



Molecular tools being applied in the SA HAB event

Dr Sarah Catalano, Dr K. Wiltshire,
R. Nicholls

2 June 2026

OFFICE FOR ALGAL BLOOM RESEARCH



Government of South Australia
Department of Primary Industries
and Regions



SARDI SOUTH AUSTRALIAN
RESEARCH AND
DEVELOPMENT
INSTITUTE



Australian Government

Type and source of samples

- **eDNA (environmental) – filtered water samples (field)**

SARDI teams: Apalie day trips, Southern Ranger voyage (live cultures; S. Nayar & team), Ngerin voyage (benthic monitoring; S. Smith & team), water sampling team (monthly & ad hoc; C. White, V. Silva, A. Ivey & team), Offshore Sampling voyage (*Karenia* hotspots; M. Doubell, I. Moody & team), Oceanographic moorings (M. Doubell & team)

DEW: GAB voyage, southern KI voyage

Flinders University: GAB voyage

- **Pure cultures**

SARDI teams: Established from SA HAB bloom event, single cell isolates grown under different conditions

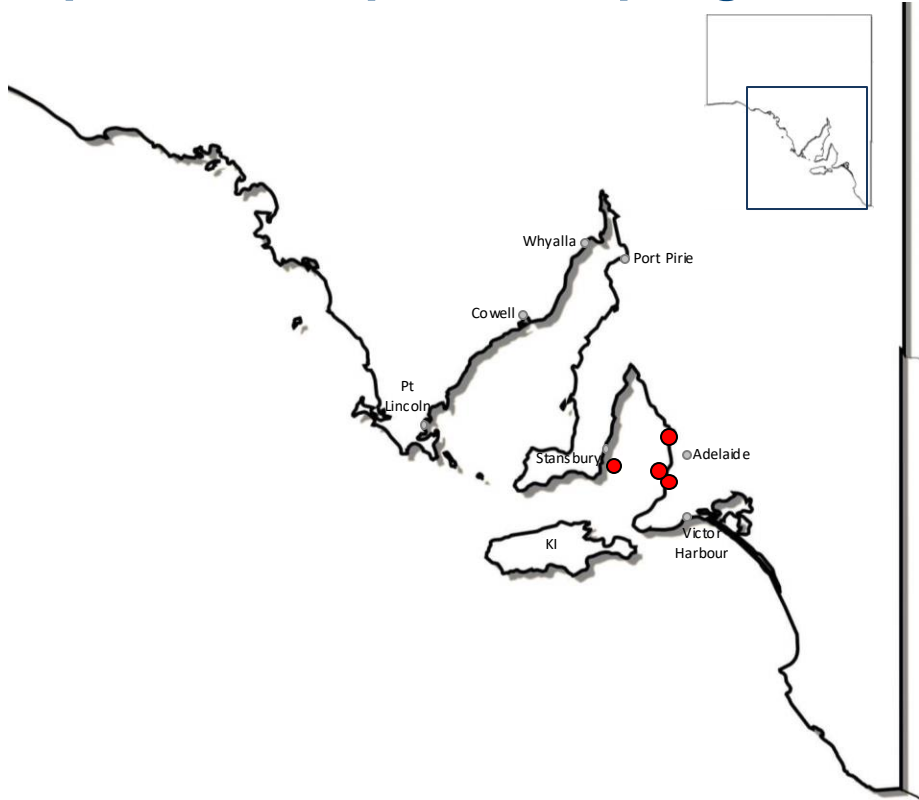
Cawthorn Culture Collection: Historical bloom samples

- **Fresh and Lugol's preserved (laboratory)**

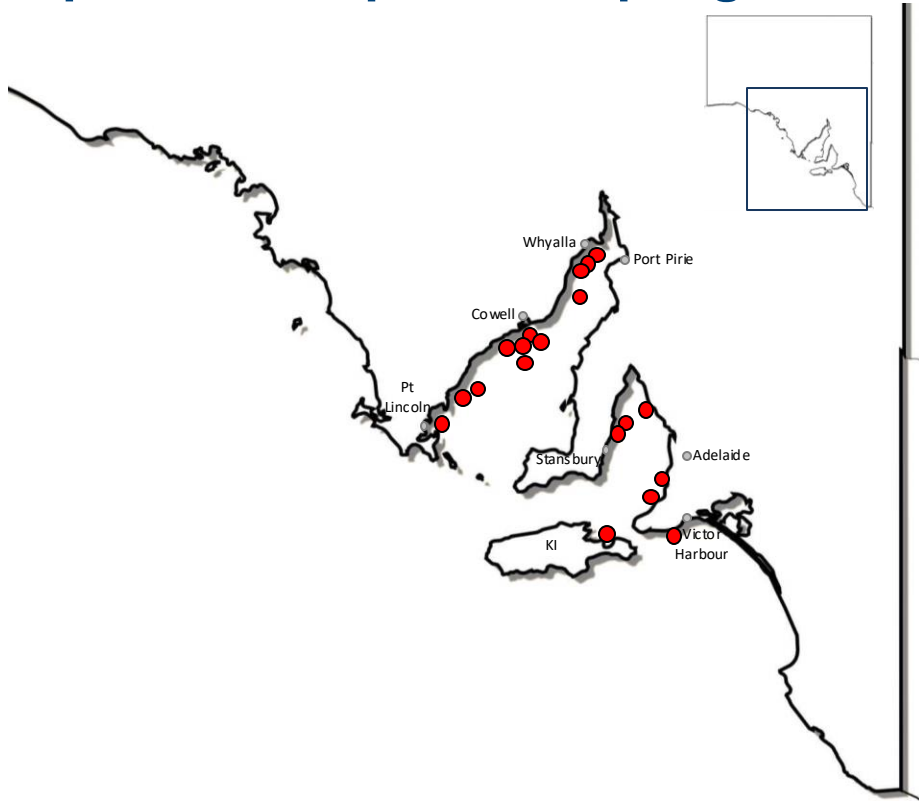
Collaborative water sampling team

Collection locations – spatial & temporal sampling

Aug25: Apalie, ad hoc. n=4 sites
(3x GSV east, 1x GSV west), 5 reps/site



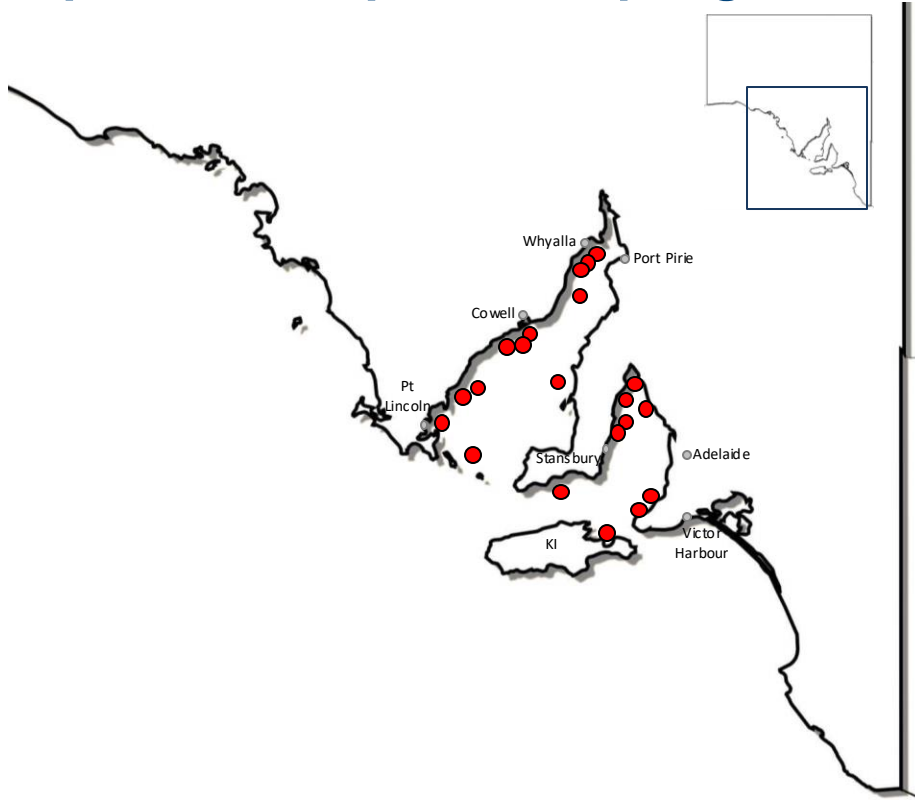
Collection locations – spatial & temporal sampling



Aug25: Apalie, ad hoc. n=4 sites
(3x GSV east, 1x GSV west), 5 reps/site

Sept25: Southern Ranger. n=19 sites (5x GSV, 1x NE BP, 1x
KI, 12x SG), 5 reps/site, pump v. Smith-root auto sampler

Collection locations – spatial & temporal sampling

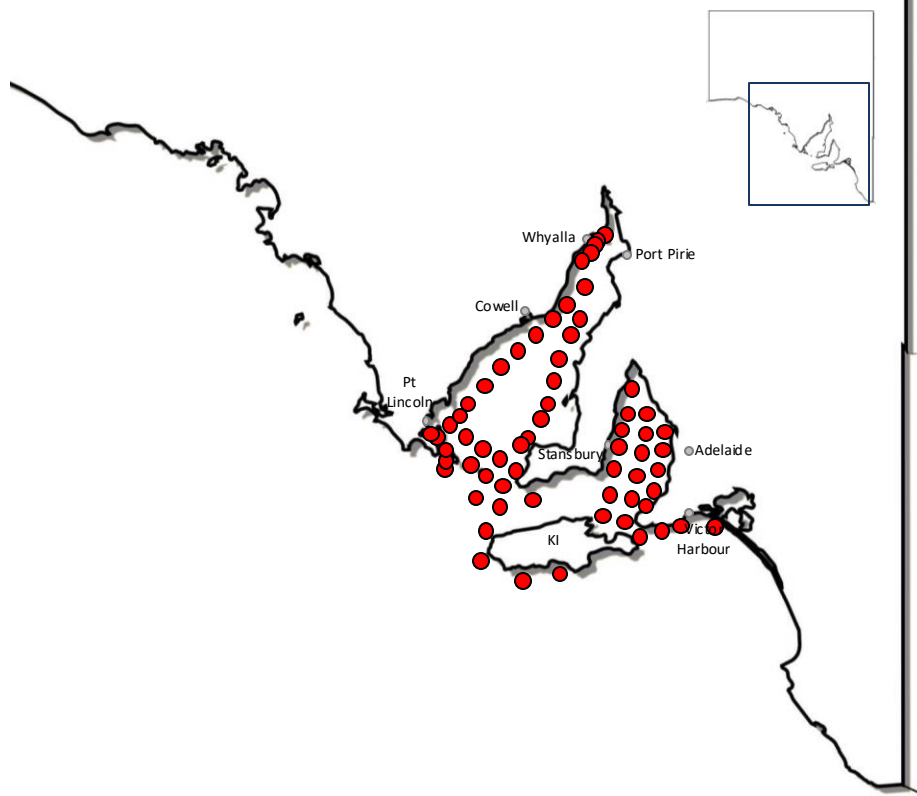


Aug25: Apalie, ad hoc. n=4 sites
(3x GSV east, 1x GSV west), 5 reps/site

Sept25: Southern Ranger. n=19 sites (5x GSV, 1x NE BP, 1x KI, 12x SG), 5 reps/site, pump v. Smith-root auto sampler

Oct25: Ngerin. n=21 sites; 15/19 sites repeated Sept25
(7x GSV, 1x KI, 1x IS, 12x SG), 3 reps/site

Collection locations – spatial & temporal sampling



Aug25: **Apalie, ad hoc.** n=4 sites
(3x GSV east, 1x GSV west), 5 reps/site

Sept25: **Southern Ranger.** n=19 sites (5x GSV, 1x NE BP, 1x KI, 12x SG), 5 reps/site, pump v. Smith-root auto sampler

Oct25: **Ngerin.** n=21 sites; 15/19 sites repeated Sept25
(7x GSV, 1x KI, 1x IS, 12x SG), 3 reps/site

Nov25: **Monthly, ad hoc.** n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Dec25: **Monthly, ad hoc.** n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Jan26: **Monthly, ad hoc.** n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

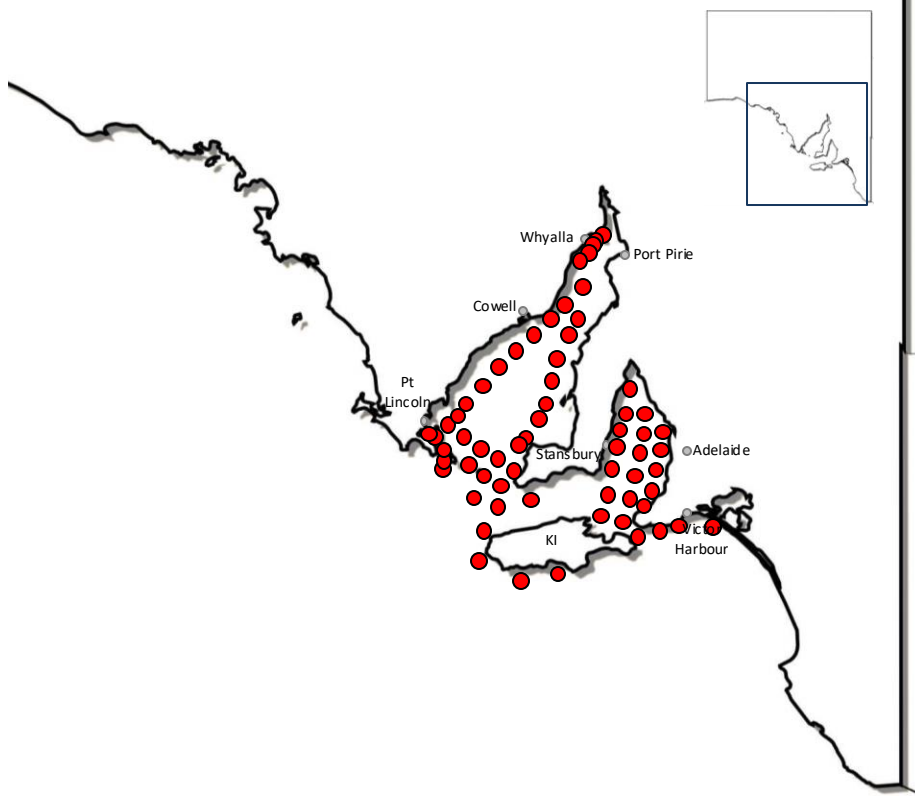
Feb26: **Monthly, ad hoc.** n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Mar26: **Monthly, ad hoc.** n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Apr26: **Monthly, ad hoc.** n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

May26: **Monthly, ad hoc.** n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Collection locations – spatial & temporal sampling



Aug25: Apalie, ad hoc. n=4 sites
(3x GSV east, 1x GSV west), 5 reps/site

Sept25: Southern Ranger. n=19 sites (5x GSV, 1x NE BP, 1x KI, 12x SG), 5 reps/site, pump v. Smith-root auto sampler

Oct25: Ngerin. n=21 sites; 15/19 sites repeated Sept25
(7x GSV, 1x KI, 1x IS, 12x SG), 3 reps/site

Nov25: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Dec25: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Jan26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Feb26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

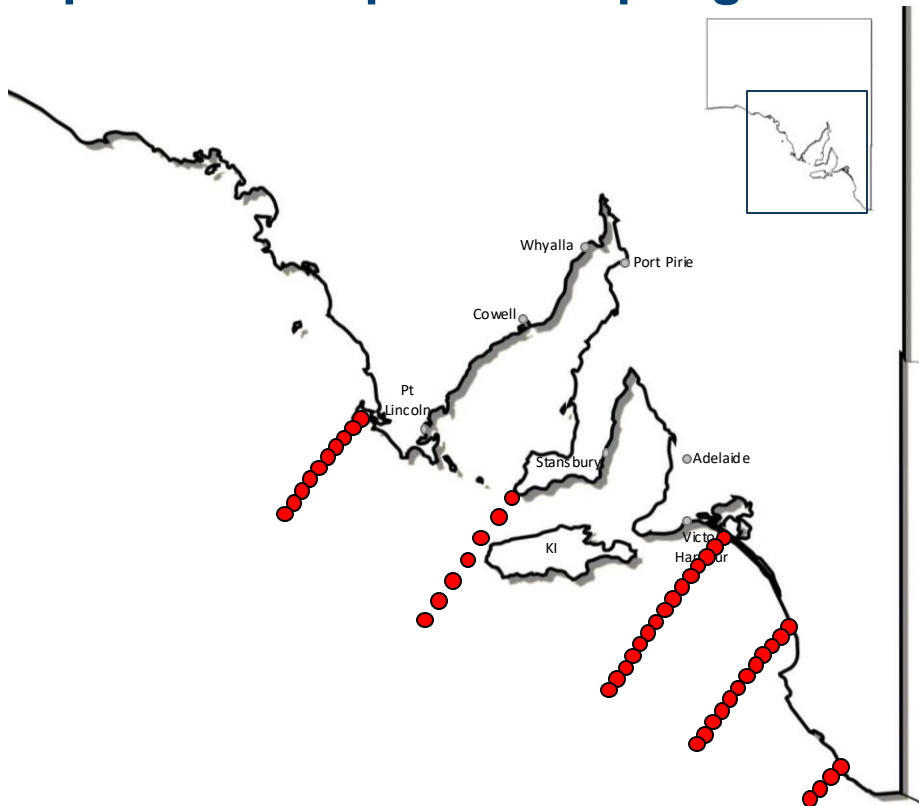
Mar26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Apr26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

May26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

+ morphology - *Karenia* spp.
counts + full community counts

Collection locations – spatial & temporal sampling



Aug25: Apalie, ad hoc. n=4 sites
(3x GSV east, 1x GSV west), 5 reps/site

Sept25: Southern Ranger. n=19 sites (5x GSV, 1x NE BP, 1x KI, 12x SG), 5 reps/site, pump v. Smith-root auto sampler

Oct25: Ngerin. n=21 sites; 15/19 sites repeated Sept25
(7x GSV, 1x KI, 1x IS, 12x SG), 3 reps/site

Nov25: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Dec25: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

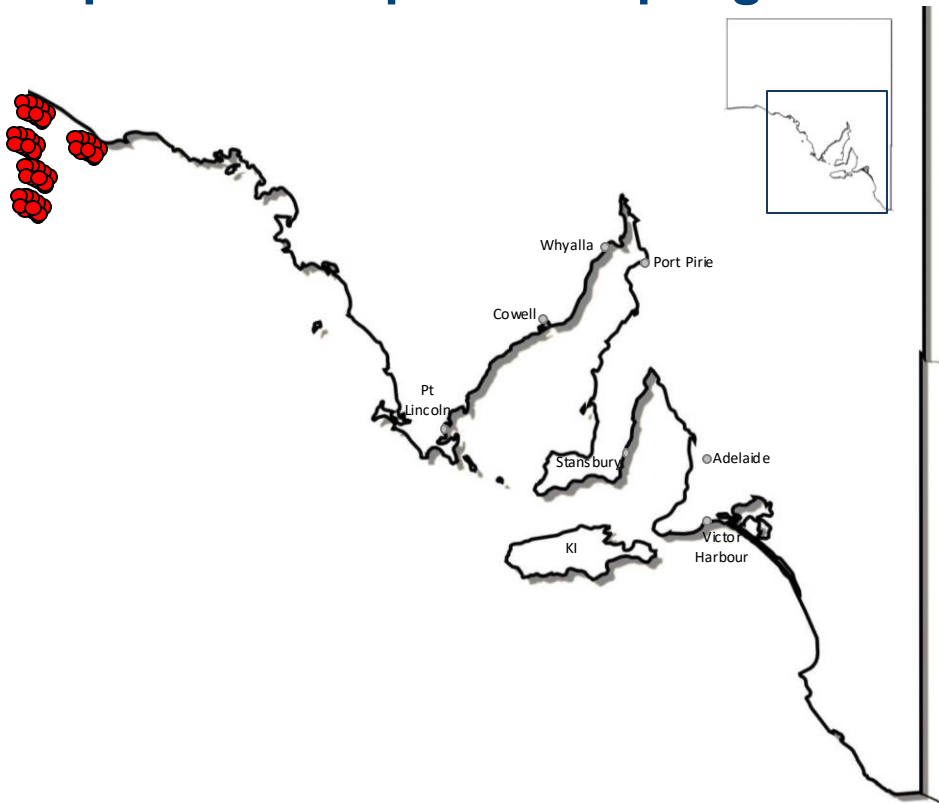
Jan26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Feb26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Mar26: Offshore Sampling voyage 1. n=49 sites, 5 transects
3 depths per site, 3 reps per depth
(voyage 2 ~Aug26, voyage 3 ~Mar27, voyage 4 ~Aug27)

(GSV, NE BP, KI, IS, SG), surface & bottom

Collection locations – spatial & temporal sampling



Aug25: Apalie, ad hoc. n=4 sites
(3x GSV east, 1x GSV west), 5 reps/site

Sept25: Southern Ranger. n=19 sites (5x GSV, 1x NE BP, 1x KI, 12x SG), 5 reps/site, pump v. Smith-root auto sampler

Oct25: Ngerin. n=21 sites; 15/19 sites repeated Sept25
(7x GSV, 1x KI, 1x IS, 12x SG), 3 reps/site

Nov25: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Dec25: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

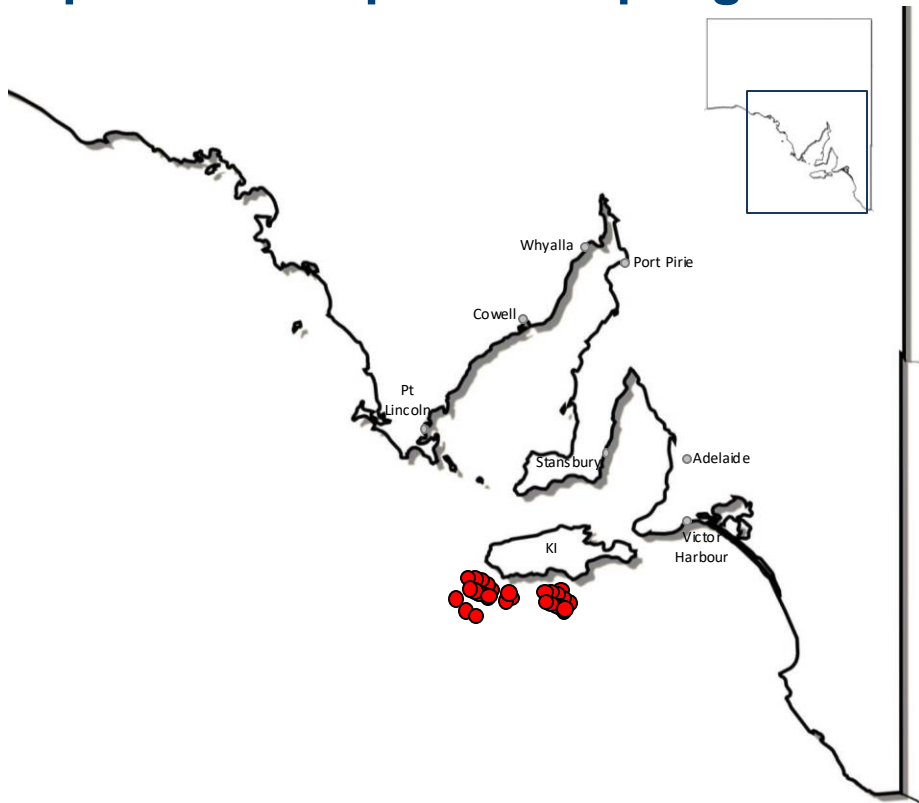
Jan26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Feb26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Apr26: GAB voyage. n=52 sites
surface & bottom

(GSV, NE BP, KI, IS, SG), surface & bottom

Collection locations – spatial & temporal sampling



Aug25: Apalie, ad hoc. n=4 sites
(3x GSV east, 1x GSV west), 5 reps/site

Sept25: Southern Ranger. n=19 sites (5x GSV, 1x NE BP, 1x KI, 12x SG), 5 reps/site, pump v. Smith-root auto sampler

Oct25: Ngerin. n=21 sites; 15/19 sites repeated Sept25
(7x GSV, 1x KI, 1x IS, 12x SG), 3 reps/site

Nov25: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Dec25: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Jan26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Feb26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Apr26: GAB Voyage. n=52 sites
surface & bottom

(GSV, NE BP, KI, IS, SG), surface & bottom

Jun26: Southern KI voyage. n=~36 sites

Collection locations – spatial & temporal sampling



Aug25: Apalie, ad hoc. n=4 sites
(3x GSV east, 1x GSV west), 5 reps/site

Sept25: Southern Ranger. n=19 sites (5x GSV, 1x NE BP, 1x KI, 12x SG), 5 reps/site, pump v. Smith-root auto sampler

Oct25: Ngerin. n=21 sites; 15/19 sites repeated Sept25
(7x GSV, 1x KI, 1x IS, 12x SG), 3 reps/site

Nov25: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Dec25: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Jan26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

Feb26: Monthly, ad hoc. n=37-65 sites/month
(GSV, NE BP, KI, IS, SG), surface & bottom

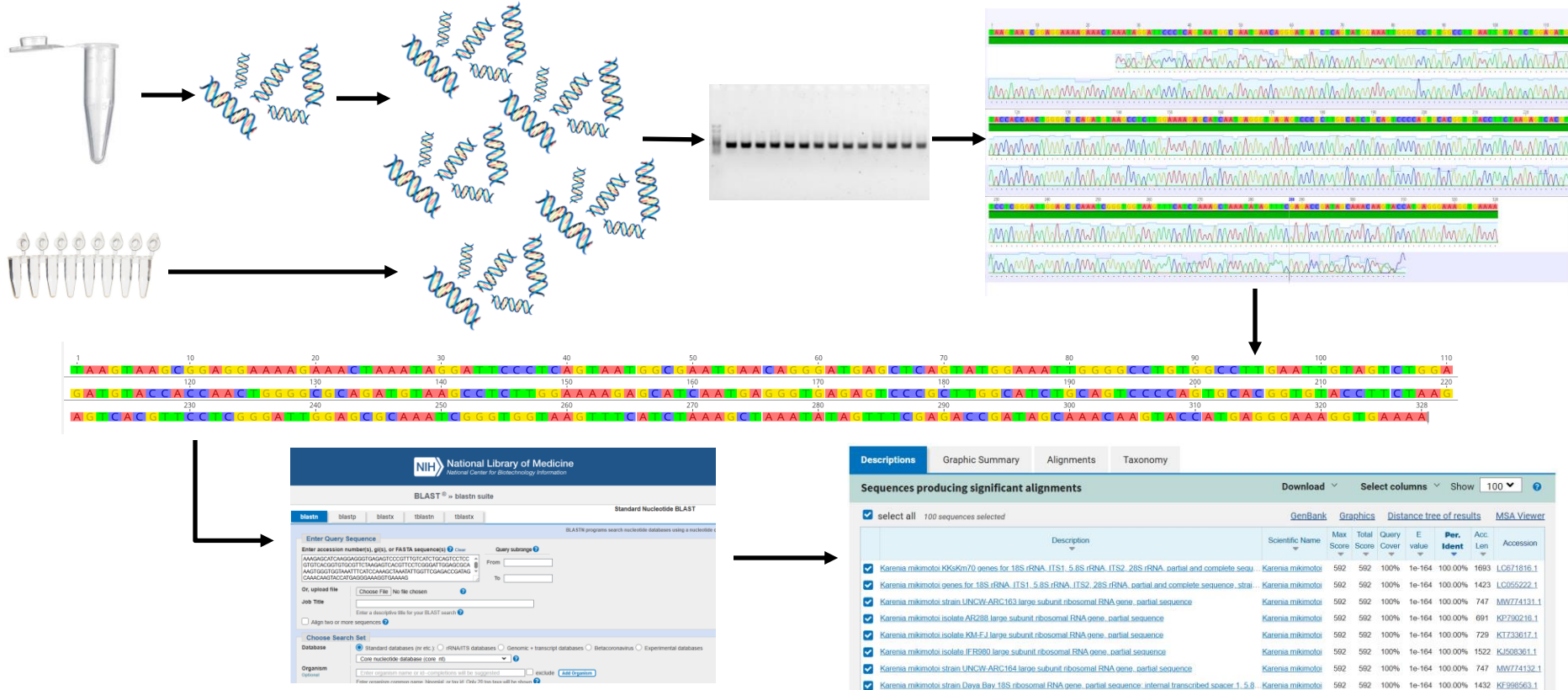
Apr26: GAB Voyage. n=52 sites
surface & bottom

(GSV, NE BP, KI, IS, SG), surface & bottom

Jun26: Southern KI voyage. n=~36 sites

Molecular tools

- **DNA BARCODING: Single species identification** – pure cultures, single cells from fresh and Lugol's preserved samples (disruption method & time post-Lugol's experiment)



Molecular tools

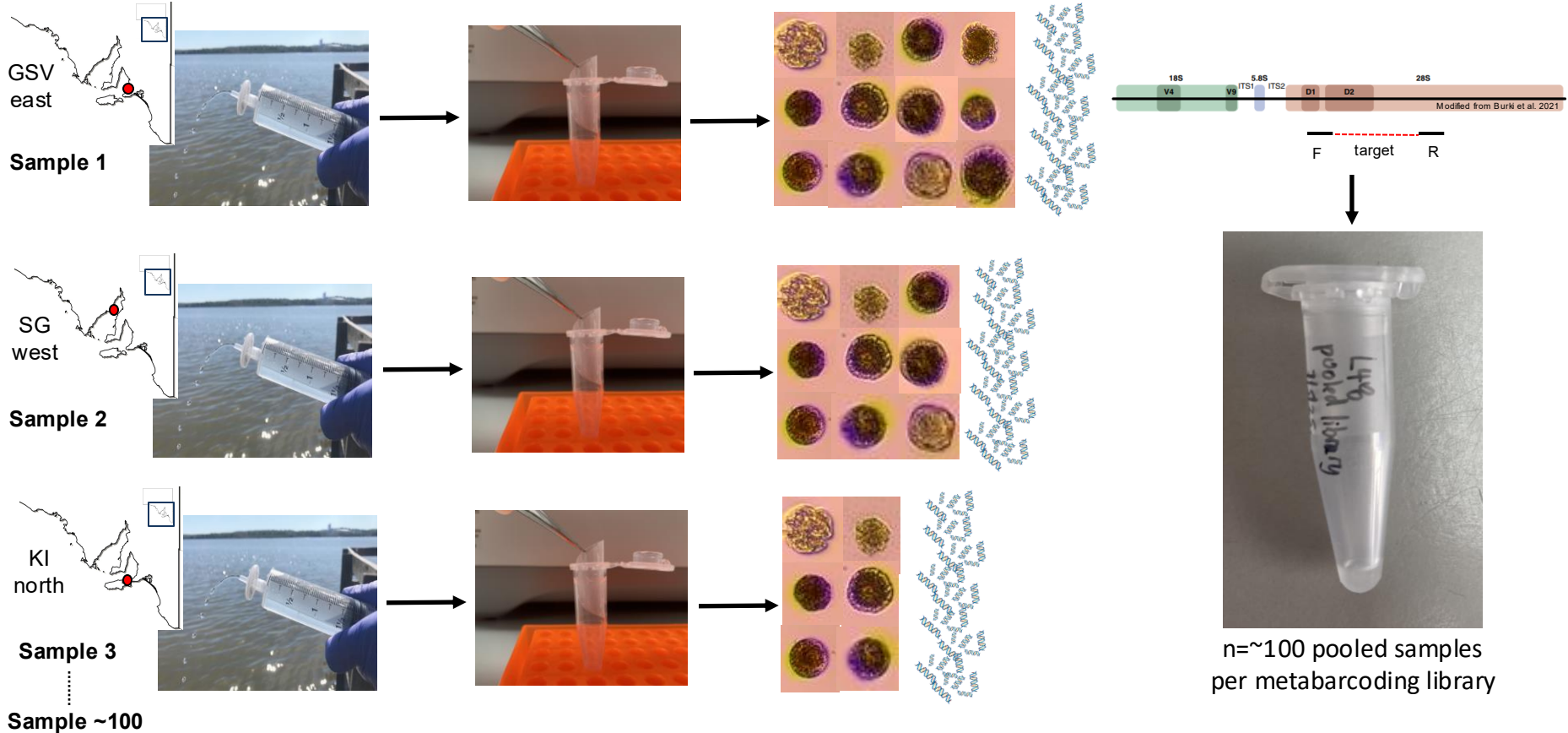
- **DNA BARCODING: Single species identification** – pure cultures (~328bp 28S)

Taxa no.	Species ID	No. of samples
1	Biecheleriopsis adriatica	12
2	Gymnodinium aureolum	7
3	Heterocapsa minima	2
4	Karenia cristata	3
5	Karenia mikimotoi	2
6	Karlodinium veneficum	11
7	Prorocentrum bidens	2
8	Scrippsiella acuminata	8
9	Scrippsiella aff. acuminata	1
10	Takayama helix	10
11	Heterocapsa borneoensis	1
12	Heterocapsa horiguchii	1
13	Pelagodinium bei	3
TOTAL CULTURES BARCODED:		63

Application/output: Confirmed species ID pure cultures used across groups i) Molecular: qPCR rapid diagnostic assays, DNA metabarcoding mock communities and quantification assessments; ii) Oceanography: sensor calibration; iii) Algal: toxin production studies, experimental and mitigation trials.

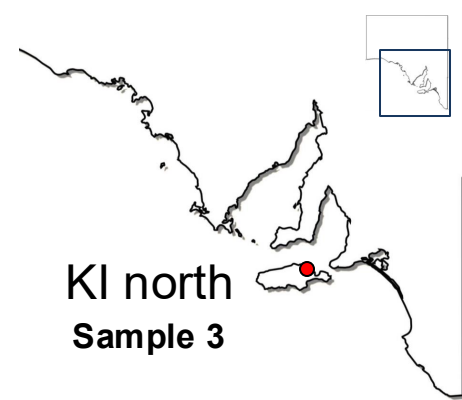
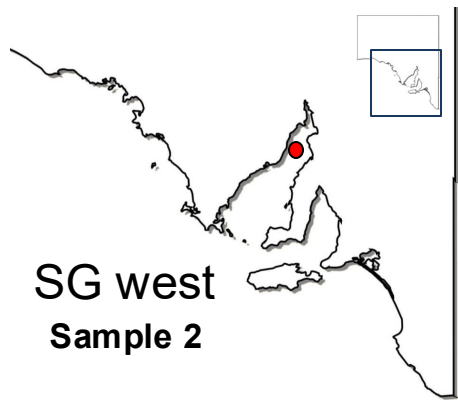
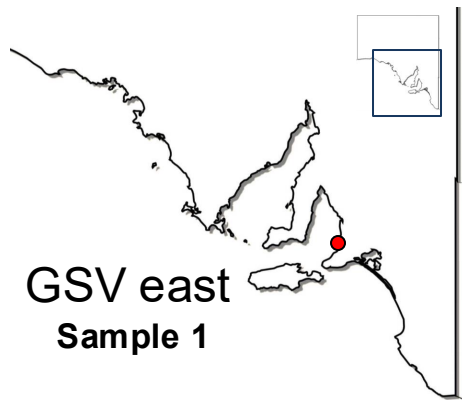
Molecular tools

- **DNA METABARCODING: Community dynamics** – environmental samples, multiplexing



Molecular tools

- DNA METABARCODING: Community dynamics – spatial scale, Sept25



Read number (>10)	Standardised - relative abundance (1-100%)	% identity	Species
24464	87.90513834	100	<i>Karenia cristata</i>
1310	4.707150557	100	<i>Karenia mikimotoi</i>
295	1.060007186	100	<i>Lepidodinium chlorophorum</i>
165		100	<i>Lebouridinium glaucum</i>
160		100	<i>Levanderina fissa_B</i>
147		97.26	<i>Tripos sp. 2</i>
139		100	<i>Azadinium dalianense_B</i>
106		92.38	<i>Karenia</i> sp. 3
82		100	<i>Gymnodinium agaricoides</i>
72		98.47	<i>Gyrodinium rubrum</i>
65		99.69	<i>Gyrodinium dominans</i>
64		100	<i>Biecheleria brevisulcata/B. cincta</i>
63		100	<i>Dinophysis caudata</i>
59		96.96	<i>Azadinium sp. 1</i>
57		99.7	<i>Azadinium anteroporum/A. trinitatum</i>
50		98.48	<i>Karlodinium veneficum_B</i>

Read number (>10)	Standardised - relative abundance (1-100%)	% identity	Species
15964	47.29933928	100	<i>Karenia mikimotoi</i>
1262	3.73914847	100	<i>Noctiluca scintillans</i>
1173	3.475452579	98.47	<i>Gyrodinium rubrum</i>
1136	3.365826198	94.64	<i>Amoebophryidae sp.</i>
1001	2.965838049	100	<i>Biecheleria brevisulcata/B. cincta</i>
959	2.841397292	100	<i>Dissodinium pseudolunula</i>
756	2.239933632	98.48	<i>Karlodinium veneficum_B</i>
699	2.071049747	95.75	<i>Heterocapsa sp.</i>
664	1.967349116	100	<i>Karlodinium elegans</i>
616	1.825131107	100	<i>Oxytoxum lohmannii</i>
567	1.679950224	100	<i>Pelagodinium bei</i>
546	1.617729845	100	<i>Lebouridinium glaucum</i>
512	1.516992089	99.69	<i>Prorocentrum thathymum</i>
503	1.490326213	100	<i>Kapelodiniopsis flava</i>
468	1.386625581	95.43	<i>Paragymnodinium sp. 1</i>

Read number (>10)	Standardised - relative abundance (1-100%)	% identity	Species
3361	14.0404378	100	<i>Warnowia sp. 1</i>
2335	9.754365444	100	<i>Levanderina fissa_B</i>
2087	8.718355752	96.95	<i>Nematodinium sp.</i>
1284	5.36385663	99.7	<i>Gyrodinium shiwhaense_A</i>
1049	4.382153898	100	<i>Lebouridinium glaucum</i>
999	4.173280976	94.64	<i>Amoebophryidae sp.</i>
920	3.84326176	98.48	<i>Karlodinium veneficum_B</i>
856	3.57590442	92.38	<i>Karenia</i> sp. 3
815	3.404628624	100	<i>Biecheleria brevisulcata/B. cincta</i>
738	3.082964325	100	<i>Levanderina fissa_A</i>
701	2.928398362	99.69	<i>Gyrodinium moestrupii</i>
669	2.794719693	99.69	<i>Gyrodinium dominans</i>
631	2.635976272	100	<i>Gymnodinium agaricoides</i>

Molecular tools

- **DNA METABARCODING: Dinoflagellate community dynamics** – environmental samples

DNAmt library	Samples	Application/output
HAB1	Aug25, Sept25	Dinoflagellate community dynamics - spatial and temporal tracking, how community is changing through time and across locations; validation of DNA mtbarcoding with morphology (count data)
HAB2	Oct25, Nov25, Dec25, Jan26	
HAB3	Feb26, Mar26, Apr26	
HAB4	Offshore sampling voyage	<i>Karenia</i> spp. hotspots offshore
HAB5	GAB voyage	Community dynamics in HAB-free (?) region
HAB6	Southern KI voyage	Community dynamics in relatively unsampled area

Molecular tools

- **DNA METABARCODING: Dinoflagellate community dynamics** – environmental samples

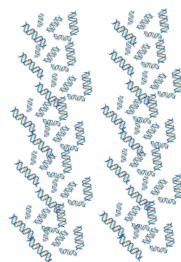
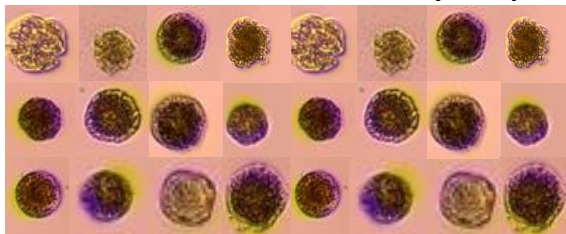
DNAmt library	Samples	Application/output	
HAB1	Aug25, Sept25	Dinoflagellate community dynamics - spatial and temporal tracking, how community is changing through time and across locations; validation of DNA mtbarcoding with morphology (count data)	Data received
HAB2	Oct25, Nov25, Dec25, Jan26		
HAB3	Feb26, Mar26, Apr26		Library being prepared in the lab
HAB4	Offshore sampling voyage	<i>Karenia</i> spp. hotspots offshore	Libraries to be prepared in coming months
HAB5	GAB voyage	Community dynamics in HAB-free (?) region	
HAB6	Southern KI voyage	Community dynamics in relatively unsampled area	

Integrate with oceanographic data (temp, salinity, pH, dissolved oxygen, chlorophyll-a), biogeochemical data, nutrient analysis, etc: associations/drivers

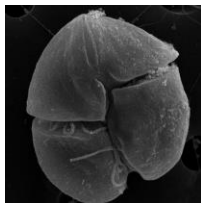
Molecular tools

- **qPCR: Rapid, targeted detection and quantification of a single species** – pure cultures, oceanographic moorings for sensor validation, environmental samples

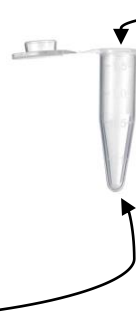
New bloom – mixed community sample



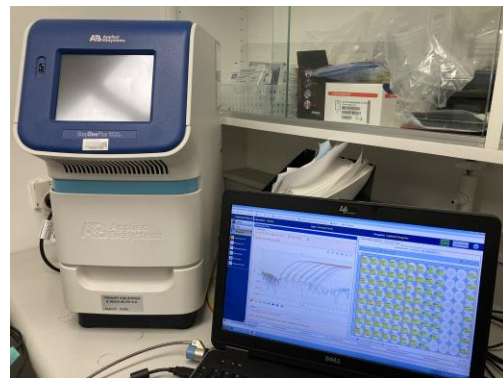
Karenia mikimotoi ??



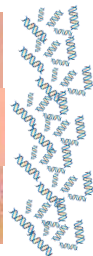
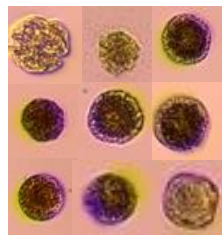
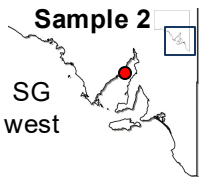
northseabio.whoi.edu – photo by FWCFWR



K. mikimotoi specific
primers & probe
+ polymerase
+ buffers
+ dNTPs
+ dye



Fluorescence intensity directly proportional to amount of amplicon produced – detect AND quantify amount of template DNA (e.g. *K. mikimotoi*) in the sample



Read number (>10)	Standardised - relative abundance (1-100%)	% identity	Species
15964	47.29933928	100	<i>Karenia mikimotoi</i>
1262	3.73914847	100	<i>Noctiluca scintillans</i>
1173	3.475452579	98.47	<i>Gyrodinium rubrum</i>
1136	3.365826198	94.64	<i>Amoebophryidae</i> sp.
1001	2.965838049	100	<i>Biecheleria brevisulcata/B. cincta</i>
959	2.841397292	100	<i>Dissodinium pseudolunula</i>
756	2.239933632	98.48	<i>Karlodinium veneficum_B</i>
699	2.071049747	95.75	<i>Heterocapsa</i> sp.
664	1.967349116	100	<i>Karlodinium elegans</i>
616	1.825131107	100	<i>Oxytoxum lohmannii</i>
567	1.679950224	100	<i>Pelagodinium bei</i>
546	1.617729845	100	<i>Lebouridinium glaucum</i>
512	1.516992089	99.69	<i>Prorocentrum rhathymum</i>
503	1.490326213	100	<i>Kapelodiniopsis flava</i>
468	1.386625581	95.43	<i>Paragymnodinium</i> sp. 1

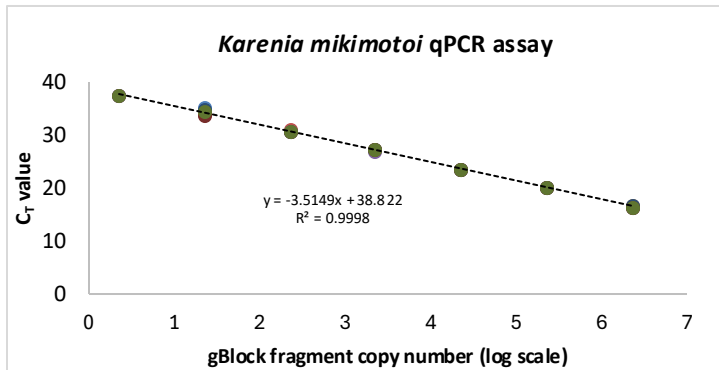
Quantification of
read no.



Molecular tools

- **qPCR: Rapid, targeted detection and quantification of a single species** – pure cultures, oceanographic moorings for sensor validation, environmental samples

Assessing published qPCR assays (n=8) implementing into MSL workflow + designing new assay (Molecular Diagnostic Laboratory)

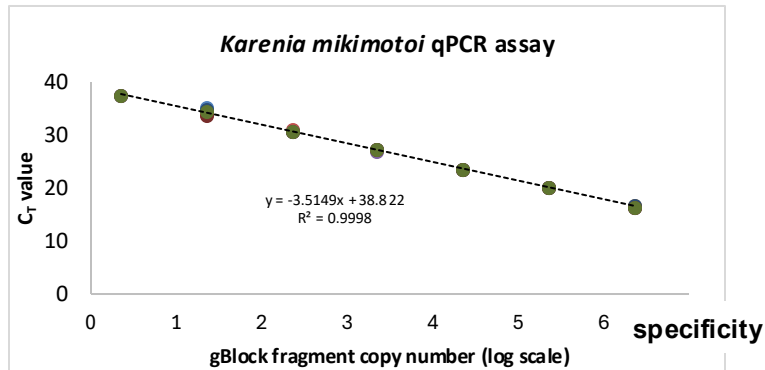


	Copies (uL-1)	detections	CT (mean +/- SE)	%CV	Δ CT (slope)
sensitivity	2,300,000	8/8	16.42 $\pm$ 0.01	0.22	
	230,000	8/8	19.97 $\pm$ 0.01	0.17	-3.55
	23,000	8/8	23.49 $\pm$ 0.01	0.10	-3.52
	2,300	8/8	27.04 $\pm$ 0.02	0.17	-3.55
	230	8/8	30.54 $\pm$ 0.05	0.47	-3.49
	23	8/8	34.20 $\pm$ 0.18	1.53	-3.67
	2.3	4/8	37.01 $\pm$ 0.03	0.14	-3.18
	0	0/8	ND		

Molecular tools

- **qPCR: Rapid, targeted detection and quantification of a single species** – pure cultures, oceanographic moorings for sensor validation, environmental samples

Assessing published qPCR assays (n=8) implementing into MSL workflow + designing new assay (Molecular Diagnostic Laboratory)

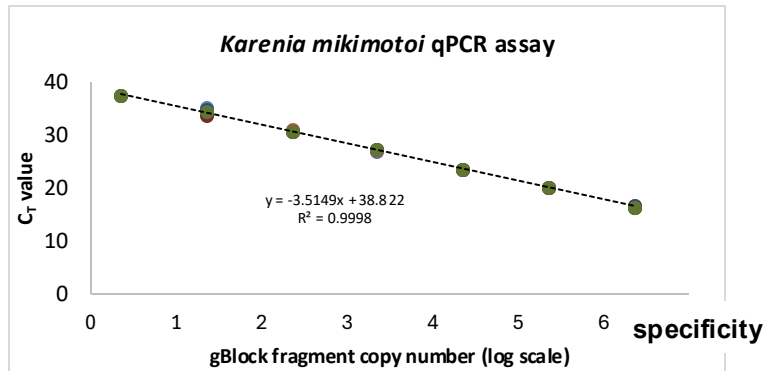


	Copies (uL-1)	detections	CT (mean +/- SE)	%CV	ΔCT (slope)
sensitivity	2,300,000	8/8	16.42 ± 0.01	0.22	
	230,000	8/8	19.97 ± 0.01	0.17	-3.55
	23,000	8/8	23.49 ± 0.01	0.10	-3.52
	2,300	8/8	27.04 ± 0.02	0.17	-3.55
	230	8/8	30.54 ± 0.05	0.47	-3.49
	23	8/8	34.20 ± 0.18	1.53	-3.67
	2.3	4/8	37.01 ± 0.03	0.14	-3.18
	0	0/8	ND		
<i>Karenia mikimotoi</i> (HAB187)		3/3	26.72 ± 0.08		
<i>Karenia cristata</i> (HAB186)		0/3	ND	ND	ND
<i>Karenia</i> sp. (HAB218)		0/3	ND	ND	ND
<i>Karenia</i> sp. (HAB222)		0/3	ND	ND	ND

Molecular tools

- **qPCR: Rapid, targeted detection and quantification of a single species** – pure cultures, oceanographic moorings for sensor validation, environmental samples

Assessing published qPCR assays (n=8) implementing into MSL workflow + designing new assay (Molecular Diagnostic Laboratory)



	Copies (uL-1)	detections	CT (mean +/- SE)	%CV	ΔCT (slope)
sensitivity	2,300,000	8/8	16.42 ± 0.01	0.22	
	230,000	8/8	19.97 ± 0.01	0.17	-3.55
	23,000	8/8	23.49 ± 0.01	0.10	-3.52
	2,300	8/8	27.04 ± 0.02	0.17	-3.55
	230	8/8	30.54 ± 0.05	0.47	-3.49
	23	8/8	34.20 ± 0.18	1.53	-3.67
	2.3	4/8	37.01 ± 0.03	0.14	-3.18
	0	0/8	ND		
	<i>Karenia mikimotoi</i> (HAB187)	3/3	26.72 ± 0.08		
	<i>Karenia cristata</i> (HAB186)	0/3	ND	ND	ND
	<i>Karenia</i> sp. (HAB218)	0/3	ND	ND	ND
	<i>Karenia</i> sp. (HAB222)	0/3	ND	ND	ND

Application/output: Rapid assay, quantification and confirmation of species identity for key taxa of interest from mixed community sample; early detection - early response; pure cultures to further quantify and assess relationship between copies/uL and Ct values to cells/uL and explore quantification factor in DNA metabarcoding

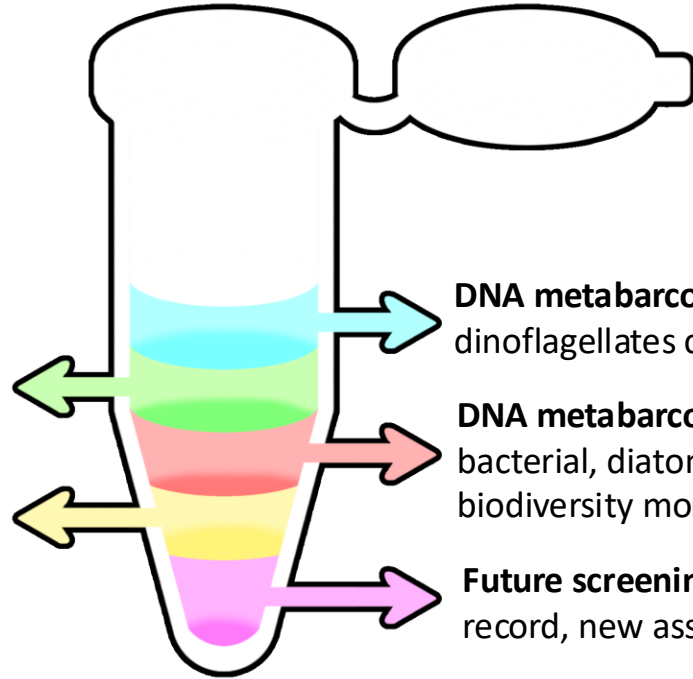
Importance of on-going sample collections

MOLECULAR TOOL KIT



qPCR – *Karenia cristata*, *K. mikimotoi*, *K. brevis*, *K. selliformis*

ONT MinION sequencing –
long reads ~3000bp



DNA metabarcoding, 28S –
dinoflagellates community dynamics

DNA metabarcoding, 16S, rbcL, etc –
bacterial, diatom, ecosystem
biodiversity monitoring

Future screening – archived, historical
record, new assays later