Combining metabarcoding and morphological approaches to identify phytoplankton taxa associated with harmful algal blooms

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ABSTRACT

Impacts of harmful algal blooms (HABs) have increased in frequency, intensity, and geographical distribution in the last several decades. Detection methods to date largely depend on microscopic observations which require expertise and time-intensive processes. In this study, we apply microscopic observational methods, quantitative real-time polymerase chain reaction (qPCR), and metabarcoding with multiple markers (*i.e.* 16S, 18S-dinoflagellate and 18S-diatom, and large subunit (LSU) rDNA) on cultured (N=30) and field (N=24) samples containing suspected harmful algae (*e.g.*, *Alexandrium* spp., *Chattonella* sp., *Chrysochromulina* spp., *Dictyocha* spp., *Heterosigma akashiwo*, *Protoceratium reticulatum*, *Pseudochattonella verruculosa*, *Pseudo-nitzschia* spp., and *Pseudopedinella* sp). Good detectability was found using previously published TaqMan assays for *A. tamarense*, *H. akashiwo*, and *P. verruculosa*. Overall, the multiple marker metabarcoding results were superior to the morphology-based method for detection and identification of harmful taxa, with the notable exception of taxa from the silicoflagellate group (*e.g. Dictyocha* spp.), which had better detection by morphology. Metabarcoding results depended greatly on the marker type applied, which highlights the value of a multiple-marker approach. The combined results of the 18S and the LSU markers closely corresponded with morphological identification of targeted species and provided the best overall taxonomic coverage and resolution. The most numerous unique taxa were identified using 18S-dinoflagellate amplicon (N=167) and the best resolution to species level occurred using LSU (N=60). This work is the first report of HAB species identification in Canada using a combination of morphological, metabarcoding, and qPCR approaches. These results emphasize the benefit of applying molecular techniques to detect HAB taxa and highlight the current necessity of using multiple markers for accurate detection of the diverse groups that cause HABs.

Keywords: Harmful algal blooms, Next-Generation Sequencing, Metabarcoding, *Alexandrium*, *Heterosigma*, *Pseudochattonella*

INTRODUCTION

Phytoplankton form the base of the marine food web and are required to support healthy aquatic ecosystems. In some circumstances, however, high-biomass events and/or proliferation of certain algal species can cause harm to aquatic animals through a variety of means including disruption of the food web, shellfish poisoning, the development of low oxygen 'dead zones' after bloom degradation, and fish kills through toxins, gill damage, or hypoxia (Rensel and Whyte, 2004). Collectively, these events are termed harmful algal blooms (HABs). Importantly, there is a general scientific consensus that public health, fisheries, and ecosystem impacts from HABs have all increased over the past few decades (*e.g.* Andersen, 2012; Hallegraeff, 2004).

The coastal waters of British Columbia (BC), Canada, in the northeast Pacific Ocean, have one of the longest documented histories of severe HABs going back to the first reported case in 1793 (Vancouver, 1798). A government program for monitoring the presence of toxins in shellfish was established in the early 1940s (Taylor and Harrison, 2002) and since then paralytic shellfish poisoning (PSP) closures have occurred every year. The BC salmon aquaculture industry initiated and has been supporting the Harmful Algae Monitoring Program (HAMP) since the 1990s due to the devastating effects of harmful algae on farmed fish (Horner et al., 1997; Rensel and Whyte, 2004). During 2009–2012, direct losses to the BC salmon aquaculture industry from HABs were ~13 M USD (Haigh and Esenkulova, 2014). HABs are currently one of the most significant risks for the BC aquaculture industry, regularly causing severe economic losses through finfish/shellfish mortalities and shellfish harvest closures due to toxin accumulation (Whyte et al., 1997). Therefore, there is an ongoing and pressing need for monitoring and research on HABs phenomena in coastal BC.

Monitoring HABs typically depends on the effective identification and enumeration of species of concern in water samples. Algal cell identification has long been accomplished based on morphology revealed through visual microscopic examination. Although traditional light microscopy currently remains the standard, it has limitations when it comes to certain species and strains that cannot be easily visualized or cannot be discriminated between harmful and benign variants based on morphological characteristics alone (Hallegraeff, 2004). Moreover, microscopic identification is highly dependent on the level of expertise and experience of the individual analyzing the samples and with fewer morphological taxonomists being trained, it is increasingly difficult to keep up with the demand. In recent years, studying HABs with

molecular techniques, either in tandem with morphological methods or independently, has become increasingly popular. Quantitative real-time polymerase chain reaction (qPCR) is a powerful method for detecting and quantifying DNA over a broad dynamic range (Livak and Schmittgen, 2001). This method has been used for species-specific harmful algal detection and enumeration (*e.g.* Antonella and Luca, 2013; Eckford-Soper and Daugbjerg, 2015; Scholin et al., 2011). High-throughput platforms, such as the Fluidigm BioMark, allow for the use of multiple species-specific probes for simultaneous detection and enumeration of multiple taxa (Medlin and Orozco, 2017) with the potential for both time and cost savings, as well as the additional ability to identify cryptic species, compared to traditional light microscopy. Next-generation sequencing (NGS) methods, as applied through metabarcoding, allow for millions of sequencing reactions to be performed in parallel, resulting in the ability to generate massive amounts of sequencing data (Goodwin et al., 2016; Valentini et al., 2016). Sequence-based taxonomic approaches can allow for the identification of multiple species of interest, including nano- and picoplankton, rare and fragile taxa, and cryptic species, in a reproducible and cost effective manner (*e.g.* Eiler et al., 2013).

The objective of the present study was to obtain microalgal taxa that are known or suspected to be harmful to cultured fish and shellfish in BC and to identify these taxa through light microscopy as well as genetic methods. Fish-killing algae targeted included *Chaetoceros concavicorne*, *C. convolutus*, *Chattonella* sp., *Chrysochromulina* spp., *Cochlodinium fulvescens*, *Dictyocha* spp., *Heterosigma akashiwo*, *Karenia mikimotoi*, and *Pseudochattonella verruculosa.* Shellfish-poisoning algae included *Alexandrium* spp., *Dinophysis* spp., *Protoceratium reticulatum*, and *Pseudo-nitzschia* spp. During this study, cultures and water samples for identification of these species were primarily acquired from HABs occurring in coastal BC. Here we describe, compare, and cross validate coastal BC harmful algae identification based on morphology, qPCR, and metabarcoding.

METHODS

Ethics Statement

No permits were required for collection of water samples in Canadian coastal waters.

Sample Collection

All samples used in this study were collected in coastal BC waters (Table 1), except for two that were obtained from the Provasoli-Guillard National Center for Marine Algae and Microbiota (Samples s01, s02) that were used to test primer specificity and to optimize qPCR reactions. All samples (N=54), with their respective sampling locations, are summarized in Table 1 and Fig. 1.

Field samples of algae were obtained by collecting seawater with a 1 L sampling bottle (Venrick, 1978). Samples to be used for culturing and genetic analyses were kept cool and dark until processed. Samples for microscopic identification were immediately preserved with Lugol's iodine (Andersen and Throndsen, 2004) and then shipped to the laboratory for taxonomic analysis. Identification and enumeration of phytoplankton based on morphology was done with a compound microscope using a Sedgewick-Rafter slide (Guillard, 1978). Identification based on morphology was done to the lowest possible taxonomic level (Hasle, 1978), by an experienced phytoplankton taxonomist.

Cultures

Cultures for this study were isolated by serial dilution from the unpreserved field water samples and from sediments collected from sites with HABs observed in the past (see Table 1 for sample origins), as some harmful algae produce cysts that play an important role in initiating subsequent blooms (Anderson et al., 2003). Several sediment samples were taken from three areas around Vancouver Island (*i.e.* Baynes Sound, Okeover Inlet, Quatsino Sound; Fig. 1) in late winter and spring 2013. Target species for isolation and germination were *Alexandrium* spp. due to the numerous PSP closures recorded in Baynes Sound and Okeover Inlet in summer 2012 (DFO, 2012), as well as *H. akashiwo* and suspected *Chattonella* spp. that caused fish mortalities in late summer 2012 in Quatsino Sound (Haigh, personal observation). Sampling was done using van Veen and Ekman grab samplers. Sediment samples (3 – 5 L) were taken from depths of 5 – 45 m. Sites were chosen based on the assumption that cysts accumulate in the same areas as silt and clay (Wall and Dale, 1967). The upper 3 cm of sediments in the grab samples were collected, mixed well, and immediately placed in containers and kept cool and dark.

Culture establishment from the collected samples occurred as described in detail previously (Esenkulova et al., 2015). In brief, sediments were sieved to produce a 20 – 120-µm fraction and incubated in enriched natural seawater medium (Harrison et al., 1980) at 18 °C

under continuous illumination (20 µmol photons m^{-2} s^{-1} provided by full-spectrum fluorescent lamps). Serial dilutions were performed in a laminar flow hood using a micropipette and sterile 24-well plates. Several cultures of *A. tamarense*, *H. akashiwo*, and *Chattonella* spp.? (question mark indicates that taxon is suspected, but not positively identified by light microscopy) were successfully established. Isolation from water samples produced cultures of *Alexandrium* sp., *Chaetoceros eibenii* (a non-harmful species), *Chattonella* sp.?, *H. akashiwo*, *Protoceratium reticulatum*, *Pseudochattonella* cf. *verruculosa*, and *Pseudo-nitzschia* spp. Some cultures were pure (appeared to contain a single species) and some were mixed (contained multiple species).

DNA Extraction and Purification

Water samples for genetic analyses were either filtered or pelleted. For filtered water samples, 60–100 mL of water was filtered through 25-mm or 47-mm GF/F Whatman filters and then frozen at -20 °C or stored in 75–95% ethanol until DNA extraction. A section of ¼ of the 47-mm filters or ½ of the 25-mm filters were used for the extraction. Water samples concentrated by centrifugation were subsampled and 1.5 – 40 mL centrifuged at 8,000 x g, the supernatant removed, and the resultant pellet stored in 95% ethanol until DNA extraction. The variation in sampling volumes and sample methods occurred due to the large variation of suggested methods in the available literature. Cultured samples for molecular analyses were taken from ~200-mL cultures in a laminar flow hood, whereby samples of well-mixed cultures were divided in 2 x 20-mL subsamples and centrifuged for 15 min at 5,000 x g for 10 min. The supernatant was decanted, samples were re-suspended in 1 mL of phosphate buffered saline, transferred to 2-mL microfuge tubes, and then centrifuged again at 4,000 g for 10 min. Finally, the supernatant was again removed and cell pellets frozen at -20 °C until extraction.

DNA was extracted and purified from all samples using the DNeasy Blood and Tissue kit (Qiagen, Toronto, Canada) as per manufacturer's instructions, with the addition of a homogenization step using a TissueLyser 2 homogenizer (Qiagen) with a 4-mm steel bead. DNA concentration was measured by spectrophotometry (NanoDrop, ND-1000).

qPCR Assays

A literature search was undertaken to identify published TaqMan-based qPCR assays for harmful algal taxa of interest in the northeast Pacific Ocean. TaqMan assays were found for five targeted

taxa: six assays for *Alexandrium* spp., two for *Chattonella* spp., three for *H. akashiwo*, two for *K. mikimotoi*, and two for *P. verruculosa*. Additional published TaqMan assays for known harmful algae from other parts of the world were also included, bringing the total number of target algal taxa to 28 and the number of assays to 39 (Table 2).

The qPCR reactions using all assays (Table 2) were conducted on a Fluidigm BioMark™ platform (Fluidigm Corporation, San Francisco, CA, USA) using a 96x96 dynamic array to run 9,216 reactions simultaneously (96 samples with 96 assays) as described in detail in Miller et al. (2016). To reduce the effect of PCR inhibitors that can be problematic in algae, samples were tested at concentrations of 10 and 2.5 ng uL^{-1} or, if the DNA concentration was below that, at the highest available concentration. Each assay was run in duplicate.

Briefly, a pre-amplification (STA) step of 14 cycles using dilute (50 nM in a 5-uL reaction) primer pairs of each assay with TaqMan Preamp MasterMix (Applied Biosystems, Foster City, CA, USA) was performed according to the BioMark protocol (Applied Biosystems). Unincorporated primers were removed with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and samples were then diluted 1:5 in DNA Suspension Buffer (Teknova, Hollister, CA, USA). A 5-µL sample mix was prepared for each pre-amplified sample with TaqMan Universal Master Mix (Life Technologies Corporation, Carlsbad, CA, USA) and GE Sample Loading Reagent (Fluidigm Corporation) and a 5-µL aliquot of assay mix was prepared containing 10-µM primers and 3-µM probes for each separate TaqMan assay and each was loaded onto a pre-primed dynamic array. An IFC controller HX pressurized and mixed the assays and samples from their individual inlets on the dynamic array and the PCR was run on the BioMark with the following conditions: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Output data was analyzed and the cycle threshold (Ct) per sample determined using Fluidigm Real Time PCR Analysis software (Fluidigm Corporation). Cycle threshold values for qPCR replicates were averaged in the final results.

Metabarcoding and Sequencing

Four primer pairs were selected for use in NGS metabarcoding from published studies with the goal of amplifying a broad range of algal taxa (see Table 2). Illumina adaptors were incorporated onto the 5' end of each primer for the attachment of Nextera XT Illumina indices (Illumina, Inc., San Diego, CA, USA) in the second round of PCR. Input sample DNA was normalized to 5 ng

μL^{-1} when starting concentrations allowed. The primer sets were used as the initial primers in a two-step PCR process to construct and sequence four Nextera XT libraries according to the Illumina 16S Metagenomic Sequencing Library Preparation protocol (15044223, Rev. B). Library quantification, normalization, pooling, and sequencing on a MiSeq with a 600-cycle flow cell (MiSeq Sequencing Kit v3, 600 bp, Illumina, Inc.) were performed according to the manufacturer's protocols, with the only modification being that the final, pooled library was run at a concentration of 16 pM with 10% PhiX control.

Bioinformatics

All sequence data was de-multiplexed using input sample barcodes during file export from the sequencer (Illumina, Inc.), resulting in a pair of fastq files for each individual sample. Quality of raw sequence fastq files was evaluated in FastQC (Andrews and Babraham Bioinformatics, 2010) with results aggregated using MultiQC (Ewels et al., 2016). In general, the OBITools package (Boyer et al., 2016) was used for the analysis of the different amplicons, but each amplicon required specific inputs due to different features of the data. All bioinformatics steps are outlined in detail on GitHub (see *Data Availability*), and explained here per marker type (*i.e.* LSU, 18S, 16S). For LSU and 18S, due to the larger size of amplicons and the lack of read merging for these paired-end datasets, only the forward reads were used. For 16S, paired-end data were used as most reads overlapped and therefore were able to be merged.

For LSU (single-end data), primers were removed using cutadapt (Martin, 2011) and then reads were formatted for the OBITools pipeline using *ngsfilter* (OBITools) on each sample without any input barcodes. Each formatted input sample file was then annotated with a sample identifier in read headers with *obiannotate*. Sample labeled fastq files were then combined to a single file, with reads cut to a uniform size (*i.e.* 230 bp) to reduce singleton records due to slight differences in read length for single-end data. Subsequently, this file was moved into the standard OBITools pipeline (see below).

For 18S (single-end data), there were two different amplicons, one for dinoflagellates and one for diatoms (*i.e.* 18S-dinoflagellate, 18S-diatom). These two amplicon types were de-multiplexed using *ngsfilter*, which output a single fastq file per sample, but from both amplicon types. A custom script separated the two types of amplicons (see *Data Availability*). Obiannotate was used to annotate each sample-amplicon type with sample and amplicon identifiers. All data

was then merged into a single file, cut to a uniform size using cutadapt (as above), and then moved into the standard OBITools pipeline.

For 16S (paired-end data), primers were removed using cutadapt, overlapping reads were merged using *illuminapairedend* retaining merged data with an overlap score ≥ 40 (OBITools), samples were annotated with a sample identifier, and then combined to a single file and moved into the standard OBITools pipeline.

Once all data were input in a uniform format into the main pipeline as described above, the OBITools package was used to retain a single representative accession per unique amplicon sequence, keeping record of the number of reads per sample for the accession in the accession header using *obiuniq*. Subsequently, the data were de-noised using a size filter and a low count threshold (*obigrep*) and by removing 'internal' sequences (*i.e.* probable PCR/sequencing errors; *obiclean*), as per standard OBITools approaches (Boyer et al., 2016). The counts per representative unique amplicon were exported using *obitab*.

The unique amplicon file for each amplicon type was annotated using BLAST (Altschul et al., 1997) run in parallel (Tange, 2011), receiving ten alignments per BLAST query. For each unique amplicon accession, taxa were assigned using MEGAN (Huson et al., 2016) using the Lowest Common Ancestor (LCA) algorithm with the following parameters: min score = 100; max expected = 10^{-9}; min % ID = 97; top % 10; min support % (off); and min support = 1. Any amplicon that received a BLAST result, but was not assigned using MEGAN due to parameters set within MEGAN was put in the 'Not assigned' category. Any amplicon without a BLAST result at all was put in the 'Unknown' category. Read counts were connected to annotations from the MEGAN output using custom R ("R Core Team.," 2018) scripts (see *Data Availability*). A threshold of at least 10 reads per sample-taxon combination was applied to reduce the potential for false positive detection and any sample-taxon combination with fewer than this was transformed to 0. Taxa proportions were calculated by dividing the count per taxon for a sample by the total number of reads for the sample. Taxon ranks and the classification of identified taxa was enabled using *taxize* (Chamberlain and Szöcs, 2013; Scott et al., 2019) in R, and a custom database was created to contain assembly taxonomy, read count, and sample information. Bar plots were constructed using ggplot2 in R (Wickham, 2016) and pie charts constructed using Krona (Ondov et al., 2011).

RESULTS

Morphological Identification

In total, 54 samples containing either cultured (N=30) or field (N=24) collections of ten suspected harmful algal taxa identified by morphology were obtained during this study, including *Alexandrium* spp., *Chattonella* sp., *Chrysochromulina* spp., *Cochlodinium fulvescens*, *Dictyocha* spp., *Heterosigma akashiwo*, *Protoceratium reticulatum*, *Pseudochattonella verruculosa*, *Pseudo-nitzschia* spp., and *Pseudopedinella* sp. (note: *Pseudopedinella* was not initially targeted, but was acquired opportunistically at a suspected HAB event). Although other species were initially targeted, they were unable to be acquired for complete analysis, due to either low cell concentrations in field samples (*i.e. C. concavicornis*, *C. convolutus*, and *Dinophysis* spp.) or due to a general absence in field samples (*i.e. K. mikimotoi*). All samples obtained for this study are listed in Table 1.

Extraction Efficiency for Molecular Methods

Sample collection volumes, types, and preservation methods varied throughout the collection time period as sampling and extraction protocols were still being optimized. As such, DNA quantity in extractions was highly variable due to the variety in the sample state, density, and preservation methods applied. Cultured samples gave the highest yields. Filtered field samples generally resulted in lower, but adequate yields. Pelleted field samples resulted in low and often unusable yields. After extractions, 46 of the 54 samples had sufficient high-quality DNA for molecular analyses. Samples with insufficient DNA (*i.e.* s025, s026, s027, s029, s039, s051, s052, s054) were not run on qPCR, but two of these samples (*i.e.* s029 and s054) were run through NGS and returned OTUs (<25 and <1,350, respectively). Five samples (*i.e.* s034, s045, s046, s050, s053) had low DNA concentration, but returned positive results with qPCR and NGS.

Taxa Identified by qPCR

A total of 46 samples with sufficient DNA concentrations were run using qPCR. However, only eight of 39 applied TaqMan assays provided amplification with one or more of the samples (Table 3), and the rest (N=31 assays) did not return positive results in any samples. These eight assays amplified within 44 of the samples. Targeted species that were detected via qPCR

included *Alexandrium* spp./*A. tamarense*, *H. akashiwo*, and *P. verruculosa*. The targeted *Chattonella* spp. had four different qPCR assays, but none resulted in detections. Other species that are potentially harmful were identified by qPCR, but were not specifically identified by microscopy. For example, cyanobacteria species were detected in nine samples, *G. instriatum* in three and *Karlodinium micrum/veneficum* in eleven samples. Nine targeted taxa were not quantifiable by qPCR due to a lack of published TaqMan assays: *C. concavicornis*, *C. convolutus*, *Chrysochromulina* spp., *Cochlodinium fulvescens*, *Dictyocha* spp., *Dinophysis* spp., *P. reticulatum*, and *Pseudo-nitzschia* spp. Due to the fact that this study ran the assays under different conditions than they were designed for, and due to algae strain variability, the possibility for false negatives or positives cannot be excluded. To confirm if the sample contained a target species, and therefore to determine the effectiveness of the tested assays and correspondence to microscopy, NGS was conducted. The relative effectiveness of the qPCR results in comparison to microscopy and metabarcoding is provided in the *Discussion*.

Metabarcoding Overview

In total, over 350 individual taxa were detected by metabarcoding and NGS. NGS returned results for 48 of 48 sequenced samples. Detailed results of the analysis including all taxa and associated read counts are provided in Supplemental Tables S1-4.

The most numerous unique taxa occurred through using the 18S-dinoflagellate amplicon (Table 4), which returned 167 individual taxa, including 49 to the species level. For comparison, the 16S amplicon identified 69 individual taxa (21 to the species level), and 18S-diatom 78 taxa (18 to the species level). The LSU data identified 136 individual taxa and was the most effective at resolving data to the species level (N=60 species), which is noteworthy given the importance of species-level identification when identifying harmful algae. Only one taxon, *H. akashiwo*, was identified by all four amplicons. Many similar taxa (N=20) were detected by 18S-diatom, 18S-dinoflagellate, and LSU amplicons, but only three were identified to the species level.

The numbers of reads per taxonomic category, with an emphasis on microalgal groups, are listed in Table 5. Total microalgae reads detected by 18S-diatom, 18S-dinoflagellate, and LSU runs were more than 85% of all reads (not including unknowns) for each amplicon, whereas microalgae reads from 16S run comprised less than 50%. The majority of microalgal reads for all amplicons belonged to raphidophytes. The 18S-dinoflagellate amplicon detected the most

microalgal groups and the 16S amplicon detected the fewest microalgal groups. Silicoflagellates (Dictyochophyceae), an important group that includes several harmful and potentially harmful species, were detected only by 18S-diatom and 18S-dinoflagellate amplicons.

All harmful and potentially harmful algal taxa reads (*i.e.* at the species, genus, and family level) detected by all amplicons are provided in Table 6. For a more convenient comparison between results employing different amplicons, grouping of harmful and potentially harmful algae species to genus level was done for *Alexandrium* spp., *Chattonella* spp., *Dinophysis* spp., *Karlodinium* spp., *Phalacroma* spp., *Prymnesium* spp., *Pseudo-nitzschia* spp., and *Pseudochattonella* spp.

Overall, most of the reads of harmful and potentially harmful algae were detected by LSU and 18S-dinoflagellate amplicons (Table 6). At the species and genus levels, the LSU amplicon provided the most reads for dinoflagellate species within the *Cochlodinium*, *Dinophysis*, *Karlodinium*, and *Phalacroma* genera, as well as haptophytes in the *Chrysochromulina* and *Prymnesium* genera. The 18S-dinoflagellate amplicon detected the most reads for species within the *Alexandrium*, *Pseudochattonella*, and *P. reticulatum* genera, whereas the 18S-diatom amplicon detected the most reads for *Pseudo-nitzschia* spp. as well as the raphidophytes *Chattonella* sp. and *H. akashiwo*. There were no instances when 16S detected the most reads for any of the listed taxa.

The total reads and percentages per sample detected by different amplicons are shown in Figure 2. Specifically in this plot, when a taxon of harmful or potentially harmful algae had less than 100 reads, it was grouped within an appropriate, larger algae category, *e.g. Karlodinium* spp. with 77 reads were included in the dinoflagellates counts; *Prymnesium* spp. with 20 reads and Prymnesiaceae with 11 reads were included in haptophytes (Figure 2). Overall, based on the results for all four amplicons, culture samples had a higher number of reads, by an order of magnitude, and lower algal species diversity than field samples (Figure 2, Supplement 5).

A comparison of microscopy and NGS taxonomic identification in each of the 48 samples revealed that the majority of the taxa detected by microscopy also were identified in the NGS results (Supplement 6). Species and genera that were positively identified by both microscopy and NGS included: *Alexandrium, Chaetoceros, Cochlodinium, H. akashiwo, P. reticulatum, Pseudochattonella*, and *Pseudo-nitzschia*. There were a few notable exceptions, with the two most consistently observed mismatches being (i) all suspected *Chattonella* spp. (10 field and

cultures) were identified by NGS as *H. akashiwo*; and (ii) *Dictyocha* spp., *P. verruculosa*, *Pseudochattonella* sp., and *Pseudopedinella* spp. (*i.e.* all three silicoflagellate genera) were not detected in 11 out of 15 samples by any of the amplicons. Although these samples had unknown and unassigned reads in NGS results (Supplement 6), the sequences for all of the species not identified by NGS but identified with microscopy were in fact present in the Gen Bank database, and therefore the reason for the lack of these taxa, as well as the identity of these unknown reads remains unknown.

DISCUSSION

Harmful Algae: Comparing Microscopy and Molecular Approaches

The term 'algae' comprises a diverse, polyphyletic group that encompasses organisms from widely different taxonomic domains and includes eukaryotes and prokaryotes. Major algal groups include diatoms, dinoflagellates, raphidophytes, silicoflagellates, haptophytes, and cyanobacteria. Identification methods applied to each of these groups will be outlined in detail below.

Diatoms

Diatoms are the major group of microalgae with approximately 250 modern genera and thousands of species (Hasle and Syvertesen, 1996). There are only a few diatom taxa that are harmful due to toxin production (*i.e.* several species from the *Pseudo-nitzschia* genus) or cell structures that cause mechanical damage to gills (*e.g. C. concavicornis* and *C. convolutus*). In our study, only one sample contained a potentially harmful diatom – the culture of *Pseudo-nitzschia* sp. Positive identification of *Pseudo-nitzschia* to the species level with microscopy can be ensured only with scanning electron microscopy (SEM), which is a very costly and time-consuming technique not generally implemented in routine monitoring. In our study, both the 18S-diatom and LSU amplicons showed promising results by confirming *Pseudo-nitzschia* presence (>10,000 reads, >98% of the total reads in the cultured sample). However, it also did not discriminate to the species level. The same amplicons detected very low presence (<1%) of *Pseudo-nitzschia* spp. in five other samples where cell presence was not detected using microscopy. Most of these samples were presumed monocultures of other species that were

processed on the same day (*i.e.* culture subsampled, DNA extracted and purified), and NGS results may indicate possible low-level cross contamination from high concentration, pure cultures that occurred in the lab in the same flow hood.

Dinoflagellates

Dinoflagellates are second to diatoms in terms of importance to marine primary production, with about 2000 described extant species (Taylor et al., 2008). This group is particularly important for HABs, as about 75–80% of known toxic phytoplankton species belong to this group (Cembella, 2003). Microscopy indicated that there were nine samples containing *Alexandrium* spp., *Cochlodinium* sp., or *P. reticulatum*. However, based on molecular techniques, additional potentially harmful dinoflagellate species were detected (*Dinophysis*, *Karlodinium*, and *Phalacroma*) and considerably higher numbers of samples containing them were identified. For dinoflagellates, as expected, the most conclusive results were obtained using the 18S-dinoflagellate and LSU amplicons.

Dinoflagellates: Alexandrium

Many species of *Alexandrium* produce toxins that cause paralytic shellfish poisoning (PSP). In BC, since the establishment of the monitoring program for toxins in shellfish in the 1940s (Taylor and Harrison, 2002), PSP closures have occurred every year, and this negatively affects shellfish aquaculture and recreational harvesting. Six cultured samples of *Alexandrium* were included in this study. One was a purchased culture of *Alexandrium* cf. *catenella* and five were locally established cultures microscopically identified as *Alexandrium* sp. and *A. tamarense;* four of these cultures were monocultures and one was a mixed culture that also contained green algae and flagellates. The most effective metabarcoding results for *Alexandrium* detection were obtained using the 18S-dinoflagellate amplicon, closely followed by LSU. Both amplicons provided comparable results with a very high percentage of total *Alexandrium* reads per sample for all five of the culture samples (>90% of reads on average) and a low percentage in the mixed culture (<4%). All six of these samples had highly positive qPCR results using an assay targeting the toxic North American strain of *A. tamarense* (Toebe et al., 2013).

Detections of *Alexandrium* in samples where it was not identified based on microscopy were less conclusive. There were 19 samples where *Alexandrium* presence was suggested by

18S-dinoflagellate and LSU amplicons but not by microscopy. In all cases these were very low counts of *Alexandrium* (*i.e.* very low percent of reads per sample, ranging from 0.02% to 4.72%). However, six of these 19 samples also had positive qPCR results with the *A. tamarense* assay, suggesting that the positives were not completely attributable to false positives from metabarcoding methods. In addition, there were 10 samples with *A. tamarense* qPCR detection that were not confirmed by microscopy or metabarcoding. Future work is needed to clarify exactly which results are correct, the microscopy or the molecular (qPCR and metabarcoding) for this taxon, and how to best score these detections (*e.g.* weak positives). With the expected higher sensitivity of molecular assays as compared to microscopy, it is not implausible that low copy numbers of a HAB species may exist in many mixed cultures, but whether these were introduced by cross-contamination of cultures, as well as the biological relevance of such levels also would need further study.

While *Alexandrium* presence detected by 18S-dinoflagellate and LSU amplicons was similar, the biggest difference in these amplicons was in the taxonomic level of identification. In the 18S-dinoflagellate amplicon, more than 99.50% of *Alexandrium* OTUs were identified to genus level only, while the very small remainder was assigned to *A. tamarense* (0.46%) and *A. andersonii* (<0.01%). In contrast, the LSU results indicated that 97.31% of total *Alexandrium* reads belonged to the species *A. fundyense*, 0.05% to *A. tamarense*, with the remainder 2.64% assigned to the genus level. Based on morphological characteristics, three species (*A. catenella*, *A. tamarense*, and *A. fundyense*) comprise the *A. tamarense* species complex (Balech, 1985). These morphospecies are quite similar in appearance and are distinguished mainly based on formation of chains of cells and features of the cell theca: presence and shape of a ventral pore between plates 1' and 4', and differences in the shapes of plates. However, recent rDNA sequencing studies (John et al., 2014; Lilly et al., 2007) found that these three morphospecies did not satisfy requirements for biological species definition. They suggested that these groups be re-evaluated and a new species definition was proposed. In our study, clonal cultures that were morphologically identified as *A. catenella*, *A. tamarense*, and *Alexandrium* spp. were all assigned to *A. tamarense* by TaqMan and to *A. tamarense* or *A. fundyense* species by metabarcoding, depending on the amplicon. This ambiguity further supports the proposition that the taxonomy of the *A. tamarense* species complex should be re-evaluated, with consensus classifications by molecular methods needing to be updated in sequence databases (*e.g.* GenBank).

453

454 ***Dinoflagellates: Cochlodinium***

455 Two out of approximately 40 known *Cochlodinium* species (*i.e. C. polykrikoides* and *C.*
456 *fulvescens*) form HABs and can cause fish kills (Kudela and Gobler, 2012). In BC, blooms of *C.*
457 *fulvescens* were implicated in farmed salmon kills that caused ~1.5 M USD in economic losses
458 (Whyte et al., 2001). Here, *Cochlodinium* was observed by microscopy in two field samples. Its
459 presence (24 and 992 reads) was detected at the genus level in both of these field samples, but
460 only by the LSU amplicon. This identification was very conclusive and OTUs of *Cochlodinium*
461 at the species or genus level were not observed in any of the other 44 samples.

462

463 ***Dinoflagellates: Protoceratium reticulatum***

464 *Protoceratium reticulatum* produces yessotoxins, which can be bioaccumulated by shellfish and
465 have been associated with diarrhetic shellfish poisoning (DSP) (Satake et al., 1998).
466 Metabarcoding was very effective for identification of this species. The sample of *P. reticulatum*
467 from an established culture with identification based on morphology had a positive identification
468 to the species level when using both the 18S-dinoflagellate and LSU amplicons (143,019, 42,069
469 reads). The 18S-dinoflagellate amplicon also detected very low presence (<2% of the total reads
470 per sample) of *P. reticulatum* in another three culture samples and one field sample (s34).
471 Although the LSU amplicon had comparable readings and percentages to the 18S-dinoflagellate
472 amplicon, it had lower detections in the unexpected samples (*i.e.* two out of four samples were
473 positive by LSU).

474

475 ***Dinoflagellates: Dinophysis, Gymnodinium, Karlodinium,* and *Phalacroma***

476 Algae from the *Dinophysis*, *Gymnodinium*, *Karlodinium*, or *Phalacroma* genera were not
477 identified in the samples using microscopy, but were detected using molecular methods. Certain
478 *Dinophysis* and *Phalacroma* species produce toxins that cause DSP, and thus are important to
479 shellfish aquaculture. In BC, the first reported DSP outbreak was associated with elevated
480 numbers of *Dinophysis* spp. (Esenkulova and Haigh, 2012). In the present study, a low number
481 of reads (<200 in total) of *D. parvula*, *P. rapa* (previously known as *D. rapa*), as well as
482 *Dinophysis* spp. were detected in one field sample using the LSU amplicon. The *Karlodinium*
483 genus includes several toxin-producing species; for example, *K. veneficum* blooms have been

associated with aquatic faunal mortalities for decades (Place et al., 2012). A small number of reads to the genus and species level (<100 in total) were detected in one field sample by LSU. Similar results for both *Dinophysis* and *Karlodinium* were obtained using the 18S-dinoflagellate amplicon, but were only annotated to the genus level. However, metabarcoding results did not confirm *Karlodinium* presence in ten other samples with positive qPCR results (range: 14 to 29 Ct).

Some *Gymnodinium* species produce toxins (Moestrup et al., 2008) and in the present study there were very weak detections of *G. instriatum* in three samples by qPCR (Ct >30), but none were found positive by metabarcoding at the species, genus, or order level.

Raphidophytes

Raphidophytes are a group of algae with very few species. However, these species include several taxa that pose some of the most serious threats to finfish aquaculture around the world, such as *H. akashiwo* and those in the *Chattonella* genus (Hallegraeff, 2004). In our work, the best results for raphidophytes were obtained using 18S-diatom and LSU amplicons.

Raphidophytes: Heterosigma akashiwo

Heterosigma akashiwo is a major fish killer in BC, causing economic losses to the BC salmon aquaculture industry of about ~3.5 M USD per year (Haigh and Esenkulova, 2014). All 24 samples (18 cultures and 6 field) where *H. akashiwo* presence was observed with microscopy returned positive detections with qPCR and with three of the amplicons. The amplicon with weaker detection ability was the 18S-dinoflagellate, which identified *H. akashiwo* in only 19 out of 24 samples. The highest metabarcoding reads were detected by the 18S-diatom amplicon. In the rest of the samples (N=24), *H. akashiwo* presence was not noted by microscopy observations, but *H. akashiwo* reads were found in more than 20 of these samples by the 18S-diatom, 16S, and LSU amplicons, in six using the 18S-dinoflagellate amplicon, and in 11 of these samples by qPCR. For most of the samples there was a general agreement between *H. akashiwo* read numbers by the different techniques (*e.g.* all 18 samples where *H. akashiwo* was not detected by 18S-dinoflagellate had <100 reads per sample by all other techniques; most of the 15 samples with the highest *H. akashiwo* reads detected by 18S-diatom had the highest levels with other techniques). One exception to this trend was in the purchased culture of *Chattonella subsalsa*,

where a high load of *H. akashiwo* was identified solely by the 16S amplicon. *C. subsalsa* identification in this case was confirmed by microscopy and other amplicons, so this clearly indicates a 16S database issue (*i.e.* mis-representation of *C. subsalsa* species as *H. akashiwo*). In 35 samples where *H. akashiwo* presence was suggested based on qPCR results, there was a general agreement of higher loads (*e.g.* Ct<20) with high OTU read counts (>4,000) in the 18S-diatom amplicon, but not in other amplicons. However, some samples with lower qPCR loads (CT=20–29.5) also had very high OTU read counts, with nine samples containing over 10,000 reads. Therefore the quantitation between the read counts and the qPCR was not always congruent. It is possible that this could be due to mismatches in specificity for the applied assay, but further work would be needed to better understand this difference.

Raphidophytes: Chattonella

Globally, many species of *Chattonella* have been associated with fish kills (Moestrup et al., 2008) and blooms of *Chattonella* sp. have caused farmed fish mortalities in BC (Haigh and Esenkulova, 2014). Here, all ten local samples with positive microscopy identification of *Chattonella* spp. did not return results for this genus by sequencing. Almost all (>99.99%) of the Raphidophytes reads in these ten samples (*i.e.* one culture, five mixed cultures, and four field samples) where *Chattonella* presence was suspected based on microscopy, were assigned to *H. akashiwo* by all four amplicons. It is possible that large *Heterosigma* cells were misidentified as *Chattonella* during microscopy. The purchased culture of *Chattonella subsalsa* was positively confirmed as *Chattonella* (>99% of total reads) by 18S-diatom and LSU amplicons with a very small portion (0.04%) identified to the species level (*C. subsalsa*) using the LSU amplicon, suggesting that the amplicons could identify *Chattonella* if it was present. Therefore, for this species the NGS approach appeared to be superior to light microscopy identification.

A very low number of reads (<30) of *Chattonella* spp. were also detected in four culture samples of *Alexandrium* spp. and *H. akashiwo* by the 18S-diatom amplicon. This small number is most likely also an artefact of the subsampling process (*see above*). Only a small portion of the purchased *C. subsalsa* culture was identified to the species level, and the rest of the non-purchased samples was identified to genus only, which suggests the difference in resolution may be a result of species sequence variation that was not reflected in the NCBI database. TaqMan

545 assays for *C. subsalsa* and *C. marina/ovata/antiqua* did not provide positive results with any of
546 the samples.
547
548 ***Silicoflagellates (Dictyochales)***
549 Silicoflagellates are a small group of algae, many of which possess a siliceous skeleton at certain
550 stages of their life cycle. Some species from this group cause fish kills (Henriksen et al., 1993),
551 including farmed salmon mortality events in BC (Haigh and Esenkulova, 2014; Haigh et al.,
552 2014, Haigh et al. 2019). All four amplicons underperformed in detecting algal species and
553 genera from the silicoflagellate group. Most of the samples (11 out of 15) where taxa from this
554 group were observed by microscopy did not have reads annotated as silicoflagellates. The
555 definitive reason for poor metabarcoding identification performance to this target remains
556 unknown, however it could be related to the extraction process, to the primers or amplification
557 process, or due to incomplete databases.
558
559 ***Silicoflagellates (Dictyochales): Dictyocha***
560 Skeleton-containing cells of *D. speculum* are very easily identified by microscopy based on their
561 unique shape and size. Blooms of this species have been linked with fish kills around the world
562 (*e.g.* Henriksen et al., 1993) including in BC (Haigh et al., 2019; Haigh and Esenkulova, 2014).
563 In our study, six field samples contained *D. speculum* cells, based on microscopy observations,
564 but there were no reads assigned to the *Dictyocha* species, genus, or family by any of the NGS
565 amplicons. One of these samples had a very low number of reads (*i.e.* 12 reads) detected by the
566 18S-diatom amplicon, but was assigned only to the order level. These results show that with the
567 current sequencing database, the NGS approach can miss targets at the species, genus, and even
568 family levels. Further work is needed to improve the sequencing database with sequences from
569 voucher specimens to determine which assays are capable of differentiating this genus, or species
570 within this genus, from other closely related taxa.
571
572 ***Silicoflagellates (Dictyochales): Pseudochattonella verruculosa***
573 Both species in the *Pseudochattonella* genus (*i.e. P. farcimen* and *P. verruculosa*) have been
574 implicated in wild and farmed fish kills (*e.g.* Jakobsen et al., 2012). In BC, *Pseudochattonella* sp.
575 blooms have been associated with farmed fish mortalities since 2007 (HAMP, unpublished data).

For this project, one culture from a fish-killing event (Haigh et al., 2014) was established and five field samples were collected. High and comparable loads (>2,000 reads) of *Pseudochattonella* genus were detected in the culture sample by 18S-diatom and 18S-dinoflagellate amplicons. For the former, ~1% of the total reads were assigned to the species level for *P. farcimen*. This culture sample was also the only one that provided qPCR amplification results (Ct=14) with the *P. verruculosa* assay. A very low number of reads (<20 reads) of *Pseudochattonella* were detected in one of the field samples, where its presence was indicated based on microscopy and by 18S-diatom and 18S-dinoflagellate runs, but not by qPCR. This is the first published confirmation of both qPCR and metabarcoding identification of *Pseudochattonella* linked to a fish-killing event in Canada.

Silicoflagellates (Dictyochales): Pseudopedinella

Toxicity of *P. pyriformis* (previously known as *P. pyriforme*) was recently discovered in laboratory studies (Skjelbred et al., 2011). Based on microscopic identification, this species has been linked to farmed salmon mortality in BC (HAMP, unpublished data). In this work, one culture and two field samples were available for molecular analysis. All these samples were obtained from a fish-killing event on the west coast of Vancouver Island. To our knowledge, this is the first published report of ichthyotoxic effects of *Pseudopedinella* species in the field. Metabarcoding did not detect *Pseudopedinella* in the available samples, and it did not identify any reads from the silicoflagellates taxon. The majority of reads in the culture of suspected *Pseudopedinella* sp. was the diatom *Plagiostriata goreensis* by the 18S-diatom amplicon (98%) and "not assigned" in the 18S-dinoflagellate and LSU amplicons (94%, 99%, respectively). While there was therefore no molecular confirmation of the microscopic identification of *Pseudopedinella* in the fish-kill-related sample, the established culture was certainly not a diatom and contained *Pseudopedinella*-like cells (small flagellates). To improve this detection method, a positive control would need to be included, such as a purchased *Pseudopedinella* culture, in order to ensure that the selected amplicons can identify this genus. However, given the putative misidentification of the culture of suspected *Pseudopedinella*, it is also possible that this is a database issue.

605 ***Haptophytes***

606 Toxic haptophytes in the *Prymnesium* and *Chrysochromulina* genera are known to cause fish
607 mortality (Moestrup, 2003). In our work, the best results were obtained using the 18S-
608 dinoflagellate and LSU amplicons.

609

610 ***Haptophytes: Chrysochromulina***

611 Haptophytes may be identified with light microscopy to the genus level, but this is challenging
612 due to small cell size, and species level identification is only possible with scanning electron
613 microscopy. In BC, blooms of suspected *Chrysochromulina* spp. have been linked to fish kills on
614 salmon farms since 2000 (HAMP, unpublished data). Our study had four field samples where
615 *Chrysochromulina* presence was suspected based on microscopy, but two of these samples had
616 low DNA yields and resulted in <200 total reads per sample. The 18S-dinoflagellate amplicon
617 detected *Haptolina fragaria* (>100 reads) in the other two samples. *Haptolina fragaria*
618 (presumably a non-toxic Prymnesiales species), originally described as *Chrysochromulina*
619 *fragaria* (Eikrem and Edvardsen, 1999) but later described to a new genus from ribosomal DNA
620 phylogenetics (Edvardsen et al., 2011). Low levels of *Chrysochromulina* spp. were also detected
621 by the LSU amplicon (220 reads, 0.35%) in one field sample where its presence was not
622 indicated based on microscopy. With the importance of haptophyte species in fish-kills, and the
623 difficulty in microscopic identification of these species, this is an area where better detection by
624 molecular methods would be of strong practical use.

625

626 ***Cyanobacteria***

627 Cyanobacteria, or blue-green algae, are highly diverse aquatic bacteria with over 2,000 species
628 (Nabout et al., 2013). More than 55 of them have been shown to produce toxins that are harmful
629 to humans, as well as other terrestrial and aquatic life (Cronberg, 2003). Due to their generally
630 small size and limited significance to marine finfish, cyanobacteria are not recorded and
631 identified during routine harmful algae analysis in BC by HAMP, unless they appear to dominate
632 in the sample. Here, only one sample (mixed culture) had evidence of possible cyanobacteria
633 presence (based on microscopy), and was mixed with green algae. The cyanobacteria presence in
634 this sample was not confirmed by molecular techniques, but cyanobacteria were detected in other
635 samples by molecular techniques where its presence was not described based on microscopy.

Nine samples provided amplification results with qPCR (Ct=17–29), but only one of these samples had evidence for cyanobacteria presence based on metabarcoding, using the 16S amplicon (Synechococcaceae, 101 reads, 0.1%). Whether the qPCR results are species specific, or whether the 16S metabarcoding was missing these detections remains unknown, and requires further study.

Summary of Methods Evaluation

Development of molecular methods for harmful taxa detection and abundance estimation is an active area of research. The present study allowed for an evaluation and cross validation of traditional microscopy and current molecular techniques and assays for each taxon relevant to BC HABs (Table 7). The best molecular technique, as determined by highest detection rate using currently available assays was identified based on the results such as taxon resolution, the conclusiveness of the detection, and whether it was confirmatory of microscopy results. Molecular techniques, and metabarcoding in particular, proved to be a very promising complement, and in some cases potentially an alternative given additional benchmarking, to standard microscopy. However, there is still much work to be done to develop a curated database with voucher specimens to ensure adequate representation and detectability of all of the important HABs forming species. The comprehensive nature of the metabarcoding approach is another benefit, where many species can be simultaneously detected even from different taxonomic levels. Information obtained during this research provides a foundation to build upon, for example, by using the sequencing results to identify species presence, to develop specific qPCR primers, and to identify existing gaps in sequence databases.

Quantitative PCR is a cost effective approach and can be used for presence/absence as well as quantitative estimates of targeted species, however it requires existing knowledge of potential HABs taxa that may be present in monitored samples. In our study, existing TaqMan assays were found for only four out of 14 targeted taxa. Assays for detection of *A. tamarense* (A.tam1), *H. akashiwo* (H.aka1), and *P. verruculosa* (P.ver1) performed well and could be implemented in routine monitoring. Ten taxa that were identified by microscopy did not have a molecular assay available during the time of this study, including *Chrysochromulina* spp., *Cochlodinium fulvescens*, *C. concavicornis*, *C. convolutus*, *Dictyocha* spp., *Dinophysis* spp., *K. mikimotoi*, *Protoceratium reticulatum*, and *Pseudo-nitzschia* spp. These particular taxa are an

667 important priority for assay development for application in BC. Sequencing data, acquired during
668 this work, provides an ability to develop tailored qPCR assays.

669 The metabarcoding approach offered accurate identification of multiple species and was
670 superior when applied to the detection and identification of cryptic species that cannot be
671 differentiated to the species level by light microscopy (*e.g. Chrysochromulina*, *Prymnesium*). It
672 generated high-throughput data, providing community composition diversity information for the
673 samples, including capturing some potentially harmful taxa that were not originally targeted, as
674 well as providing some cursory information on relative abundance, although the reliability of
675 abundance estimates has not yet been conducted. The presence of a large number of unannotated
676 reads in a number of samples suggests that the sequences of a number of species are still missing
677 from the public databases. This will improve with curated databases, which will be generated as
678 this method and application continues to be developed.

679 The highly sensitive nature of this technique emphasised the need for extremely careful
680 sample handling, as detections of pure cultures transferring small proportions of taxa between
681 samples was detected, probably caused during subsampling of cultures. Since the laboratory
682 component of this study, the increased use of metabarcoding in many areas of research (*e.g.*
683 environmental DNA, or eDNA) applications, has resulted in specialized sample collection,
684 processing workflows, laboratory environments, and establishing threshold detections designed
685 to reduce contamination risks.

686 Although metabarcoding processing time and costs may limit uptake currently, with
687 increased development it is highly likely to become a useful technique for analysing samples
688 collected during active HABs, leading to an unprecedented monitoring opportunity of HABs in
689 terms of sensitivity and precision.

CONCLUSIONS

Applying light microscopy and various molecular techniques to culture and field samples containing multiple harmful algal species allowed cross validation of these techniques and offered a significant foundation for choosing appropriate techniques for targeted taxa in the future. DNA yields were considerably higher for the cultures and filtered field samples, whereas pelleted samples were often unusable. While TaqMan assays were available only for four out of 14 HAB taxa of concern in BC, assays for detection of *A. tamarense*, *H. akashiwo*, and *P. verruculosa* provided adequate identification results. This indicates a need for the development of primers and probes for the rest of the harmful species to allow cost effective detection of many species simultaneously. Sequencing data obtained during this study enabled the development of new qPCR assays tailored for species within the northeastern Pacific Ocean.

Metabarcoding with a combination of markers (*i.e.* 16S, 18S-diatom, 18S-dinoflagellate, and LSU) allowed the identification of over 350 taxa and proved to be an unmatched technique for phytoplankton community structure analysis. Different markers had different strengths for particular taxa, although result congruence was observed among amplicons. The 18S-diatom amplicon identified harmful taxa from the diatom and raphidophyte groups, and the 18S-dinoflagellate and LSU amplicons, producing similar results to each other, overall provided the best identification for harmful algae from dinoflagellate and haptophytes groups, as well as the raphidophyte *H. akashiwo*. Although cyanobacteria were detected by only the 16S amplicon, this amplicon was weak for most of the HABs species. All amplicons appeared to underperform for identification of silicoflagellate algae, although the reason for this remains unknown. The isolated culture from the fish-killing event associated with a *P. verruculosa* bloom (morphology-based identification) was confirmed as *P. verruculosa* by qPCR and *Pseudochattonella* spp. by NGS. This is the first record of both PCR and metabarcoding confirmation of *Pseudochattonella* associated with a fish kill in Canada. Overall, the combination of morphology and molecular-based identification, if implemented, will greatly improve HABs monitoring, help mitigate issues caused by HABs, and aid in better understanding the dynamics of the phenomenon. This work demonstrates a pressing need to tailor qPCR assays, to improve reference databases, and apply a multiple marker approach for metabarcoding of diverse taxa.

DATA AVAILABILITY

Analysis pipeline: https://github.com/bensutherland/eDNA_metabarcoding

Raw data: raw sequence was uploaded to SRA under BioProject PRJNA544881 within BioSample accessions SAMN11865982-SAMN11866125.

SUPPLEMENTAL INFORMATION

Supplemental File S1. Number of reads for each taxonomic group identified with the 16S amplicon.

Supplemental File S2. Number of reads for each taxonomic group identified with the 18S diatom amplicon.

Supplemental File S3. Number of reads for each taxonomic group identified with the 18S dinoflagellate amplicon.

Supplemental File S4. Number of reads for each taxonomic group identified with the LSU amplicon.

Supplemental File S5. Average number of algae taxa (t) and algae species (s) detected by the different amplicons.

Supplemental File S6. Taxa detected by microscopy, the closest match as detected by NGS, the percentage of total reads for that sample represented by the match, and the percentage of unknown reads in the sample.

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751

752 ## AUTHOR CONTRIBUTIONS

753 Wrote the proposal to obtain funding: KM, CP, NH. Conceived and designed the experiments:
754 NH, SE, KM, AT, CP. Performed the experiments: SE, NH, AT. Bioinformatics: BS. Analyzed
755 the data: SE, AT, BS, NH. Contributed reagents/materials/analysis tools: KM, CP, NH. Wrote
756 the manuscript: SE, BS, AT. Revised and approved the manuscript: SE, BS, AT, KM, CP, NH.
757

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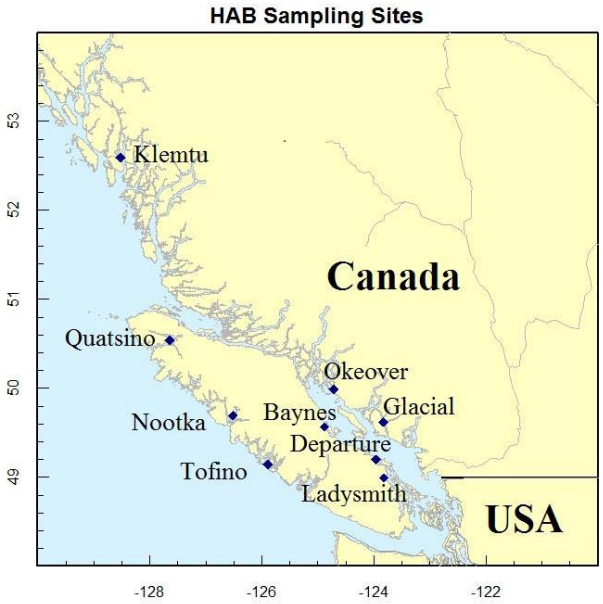
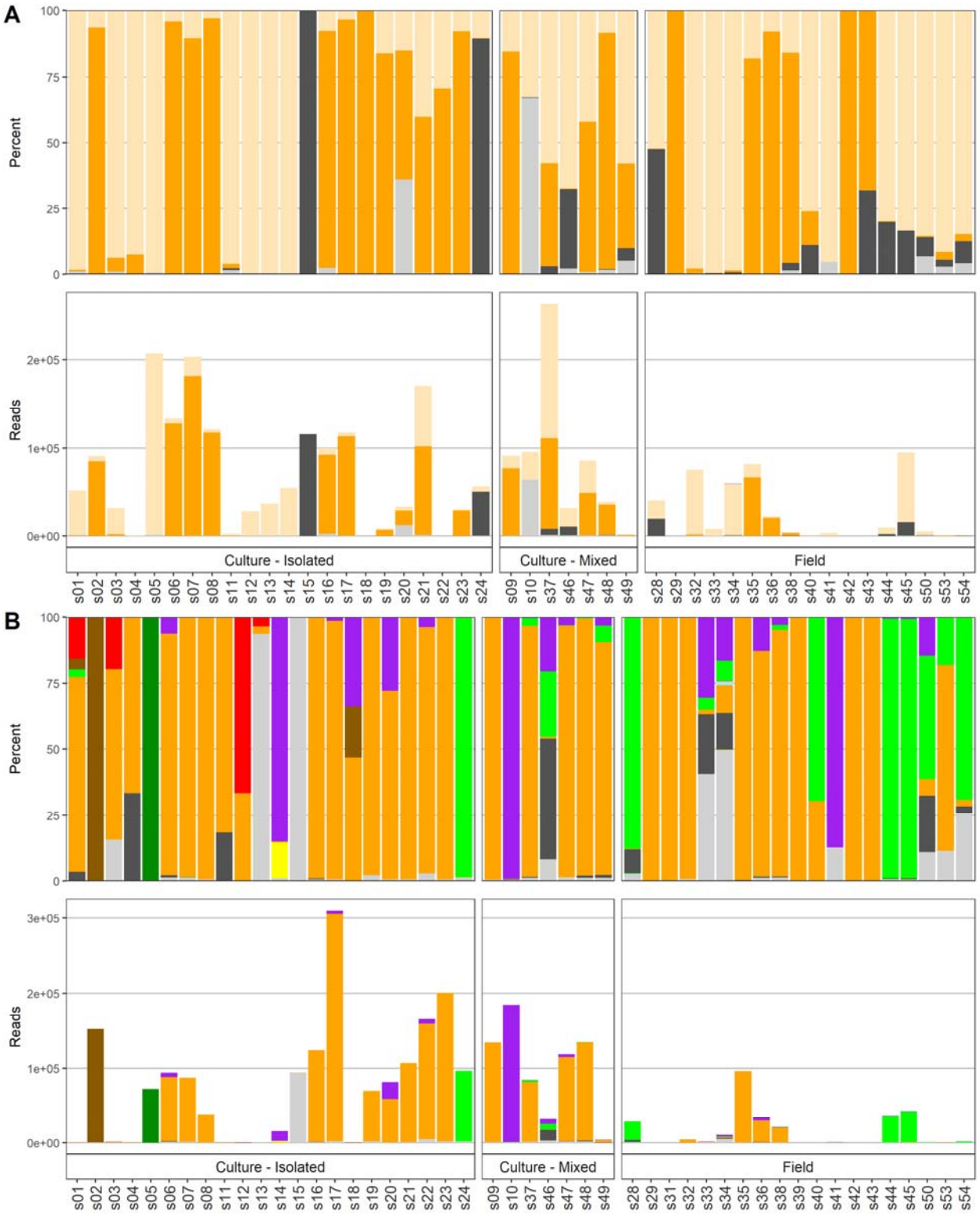
FIGURES AND TABLES

984



986 **Figure 1.** Map of Vancouver Island and British Columbia coast with locations of the sampling
987 stations.



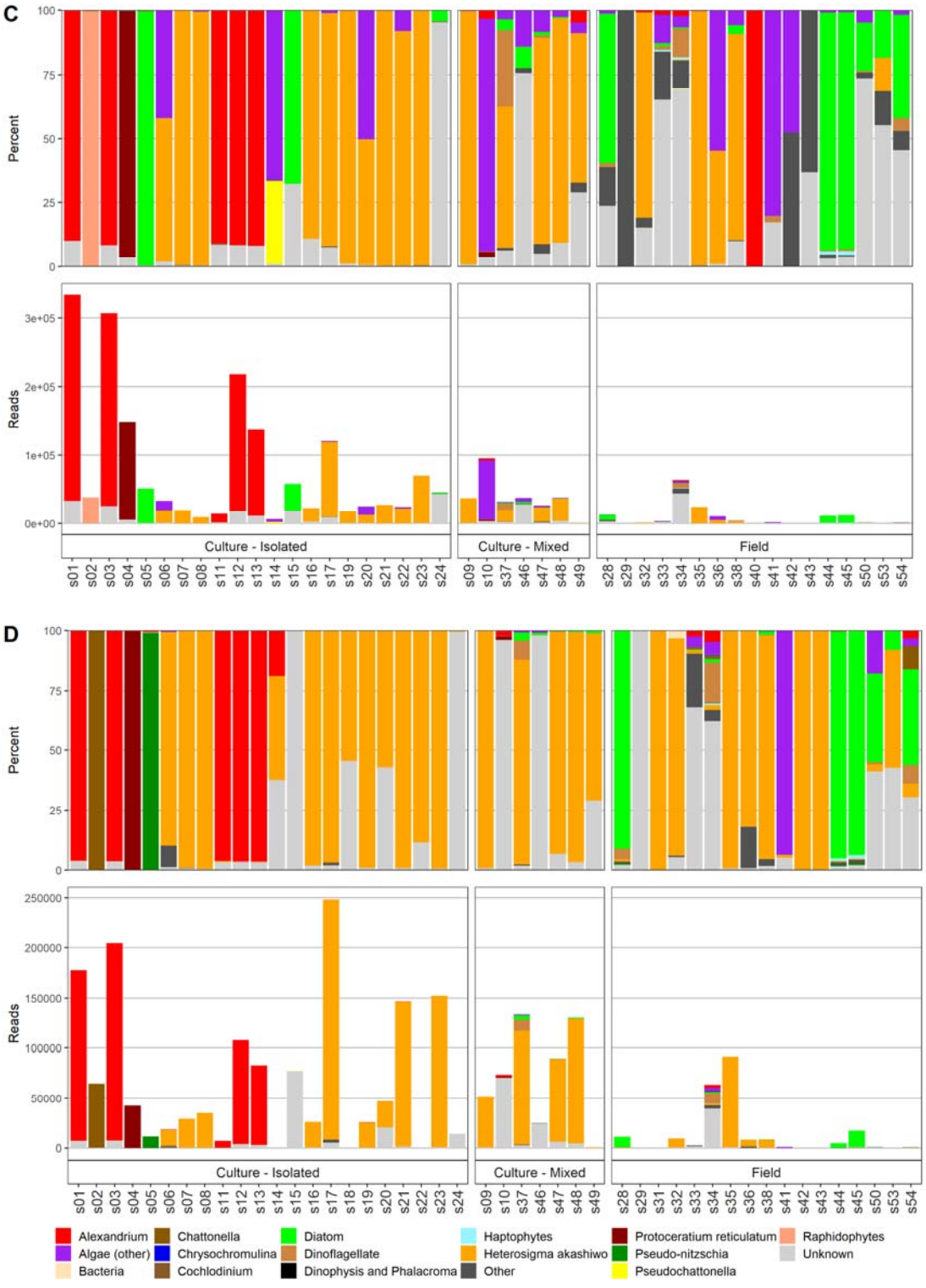


Figure 2. Percentages and number of reads per sample with emphasis on harmful and potentially harmful algal species: (A) 16S, (B) 18S-Diatom, (C) 18S-Dinoflagellate, and (D) LSU.

995 **Table 1.** Sample identifiers, the microscopic observations, and their sampling origin and
996 collection location. Cultures from USA are denoted by an asterisk, with s01 from North Pacific
997 Ocean, Sequim Bay, Washington and s02 from North Atlantic Ocean, Indian River Bay,
998 Delaware.

ID	Microscopy observations	Sample origin	Sampling location
s01	*Alexandrium* cf *catenella*	PC	Sequim Bay*
s02	*Chattonella subsalsa*	PC	Indian River*
s03	*Alexandrium* sp.	IC	Okeover Inlet
s04	*Protoceratium reticulatum*	IC	Okeover Inlet
s05	*Pseudo-nitzschia* spp.	IC	Quatsino Sound
s06	*Heterosigma akashiwo*	IC	Quatsino Sound
s07	*Heterosigma akashiwo*	IC	Quatsino Sound
s08	*Heterosigma akashiwo*	IC	Quatsino Sound
s09	*Heterosigma akashiwo*, Raphidophyte (*Chattonella* sp.?), nanoflagellates	MC	Quatsino Sound
s10	*Alexandrium* sp., flagellates, green algae	MC	Okeover Inlet
s11	*Alexandrium* sp.	IC	Okeover Inlet
s12	*Alexandrium tamarense*	IC	Okeover Inlet
s13	*Alexandrium* sp.	IC	Okeover Inlet
s14	*Pseudochattonella* cf. *verruculosa*	IC	Quatsino Sound
s15	*Chaetoceros eibenii*	IC	Departure Bay
s16	*Heterosigma akashiwo*	IC	Quatsino Sound
s17	*Heterosigma akashiwo, Chattonella* sp.?	IC	Quatsino Sound
s18	*Heterosigma akashiwo*	IC	Quatsino Sound
s19	*Heterosigma akashiwo*	IC	Quatsino Sound
s20	*Heterosigma akashiwo*	IC	Ladysmith
s21	*Heterosigma akashiwo*	IC	Departure Bay
s22	*Heterosigma akashiwo*	IC	Klemtu
s23	*Heterosigma akashiwo*	IC	Glacial
s24	*Pseudopedinella* sp.	IC	Klemtu
s25	*Pseudopedinella* sp.	–	Quatsino Sound
s26	*Pseudopedinella* sp.	PWS	Quatsino Sound
s27	*Pseudopedinella* sp.	PWS	Quatsino Sound
s28	*Pseudopedinella* sp.	PWS	Quatsino Sound
s29	*Chattonella* sp., *Dictyocha* spp., *Heterosigma akashiwo, Prorocentrum gracile*	PWS	Nootka Sound
s30	*Chattonella* sp., *Dictyocha* spp., *Heterosigma akashiwo, Prorocentrum gracile*	FWS	Nootka Sound
s31	*Chattonella* sp., *Dictyocha* spp., *Heterosigma akashiwo, Prorocentrum gracile*	FWS	Nootka Sound
s32	*Pseudopedinella* sp.?	FWS	Quatsino Sound
s33	*Chattonella* sp., *Pseudochattonella* sp., *Dictyocha* spp., *Cochlodinium fulvescens*	FWS	Quatsino Sound
s34	*Chattonella* sp., *Pseudochattonella* sp., *Dictyocha* spp., *Cochlodinium fulvescens*	FWS	Quatsino Sound
s35	*Heterosigma akashiwo*	FWS	Quatsino Sound

s36	*Heterosigma akashiwo*	FWS	Quatsino Sound
s37	*Heterosigma akashiwo*, nanoflagellates, big Raphidophyte	MC	Baynes Sound
s38	*Heterosigma akashiwo* (various morphotypes), nanoflagellates	FWS	Quatsino Sound
s39	*Chaetoceros* spp., *Skeletonema costatum*, *Dictyocha* spp., *Chrysochromulina* sp.?	FWS	Quatsino Sound
s40	Diatoms, *Dictyocha* spp., *Chrysochromulina* sp.?	FWS	Quatsino Sound
s41	Green algae, Cyanobacteria	FWS	Quatsino Sound
s42	*Heterosigma akashiwo*	FWS	Tofino Inlet
s43	*Heterosigma akashiwo*	FWS	Tofino Inlet
s44	*Chrysochromulina* sp.?	FWS	Kunechin Point
s45	*Chrysochromulina* sp.?	FWS	Quatsino Sound
s46	*Heterosigma akashiwo*, nanoflagellates, *Chattonella* sp.?, diatoms	MC	Quatsino Sound
s47	*Heterosigma akashiwo*, nanoflagellates, *Chattonella* sp.?, diatoms	MC	Quatsino Sound
s48	*Heterosigma akashiwo*, nanoflagellates, *Chattonella* sp.?, diatoms	MC	Quatsino Sound
s49	*Heterosigma akashiwo*, nanoflagellates, *Chattonella* sp.?, diatoms	MC	Quatsino Sound
s50	*Pseudochattonella* cf. *verruculosa*	PWS	Quatsino Sound
s51	*Pseudochattonella* cf. *verruculosa*	PWS	Quatsino Sound
s52	*Pseudochattonella* cf. *verruculosa*	PWS	Quatsino Sound
s53	*Pseudochattonella* cf. *verruculosa*	PWS	Quatsino Sound
s54	*Pseudochattonella* cf. *verruculosa*	PWS	Quatsino Sound

999

1000 ? = suspected species; PC = purchased culture; IC = isolated culture; MC = mixed culture; FWS
1001 = filtered water sample; PWS – pelleted water sample.

1002 **Table 2.** Overview of the algal taxa and qPCR primers included in this study.

Family	Genus	Species	Assay	Size (bp)	Citation for the primer	Forward Name	Forward Sequence	Reverse Name	Reverse Sequence	Probe Name	Probe Sequence
Bacillariophyceae	*Chaetoceros*	*tenuissimus*	C.ten	64	Toyoda et al., 2010	2–10_EF	TTG TGG AGA GGT ACG CTT GTC TT	2–10_ER	CCC TCA TAG GCA CCC TGT TC	2–10_Probe	CCT TAG CTT AAA TCT CT
	Pseudo-nitzschia	*multiseries*	P.mul	106	Delaney et al., 2011	Pmulti_F	AGATTTAACTGATG AACAAA	Pmulti_R	GTAACTATTACGT GGGTGT	Pmulti_P	CTATTAGCCGCGGTTTA
Cyanobacteria	*Cyanobacteria*	General	Cyan		Rinta-Kanto et al., 2005	CYAN-108-F	ACGGGTGAGTAAC RCGTRA	CYAN-377-R	CCATGGCGGAAAA TTCCCC	CYAN-328-P	CTCAGTCCCAGTGTGGC TGNTC
	Microcystis	spp.	Micr		Rinta-Kanto et al., 2005	Micr-184-F	GCCGCRAGGTGAA AMCTAA	Micr-431-R	AATCCAAARACCT TCCTCCC	Micr-228-P	AAGAGCTTGCGTCTGAT TAGCTAGT
Dictyochophyceae	*Pseudochattonella*	*verruculosa*	P.ver1	227	Bowers et al., 2006	ChattaqFor	CCGTAGTAATTCTA GAGCTAATACRTG CA	ChattaqRev	AATTCTCCGTTACC CGTTAAAGCCAT	CVerrProbe	AATGGCGCGCAAGCGTG TATTATG
			P.ver2	239	Handy et al., 2006	Cv1561F	ATGCATACAGCGA GTCTAGA	Cv1780R	TCACTCCGAAAAG TGTCAAC	CvProbe	CAAGAGTACCCAGGCCT CTCGACC
Dinoflagellate phycea e	*Alexandrium*	*catenella*	A.cat	160	Hosoi-Tanabe and Sako, 2005	catF	CCTCAGTGAGATTG TAGTGC	catR	GTGCAAAGGTAAT CAAATGTCC	Taq man cat	ATGGGTTTTGGCTGCAA GTGCA
		minutum	A.min	89	Toebe et al., 2013	Amin-F	ACATGGATAACTG TGGTAATTCTATAG CT	Amin-R	GTTGGTTCTGTAA CTAATGACCACA	Amin-P	CATCCAAACCTGACTTC
		tamarense	A.tam1	131	Toebe et al., 2013	At-NA-1-F	CCACAGCCCAAAG CTCTTG	At-NA-1-R	CCATGAGGGAAAT ATGAAAAGG	At-NA-1-P	AACACTCCCACCAAGCA
			A.tam2	108	Toebe et al., 2013	At-ME-2-F	GCATGCCAAGAAA GAATCATTGT	At-ME-2-R	TGTATTTGCTGAA CAAAAAGTAAACA GA	At-ME-2-P	AGCACAACAACCTCACC A
			A.tam3	82	Toebe et al., 2013	At-WE-3-F	TTGTGTGTGTCAGG GCTTGTA	At-WE-3-R	GAATGTGTCTGGT GTATCTGTT	At-WE-3-P	CAACCTCAAACACATGG A
			A.tam4	230	Hosoi-Tanabe and Sako, 2005	tamF	TGCTTGGTGGGAGT GTTGCA	tamR	TAAGTCCAAGGAA GGAAGCATC	Taqman tam	AGAGCTTTGGGCTGTGG GTGTA
		tamutum	A.tamu	90	Toebe et al., 2013	Amut-F	GAACATTTTGCAGC AACATTGTG	Amut-R	GTGGTACTTGTGT GTGTTCCTTTG	Amut-P	CTCAAGGAAGCAAACC
	Azadinium	*Obesum*	A.obe	74	Toebe et al., 2012	Aob134 F	AGG GAT CGA TAC ACA AAT GAG TAC TG	Aob208R	AAA CTC CAG GGA CAT GGT AGT CTT A	Aob163	AAG ACA TTC GAC CTA CCG T
		poporum	A.pop	68	Toebe et al., 2012	Apop62 F	GAT GCT CAA GGT GCC TAG AAA GTC	Apop148 R	CCT GCG TGT CTG GTT GCA	Apop112	TTC CAG ACG ACT CAA A
		spinosum	A.spi	72	Toebe et al., 2012	Asp48F	TCG TCT TTG TGT CAG GGA GAT G	Asp120R	GGA AAC TCC TGA AGG GCT TGT	Aspin77 T	CGC CCA AAA GGA CTC CT
	Cochlodinium	*polykrikoides*	C.pol	148	Park et al., 2009	CPITSF	CGGCAACCTTTGTC AAACA	CPITSR2	GGTTTGCTGATCT AACTTCATGTCT	CPITSP	CAACCGTGATACCCGCT AGCTTTGC
			Cpoly		Kamikawa et al., 2006	Cpoly-F	CCACACGGTGAGA TTGTTGG	Cpoly-R	GCCACCGAAGTCG TTCG	Cpoly-P	CCACCAATCACCAATGA CC
	Gyrodiniu	*impudicum*	G.imp	105	Park et al.,	GIITSF	GAGTGCTTGTGCCT	GIITSR	CGCTTAGCCAGTT	GIITSP	CCTCAGGAGCGCCTCGA

	m				2009		CAGGAT		GGTTGAC		GAACA
		instriatum	G.ins	108	Handy et al., 2008	Gi 166F	GCACAAATTCCCA ACTTCGCGG	Gi 274R	GCTCGAATGATTC ATCGCCAGCA	GiProbe	CCAACACAGGCTCTGCC TGTTCTC
	Heterocapsa	*circularisquama*	H.cir		Kamikawa et al., 2006	HcircF	GTTTGCCTATGGGT GAGC	HcircR	CATTGTGTCAGGG AGGAG	Taq-Circ	CACCACAAGGTCATGAG GACACA
	Karenia	*Brevis*	K.bre	91	Gray et al., 2003		TGAAACGTTATTGG GTCTGT		AGGTACACACTTT CGTAAACTA		TTAACCTTAGTCTCGGGT A
		mikimotoi	K.mik1		Kamikawa et al., 2006	KmikiF	GTCTGGTAGCACTG CTTCA	KmikiR	GTCGGTTGCTGGT GCAAAA	Taq-miki	TGCTCCTGGCACCAACA AC
			K.mik2		Yuan et al., 2012	KMF	CTTTGTGTGTAACC TGTTGCTTTGT	KMR	TCAGCGGGTTTGC TTACCT	KMP	ACCTGTCCTCCTGTCTGC CACTTCATTTGT
	Karlodinium	*micrum/veneficum*	K.ven1	~150	Handy et al., 2008	Dino 1662F	CCGATTGAGTGWT CCGGTGAATAA	Euk B	GATCCWTCTGCAG GTTCACCTAC	KvProbe	CCGCTGCAACGTTCAGG AACTAAACACTG
		veneficum	K.ven2	120	Park et al., 2009	KVITSF 3	CTGTGAACTTCTTT GTGAGCTCTT	KVITSR 3	TAGCGATAGCTTC GCAGACA	KVITSP 3	AGGTGAATCCCAATGCT GCTCCACTA
	Pfiesteria	*piscicida*	P. pi	213	Bowers et al., 2000	107	CAGTTAGATTGTCT TTGGTGGTCAA	320	TGATAGGTCAGAA AGTGATATGGTA	Probe	CATGCACCAAAGCCCGA CTTCTCG
	Prorocentrum	*minimum*	P.min	325	Handy et al., 2008	Pm 200F	TGTGTTTATTAGTT ACAGAACCAGC	Pm 525R	AATTCTACTCATTC CAATTACAAGACA AT	PmProbe	CCGCCTGGTCCTTTGGTG ATTCATAATAAC
	Pseudopfiesteria	*shumwayae*	P.shu	78	Bowers et al., 2000	PSITSF1	TTGACGCATTGAG GCTATGG	PSITSR	AAACGGATGCAAA CGAGTTG	PSITSP1	CGGCTTCTCTGGCGACG AATCAC
Haptophyceae/ Prymnesiophyceae	*Prymnesium*	*Parvum*	P.par1	195	Manning and La Claire, 2010	FucoF	CTGCAGATGGTCTT CCTCATC	FucoR	AACCCTGGAGAGA CGAAGTTC	0	AAGCCTGACCTTGACGC TGATGAG
			P.par2	132	Zamor et al., 2012	PrymF	TGTCTGCCGTGGAC TTAGTGCT	PrymR-3	ATGGCACAACGAC TTGGT	PrymP	ACGTGTGCCGACGTGCT AGTAG
Raphidophyceae	*Chattonella*	*marina/ovata/antiqua*	C.mar1		Kamikawa et al., 2006	ChattF	GCTCTTTGTTCTCT GCATCC	ChattR	CAGTCCAATCACT GCAACC	Taq-Chatt	GCAACGGCAAGATGGAA CC
			C.mar2	224	Bowers et al., 2006	Cmarina For	GGTAGTTGCCGTAC ATTTTGCTCTT	Cmarina Rev	AAAAGTGGATTCA GCCGAAGCTTC	Cmarina Probe	TTGAGTTCAACGGGCGT GGTAGC
		subsalsa	C.sub1	156	Bowers et al., 2006	Subsalsa For	TTGGATTCCGACGG GC	Subsalsa Rev	ATATGCTTAAATT CAGCGGGTTTT	Subsalsa Probe	TTCGGCCAAGCACACAT TTCGGCCAAGCACACAT CCTC
			C.sub2	~350	Coyne et al., 2005	Cs 1350F	CTAAATAGTGTGG GTAATGCTTAC	Cs 1705R	GGCAAGTCACAAT AAAGTTCCAA	Raph Probe	CAACGAGTACTTTCCTTG GCCGGAA
	Fibrocapsa	*japonica*	F.jap	167	Bowers et al., 2006	Fjap490 For	TGAAAACGGCCCG TACACA	Fjap657R ev	CGGGAACAGCTCA TGATGT	Fjap578P robe	CGGCTGGACACGCTTCT G
	Heterosigma	*akashiwo*	H.aka1	228	Bowers et al., 2006	Haka127 For	AAAGGTGCGTGCT CAGTCGTGGT	Haka355 Rev	CAAAAGTCTTTTC ATCTTTCCCT	Haka222 Probe	TACGAGCCGTTTCCGAC GA
			H.aka2	~350	Coyne et al., 2005	Hs 1350F	CTAAATAGTGTCG GTAATGCTTCT	Hs 1705R	GGCAAGTCACAAT AAAGTTCCAT	Hs Probe	CAACGAGTAACGACCTT TGCCGGAA
			H.aka3		Kamikawa et al., 2006	HakaF	CTGCTGAAGGAAG CGATTG	HakaR	GGAACGCGCAGTC AAAG	Taq-aka	GGAGCGTTTCAACATGC GTTC

1003 **Table 3.** TaqMan assay amplification results (Ct values).

Sample #	Microscopy observations	A.tam1	Cyan	G.ins_	H.aka1	H.aka3	K.ven1	K.ven2	P.ver1
s01	*Alexandrium* cf *catenella*	8.0			24.2				
s02	*Chattonella subsalsa*								
s03	*Alexandrium* sp.	9.8			23.2				
s04	*Protoceratium reticulatum*	21.7	26.5		25.3				
s05	*Pseudo-nitzschia* spp.	25.1			26.1				
s06	*Heterosigma akashiwo*				25.2				
s07	*Heterosigma akashiwo*				28.6				
s08	*Heterosigma akashiwo*	36.2			18.6				
s09	*Heterosigma akashiwo*, big Raphidophyte (*Chattonella* sp.?), nanoflagellates				11.7				
s10	*Alexandrium* sp., flagellates, green algae	22.7	18.4		28.7				
s11	*Alexandrium* sp.	17.2			25.4				
s12	*Alexandrium tamarense*	8.7							
s13	*Alexandrium* sp.	8.4	24.4						
s14	*Pseudochattonella* cf *verruculosa*	25.7			26.6				14.0
s15	*Chaetoceros eibenii*	23.9			23.6	27.1			
s16	*Heterosigma akashiwo*				26.1				
s17	*Heterosigma akashiwo*, *Chattonella* sp.?								
s18	*Heterosigma akashiwo*				26.4				
s19	*Heterosigma akashiwo*				25.8				
s20	*Heterosigma akashiwo*				26.2				
s21	*Heterosigma akashiwo*				28.9				
s22	*Heterosigma akashiwo*				17.1				
s23	*Heterosigma akashiwo*				10.3				
s24	*Pseudopedinella* sp.	27.1							
s28	*Pseudopedinella* sp.						16.3		
s31	*Chattonella* sp., *Dictyocha* spp., *Heterosigma akashiwo*, *Prorocentrum gracile*		29.1	30.9	24.2		14.2	29.1	
s32	*Pseudopedinella* sp.?	35.2			17.6		22.9		
s33	*Chattonella* sp., *Pseudochattonella* sp., *Dictyocha* spp., *Cochlodinium fulvescens*	25.7	18.8		23.9		21.1		

s34	*Chattonella* sp., *Pseudochattonella* sp., *Dictyocha* spp., *Cochlodinium fulvescens*	21.2	16.9		20.5	13.4	29.2
s35	*Heterosigma akashiwo*				23.6		
s36	*Heterosigma akashiwo*				19.8		
s37	*Heterosigma akashiwo*, nanoflagellates, big Raphidophyte				13.7	25.6	
s38	*Heterosigma akashiwo* (various morphotypes), nanoflagellates				12.7		
s39	*Chaetoceros* spp., *Skeletonema costatum*, *Dictyocha* spp., *Chrysochromulina* sp.?						
s40	Diatoms, *Dictyocha* spp., *Chrysochromulina* sp.?	24.2		34.7	27.4	20.2	
s41	Green algae, Cyanobacteria					19.7	
s42	*Heterosigma akashiwo*	24.6	25.6				
s43	*Heterosigma akashiwo*	28.6	21.6				
s44	*Chrysochromulina* sp.?	24.4		31.7	27.2		
s45	*Chrysochromulina* sp.?	34.1			29.5	22.2	
s46	*Heterosigma akashiwo*, nanoflagellates, *Chattonella* sp.?, diatoms				22.1		
s47	*Heterosigma akashiwo*, nanoflagellates, *Chattonella* sp.?, diatoms				24.4		
s48	*Heterosigma akashiwo*, nanoflagellates, *Chattonella* sp.?, diatoms				25.6		
s49	*Heterosigma akashiwo*, nanoflagellates, *Chattonella* sp.?, diatoms				27.0		
s50	*Pseudochattonella* cf *verruculosa*	36.0				22.5	
s53	*Pseudochattonella* cf *verruculosa*		23.1		25.7		

Note: Full name for the primer abbreviation and its citation are listed in Table 2. Note that Ct's on the microfluidics BioMark platform are approximately 10 Ct values lower than on traditional single-assay platforms (Miller et al., 2016).

Table 4. Taxon levels and number of reads detected by the four different amplicons: 16S, 18S-Diatom, 18S-Dinoflagellate, and large subunit (LSU).

Taxon level	16S		18S Diatom		18S Dino		LSU	
	Count	Reads	Count	Reads	Count	Reads	Count	Reads
Species	21	1281094	18	1947366	49	667599	60	1819902
Genus	27	229337	32	570709	52	1005795	46	103234
Family	11	41069	10	1095	23	104880	10	14705
Order	2	100	6	17002	18	96580	6	3295
Higher taxonomic rank	5	976679	8	29705	20	2222	9	6296
N/A, unknown	3	87534	4	122585	5	270013	5	291360
Total individual taxa	**69**	**2615813**	**78**	**2688462**	**167**	**2147089**	**136**	**2238792**

Table 5. Number of reads per taxonomic category and percent of total microalgae reads.

Row Labels	16S	18S Diatom	18S Dinoflagellates	LSU
Diatoms	30	291561	128820	49063
Dinoflagellate		557	1087060	630785
Raphidophytes	1229547	1993049	509885	1249934
Silicoflagellates		12272	5992	
Cryptophytes			366	594
Haptophytes		134	962	982
Microalgae (other)	95	238460	116431	4944
Cyanobacteria	101	14	21	
Bacteria	1076778			450
Fungi		30	606	3620
N/A, unknown	87534	122585	270013	291360
Other	221728	29800	26933	7060
Percent of total reads in microalgae taxa (%)	**47**	**94**	**87**	**86**

Table 6. Number of reads for all detected harmful and potentially harmful algae species, genera, and families.

Taxonomic group	16S	18S Diatom	18S Dino	LSU	Amplicon returning maximum read
Alexandrium andersonii			97		18S Diatom
Alexandrium fundyense				549437	LSU
Alexandrium spp.		509	920011	14871	18S Dino
Alexandrium tamarense			4300	292	18S Dino
Total *Alexandrium* spp.	**0**	**509**	**924408**	**564600**	**18S Dino**
Chattonella spp.		152203	40	64380	18S Diatom
Chattonella subsalsa			58	28	18S Dino
Total *Chattonella* spp.	**0**	**152203**	**98**	**64408**	**18S Diatom**
Dinophysis parvula				13	LSU
Dinophysis spp.			119	111	18S Dino
Phalacroma rapa				44	LSU
Total *Dinophysis* and *Phalacroma* spp.	**0**	**0**	**119**	**168**	**LSU**
Karlodinium spp.			21	50	LSU
Karlodinium veneficum				27	LSU
Total *Karlodinium* spp.	**0**	**0**	**21**	**77**	**LSU**
Prymnesium kappa				20	LSU
Prymnesium spp.			10		18S Dino
Total *Prymnesium* spp.	**0**	**0**	**10**	**20**	**LSU**
Pseudo-nitzschia sp. B HAB-2017				33	LSU
Pseudo-nitzschia spp.		72978		11254	18S Diatom
Total *Pseudo-nitzschia* spp.	**0**	**72978**	**0**	**11287**	**18S Diatom**
Pseudochattonella farcimen		167			18S Diatom
Pseudochattonella spp.		1944	2104		18S Dino
Total *Pseudochattonella* spp.	**0**	**2111**	**2104**	**0**	**18S Dino**
Chattonellaceae			38486		18S Dino
Chrysochromulina spp.				220	LSU
Cochlodinium spp.				1016	LSU
Heterosigma akashiwo	1229451	1840799	471269	1185484	18S Diatom
Protoceratium reticulatum			145010	43109	18S Dino
Prymnesiaceae			11		18S Dino

Table 7. Evaluation of harmful taxa detection methods based on the present study: ** = best current technique(s) for taxa detection (at least one designated per taxon), * = technique that can detect taxa, 0 = no taxa detection, n/a = not applied.

Harmful taxa	qPCR	16S	18S-Diatom	18S-Dinoflagellate	LSU
Alexandrium tamarense complex	*	0	*	**	**
Chattonella spp.	0	0	**	*	*
Chrysochromulina spp.	n/a	0	0	**	*
Cochlodinium spp.	n/a	0	0	0	**
Cyanobacteria	*	**	0	0	0
Dictyocha spp.	n/a	0	0	0	0
Dinophysis and *Phalacroma* spp.	n/a	0	0	**	**
Heterosigma akashiwo	*	*	**	*	**
Karlodinium spp.	*	0	0	*	**
Protoceratium reticulatum	n/a	0	0	**	*
Prymnesium spp.	n/a	0	0	*	**
Pseudochattonella spp.	*	0	**	*	0
Pseudo-nitzschia spp.	n/a	0	**	0	*
Pseudopedinella spp.	n/a	0	0	0	0