples (benchtop controls), with a logarithmic dependency on increasing cell numbers. This filtering system provides simple, rapid, and consistent sample collection and concentration for further analysis, and could be applied to a wide range of different samples and target organisms in situations lacking laboratories.

1. Introduction

Algal blooms are a natural worldwide phenomenon, resulting from rapid accumulation of algal populations in marine and freshwater systems. They form the basis of production in marine food webs and are often recognised from distinct water discoloration, caused by the pigments of associated algae (Davidson et al., 2011; Smythe-Wright et al., 2010). Some algal blooms have negative effects on humans, marine mammals, fish, and the overall marine ecosystem, with the harmful impact attributed either to high biomass or the production of biotoxins (Anderson et al., 2012; Anderson et al., 2002); the latter is of particular concern due to

Abbreviations: LOC, Lab-on-a-Chip; HAB, Harmful algal blooms; IC-NASBA, nucleic acid sequence-based amplification with internal control.

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https://doi.org/10.1016/j.hal.2018.03.006
1568-9883/

toxin accumulation in seafood, which can lead to human food poisoning. Consequently, Harmful Algal Blooms (HABs) have been well studied as they have a significant impact on the global economy and public health (Backer et al., 2015; Hoagland et al., 2002). In the United States alone, they annually affect expenses in public health ($20 million), commercial fisheries ($18 million) and recreational tourism ($7 million), while monitoring and management costs account for another $2 million (Hoagland et al., 2002).

There are HAB-associated species in several phytoplankton groups, including diatoms, dictyochophyceae, dinoflagellates, haptophytes, raphidophyceae, and cyanobacteria. Dinoflagellates make up the majority of toxin producing microalgae and were even thought to be the only HAB species until the 1980s (Arff and Martin-Miguez, 2016). As of 2012, there have been 2377 described dinoflagellate species, 80 of which are listed as toxin producers (Arff and Martin-Miguez, 2016; Gómez, 2012), and responsible for poisoning of marine life, animal mortalities and respirational conditions in humans (Ferrante et al., 2013; Fleming et al., 2011; Hallett et al., 2016; Pierce and Henry, 2008; Wang, 2008).

Thousands of fish and other species are killed annually by *Karenia brevis* (*K. brevis*) red tides alone, and persistent blooms may cause widespread die-offs of benthic communities and short-term declines in local fish populations (Landsberg et al., 2009). This toxic dinoflagellate is capable of having adverse effects on human health starting from concentrations as little as 5 cells/mL (Bricelj et al., 2012) and is currently monitored by the Florida Fish and Wildlife Conservation Commission (FWRI, 2015) at concentrations between 10^3 cells/L (bloom not present) and 10^6 cells/L (bloom with high cell density). Even though there may be multiple causes of red tides, nutrients such as nitrates and phosphorus have an important role in sustaining microalgal blooms (Vargo et al., 2008). As a result, it is not surprising that areas of significant human induced pollution may lead to increased frequency of red tide outbreaks (Liu et al., 2013). Toxicity of HABs can be especially pronounced once phosphorous limitation occurs, as this has been suggested to be an important factor regulating cellular toxicity (Hardison et al., 2013). In order to adequately manage waste contamination and resulting HABs, particularly in regions of rapid economic and industrial growth, environmental monitoring is required.

Efficient sampling, sample analysis, and thus monitoring of HABs will help prevent direct or indirect damage to human health, as well as potentially significant financial losses for the fisheries and aquaculture industry. Importantly, it also serves as a means of identifying waste spills and contamination of the environment. Current methods for monitoring microalgal species using morphological assessment by microscopy or analogous techniques can be time-consuming, limiting the number of samples which can be analysed and the size of those samples. In addition, the acquired information may be limited regarding species-specific definition and toxin production. By contrast, molecular techniques, if automated, could accelerate the rate of sample analysis, while providing the benefits of increased accuracy and simultaneous examination of multiple parameters (Medlin, 2013).

This paper presents a novel filtration/concentration system, designed for the collection and concentration of seawater samples, which are characterised particularly by very low cell concentrations and therefore the requirement to process very large volumes. The system is intended primarily for manual, field sample processing of the sort required by environmental monitoring. Test samples were processed by the system and subsequently analysed using a molecular method for the detection and quantification of marine microorganisms. To demonstrate the viability of the method and to validate the operation and the detection capabilities of the system, two marine microorganisms were examined: *Tetraselmis suecica* (*T. suecica*), (Kylin) Butcher 1959 and *K. brevis*, (Davis) Hansen and Moestrup 2000.

2. Background on sample collection and molecular tools for environmental analysis

Field monitoring of ocean biology is typically done in the form of sample collection during organized cruises and sample analysis either on-board the research ship or in a laboratory at a later time. However, such research expeditions can be expensive, labour intensive and only cover a fraction of the oceans, since they follow pre-defined courses and locations. This leads to significant undersampling and, consequently, alternative sampling or monitoring methods are used in an effort to fill the gaps. Remote sensing, for instance, is a cost-effective approach for estimating phytoplankton biomass, by determining chlorophyll concentration on satellite images (Blondeau-Patissier et al., 2014; Carvalho et al., 2010). Autonomous underwater vehicles implement *in situ* and deployable sensors for the analysis of biological samples, and may be useful for getting a more complete picture of ocean biology (Schofield et al., 2013). Microfluidic biosensors and lab-on-chip technologies will also play an important part in the future of ocean monitoring; this is particularly evident when looking at projects such as the European LABONFOIL and "The Ocean of Tomorrow" initiative, both funded by the European Commission, which invested in the development of microfluidic devices for the molecular sensing of phytoplankton, among others.

Molecular tools have been employed for the study of microbial diversity and ecology in natural environments since the mid-1980s (DeLong et al., 1989). Marine biology is an interdisciplinary study of life in the world's oceans, estuaries, and inland seas (Thakur et al., 2008) and it has witnessed significant growth in the application of molecular techniques. As a result, new fields of investigation have opened (Keeling et al., 2014), the distribution and composition of microbial populations has been re-defined (Valiadi et al., 2014), and in some cases, previous studies have been re-evaluated (Burton, 1996). Marine molecular biology is constantly evolving to solve problems regarding the exploration of marine organisms for human health and welfare purposes (Thakur et al., 2008). Genomics, transcriptomics, proteomics, and metabolomics have already provided information on phylogenetic relationships among HAB taxa, pathways of toxin production, HAB diversity patterns, as well as genetic responses to grazers or inter- and intraspecies-specific competition (Anderson et al., 2012; Kohli et al., 2015).

One of the recent trends in this area, which has the potential to have a huge impact on environmental science in the future, is the use of technology to perform analysis in the field. Handheld analyzers for the detection of marine microorganisms in environmental samples, including *K. brevis,* have been investigated (Casper et al., 2007), as well as the application of biological sensors in the field of oceanography (Zehr et al., 2008). Microfluidic systems, both within and outside the field of oceanography, have been designed for numerous purposes such as molecule separation (Brody and Yager, 1997), genotyping (Rich et al., 2011) and for the performance of various biochemical and molecular assays (Lin et al., 2009). Also referred to as Lab-on-a-Chip (LOC), such systems have also been employed to monitor cell growth (Jeong et al., 2014; Lee et al., 2008), detect water-borne pathogens (Zhao et al., 2012), and observe a range cellular functions (Dimov et al., 2011) and behaviours associated with environmental toxicity (Huang et al., 2015; Zheng et al., 2014). Lab-on-a-Chip technologies provide the user with the benefits of miniaturisation, integration and automation. They therefore offer several advantages over conventional techniques: portability, speed of analysis, the ability to multiplex (Lutz et al., 2010), and platform and device compatibility with multiple molecular techniques (Loukas et al., 2017; Sun et al., 2013; Tsaloglou et al., 2013). When coupled with appropriate molecular tools, LOC devices may provide a greater understanding