THE IMAGING FLOW CYTOBOT(IFCB) AS A TOOL FOR MONITORING HARMFUL MARINE MICRO-ALGAE IN AN AQUACULTURE REGION

Lincoln MacKenzie[1], Raphael Kudela[2], Ben Knight[1.]
[1]Kirsty Smith

1. Cawthron Institute, Nelson, New Zealand
2, University of California, Santa Cruz, USA

- In 2019 we had an IFCB on loan from Prof Raphael Kudela's lab.

- The aim was to evaluate the IFCB as a routine monitoring tool against criteria (sensitivity, accuracy, sample throughput) of the current manual programme

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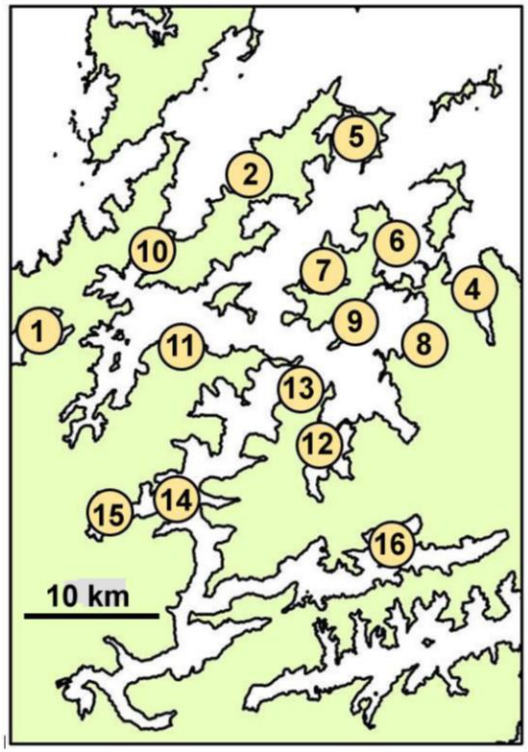
Pelorus Sound sampling sites



Alexandrium pacificum **cells x10^3 L^{-1}**

		Jan-19					Feb-19					Mar-19					Apr-19					May-19			
1	Oyster Bay	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	South Pukatea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0	0	0	0	0
3	Melville Cove	0	0	0	0	0	0	0	0	0	0	0	0.5	0.7	0.5	0.4	0	0	0.4	0	0	0	0	0
4	Anakoha Bay	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0.2	0	0	0.1	0	0	0	0
5	Cannon Hill	0	0	0	0	0	0	0	0.5	0.7	0.2	0.5	0.2	0	0.9	3.1	0.8	0.1	0.2	0.3	0	0	0	0
6	Forsyth Bay	0	0	0	0	0	0	0	0	0.1	0.1	0	0.3	0	0.2	0	0	0.2	0.4	0	0	0	0	0
7	Richmond Bay	0	0	0	0	0	0	0.1	0	0.8	0.3	0.2	0.4	0.2	1.0	0	0	0.2	1.5	0.1	0	0	0	0
8	Laverique Bay	0	0	0	0	0	0	0	0	0	0.1	0.5	0	0.1	0.1	0	1.9	0.6	0.3	1.3	0	0	0	0
9	West Beatrix	0	0	0	0	0	0.1	0	0	0	0	0.3	6.4	0.2	2.4	0.7	0.4	0.6	0.7	0.1	0	0	0	0
10	Hallam Cove	0	0	0	0	0.3	0.7	0.3	2.7	0.7	0	0	0	0.1	0	0	0	0	0.6	0.2	0	0	0.1	
11	Brightlands	0	0	0	0	0	0	0.1	0.5	0.1	0.2	0	0.2	0.1	3.2	2.2	0	0.4	1.3	0.3	0	0	0	0
12	Crail Bay	0	0	0	0	0	0	0	0	0.1	0.3	0	0	0.3	0.7	0.3	0.9	0.9	0.7	0.4	0	0	0	0
13	Capsize Point	0	0	0	0	0	0	0	0	0.5	0	6.8	0.7	19.0	1.0	1.1	0.5	0.8	0.5	0.2	0	0	0	0
14	Nydia Bay	0	0	0	0	0	0	0.2	2.7	2.2	0.3	6.2	6.5	4.6	2.0	2.1	1.9	1.8	0.4	0.3	0	0	0	0
15	Head Of Nydia	0	0	1.3	0	2.2	0	7.2	4.0	12.0	10.0	0	14.0	6.8	46.0	2.4	6.9	38.0	21.0	0.4	0	0	0	0
16	Waitaria Bay	0	0	0	0	0	0	0	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0

- *Alexandrium pacificum* blooms have become an annual problem in the mussel growing areas of the Marlborough Sounds
- > 30 samples are collected over 3 days, results are reported with 24 hrs of collection
- Phytoplankton monitoring is effective at predicting the onset of blooms, a limit of detection of 100 cells / Litre is necessary

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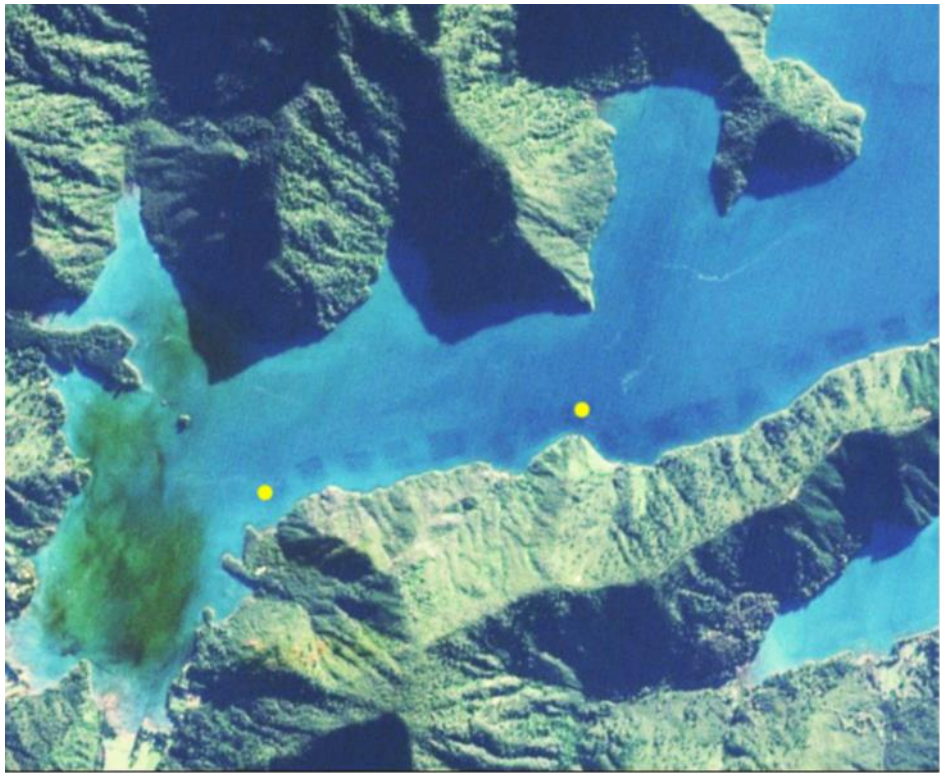
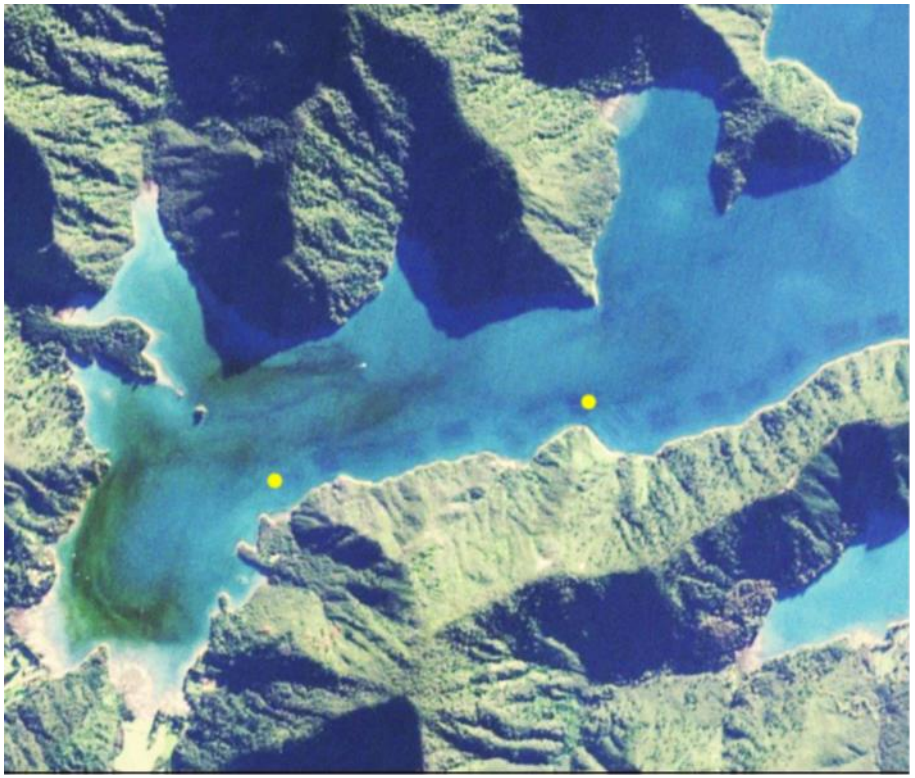
An *Alexandrium pacificum* bloom in Nydia Bay, Pelorus Sound, June 2019



18th June 2019



23rd June 2019

It is important to quickly identify the development and spread ,of blooms in isolated embayment's.

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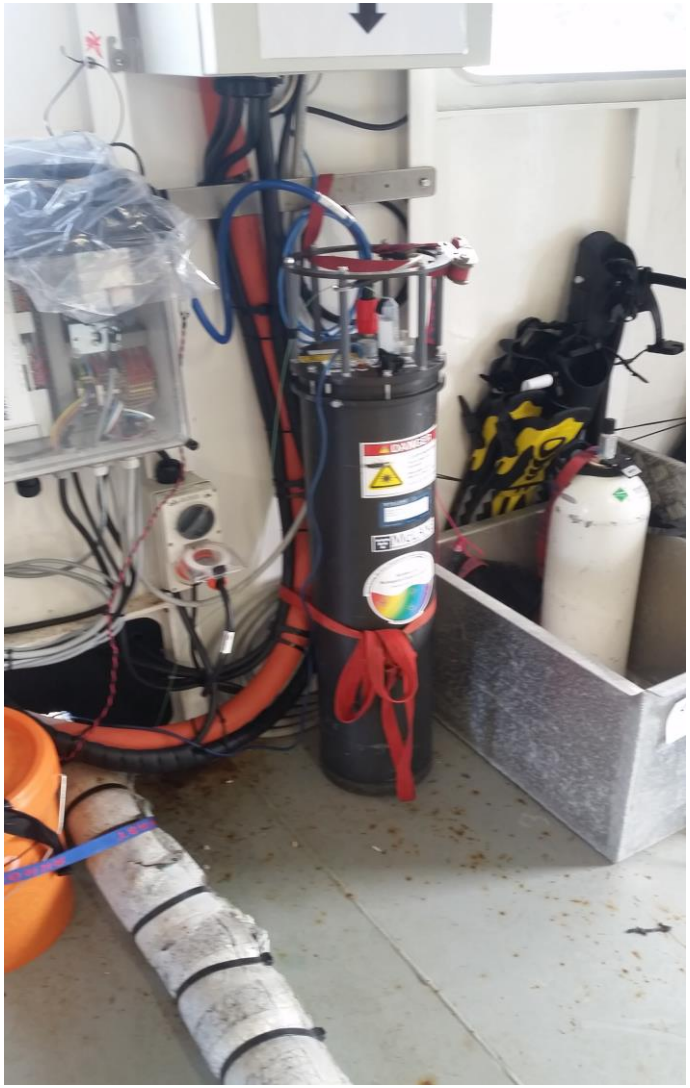
IFCB deployments

‘On the fly’ phytoplankton analysis on the sampling vessel



3 week autonomous deployment on a NZKS Pelorus Sound salmon farm

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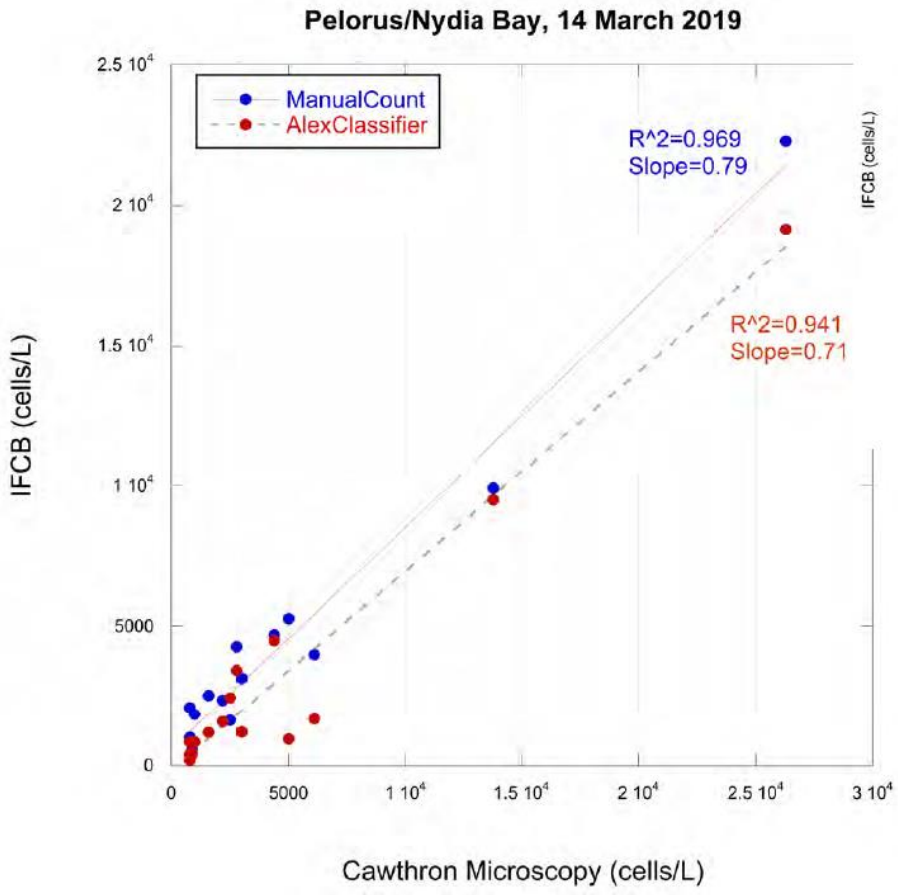
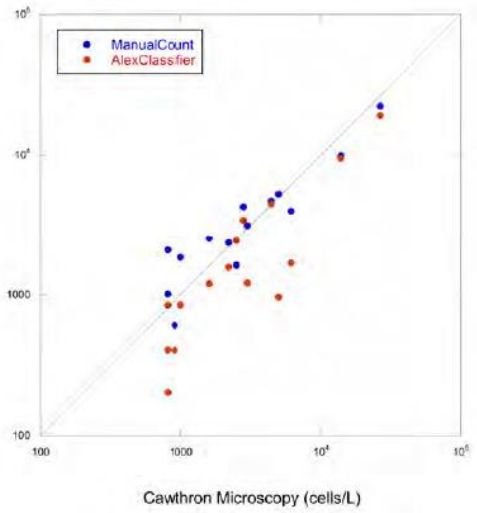
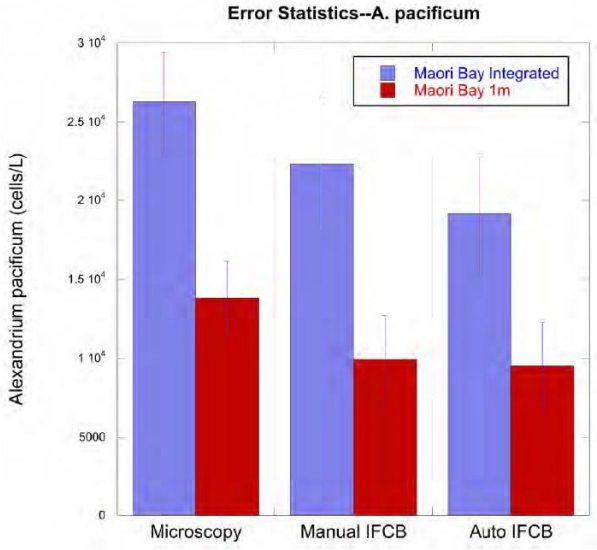
Comparing IFCB estimates of *A. pacificum* cell numbers with microscopy





Log-Transformed



There was good agreement between IFCB and manual counts of *A, pacificum*

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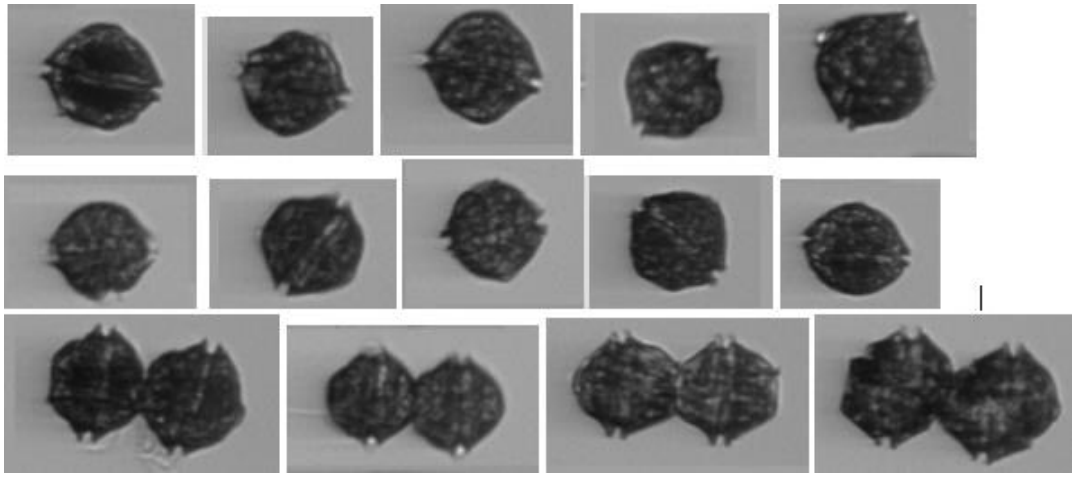
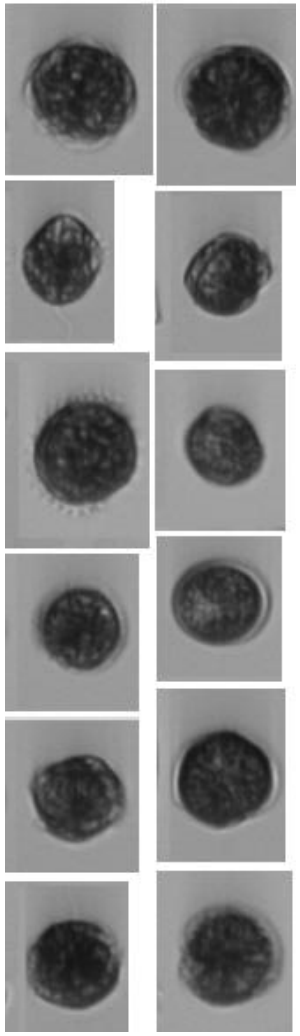
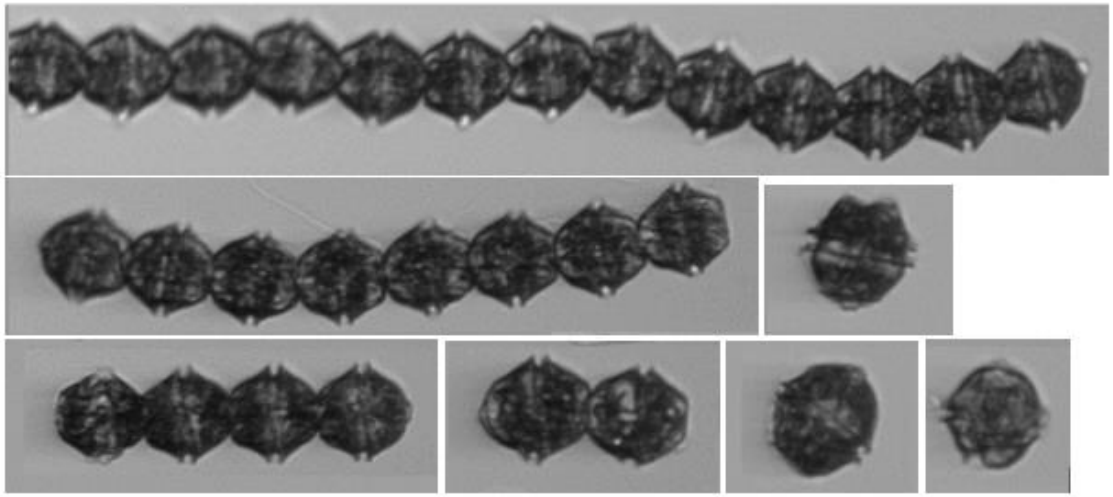
Alexandrium pacificum -toxic



Ambiguous cells



Alexandrium fraterculus - nontoxic



Toxic *A. pacificum* and non-toxic *A, fraterculus* co-occur in blooms, they can be hard to distinguish in IFCB images.

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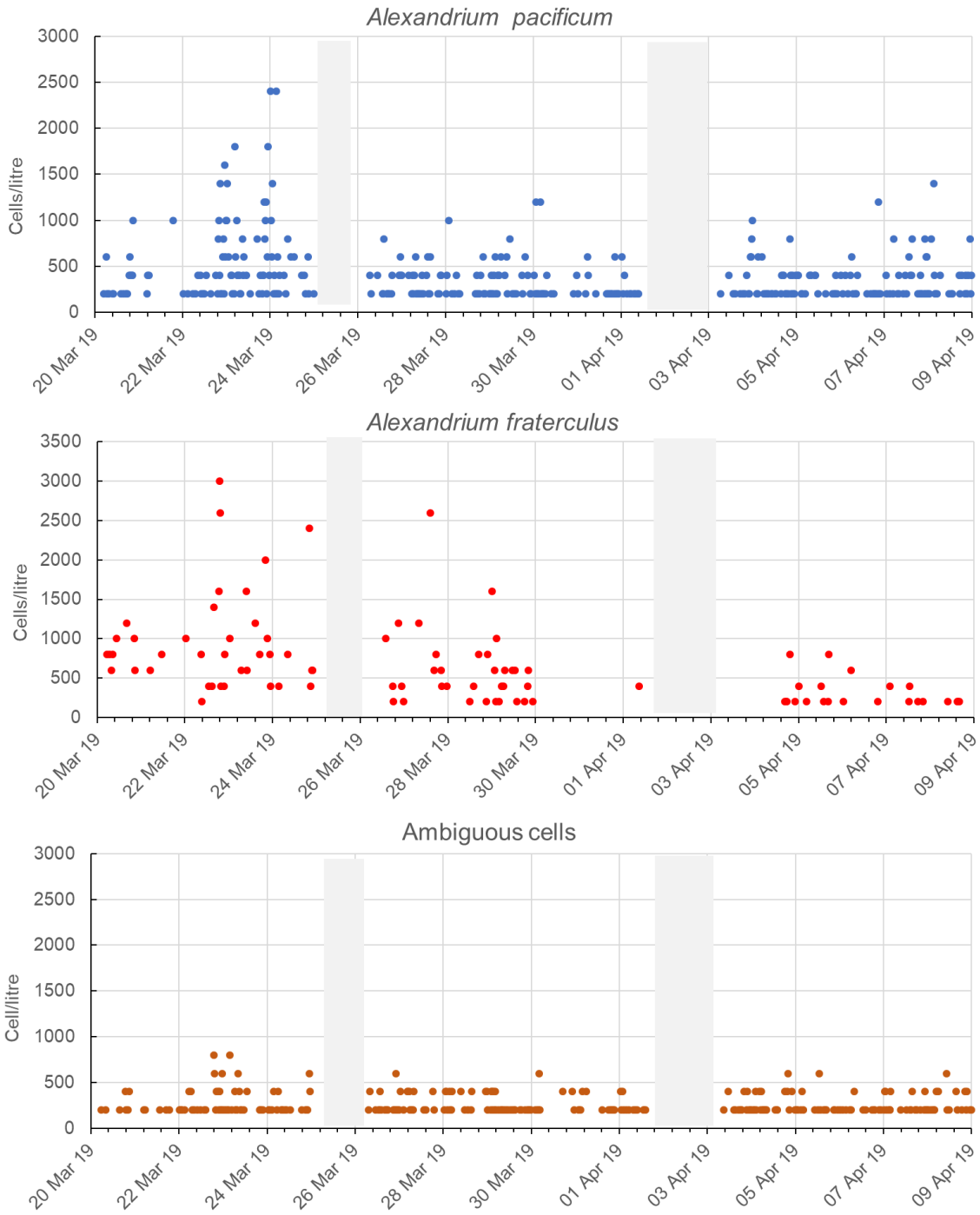
3 week salmon farm autonomous deployment of the IFCB

- 12 hourly IFCB images were manually counted
- 32% of cells were identified as *A. fraterculus,* 41% as *A. pacificum*
- 27% of cells were classified as ambiguous



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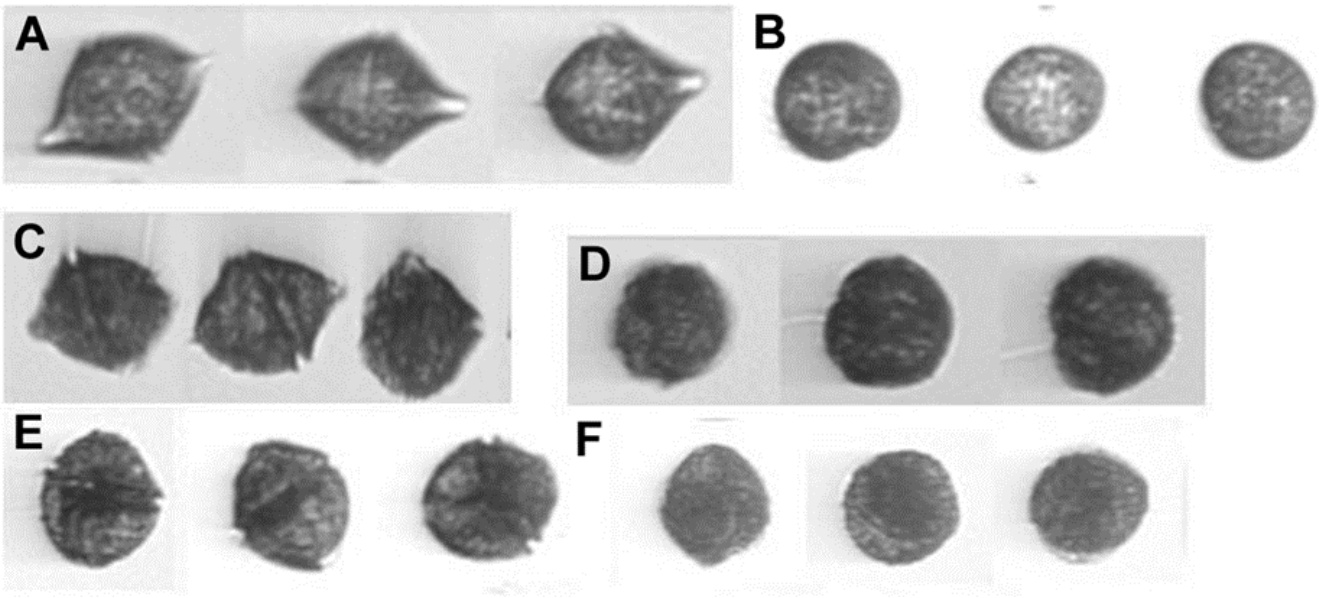
Other species can also be difficult to distinguish from *Alexandrium spp.*

Dorsal/ventral **Apical/antapical**



A & B. *Pentapharsodinium* sp.

C & D. *Gonyaulax balticum*

E & F. *Protoceratium reticulatum*

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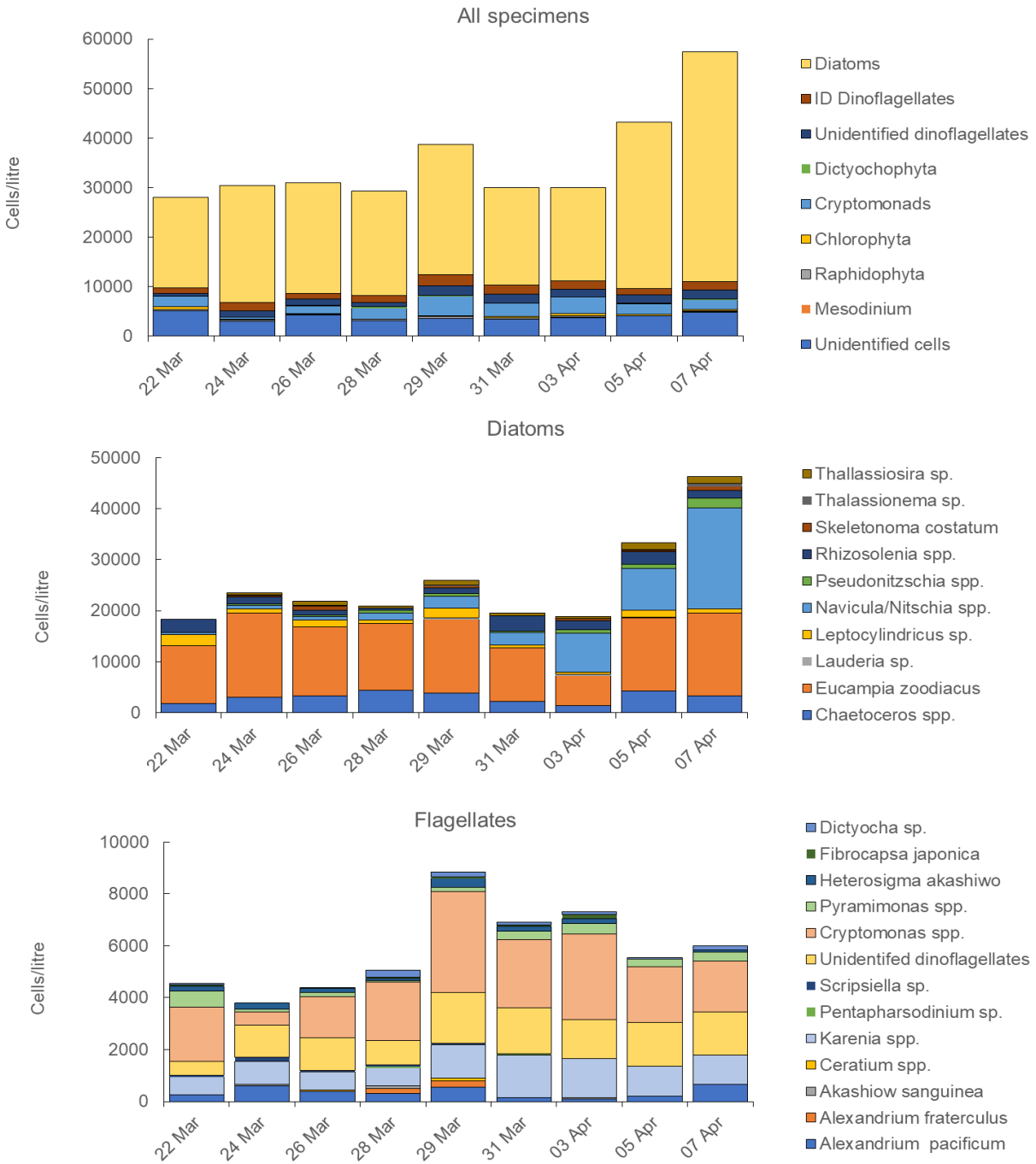
The IFCB provides comprehensive data on phytoplankton community composition.

During the autonomous deployment *A. pacificum* was a minor component of the phytoplankton



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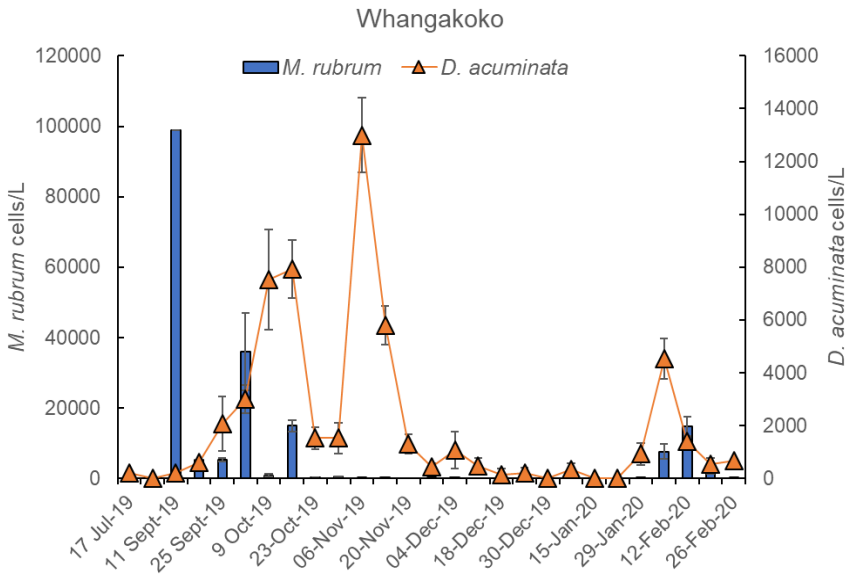
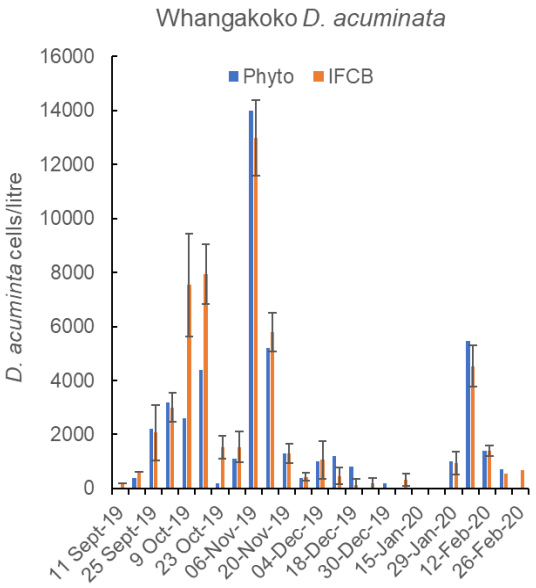
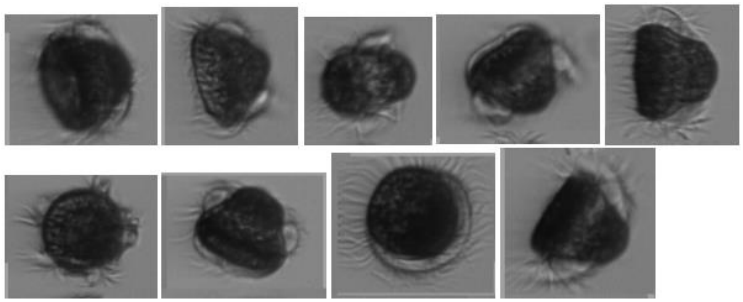
The IFCB reveals biological processes driving blooms, e.g. the relationship between *Dinophysis* (predator) and *Mesodinium* (prey) populations

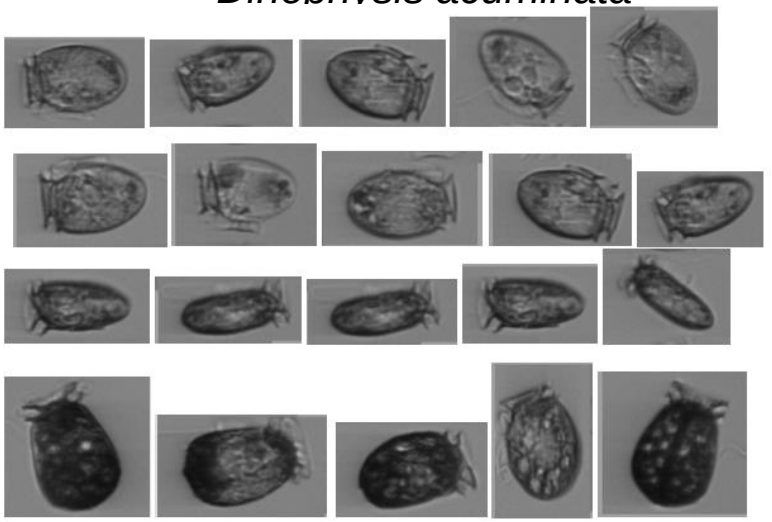




Mesodinium rubrum



Dinophysis acuminata



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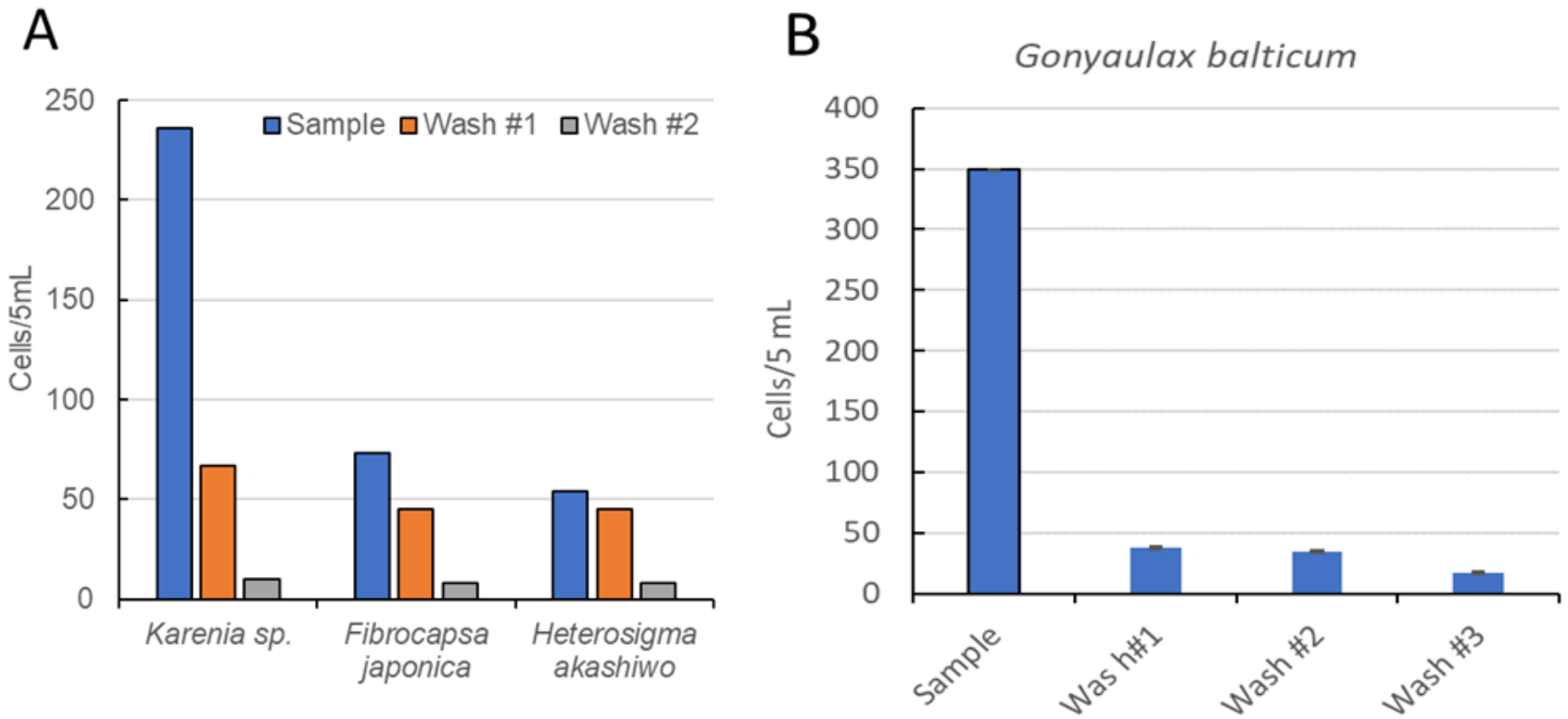
- Carry-over of specimens in successive IFCB runs can be a problem
- Repeated flushing with filtered sea-water between runs was necessary
- It is important because carry-over can result in misleading data on the spread of blooms
- This is not an issue with manual methods (e.g. Utermohl)



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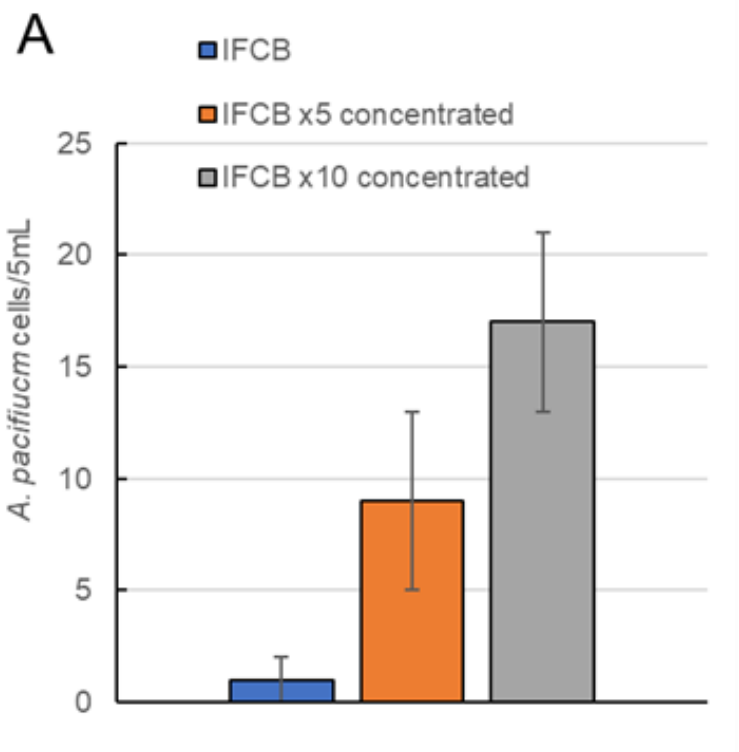
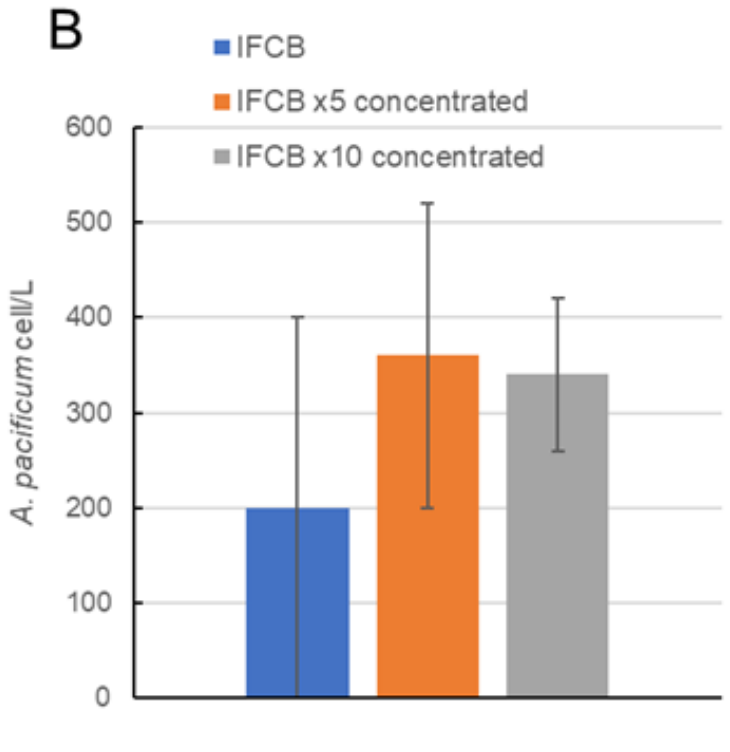
Concentration of samples using pre-screening of samples increases the sensitivity of detection





A. pacificum ells/ 5mL IFCB run

A. pacificum corrected cells/Litre

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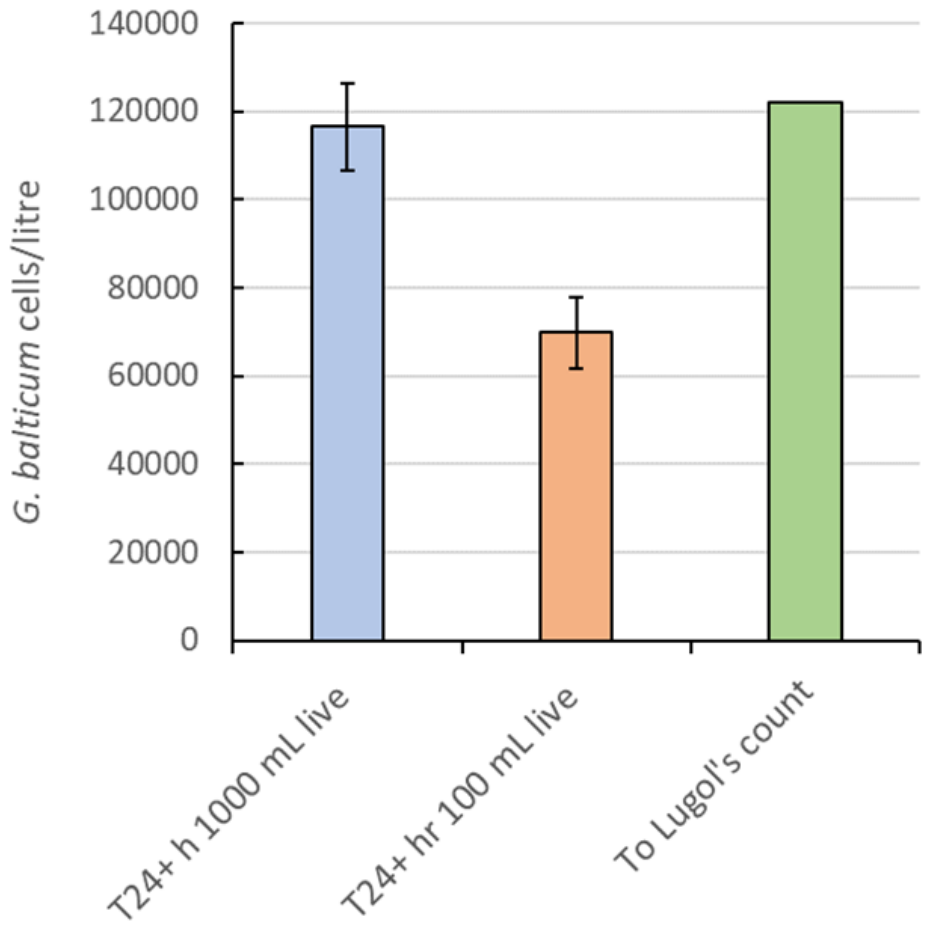
The IFCB requires live-healthy specimens so sample transport conditions are important

Larger sample size containers (1L) improved the survival of cells during overnight transport at ambient temperatures.



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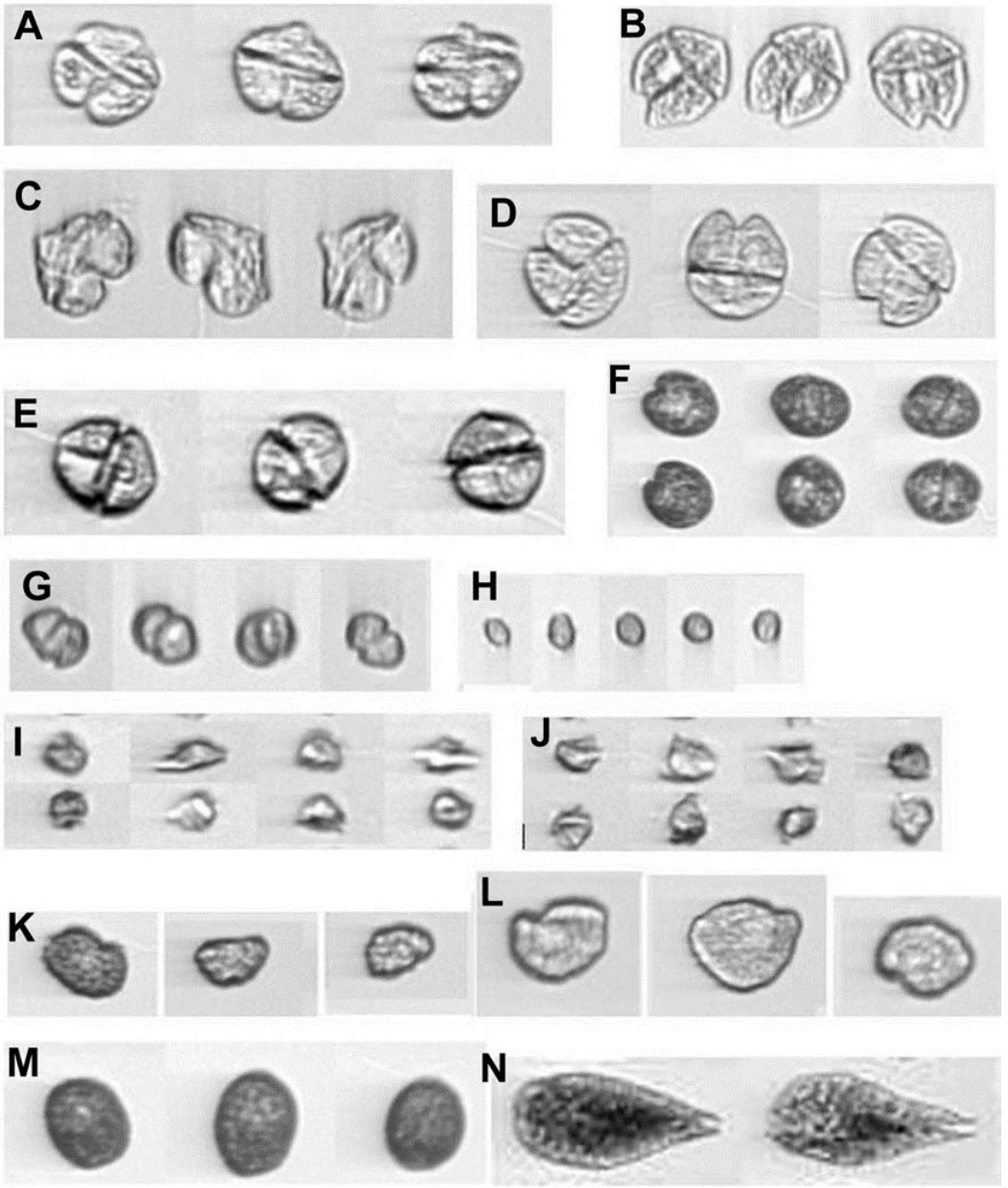
IFCB images of potential fish killing species

A. *Karenia brevis*
B. *Karenia bidigitata*
C. *Karenia papilonacae*
D. *Karebia selliformis*
E. *Katenia mikimotoi*
F. *Karenia umbella*
G. *Karlodinium veneficum*
H. *Pseudochattonella verruculosa*
I. *Chrysochromulina ericina*
J. *Chrysochromulina camella*
K. *Heterosigma akashiwo* (small)
L. *Heterosigma akashiwo* (large)
M. *Fibrocapsa japonica*
N. *Chattonella marina*



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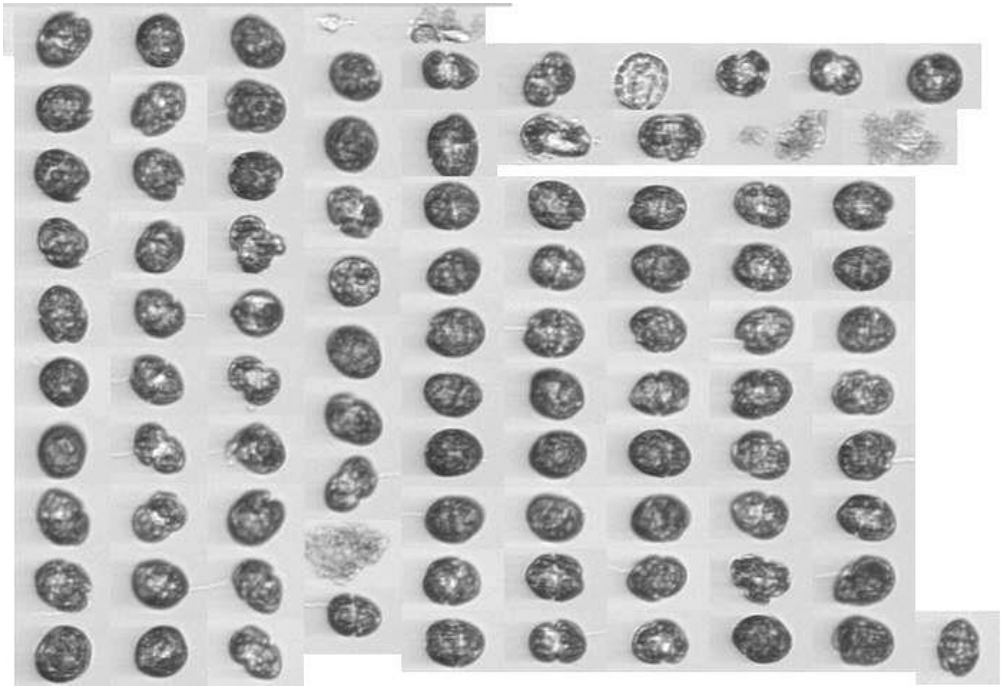
18 Feb 2020

23 Feb 2020

A *Karenia umbella* bloom impacts a salmon farm in Akaroa Harbour, February 2020.

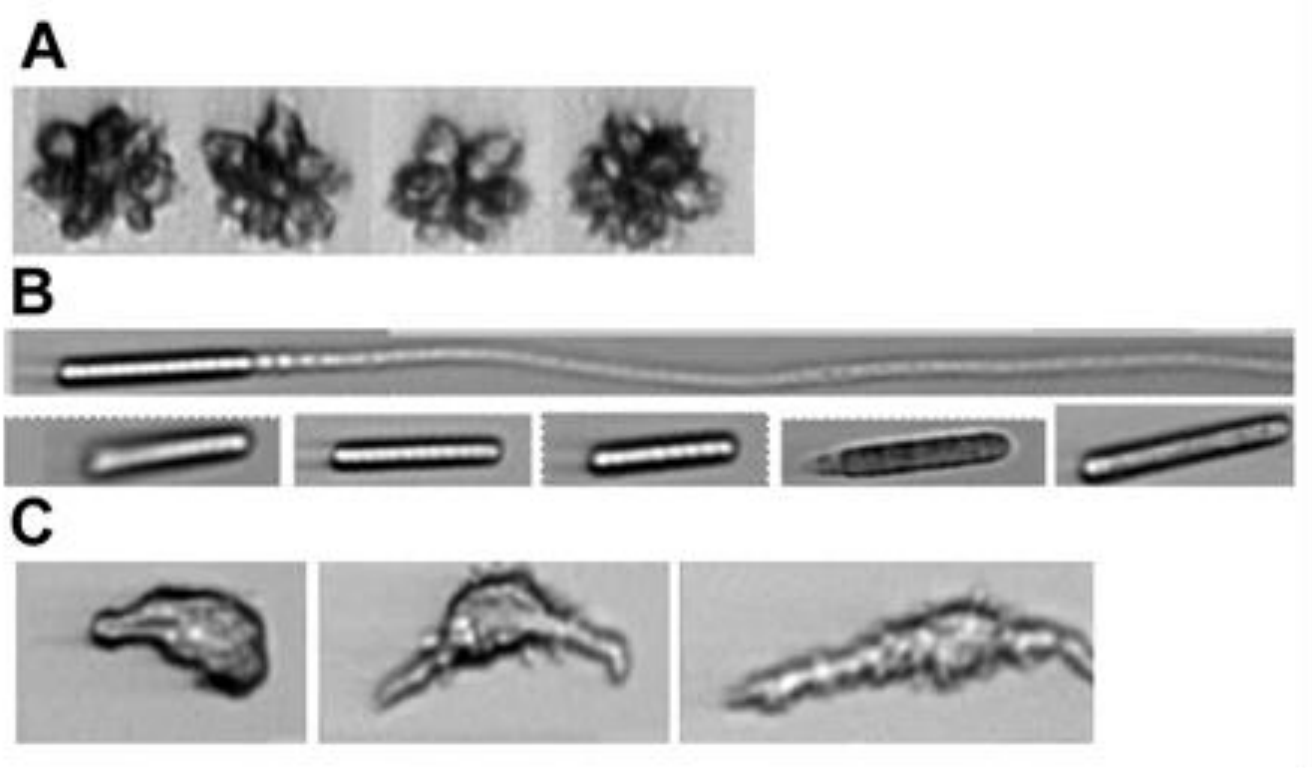


IFCB images of the *K. umbella* bloom

In situ IFCB monitoring would haveeasily detected *K. umbella* at an early stage of bloom development

Bio-discovery

The IFCB enables recognition of species not previously noticed or identified, due to the effects of preservatives (e.g. Lugol's iodine) and rapid motion of live flagellates



A. Undescribed colonial haptophyte
B. Calothrix sp.(cyanobacterium)
C. Unidentified polymorphic flagellate

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Pros and cons of the IFCB as a routine HAB monitoring tool

Pros

- Real time ID and enumeration of HAB species
- Whole community analysis
- *In situ* observations of live organisms
- High sensitivity with pre-concentration
- Automated size and biovolume sorting
- Image analysis species classifiers
- Remote access to data
- Permanent data records
- Improved understanding of bloom dynamics
- Bio-discovery
- Potential labour saving

Cons

- Only one sample can be run at a time
- 5mL samples, 20 minutes/sample: throughput and sensitivity limitations
- Ambiguity between some species
- Some small nondescript species not well resolved
- Some large species (e.g. *Pseudonitzschia*) not quantitatively sampled
- Requires live samples in good condition.
- Problem of carry-over of specimens
- Need for an external power and data transmission
- Submersible configuration not essential
- Cost (≈ $NZ 240,000)

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SUMMARY

- The IFCB is an excellent research and monitoring tool that would be a valuable supplement to the current monitoring programme
- However, its limitations (especially . sample throughput) preclude it replacing manual methods in its current configuration
- Its slow throughput cannot match the needs of a monitoring programme that requires rapid analysis of multiple samples from numerous locations.
- A multichannel instrument (to enable simultaneous running of several samples and cleaning cycles), designed to withstand the rigors of installation on the sampling vessel for real time analysis, would be ideal.

Acknowledgements

- This research was funded by the Sustainable Seas National Science Challenge, “Innovative technologies for early detection of Harmful Algal Bloom (HAB) threats” (C01X1515) and the Sea food Safety Platform (MBIE contract No. CAWX1801).

- Professor Kudela’s expenses while in New Zealand were supported by a Royal Society of New Zealand Leaders Catalyst Fellowship (contract IFL-CAW1601).

- Thanks to the NZ King Salmon Co. for assistance with the Kopaua farm IFCB deployment.

- Thanks to Marlborough Shellfish Quality Programme for access to monitoring data and Colin Johnson, Mike Williams, Noel McArthur and James Brodie (Marlborough Commercial Divers) for assistance with sampling.

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