Metabarcoding, qPCR, and microscopy identification of taxa associated with harmful algal blooms

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Fisheries and Oceans Canada

MICROTHALASSIA

Harmful algal blooms

- British Columbia – one of the most affected areas in the world, every year numerous shellfish harvest closures, aquacultured salmon fish kills

During 2009–2012, direct losses to the BC salmon aquaculture industry from HABs were ~13 M USD

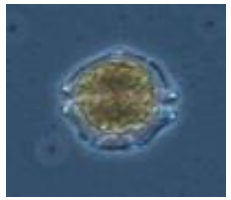
Haigh, N., Esenkulova, S., 2014. Economic losses to the British Columbia salmon aquaculture industry due to harmful algal blooms, 2009-2012. PICES Sci. Rep. 2. Photos by Michael Bahrey

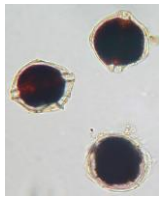
Harmful Algae Negatively Impacting Shellfish Aquaculture in British Columbia

Paralytic Shellfish Poisoning (PSP)

In BC, PSP toxins are produced by dinoflagellates in the *Alexandrium* genus, most particularly by *A. catenella* and *A. tamarense*.
PSP closures are common in summer, but may occur in winter due to re-suspension of cysts.

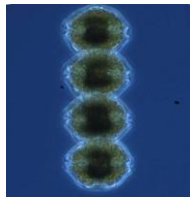
***Alexandrium* sp.**





20-50 µm

A. catenella

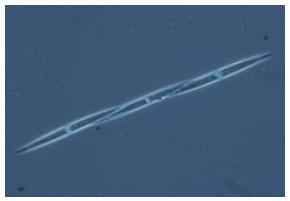


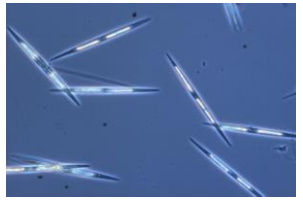
***Alexandrium* sp. cyst**

Amnesic Shellfish Poisoning (ASP)

ASP toxins are produced by diatom species in the *Pseudo-nitzschia* genus. Among more than 30 taxa, only 12 species are potential toxin producers. Identification to the species level cannot be done under light microscopy.

***Pseudo-nitzschia* sp.**



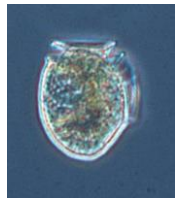


40-175 µm

Diarrhetic Shellfish Poisoning (DSP)

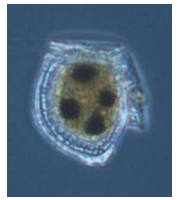
DSP toxins are produced by dinoflagellate species in the *Dinophysis* genus. In BC, the most common species that are known to produce DSP toxins are *Dinophysis acuminata*, *D. acuta*, *D. fortii*, and *D. norvegica.*

D. acuminata



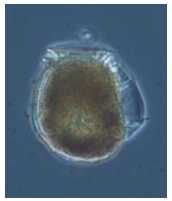
38-58 µm

D. acuta



54-94 µm

D. fortii



62-66 µm

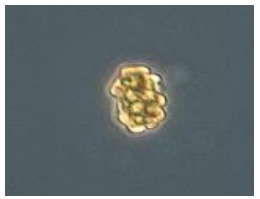
D. norvegica

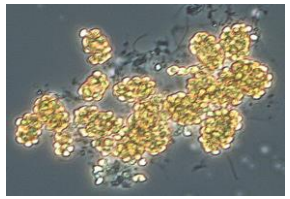
48-67 µm

Algae Causing Other Harmful Effects

Algae that may negatively impact shellfish. Blooms of these algae may cause smothering or low dissolved oxygen. *Heterosigma akashiwo*, *Cochlodinium fulvescens*, and *Dictyocha* spp. produce toxins that might harm shellfish. *Myronecta rubra* can make shellfish unappetizing.

Heterosigma akashiwo





15-40 µm

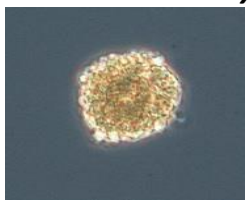
Dictyocha speculum

25-50 µm

D. fibula

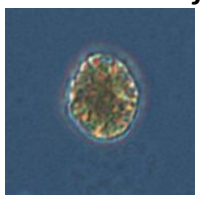
25-50 µm

Non-skeletal *Dictyocha*



25-65 µm

Cochlodinium fulvescens



25-40 µm

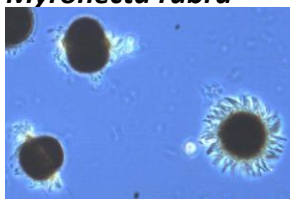
Karenia mikimotoi

18-37 µm

Noctiluca scintillans

200-2000 µm

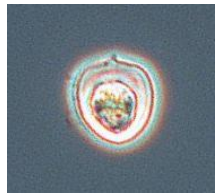
Myronecta rubra



25-50 µm

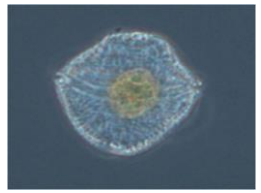
Potentially toxic species found in BC that cause shellfish poisoning with venerupin (*Prorocentrum minimum*) and yessotoxin (*Protoceratium reticulatum*) in other parts of the world.

P. minimum



10-20 µm

P. reticulatum



28-53 µm

Funding provided by: Fisheries and Oceans Canada, Aquaculture Collaborative Research and Development Program

Produced by: Nicky Haigh and Svetlana Esenkulova of Microthalassia Consultants Inc. and Dr. Chris Pearce and Laurie Keddy of Fisheries and Oceans Canada

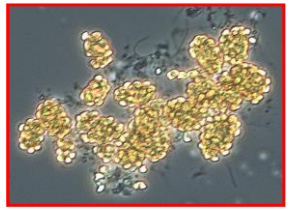
Note: All algae cells preserved with Lugol's iodine.

Harmful Algae Negatively Impacting Finfish Aquaculture in British Columbia

Photographs of algal species that produce toxins harmful to fish are framed with red; species that are mechanically harmful are framed in green; other – purple.

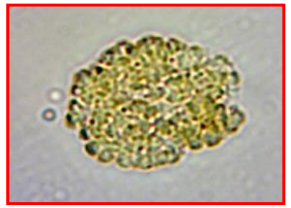
Raphidophyceae

Heterosigma akashiwo

15-40 µm

***Chattonella* sp. (live in the left image)**

30-70 µm

Dictyochophyceae

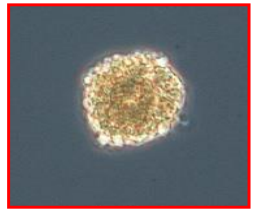
Dictyocha speculum

25-50 µm

D. fibula

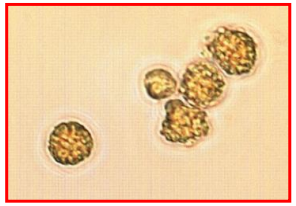
25-50 µm

Non-skeletal *Dictyocha*



25-65 µm

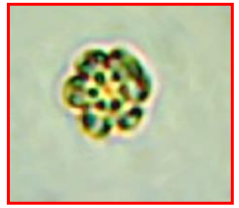
***Pseudochattonella* sp.**



(live cell)

20-70 µm

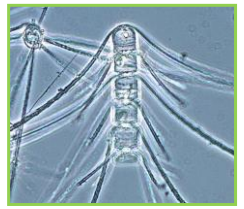
***Pseudopedinella* sp.**



3-9 µm

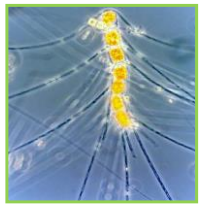
Bacillariophyceae

Chaetoceros concavicorne



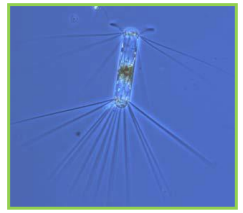
12-30 µm (valve)

C. convolutus



10-27 µm (valve)

Corethron hystrix



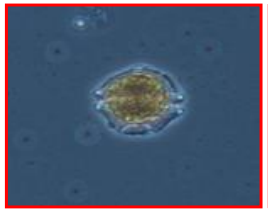
100-250 µm

Rhizosolenia setigera

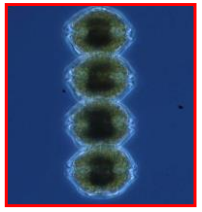
200-1000 µm

Dinophyceae

***Alexandrium* sp.**

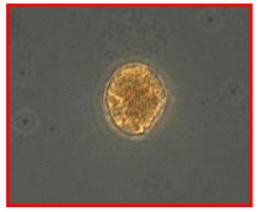
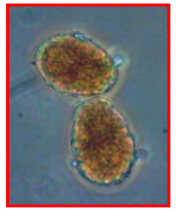


A. catenella



20-50 µm

Cochlodinium fulvescens

25-40 µm

Karenia mikimotoi

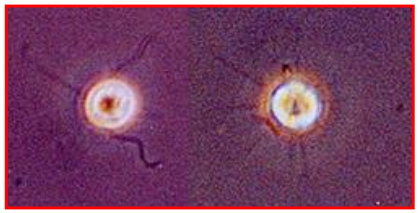
18-37 µm

Noctiluca scintillans

200-2000 µm

Prymnesiophyceae

***Chrysochromulina* spp. (live cells)**



5-15 µm

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Note: All algae cells preserved with Lugol's iodine (unless otherwise stated).

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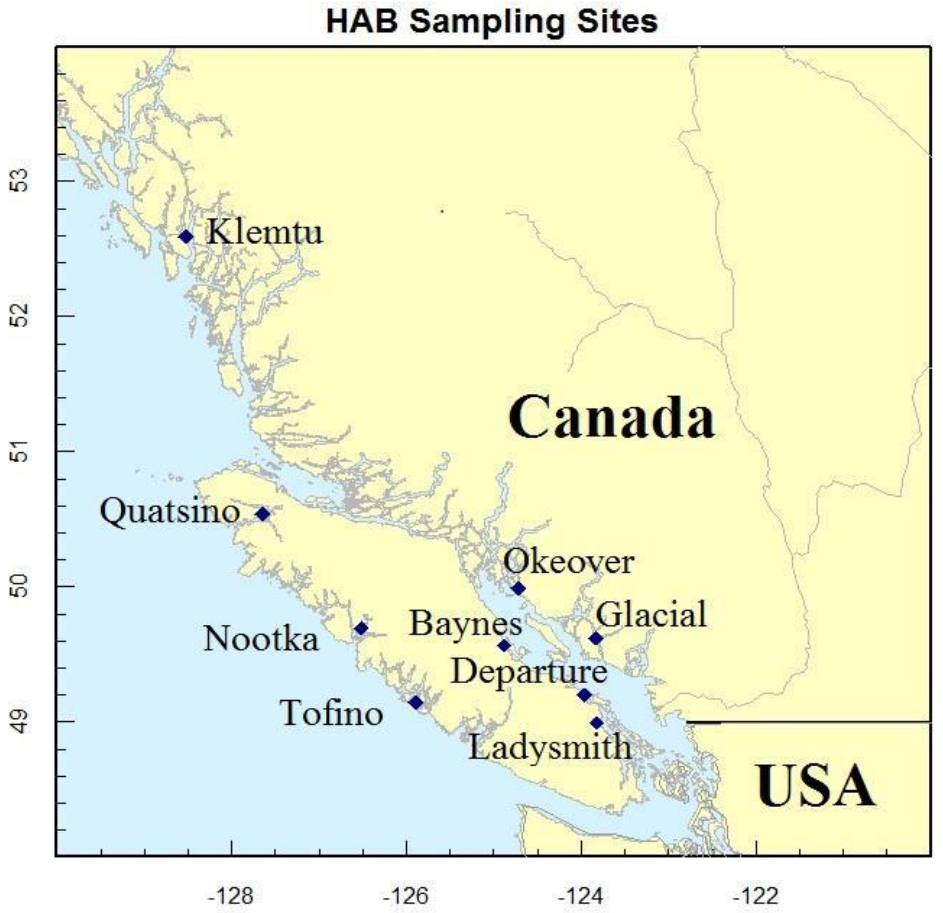
Samples

- **30 cultures** (uni-algal and mixed)

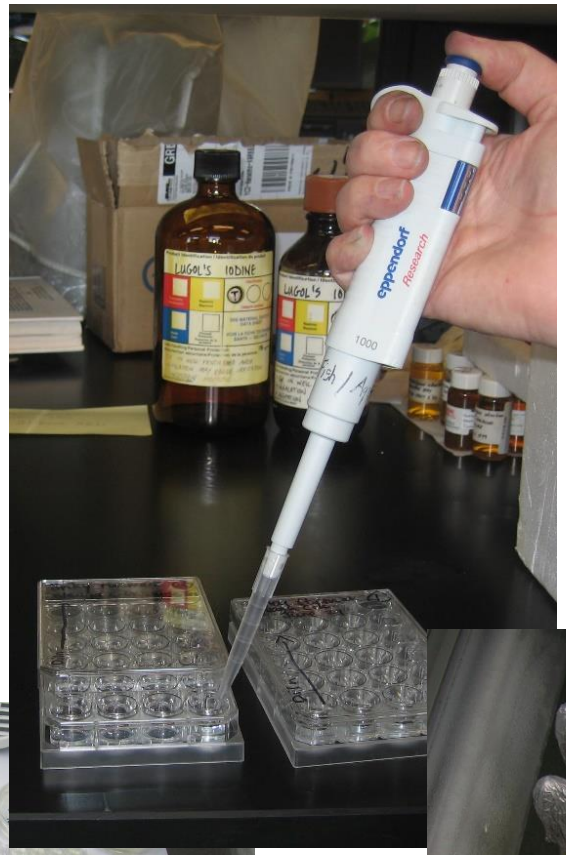
Taxa: *Alexandrium* sp., *Chaetoceros eibenii* (a non-harmful species), *Chattonella* sp.?, *H. akashiwo*, *Protoceratium reticulatum*, *Pseudochattonella* cf. *verruculosa*, and *Pseudo-nitzschia* spp.

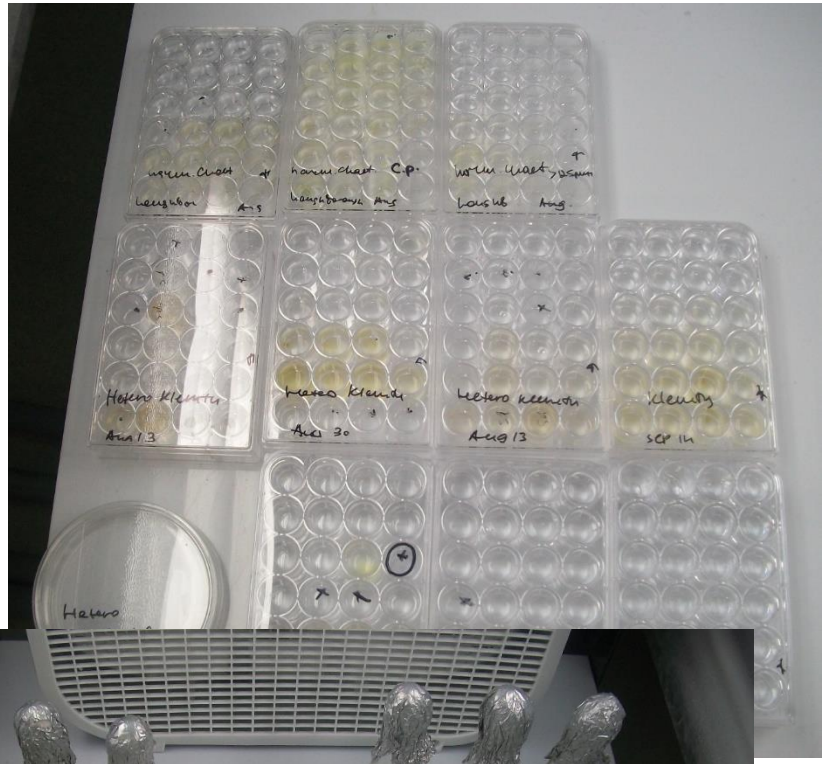
- **24 field water** samples (filtered and pelleted)

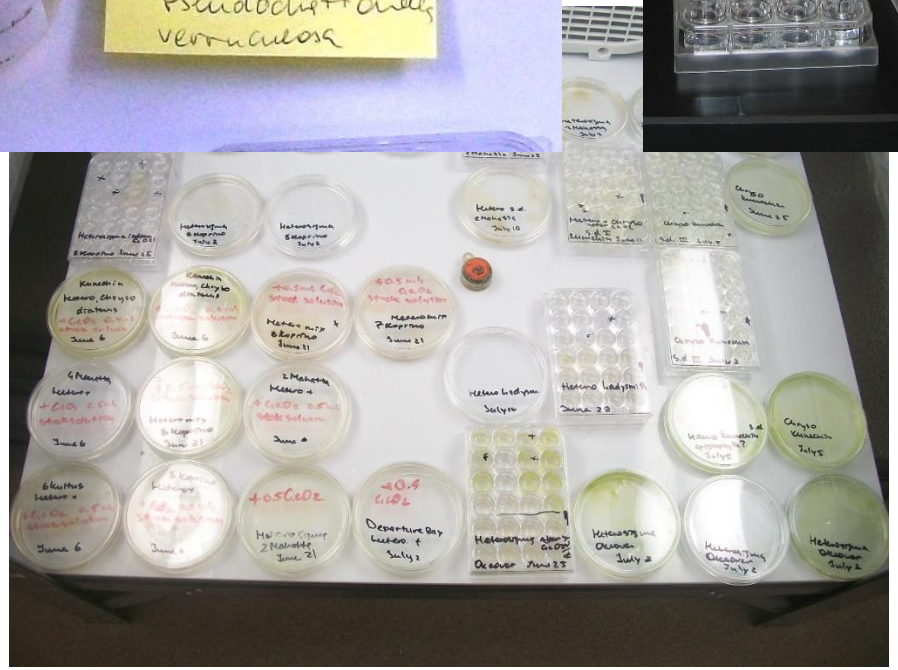
Taxa: *Chattonella* sp., *Chrysochromulina* sp., *Cochlodinium fulvescens*, *Dictyocha* spp., *Heterosigma akashiwo*, *Pseudochattonella* cf *verruculosa*, *Pseudopedinella* sp.

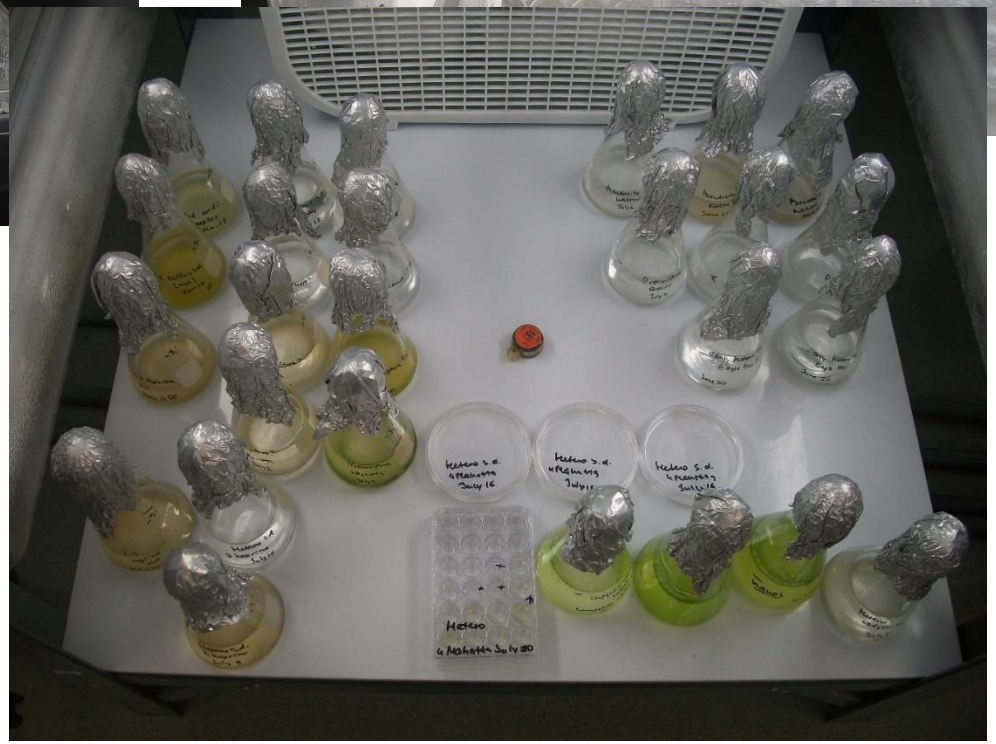



live sample
from fish
killing event
toxic algae
Pseudochattonella
verruculosa


LUGOL'S IODINE
eppendorf
Research







qPCR

- Total of 39 previously published TaqMan assays for 28 taxa were found

- 15 assays for 5 targeted taxa: 6 for *Alexandrium* spp., 2 for *Chattonella* spp., 3 for *H. akashiwo,* 2 for *K. mikimotoi*, and 2 for *P. verruculosa*.

- Additional 24 assays for another 22 taxa known to be harmful.

- The qPCR reactions using all assays were conducted on a Fluidigm BioMark™ platform (Miller et al., 2016)

qPCR results

- Out of 39 assays only 8 provided amplification results
- Targeted species that were detected via qPCR: *Alexandrium* spp./*A. tamarense*, *H. akashiwo*, and *P. verruculosa*
- Non targeted taxa detected with qPCR: cyanobacteria, *G. instriatum*, *Karlodinium micrum/veneficum*

Metabarcoding

- Muliple amplicons were used: 16S, 18S-diatom, 18S-dinoflagellate, LSU (23S)
- 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
- OBITools package (Boyer et al., 2016) was used for the analysis of the different amplicons
- The unique amplicons were annotated using BLAST, read counts were connected to annotations from the MEGAN output using custom R

Taxon levels and number of reads detected by the four different amplicons

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

~ 350 individual taxa were identified

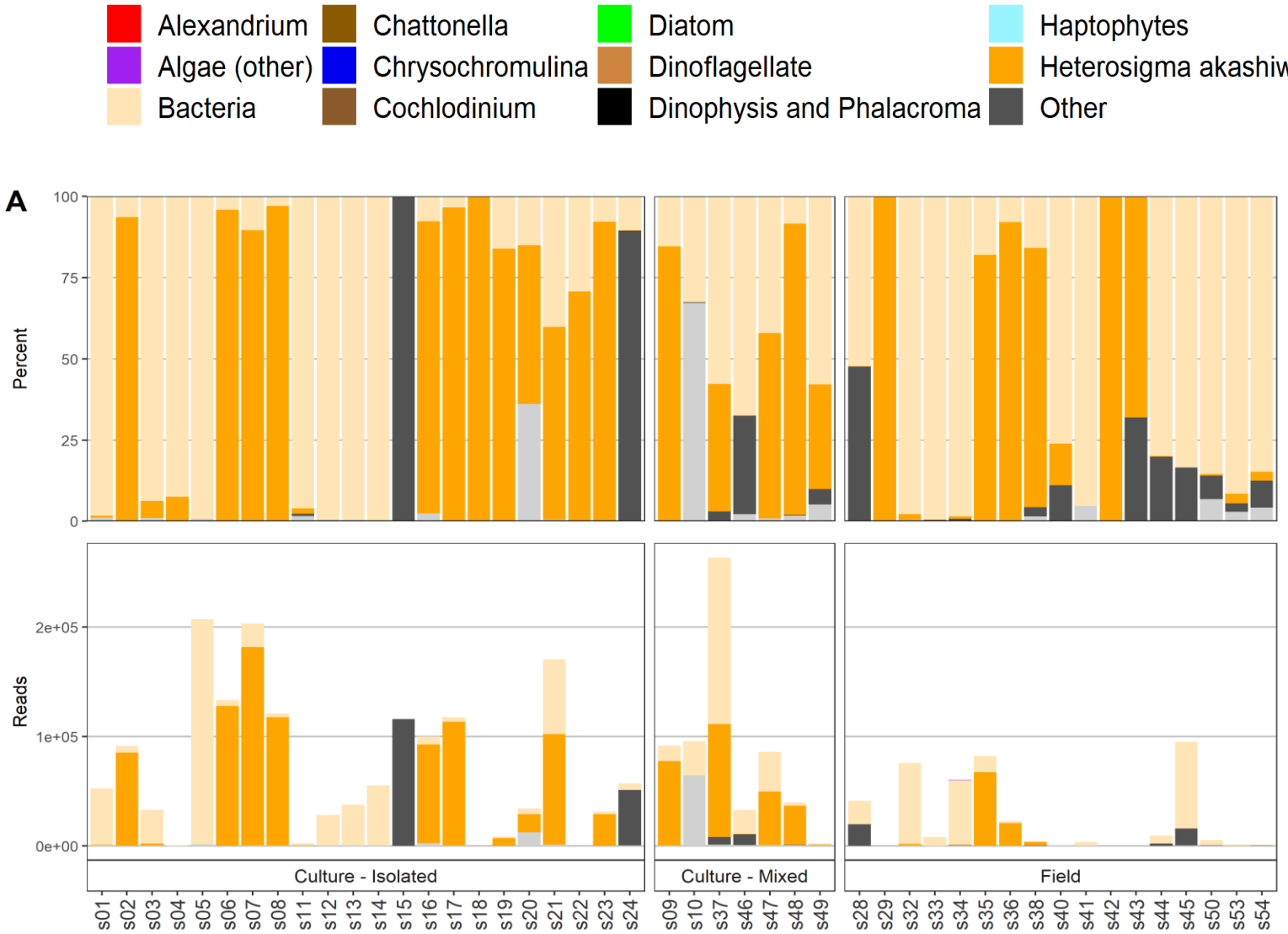
The most numerous unique taxa through 18S-dinoflagellate

the most effective at resolving data to the species level species LSU

Number of reads per taxonomic category and percent of total microalgae read

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

Metabarcoding results – 16S

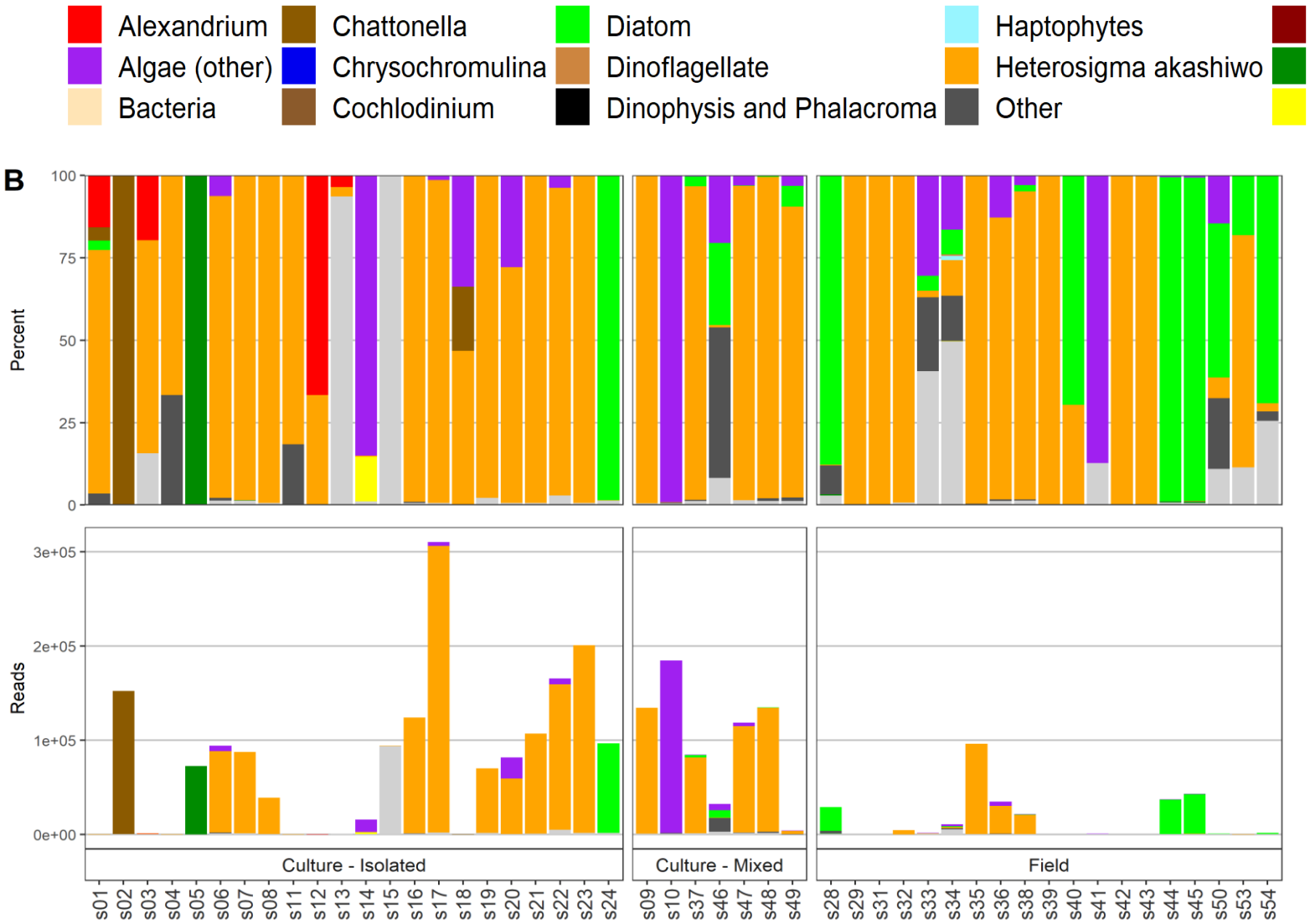


weak for detection of microalgae

the only amplicon detecting cyanobacteria

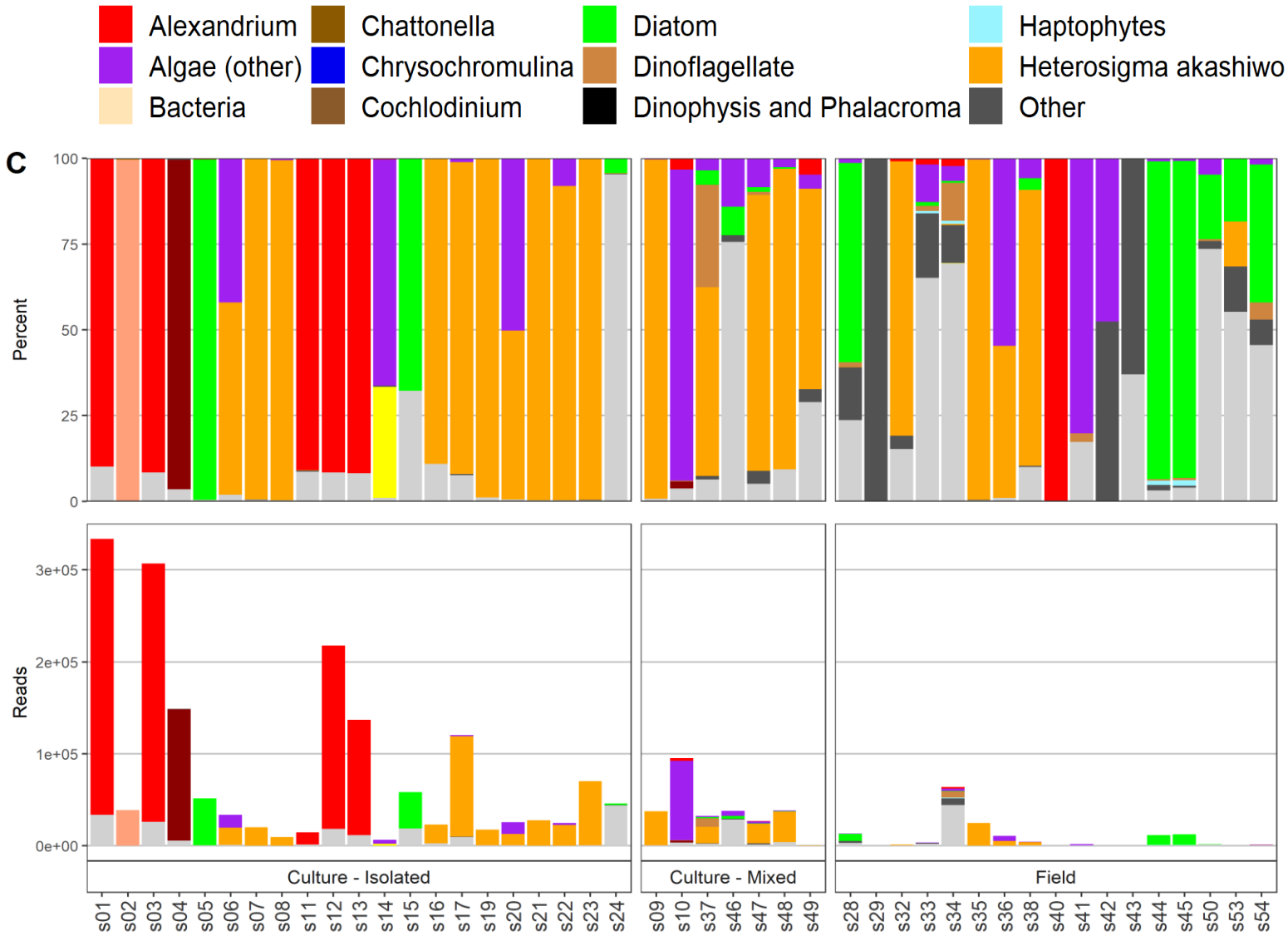
the only amplicon detecting numerous pathogens

Metabarcoding results – 18S-diatom



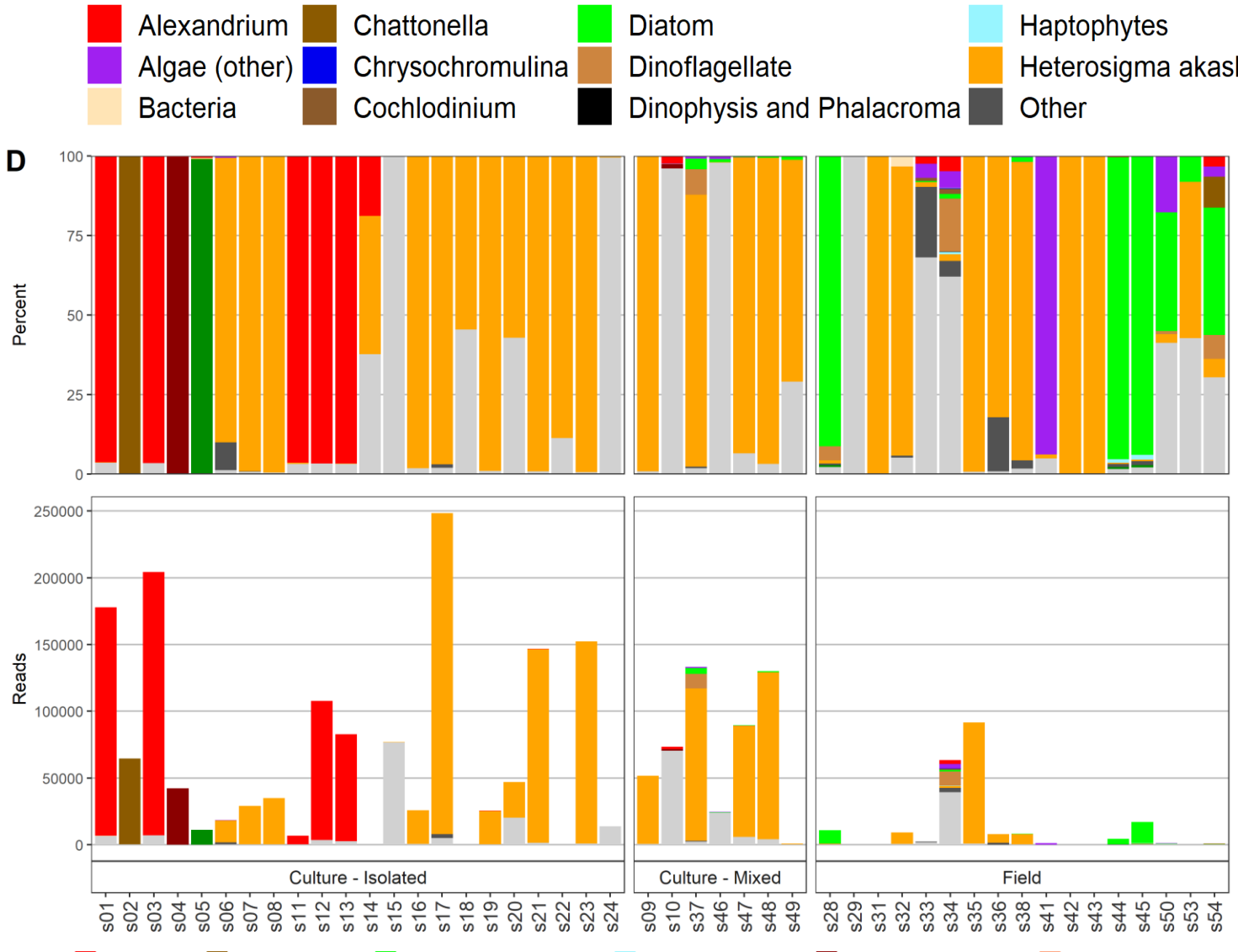
good at identification of harmful taxa from the diatom and raphidophyte groups

Metabarcoding results – 18S-dinoflagellate

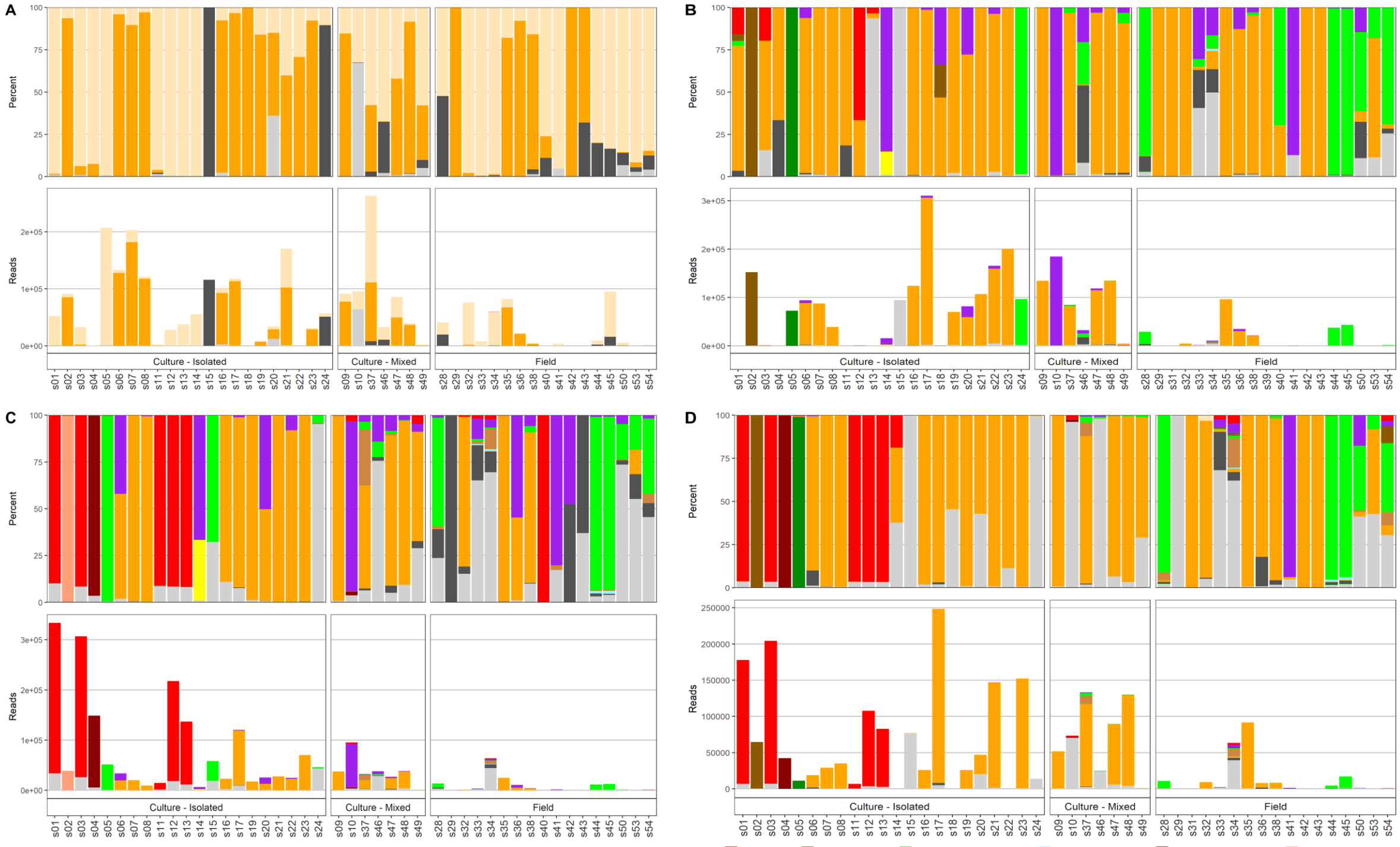


best identification for harmful algae from dinoflagellate and haptophytes groups, as well as the raphidophyte *H. akashiwo*

Metabarcoding results – LSU



18S-dinoflagellate and LSU amplicons, producing similar results to each other


A
B
C
D
Percent
Reads
Culture - Isolated
Culture - Mixed
Field
s01 s02 s03 s04 s05 s06 s07 s08 s11 s12 s13 s14 s15 s16 s17 s18 s19 s20 s21 s22 s23 s24
s09 s10 s37 s46 s47 s48 s49
s28 s29 s32 s33 s34 s35 s36 s38 s40 s41 s42 s43 s44 s45 s50 s53 s54
s31 s39

Evaluation of harmful taxa detection methods based on the present study

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

Conclusions

- Applying microscopy, qPCR, and metabarcoding on multiple HA allowed cross validation of these techniques and offered a significant foundation for choosing appropriate techniques for targeted taxa in the future.
- TaqMan assays for *A. tamarense*, *H. akashiwo*, and *P. verruculosa* provided adequate identification results. There is a need for the development of primers and probes for the rest of the harmful species; sequencing data obtained during this study allows the development of new qPCR assays tailored for species within the northeastern Pacific Ocean.
- Metabarcoding with a combination of markers proved to be an unmatched technique for phytoplankton community structure analysis.
- Different markers had different strengths for particular taxa.
- This work demonstrates a need to improve reference databases and advantages of multiple marker approach for metabarcoding of diverse taxa.

Pre-print

- https://www.biorxiv.org/content/10.1101/816926v1.abstract

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

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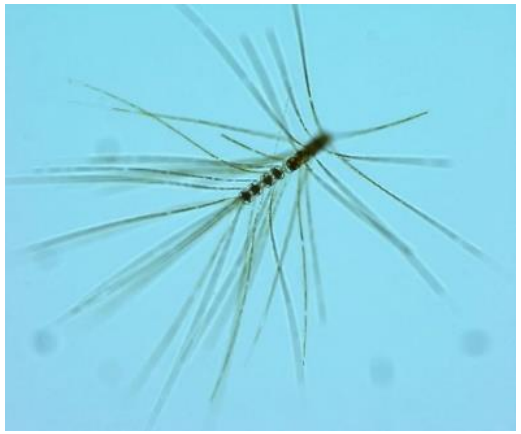
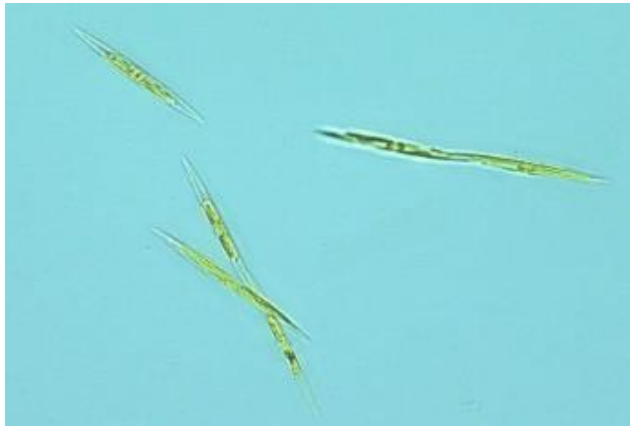


Thank you.



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