CHEMTAX: developments in interpretation of pigment field data

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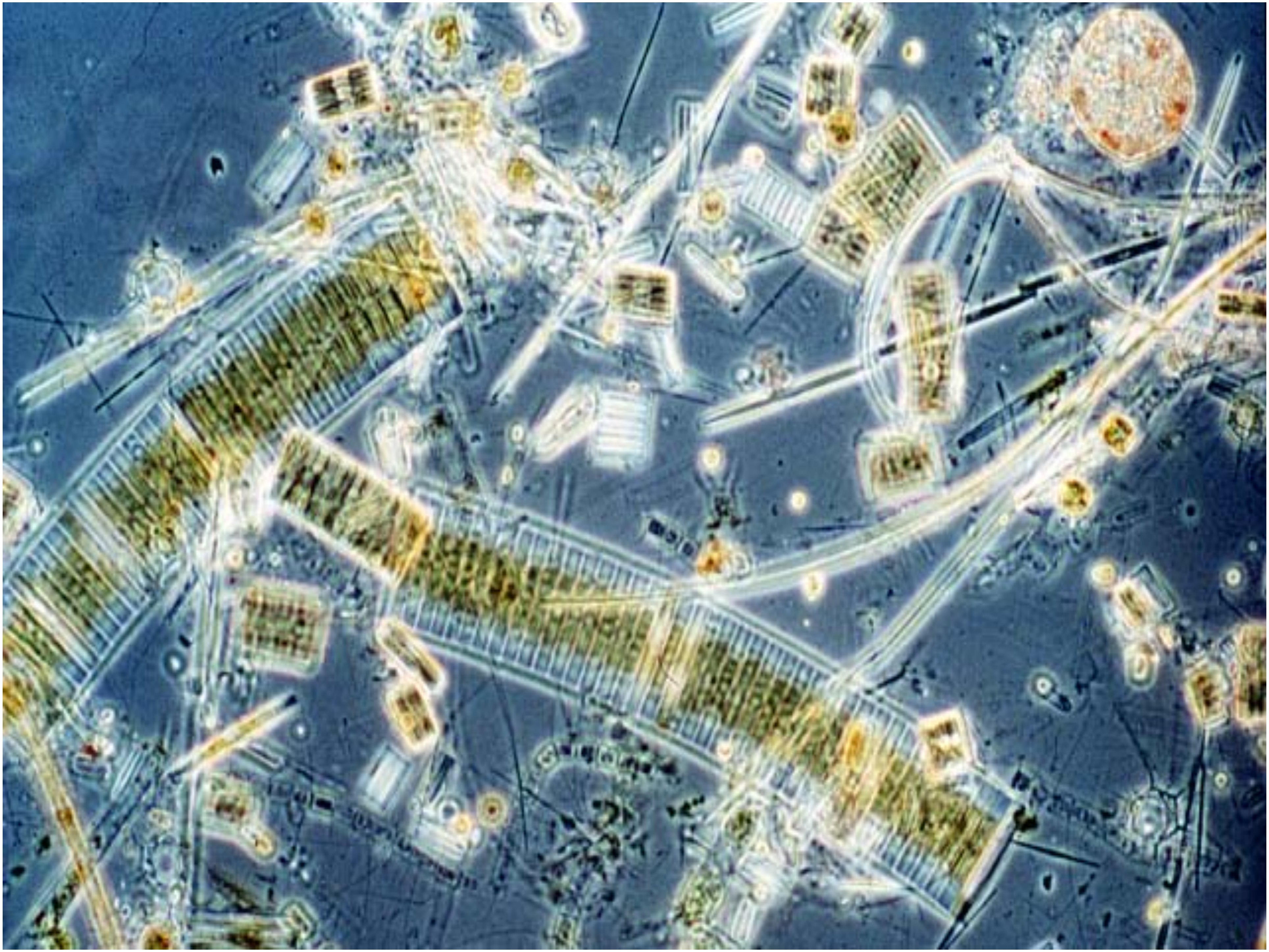
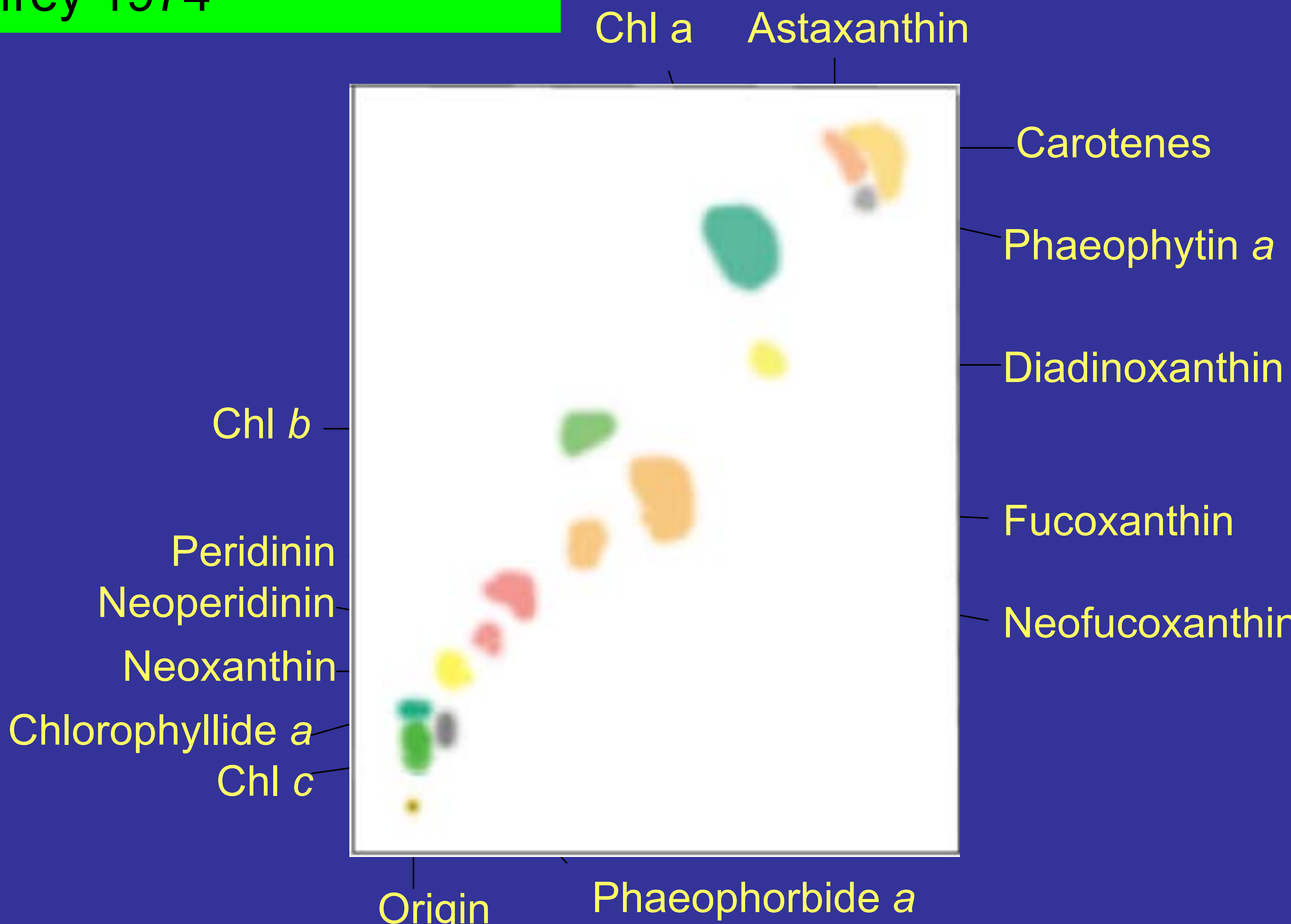
COOPERATIVE RESEARCH CENTRE



Interpretation of pigment field data

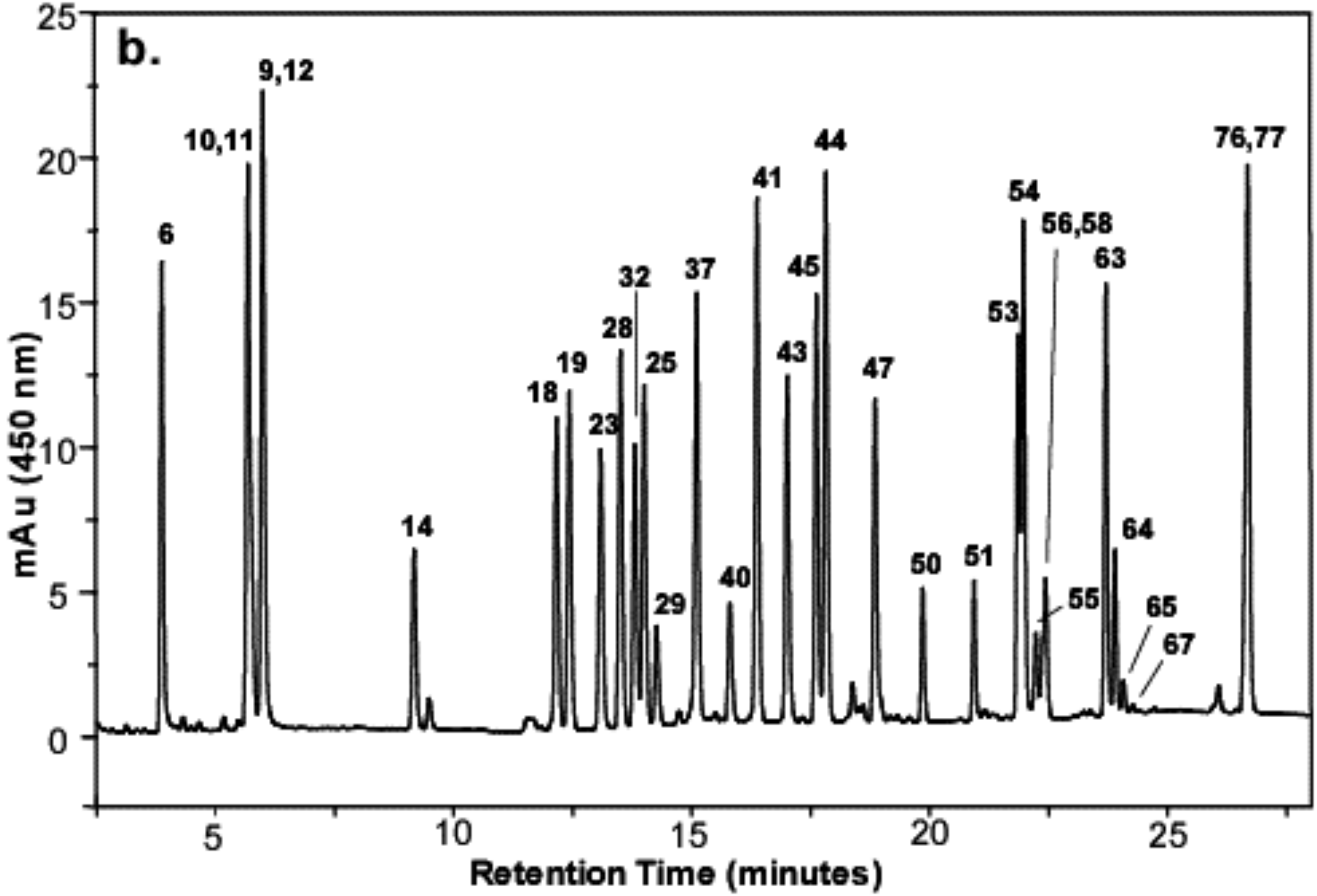
- Overview
- Developments
  - Quantitative interpretation chapter
  - Development of software
- Application

Thin layer chromatography
Jeffrey 1974



Jeffrey 1974

Pigments	Algal types or biological processes indicated
Chl *a* Chl *c* Fucoxanthin Diadinoxanthin	Diatoms and / or chrysomonads
Chl *b* Neoxanthin	Green algae
Peridinin	Dinoflagellates
Chlorophyllide *a*	Senescent diatoms (due to chlorophyllase)
Phaeophorbide *a*	Faecal pellets of copepods
Phaeophytin *a*	Us. Trace amounts on all c'grams
Astaxanthin	Copepods present
High chl *c:a* ratios	Senescent phytoplankton or detritus



HPLC analysis of pigments

HPLC data

Sample	Depth (m)	CTD NO.	Lat	Long	chl c3	peri	but	fuc	hex	pras	violax	ddx	allox	lutein	zea
98MAR151	15.8	041	48.22445	141.4017	0.031392	0.027687	0.013293	0.072922	0.064616	0.00591	0.004732	0.035452	0.00051	0.003593	0.003148
98MAR152	31.6	041	48.22445	141.4017	0.035344	0.027601	0.013483	0.071824	0.065545	0.006021	0.005369	0.037461	0.000958	0.003663	0.003272
98MAR153	44.4	041	48.22445	141.4017	0.02762	0.027792	0.012789	0.070482	0.06295	0.005781	0.003962	0.035773	0.001032	0.003611	0.002886
98MAR154	60.7	041	48.22445	141.4017	0.034245	0.028429	0.013522	0.073983	0.066931	0.006996	0.004527	0.037545	0.000895	0.003833	0.003409
98MAR155	87.5	041	48.22445	141.4017	0.031448	0.026573	0.013022	0.070428	0.062184	0.005939	0.004872	0.033211	0.000988	0.003731	0.003192
98MAR156	119.4	041	48.22445	141.4017	0.00206	0.001961	0.000882	0.005445	0.002631	0.000595	0.000181	0.000839	0.000285	0	0.000123
98MAR157	8.3	043	49.5136	141.7885	0.032184	0.031934	0.0139	0.064827	0.073772	0.006293	0.005072	0.032724	0.000727	0.00307	0.002944
98MAR158	17.6	043	49.5136	141.7885	0.02864	0.02996	0.01272	0.056662	0.065716	0.005188	0.003753	0.030954	0.001069	0.002544	0.002254
98MAR159	31.7	043	49.5136	141.7885	0.035289	0.032554	0.013662	0.062159	0.071614	0.006577	0.00505	0.032701	0.001156	0.002741	0.002603
98MAR160	49.2	043	49.5136	141.7885	0.034737	0.03299	0.013943	0.063439	0.072411	0.006559	0.00429	0.033522	0.000982	0.00305	0.002413
98MAR161	62.3	043	49.5136	141.7885	0.027936	0.033016	0.013617	0.063596	0.072187	0.006351	0.005184	0.032443	0.000334	0.002721	0.002536
98MAR162	74.4	043	49.5136	141.7885	0.029441	0.035429	0.014008	0.064851	0.073278	0.006214	0.00395	0.033054	0.000719	0.002975	0.002646
98MAR163	89.8	043	49.5136	141.7885	0.037024	0.032282	0.013194	0.061935	0.068627	0.005887	0.004741	0.030555	0.001009	0.002916	0.002022
98MAR164	104.4	043	49.5136	141.7885	0.008651	0.003389	0.004103	0.029902	0.008678	0.002161	0.000701	0.003551	0	0	0.000497
98MAR165	120.6	043	49.5136	141.7885	0.001619	0.002375	0.001465	0.007307	0.003128	0.000336	0.000449	0.001023	0.000225	0	0.000123
98MAR166	152.4	043	49.5136	141.7885	0.001276	0.002911	0.000611	0.003189	0.001495	0.000518	0	0.000829	0	0.000311	0.000127
98MAR167	205.8	043	49.5136	141.7885	0.000909	0.001968	0.000797	0.006559	0.00127	0	0	0.001219	0.000147	0	0.000329
98MAR168	250.3	043	49.5136	141.7885	0	0	0.000406	0.006389	0.001237	0	0	0	0	0	0
98MAR169	5.8	045	49.60053	141.8961	0.032677	0.040781	0.014809	0.064245	0.07578	0.007188	0.003831	0.047372	0.001394	0.003732	0.002638
98MAR170	18	045	49.60053	141.8961	0.029472	0.034098	0.01317	0.051162	0.071568	0.006875	0.0044	0.040767	0.001243	0.003521	0.002771
98MAR171	30.1	045	49.60053	141.8961	0.035932	0.037349	0.013961	0.062603	0.071785	0.006484	0.005567	0.04741	0.001365	0.003752	0.003092
98MAR172	45.8	045	49.60053	141.8961	0.035648	0.039172	0.014342	0.064738	0.073931	0.005836	0.004241	0.042785	0.000873	0.00382	0.002883
98MAR173	61.9	045	49.60053	141.8961	0.034915	0.038766	0.014196	0.056765	0.076588	0.006384	0.005122	0.033499	0.001295	0.003789	0.003005
98MAR174	76.1	045	49.60053	141.8961	0.038247	0.048277	0.016592	0.068778	0.089713	0.007747	0.006305	0.03503	0.001201	0.003451	0.002712
98MAR175	92.5	045	49.60053	141.8961	0.043461	0.04537	0.016037	0.065489	0.087526	0.0079	0.005406	0.033986	0.001375	0.003765	0.003048
98MAR176	102.2	045	49.60053	141.8961	0.044594	0.047667	0.017678	0.069405	0.089403	0.007358	0.005895	0.033466	0.001255	0.004339	0.003852
98MAR177	104.8	045	49.60053	141.8961	0.039747	0.039694	0.014335	0.057668	0.076858	0.006633	0.003966	0.027412	0.001107	0.00313	0.002533
98MAR178	119	045	49.60053	141.8961	0.006998	0.004086	0.003308	0.022623	0.009092	0.001623	0.00065	0.003255	0.000228	0.000163	0.000389
98MAR179	148.8	045	49.60053	141.8961	0.000827	0.001529	0.00052	0.004228	0.001453	0.000763	0.000283	0.000788	0	0	9.45E-05
98MAR180	6.5	046	50.5319	141.7861	0.036056	0.022301	0.012625	0.11543	0.057005	0.005664	0.003268	0.038251	0.001917	0.001998	0.001217
98MAR181	29.4	046	50.5319	141.7861	0.035386	0.021518	0.012865	0.110655	0.056887	0.007413	0.004481	0.039939	0.001911	0.003303	0.002029
98MAR182	44.7	046	50.5319	141.7861	0.033412	0.020327	0.012383	0.107556	0.055265	0.005418	0.002989	0.039279	0.002011	0.002753	0.001904
98MAR183	60.7	046	50.5319	141.7861	0.030896	0.018553	0.011819	0.105353	0.054373	0.005287	0.003228	0.032453	0.001792	0.002113	0.001428
98MAR184	93	046	50.5319	141.7861	0.028317	0.020547	0.011769	0.081506	0.05888	0.007084	0.003525	0.025059	0.000992	0.002663	0.002216

Major marker pigments

Pigment	IUPAC	Cyanophyta (cyanobacteria)[11]	Prochlorophyta	Rhodophyta	Cryptophyta	Chlorophyceae[14]	Prasinophyceae[7, 8, 9]	Euglenophyta[13]	Eustigmatophyta	Bacillariophyta	Dinophyta[1]	Prymnesiophyceae[5]	Chrysophyceae[6]	Raphidophyceae
Chlorophylls														
a		●	15	●	●	●	●	●	●	●	●	●	●	●
b			15			●	●	●			1			
c_1										3 ●	1	5 ●		●
c_2					●					●	●	●	●	●
c_3										3		5 ●	●	
Phytylated chlorophyll *c*-like												5 •		
MgDVP			•				●							
Divinyl chlorophyll *a*			●											
Divinyl chlorophyll *b*			●											
Carotenes														
Old terminology	IUPAC													
α	β,ε		●	●	●	•	•					●		
β	β,β	●	●			●	●	●	●	•	•	•	•	●
γ	β,ψ					•								
ε	ε,ε				•								•	
Lycopene	ψ,ψ	11			•		2							
Xanthophylls														
Alloxanthin					●									
Antheraxanthin						•	•	•						
Astaxanthin						2	2							
19'-Butanoyloxyfucoxanthin											1	5 ●	●	
Canthaxanthin		11				2								
Crocoxanthin					•									
Diadinoxanthin								●		●	●	●	●	●
Diatoxanthin								•		•	•	•	•	•
Dinoxanthin											●			
Echinenone		11				2	2	13						
Fucoxanthin										●	1	5 ●	●	●
19'-Hexanoyloxyfucoxanthin											1	5 ●		
Lutein						●	●							
Monadoxanthin					•									
Neoxanthin						●	●	•						
P-457 + P-468 [4]											•			
Peridinin											●			
Peridininol											•			
Prasinoxanthin							7 ●							
Pyrrhoxanthin											•			
Siphonaxanthin						14	14							
Siphonein						14	14	14						
Vaucheriaxanthin ester									●					
Violaxanthin						●	●		●					
Zeaxanthin		●	●	●		●			•					
Biliproteins														
Allophycocyanins		12 ●		12 ●										
Phycocyanins		12 ●		12 •	10 ●									
Phycoerythrins		12 ●		12 ●	10 ●									

Jeffrey and Vesk (1997)

Major marker pigments

Ubiquitous

Chl *a*

Pigment		Cyanophyta (cyanobacteria)[11]	Prochlorophyta	Rhodophyta	Cryptophyta	Chlorophyceae[14]	Prasinophyceae[7, 8, 9]	Euglenophyta[13]	Eustigmatophyta	Bacillariophyta	Dinophyta[1]	Prymnesiophyceae[5]	Chrysophyceae[6]	Raphidophyceae
Chlorophylls														
a		●	15	●	●	●	●	●	●	●	●	●	●	●
b			15			●	●	●			1			
c_1										3 ●	1	5 ●		●
c_2					●					●	●	●	●	●
c_3										3		5 ●	●	
Phytylated chlorophyll *c*-like												5 •		
MgDVP			•				●							
Divinyl chlorophyll *a*			●											
Divinyl chlorophyll *b*			●											
Carotenes														
Old terminology	IUPAC													
α	β,ε		●	●	●	•	•					•		
β	β,β	●	●			●	●	●	●	•	•	•	•	●
γ	β,ψ					•								
ε	ε,ε				•								•	
Lycopene	ψ,ψ	11			•		2							
Xanthophylls														
Alloxanthin					●									
Antheraxanthin						•	•	•						
Astaxanthin						2	2							
19'-Butanoyloxyfucoxanthin											1	5 ●	●	
Canthaxanthin		11				2								
Crocoxanthin					•									
Diadinoxanthin								●		●	●	●	●	●
Diatoxanthin								•		•	•	•	•	•
Dinoxanthin											●			
Echinenone		11				2	2	13						
Fucoxanthin										●	1	5 ●	●	●
19'-Hexanoyloxyfucoxanthin											1	5 ●		
Lutein						●	•							
Monadoxanthin					•									
Neoxanthin						●	●	•						
P-457 + P-468[4]											•			
Peridinin											●			
Peridininol											•			
Prasinoxanthin							7 ●							
Pyrrhoxanthin											•			
Siphonaxanthin						14	14							
Siphonein						14	14	14						
Vaucheriaxanthin ester									●					
Violaxanthin						●	●		●					
Zeaxanthin		●	●	●		●			•					
Biliproteins														
Allophycocyanins		12 ●		12 ●										
Phycocyanins		12 ●		12 •	10 ●									
Phycoerythrins		12 ●		12 ●	10 ●									

Major marker pigments

Ubiquitous

Chl *a*

Unambiguous

Alloxanthin

Peridinin

Prasinoxanthin

Pigment		Cyanophyta (cyanobacteria)[11]	Prochlorophyta	Rhodophyta	Cryptophyta	Chlorophyceae[14]	Prasinophyceae[7, 8, 9]	Euglenophyta[13]	Eustigmatophyta	Bacillariophyta	Dinophyta[1]	Prymnesiophyceae[5]	Chrysophyceae[6]	Raphidophyceae
Chlorophylls														
a		●	15	●	●	●	●	●	●	●	●	●	●	●
b			15			●	●	●			1			
c_1										3 ●	1	5 ●		●
c_2					●					●	●	●	●	●
c_3										3		5 ●	●	
Phytylated chlorophyll *c*-like												5 •		
MgDVP			•				●							
Divinyl chlorophyll *a*			●											
Divinyl chlorophyll *b*			●											
Carotenes														
Old terminology	IUPAC													
α	β,ε		●	●	●	•	•					•		
β	β,β	●	●			●	●	●	●	•	•	•	•	●
γ	β,ψ					•								
ε	ε,ε				•								•	
Lycopene	ψ,ψ	11			•		2							
Xanthophylls														
Alloxanthin					●									
Antheraxanthin						•	•	•						
Astaxanthin						2	2							
19'-Butanoyloxyfucoxanthin											1	5 ●	●	
Canthaxanthin		11				2								
Crocoxanthin					•									
Diadinoxanthin								●		●	●	●	●	●
Diatoxanthin								•		•	•	•	•	•
Dinoxanthin											●			
Echinenone		11				2	2	13						
Fucoxanthin										●	1	5 ●	●	●
19'-Hexanoyloxyfucoxanthin											1	5 ●		
Lutein						●	●							
Monadoxanthin					•									
Neoxanthin						●	●	•						
P-457 + P-468[4]											•			
Peridinin											●			
Peridininol											•			
Prasinoxanthin							7 ●							
Pyrrhoxanthin											•			
Siphonaxanthin						14	14							
Siphonein						14	14	14						
Vaucheriaxanthin ester									●					
Violaxanthin						●	●		●					
Zeaxanthin		●	●	●		●			•					
Biliproteins														
Allophycocyanins		12 ●		12 ●										
Phycocyanins		12 ●		12 •	10 ●									
Phycoerythrins		12 ●		12 ●	10 ●									

Major marker pigments

Ubiquitous
- Chl *a*

Unambiguous
- Alloxanthin
- Peridinin
- Prasinoxanthin

Shared
- Fucoxanthin
- Chl b
- Zeaxanthin
- Violaxanthin

Pigment		Cyanophyta (cyanobacteria)[11]	Prochlorophyta	Rhodophyta	Cryptophyta	Chlorophyceae[14]	Prasinophyceae[7, 8, 9]	Euglenophyta[13]	Eustigmatophyta	Bacillariophyta	Dinophyta[1]	Prymnesiophyceae[5]	Chrysophyceae[6]	Raphidophyceae
Chlorophylls														
a		●	[15]	●	●	●	●	●	●	●	●	●	●	●
b			[15]			●	●	●			[1]			
c_1										[3] ●	[1]	[5] ●		●
c_2					●					●	●	●	●	●
c_3										[3]		[5] ●	●	
Phytylated chlorophyll *c*-like												[5] •		
MgDVP			•				●							
Divinyl chlorophyll *a*			●											
Divinyl chlorophyll *b*			●											
Carotenes														
Old terminology	IUPAC													
α	β,ε		●	●	●	•	•					•		
β	β,β	●	●			●	●	●	●	•	•	•	•	●
γ	β,ψ					•								
ε	ε,ε				•								•	
Lycopene	ψ,ψ	[11]			•		[2]							
Xanthophylls														
Alloxanthin					●									
Antheraxanthin						•	•	•						
Astaxanthin						[2]	[2]							
19'-Butanoyloxyfucoxanthin											[1]	[5] ●	●	
Canthaxanthin		[11]				[2]								
Crocoxanthin					•									
Diadinoxanthin								●		●	●	●	●	●
Diatoxanthin								•		•	•	•	•	•
Dinoxanthin											●			
Echinenone		[11]				[2]	[2]	[13]						
Fucoxanthin										●	[1]	[5] ●	●	●
19'-Hexanoyloxyfucoxanthin											[1]	[5] ●		
Lutein						●	●							
Monadoxanthin					•									
Neoxanthin						●	●	•						
P-457 + P-468 [4]											•			
Peridinin											●			
Peridininol											•			
Prasinoxanthin							[7] ●							
Pyrrhoxanthin											•			
Siphonaxanthin						[14]	[14]							
Siphonein						[14]	[14]	[14]						
Vaucheriaxanthin ester									●					
Violaxanthin						●	●		●					
Zeaxanthin		●	●	●		•			•					
Biliproteins														
Allophycocyanins		[12] ●		[12] ●										
Phycocyanins		[12] ●		[12] •	[10] ●									
Phycoerythrins		[12] ●		[12] ●	[10] ●									

Major marker pigments

Ubiquitous

Chl *a*

Unambiguous

Alloxanthin

Peridinin

Prasinoxanthin

Shared

Fucoxanthin

Chl b

Zeaxanthin

Violaxanthin

"SUITES" of pigments

Pigment	Cyanophyta (cyanobacteria)[11]	Prochlorophyta	Rhodophyta	Cryptophyta	Chlorophyceae[14]	Prasinophyceae[7,8,9]	Euglenophyta[13]	Eustigmatophyta	Bacillariophyta	Dinophyta[1]	Prymnesiophyceae[5]	Chrysophyceae[6]	Raphidophyceae
Chlorophylls													
a	●	15	●	●	●	●	●	●	●	●	●	●	●
b		15			●	●	●			1			
c_1									3 ●	1	5 ●		●
c_2				●					●	●	●	●	●
c_3									3		5 ●	●	
Phytylated chlorophyll *c*-like											5 •		
MgDVP		•				●							
Divinyl chlorophyll *a*		●											
Divinyl chlorophyll *b*		●											
Carotenes													
Old terminology — IUPAC													
α — β,ε		●	●	●	•	•					•		
β — β,β	●	●			●	●	●	●	•	•	•	•	●
γ — β,ψ					•								
ε — ε,ε				•								•	
Lycopene — ψ,ψ	11			•		2							
Xanthophylls													
Alloxanthin				●									
Antheraxanthin					•	•	•						
Astaxanthin					2	2							
19'-Butanoyloxyfucoxanthin										1	5 ●	●	
Canthaxanthin	11				2								
Crocoxanthin				•									
Diadinoxanthin							●		●	●	●	●	●
Diatoxanthin							•		•	•	•	•	•
Dinoxanthin										●			
Echinenone	11				2	2	13						
Fucoxanthin									●	1	5 ●	●	●
19'-Hexanoyloxyfucoxanthin										1	5 ●		
Lutein					●	•							
Monadoxanthin				•									
Neoxanthin					●	●	•						
P-457 + P-468 [4]										•			
Peridinin										●			
Peridininol										•			
Prasinoxanthin						7 ●							
Pyrrhoxanthin										•			
Siphonaxanthin					14	14							
Siphonein					14	14	14						
Vaucheriaxanthin ester								●					
Violaxanthin					●	●		●					
Zeaxanthin	●	●	●		•			•					
Biliproteins													
Allophycocyanins	12 ●		12 ●										
Phycocyanins	12 ●		12 •	10 ●									
Phycoerythrins	12 ●		12 ●	10 ●									

CHEMTAX

HPLC water sample with N pigment concentrations y_i

Abundance β_j of each of M plankton species is solved by least squares

$$\min \left\| y_i - A_{ij} B_j \right\|^2, i = 1 \ldots N, j = 1 \ldots M$$

where matrix A_{ij} provides the relative proportions of pigment i in species j . The ratios are uncertain and must be determined from the data set. They may vary (data is then divided into homogenous sub-sets).

Optimised iteratively

Pigment concentrations are normalized for each sample:

$$ny_i = \frac{y_i}{\sum_{i=1}^{N} y_i}$$

CHEMTAX pigment ratios

a. Initial Ratio matrix

Class	Chl c_3	Chl c_1	Peri	Fuco	Neo	Pras	Viola	19'-Hex	Allox	Lutein	Chl *b*
Prasinophytes[1]	0	0	0	0	0.03	0.097	0.056	0	0	0.006	0.62
Chlorophytes[2]	0	0	0	0	0.062	0	0.031	0	0	0.22	0.18
Cryptophytes[3]	0	0	0	0	0	0	0	0	0.22	0	0
Diatoms-A[4]	0	0.04	0	0.52	0	0	0	0	0	0	0
Diatoms-B[5]	0.033	0	0	0.61	0	0	0	0	0	0	0
Dinoflagellates-A[6]	0	0	1.06	0	0	0	0	0	0	0	0
Haptophytes-H[7]	0.13	0	0	0.08	0	0	0	0.4	0	0	0
Haptophytes-L [7]	0.27	0	0	0.01	0	0	0	1.1	0	0	0

b. Final Ratio Matrix

Class	Chl c_3	Chl c_1	Peri	Fuco	Neo	Pras	Viola	19'-Hex	Allox	Lutein	Chl *b*
Prasinophytes	0	0	0	0	0.07	0.09	0.049	0	0	0.007	0.55
Chlorophytes	0	0	0	0	0.071	0	0.032	0	0	0.23	0.15
Cryptophytes	0	0	0	0	0	0	0	0	0.21	0	0
Diatoms-A	0	0.21	0	1.04	0	0	0	0	0	0	0
Diatoms-B	0.016	0	0	0.83	0	0	0	0	0	0	0
Dinoflagellates-A	0	0	0.82	0	0	0	0	0	0	0	0
Haptophytes-H	0.34	0	0	0.13	0	0	0	0.43	0	0	0
Haptophytes-L	0.13	0	0	0.01	0	0	0	1.21	0	0	0

[1]*Mantoniella* sp. (Latasa *et al*. 2004), [2]*Chlorella* sp. (Schlüter *et al*., 2000), [3]*Chroomonas salina* (Jeffrey and Wright 1997), [4]*Phaeodactylum tricornutum* (Wright, unpublished), [5]*Pseudonitzschia heimii* (Wright, unpublished), [6]*Amphidinium carterae* (Jeffrey and Wright 1997), [7]*Phaeocystis antarctica*, high and low Fe forms (DiTullio *et al*. 2007)

Developments: 1

QUANTITATIVE INTERPRETATION OF CHEMOTAXONOMIC PIGMENT DATA

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[2]Australian Antarctic Division and Antarctic Climate and Ecosystems CRC, Channel Hwy, Kingston, Tasmania, 7050, Australia

[3]DHI Water & Environment, Agern Allé 5, DK-2970 Hørsholm, Denmark

CHAPTER 5.

QUANTITATIVE INTERPRETATION OF CHEMOTAXONOMIC PIGMENT DATA
CHAPTER 5

- **5.1 Introduction**
- **5.2 Qualitative assessment of data**
  - **5.2.1 Specific markers for algal types**
- **5.3 Non-taxonomic interpretation of pigment data sets**
  - *a. Pigment based size classes*

Σ DPw = 1.41[Fuco] + 1:41[Peri] + 1:27[Hex-fuco] + 0:35[But-fuco] + 0:60[Allo] + 1:01[TChl*b*] + 0:86[Zea]

fmicro = (1.41[Fuco] + 1:41[Perid]) / Σ DPw

fnano = (1:27[Hex-fuco] + 0:35[But-fuco] + 0:60[Allo]) / Σ DPw

fpico = (1:01[TChl*b*] + 0:86[Zea]) / Σ DPw

QUANTITATIVE INTERPRETATION OF CHEMOTAXONOMIC PIGMENT DATA
CHAPTER 5.

- **5.1 Introduction**
- **5.2 Qualitative assessment of data**
  - **5.2.1 Specific markers for algal types**
- **5.3 Non-taxonomic interpretation of pigment data sets**
  - *a. Pigment based size classes*
  - *b. Ecological similarity indices*
- **5.4 Mathematical tools for taxonomic interpretation of pigment data sets**
  - *a. Multiple linear regression*
  - *b. Inverse simultaneous equations*
  - *c. Excel Solver*
  - *d. CHEMTAX software*
  - *e. Bayesian method*

- **5.4.3 Guide for quantitative chemotaxonomic interpretation of pigment data**
  - Step by step guide:
    - Examine the pigment data for specific markers for algal types (Section 5.2.1)
    - Examine available complementary data
      - *Microscopy data*:
      - *Flow cytometry and FlowCAM data*:
      - *In situ and in vivo fluorometry data*: *in situ* fluorescence profiles
      - *Environmental data*:
      - *Remote sensing data*:
      - *Productivity and grazing data*:
      - *Cluster analysis*
    - Pigment data exploration:
      - *Multiple linear regression (MLR)*
      - *Testing correlation*
      - *diatoxanthin + diadinoxanthin:Chl a*
    - CHEMTAX analysis
      - *Sub-grouping*
      - *Initial pigment:Chl a ratio and ratio limit matrices*
      - *Preliminary CHEMTAX analysis*
      - *Comprehensive CHEMTAX analysis*
      - *Publication of CHEMTAX (or ISE) estimates*

QUANTITATIVE INTERPRETATION OF CHEMOTAXONOMIC PIGMENT DATA

CHAPTER 5.

- **5.1 Introduction**
- **5.2 Qualitative assessment of data**
  - **5.2.1 Specific markers for algal types**
- **5.3 Non-taxonomic interpretation of pigment data sets**
- **5.4 Mathematical tools for taxonomic interpretation of pigment data sets**
  - **5.4.1 Assumptions and constraints of inverse simultaneous equations and CHEMTAX**
  - **5.4.2 Reaching the optimum solution**
  - **5.4.3 Guide for quantitative chemotaxonomic interpretation of pigment data**
- **5.5 Variability of Marker Pigment:Chl *a* and from cultures and field studies**
  - **5.5.1 Pigment:Chl a ratios in culture vs. field**
  - **5.5.2 Irradiance**

2m. Haptophytes Type 6–8

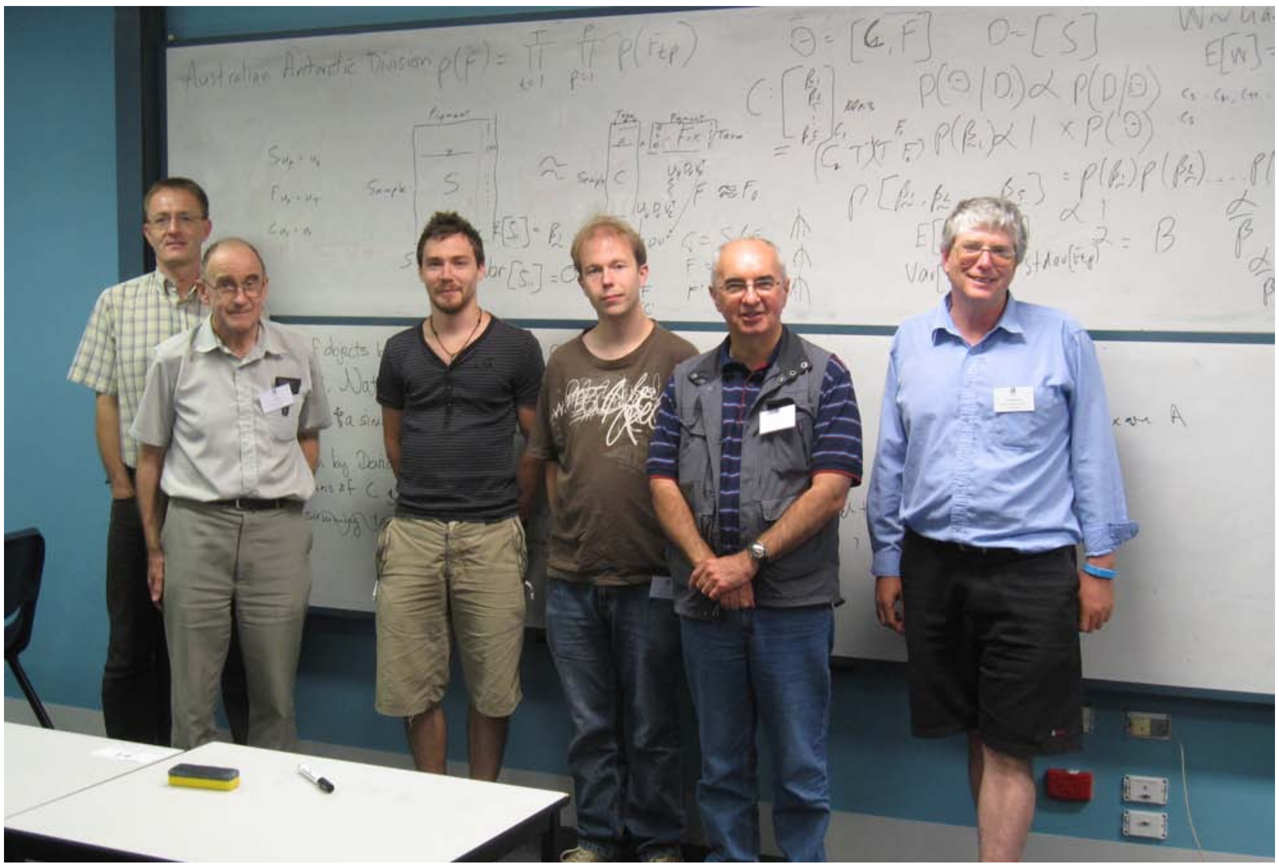
Type	Data	n	chl c3	MV-chl c3	Mg-DVP	chl c2	chl c1	BUT	FUCO	4k-HEX	HEX	DIAD	DIAT	βε-CAR	ββ-CAR	c2-MGDG [14/18]	c2-MGDG [14/14]
HAPTO-6																	
Culture	LL	4	0.229		0.018	0.341			0.340	0.125	0.234	0.094	0.011	0.013	0.023	0.099	
"	ML	21	0.176	0.016	0.007	0.192		0.005	0.263	0.149	0.562	0.127	0.036		0.025	0.089	
"	HL	6	0.130	0.042	0.022	0.290			0.072	0.029	0.248	0.102	0.051		0.020	0.043	
"	Min	34	0.110	0.005	0.002	0.145	0	0	0.006	0.029	0.037	0.026	0.004	0.013	0.008	0.043	0
"	Max	34	0.229	0.050	0.022	0.341	0	0.008	0.722	0.313	1.507	0.316	0.169	0.013	0.049	0.099	0
"	**Mean**	**34**	**0.177**	**0.018**	**0.009**	**0.209**		**0.005**	**0.229**	**0.137**	**0.470**	**0.137**	**0.047**	**0.013**	**0.026**	**0.087**	
"	Std Dev	34	0.031	0.015	0.006	0.066		0.004	0.186	0.082	0.344	0.094	0.054		0.015	0.014	
Field	LL	14	0.150			0.171		0.016	0.224		1.342	0.099				0.097	
"	ML	15	0.132			0.180	0	0.015	0.186		1.138	0.100		0.007	0.013		
"	HL	42	0.155	0.119	0	0.157	0	0.019	0.195		1.054	0.122		0.014	0.030	0.090	0
"	Min	86	0.033	0.119	0	0.080	0	0	0	0	0.228	0.015	0	0.006	0.012	0.082	0
"	Max	86	0.300	0.119	0	0.230	0	0.198	0.722	0	2.066	0.196	0	0.014	0.030	0.097	0
"	**Mean**	**86**	**0.146**	**0.119**	**0**	**0.168**	**0**	**0.015**	**0.195**		**1.214**	**0.109**		**0.010**	**0.021**	**0.091**	**0**
"	Std Dev	86	0.087			0.048	0	0.028	0.172		0.595	0.046		0.004	0.010	0.008	
References (see Table p): 13, 15, 16, 18, 19, 21–24, 26–29, 31–35, 37, 40, 42–45, 47, 61, 63–65																	
HAPTO-7																	
Culture	LL	7	0.215	0.017	0.010	0.227	0.009	0.023	0.420	0.100	0.682	0.181	0.005	0.025	0.030	0.091	0.110
"	ML	15	0.210	0.028	0.015	0.198	0.009	0.009	0.436	0.215	0.543	0.267	0.015	0.017	0.037	0.097	0.081
"	HL	6	0.171	0.030	0.020	0.251	0.025	0.013	0.259	0.037	0.491	0.167	0.086	0.007	0.022	0.085	0.131
"	Min	29	0.060	0.008	0.003	0.094	0.009	0	0.004	0.016	0.007	0.070	0.005	0.007	0.013	0.002	0.027
"	Max	29	0.346	0.041	0.045	0.400	0.025	0.024	1.184	0.674	1.315	0.644	0.365	0.045	0.068	0.285	0.300
"	**Mean**	**29**	**0.202**	**0.026**	**0.015**	**0.216**	**0.014**	**0.011**	**0.388**	**0.151**	**0.567**	**0.201**	**0.061**	**0.020**	**0.030**	**0.094**	**0.103**
"	Std Dev	29	0.052	0.011	0.011	0.071	0.009	0.008	0.365	0.160	0.309	0.136	0.115	0.011	0.015	0.084	0.078
Field	LL	2	0.079			0.144		0.009	0.125		1.077						0.134
"	ML	0															
"	HL	4	0.171			0.190	0	0.004	0.244		0.706						0.109
"	Min	8	0.058	0	0	0.110	0	0	0.009	0	0	0	0	0	0	0.034	0
"	Max	8	0.296	0	0	0.422	0	0.062	0.465	0	1.281	0	0	0	0	0.034	0.188
"	**Mean**	**8**	**0.136**			**0.198**	**0**	**0.012**	**0.199**		**0.794**					**0.034**	**0.101**
"	Std Dev	8	0.088			0.101	0	0.021	0.168		0.386						0.073
References (see Table p): 25, 54, 61, 63, 64																	
HAPTO-8																	
Culture	LL	4	0.222	0.010	0.071	0.232		0.043	0.555	0.049	0.250	0.125	0.006	0.027	0.028	0.069	

Developments: 2

CHEMTAX Development

Australian Mathematics Institute

Maths and Statistics in Industry Study Group (MISG)



CHEMTAX MISG

Tasks

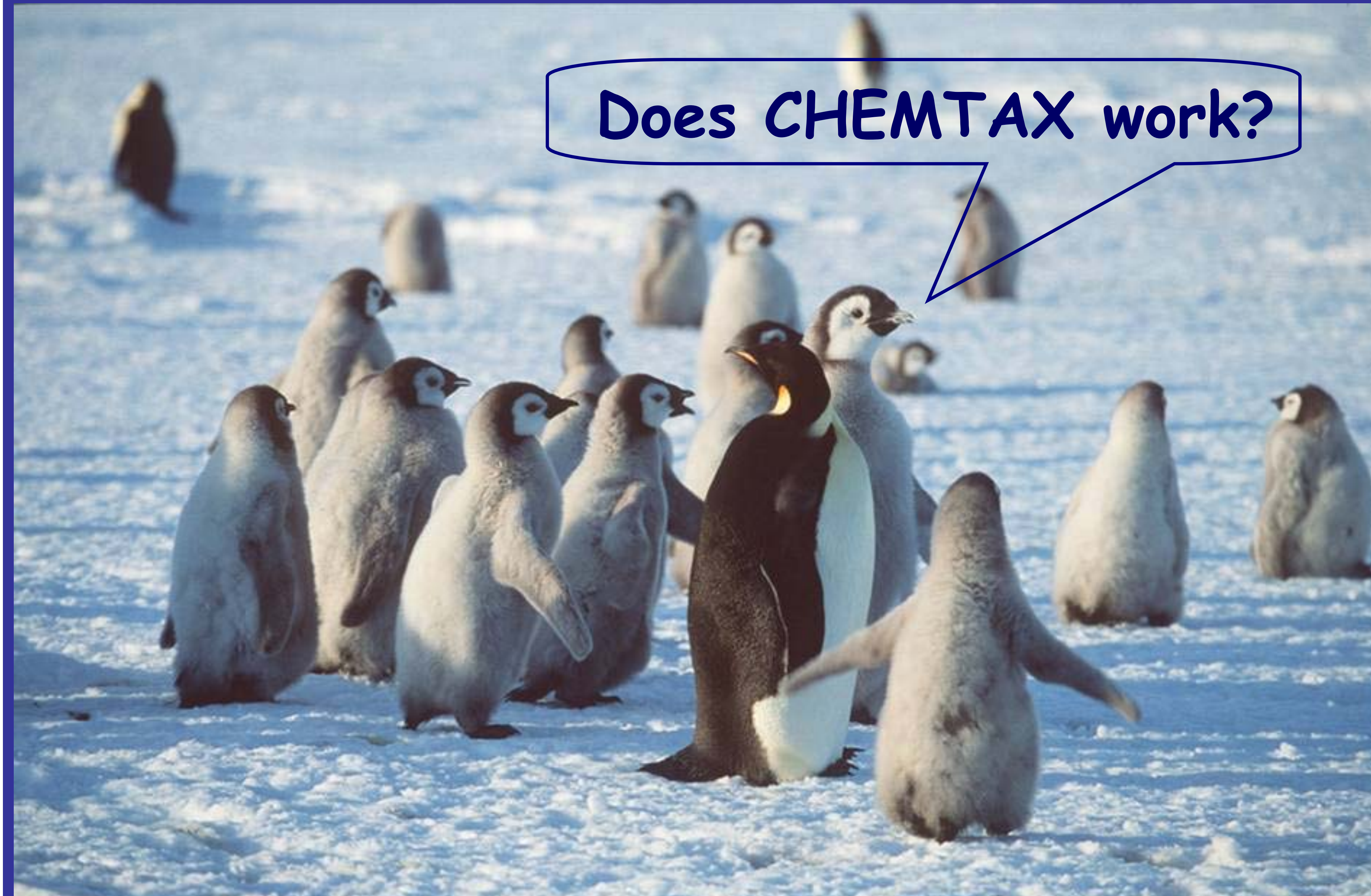
Analyse CHEMTAX operation and check its validity

Improve calculation efficiency and determine confidence limits of result

Compare CHEMTAX with Bayesian method and determine which is the better way to go.

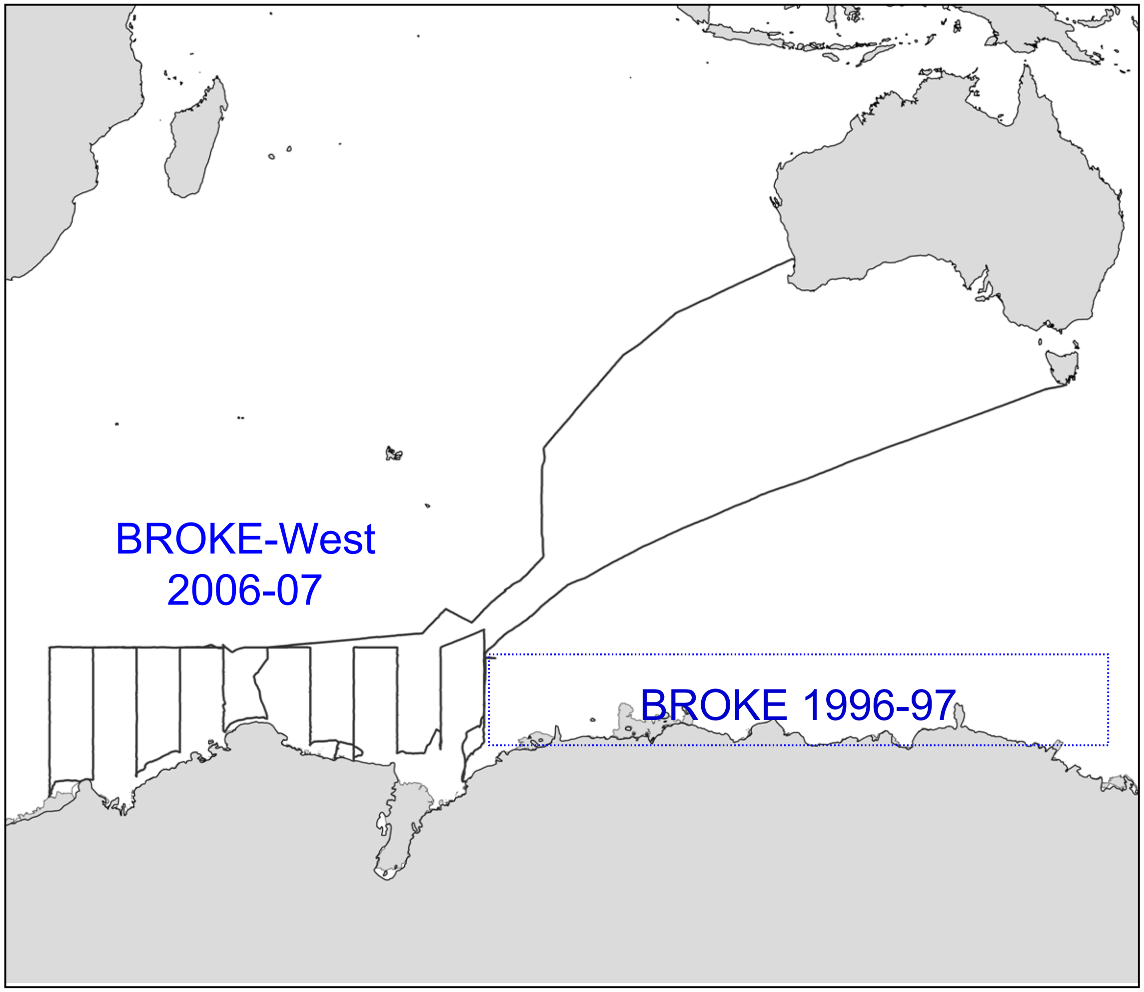
Results

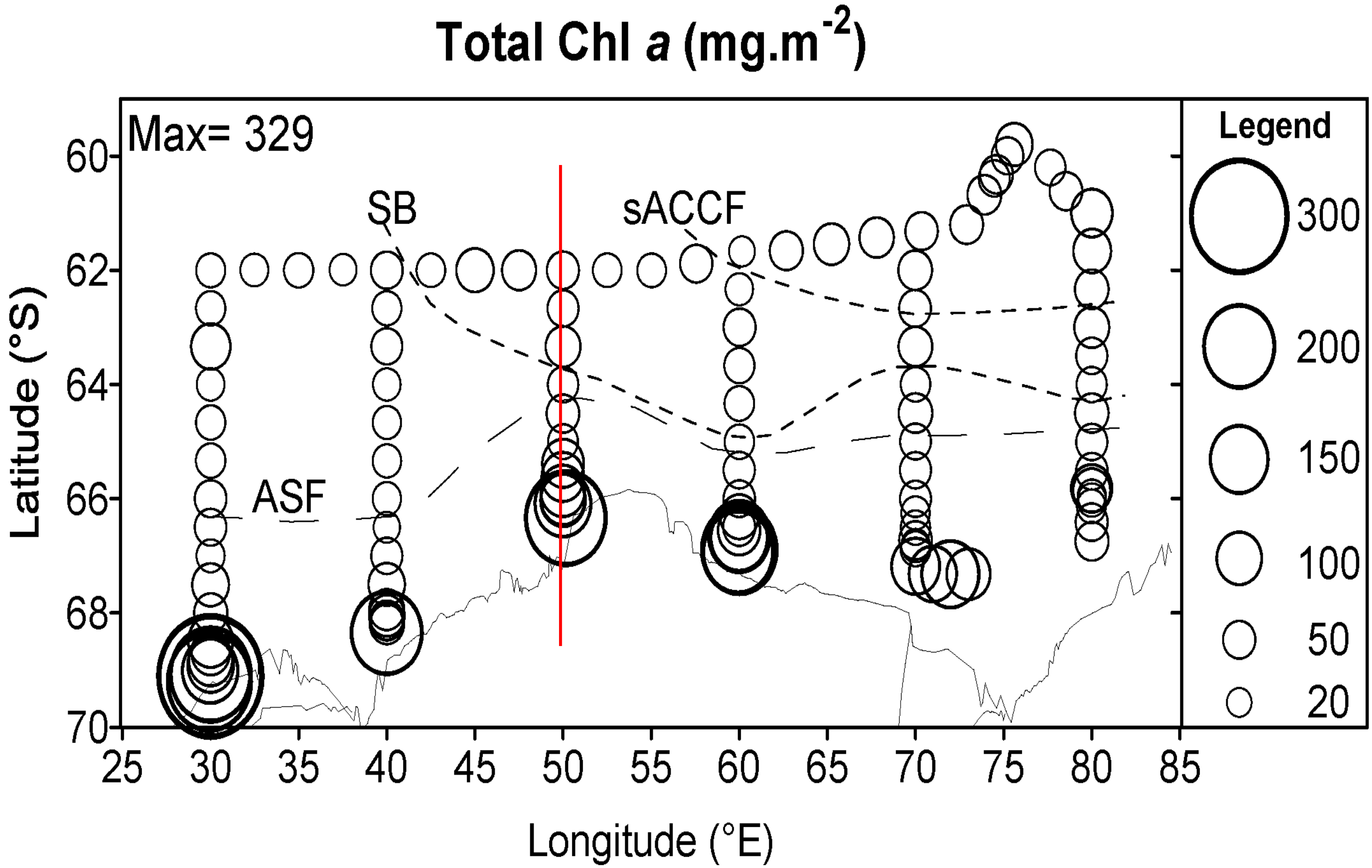
- A general proof of the factorization method was found
- Improved algorithm for CHEMTAX was developed
  - based on non-negative matrix factorization including simplified weighting of errors and prior knowledge of pigment distribution
  - Gave fastest solution to the problem
  - Methods developed to test uniqueness of the solution
  - Analysis of residual errors showed different oceanic regimes
  - Developed in Matlab, implemented in Octave, partially translated to R
  - Currently finalizing devt. and exploring ways to test it and implement it for distribution
- Bayesian method
  - Highly dependent on input ratios – requires good knowledge of local species
  - Not recommended for determining pigment ratios from field samples
  - Restricted to small sample numbers – works on 1 sample !
  - Not recommended for > 40 samples


Does CHEMTAX work?

BROKE-West cruise
(Baseline Research on Oceanography, Krill and the Environment)

- Oceanography
- Krill
- Mesozooplankton
- Seabirds
- Whales
- Fish and Squid
- Microbial loop

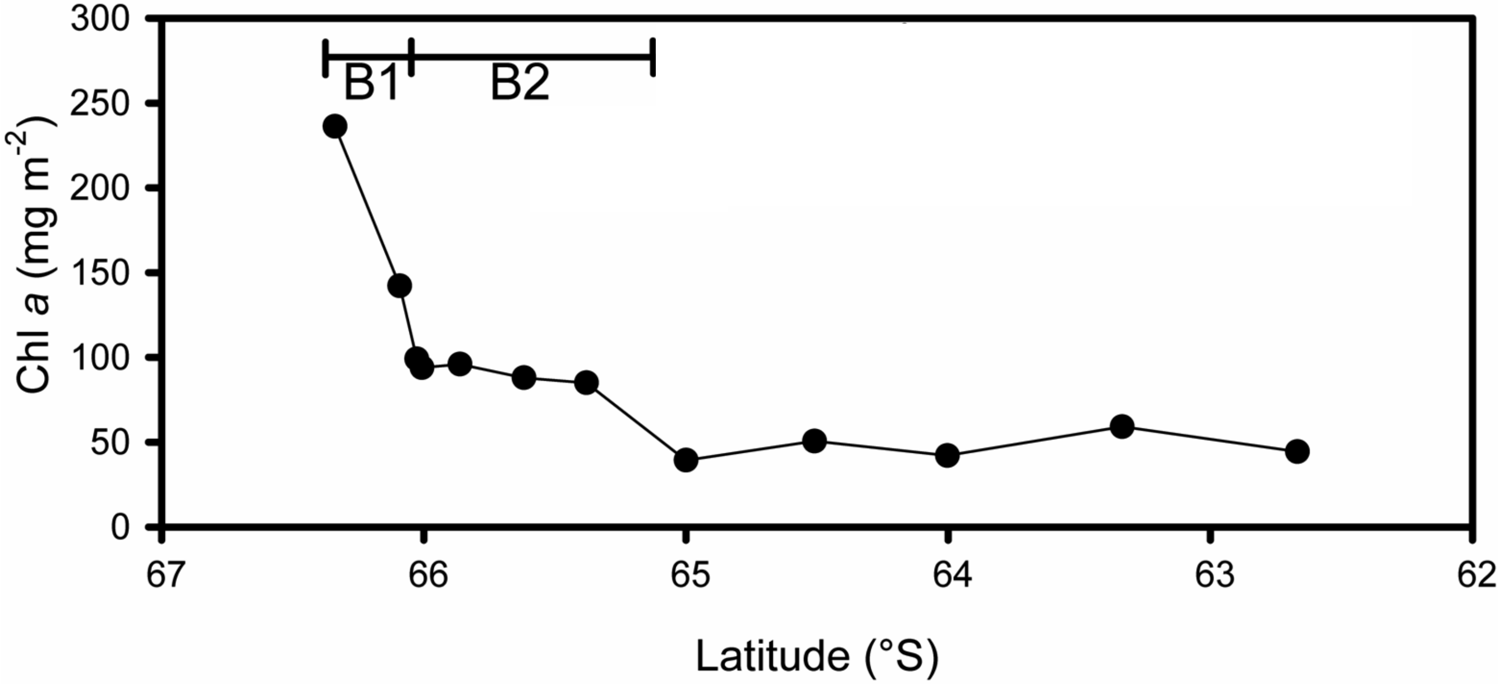




Total Chl a (mg.m-2)
Max= 329
SB
sACCF
ASF
Legend
300
200
150
100
50
20
Latitude (°S)
60
62
64
66
68
70
Longitude (°E)
25
30
35
40
45
50
55
60
65
70
75
80
85

Differentiation of bloom zones

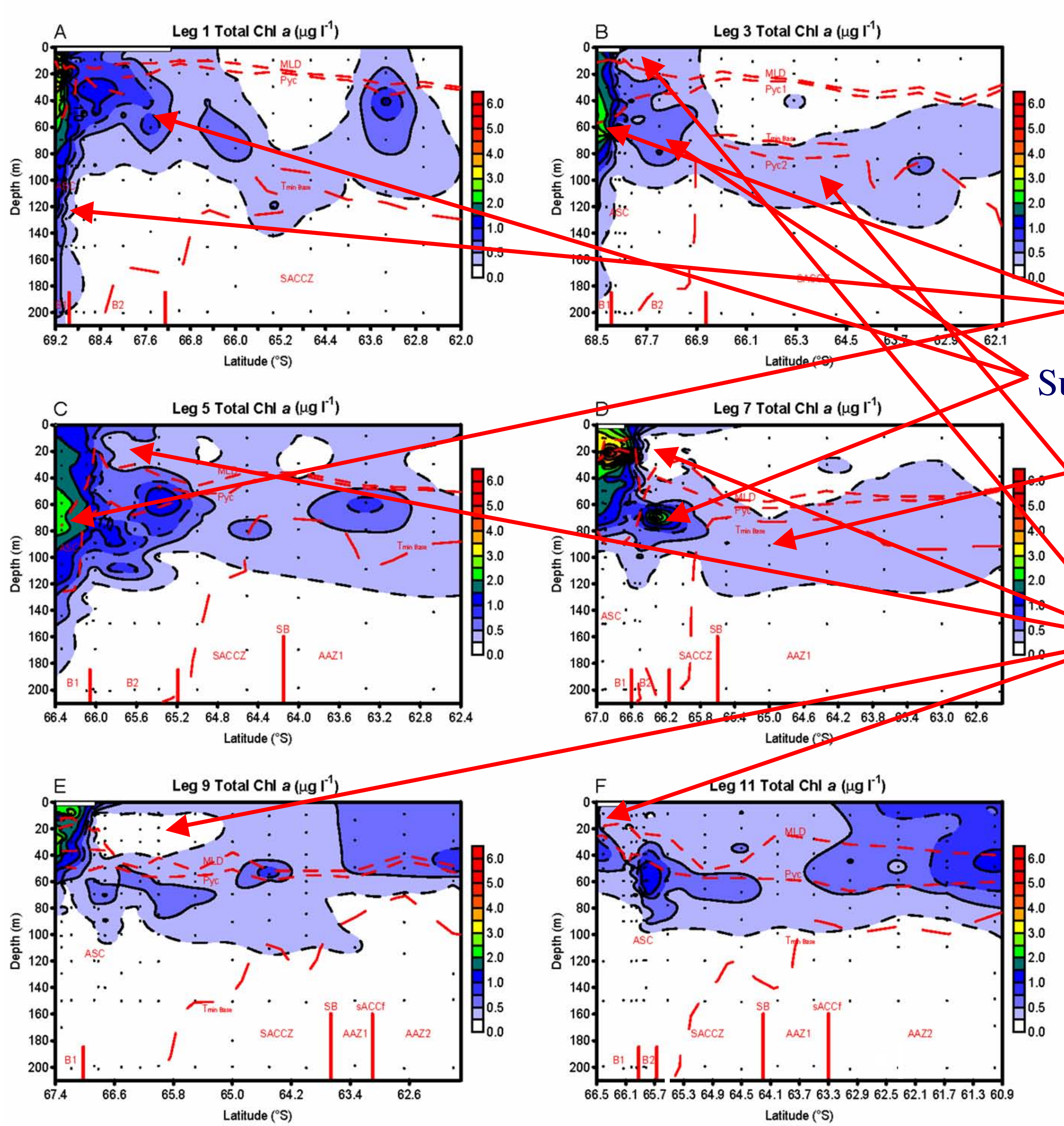
Based on integrated chl *a* stocks



Zones

- B1: Primary bloom
- B2: Secondary zone
- SACCZ: Zone south of the ACC
- AAZ1: Antarctic zone between SB and sACCf
- AAZ2: Antarctic zone north of sACCf

Total Chl *a*
along each transect



Consistent features

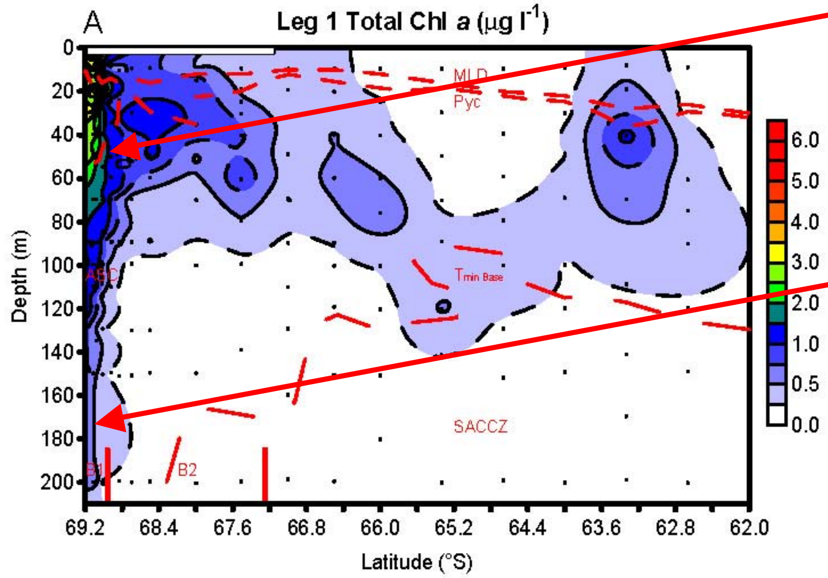
Deep bloom under ice

Subsurface secondary bloom

Deep chlorophyll maximum often below T_{min} layer

Hole near ice edge

Deep ice edge bloom (B1 zone)



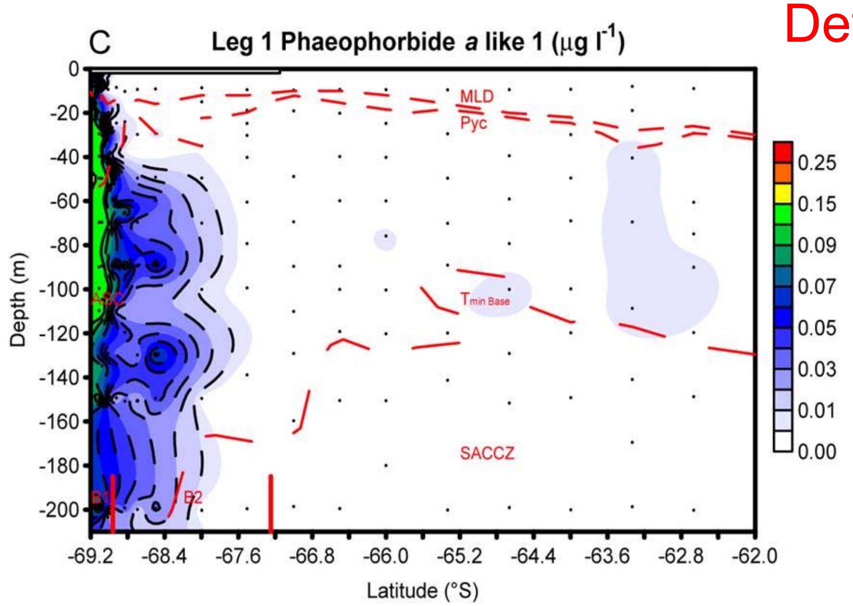
Very productive
Colonial *Phaeocystis* or gametes
Diatoms

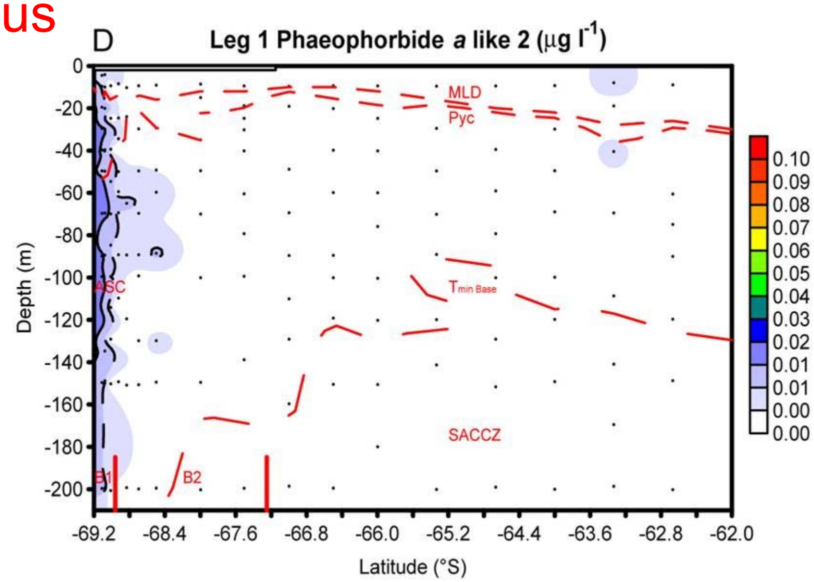
Very deep
Too deep for active photosynthesis

Sea-ice algae
+nutrients ?

+light
=> bloom

Detritus





CHEMTAX categories **(using chlorophyll and carotenoid markers plus microscopy)**

Those with **unambiguous markers**

Dinoflagellates-A **peridinin**

Prasinophytes **prasinoxanthin**

Chlorophytes **lutein**

Cryptophytes **alloxanthin**
but may include the ciliate *Myrionecta (Mesodinium) rubrum*

Two diatom categories

Diatoms-A, typical diatom pigmentation (**Chls *c*1, *c*2,** FUCO, diadinoxanthin)

Diatoms-B, typified by *Pseudonitzschia* sp (**Chls *c*2, *c*3**, FUCO, diadinoxanthin)

Two haptophyte categories (Di Tullio *et al.* 2007)

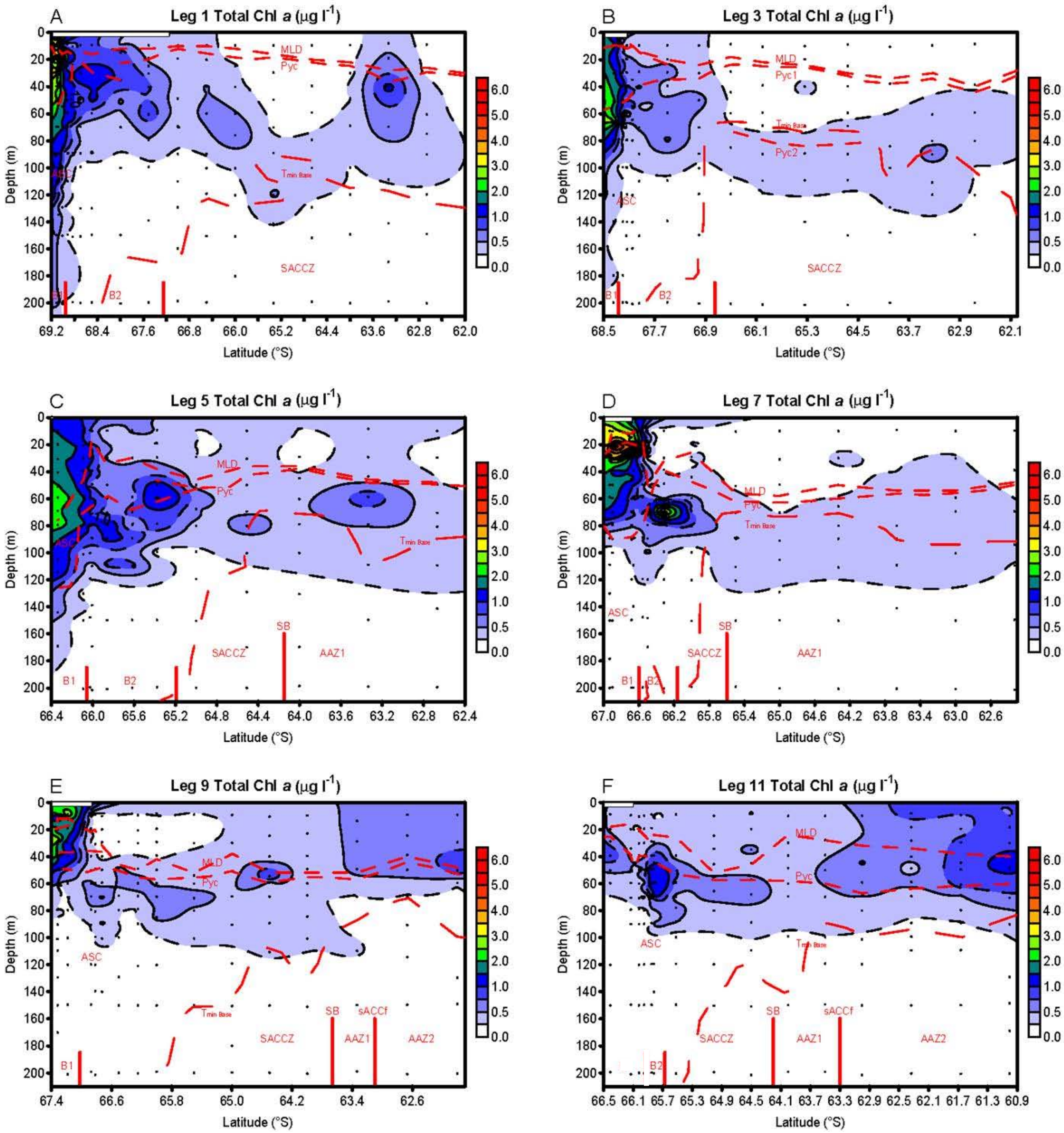
Haptophytes-H based on the **high iron form** of *P. antarctica*

Haptophytes-L based on the **low iron form** of *P. antarctica*

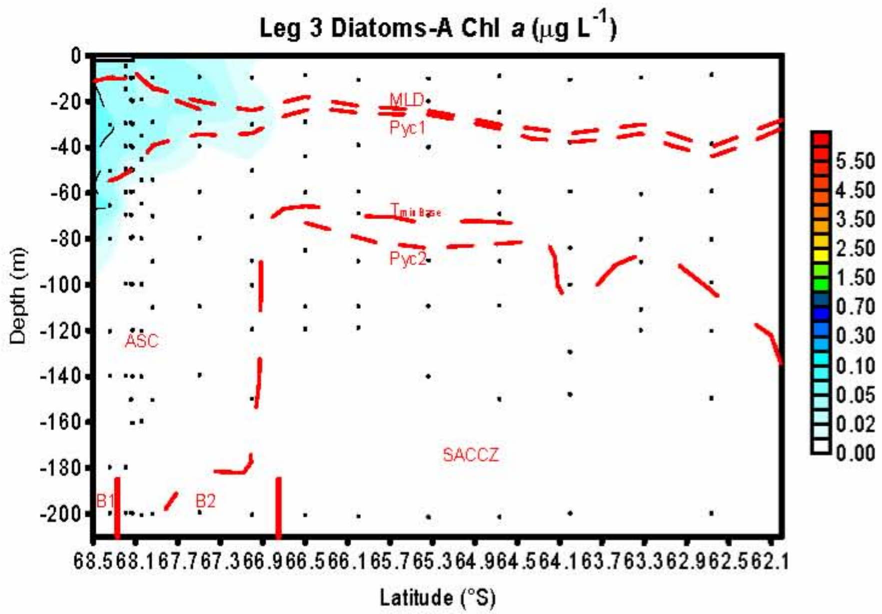
CHEMTAX workup

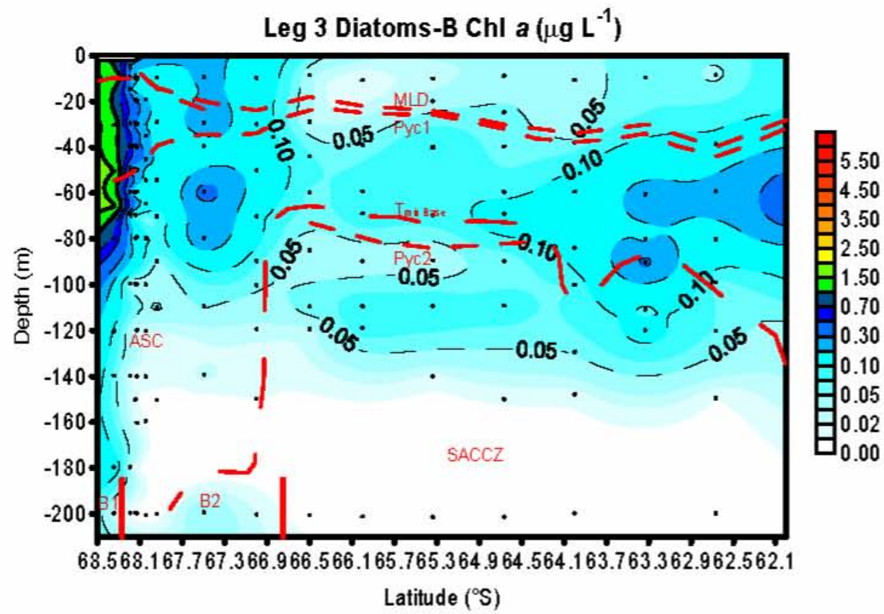
- Several scenarios tried
- 50 randomised trials per scenario
- Data were split into **five bins** according to **sample depth**
- The depth bins and sample numbers in each bin were:

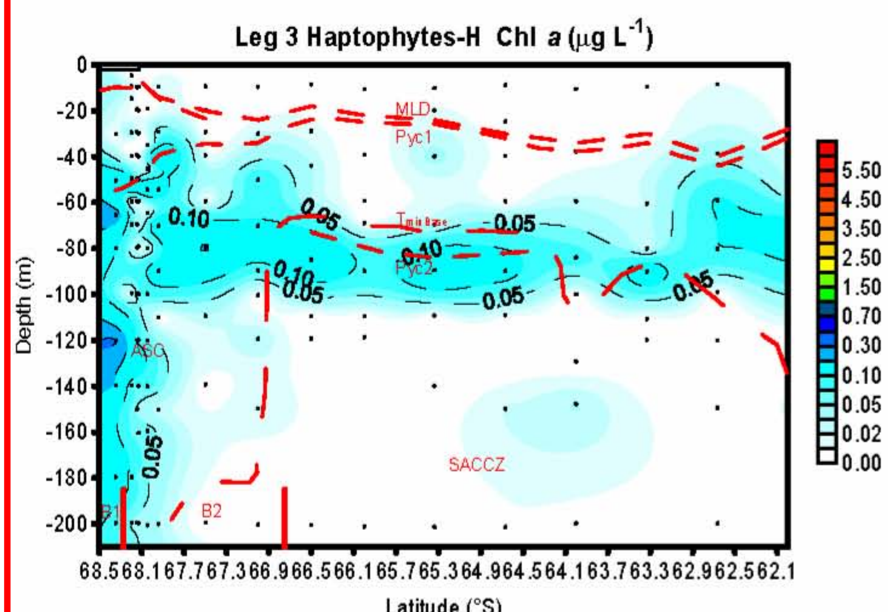
  0–15 m [129]   15–31 m [143]   31–56 m [169]
  56–92 m [282]   > 92 m [405]
  Total = 1128

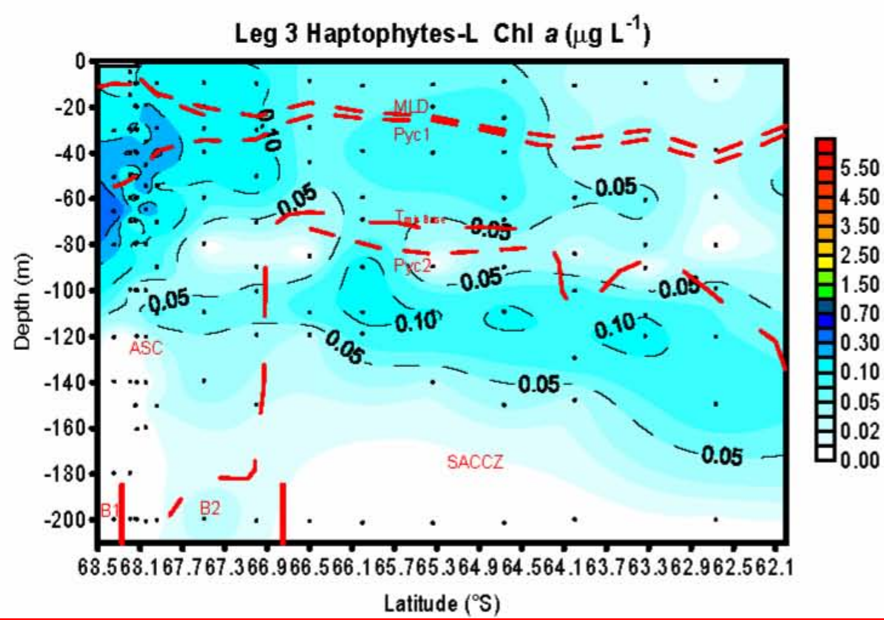

A
Leg 1 Total Chl a (μg l-1)
B
Leg 3 Total Chl a (μg l-1)
C
Leg 5 Total Chl a (μg l-1)
D
Leg 7 Total Chl a (μg l-1)
E
Leg 9 Total Chl a (μg l-1)
F
Leg 11 Total Chl a (μg l-1)
Depth (m)
Latitude (°S)
MLD
Pyc
Pyc1
Pyc2
Tmin Base
ASC
SACCZ
SB
sACCf
AAZ1
AAZ2
B1
B2
0.0
0.5
1.0
2.0
3.0
4.0
5.0
6.0

CHEMTAX analysis

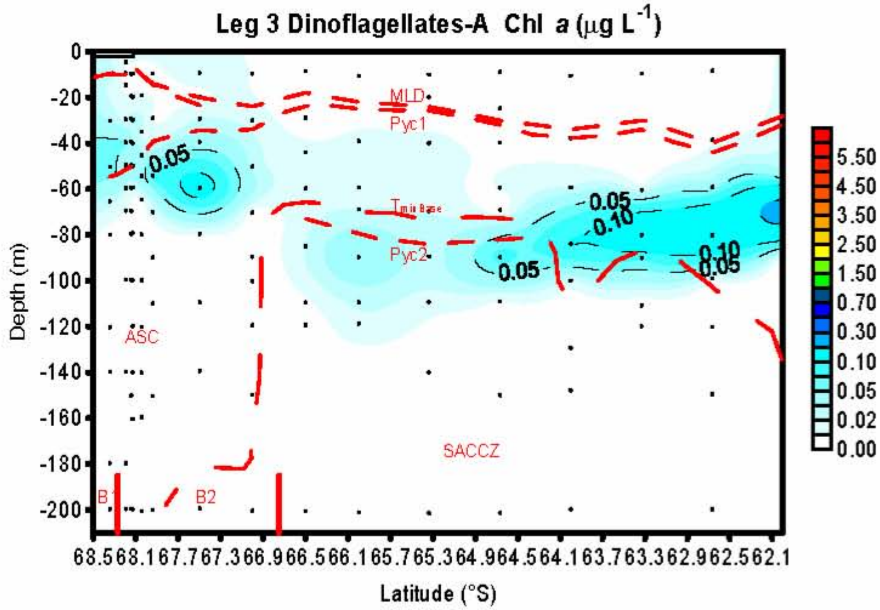


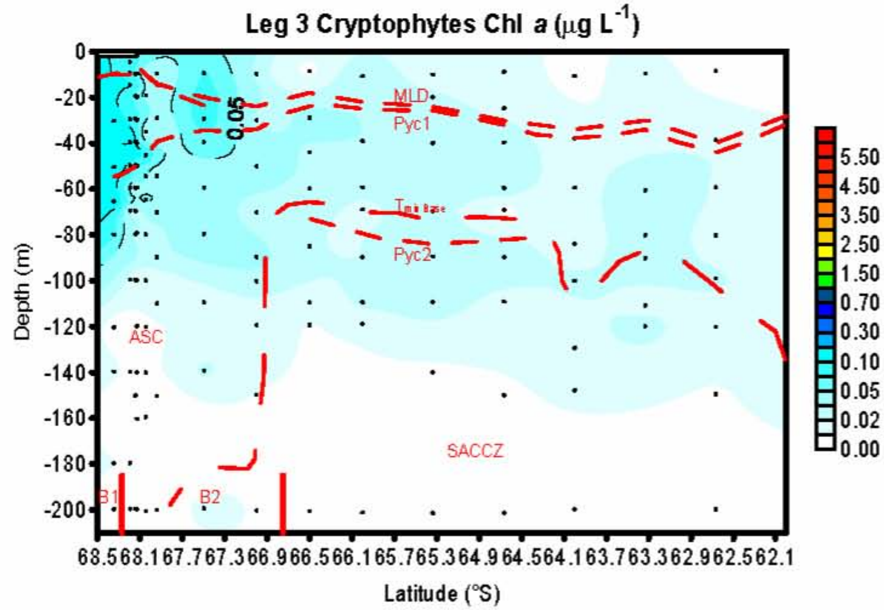


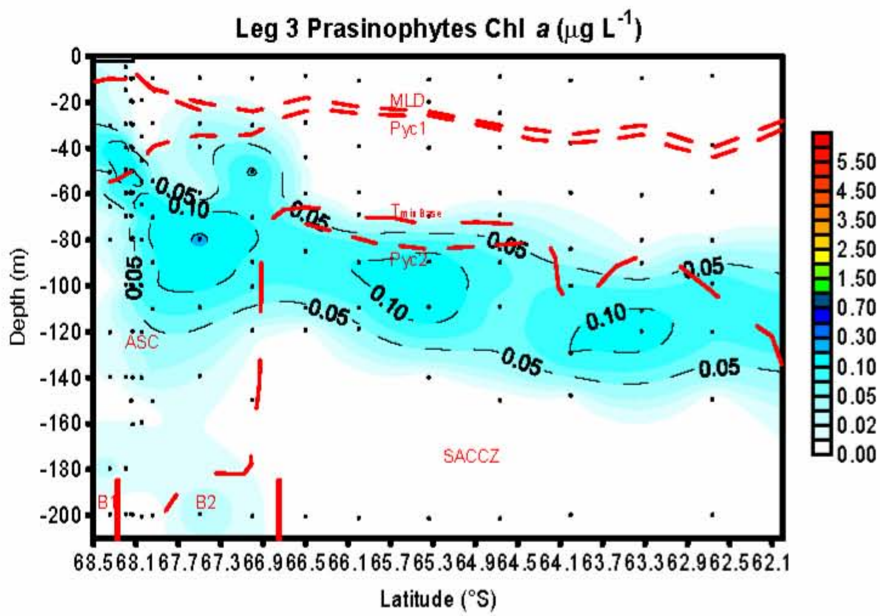


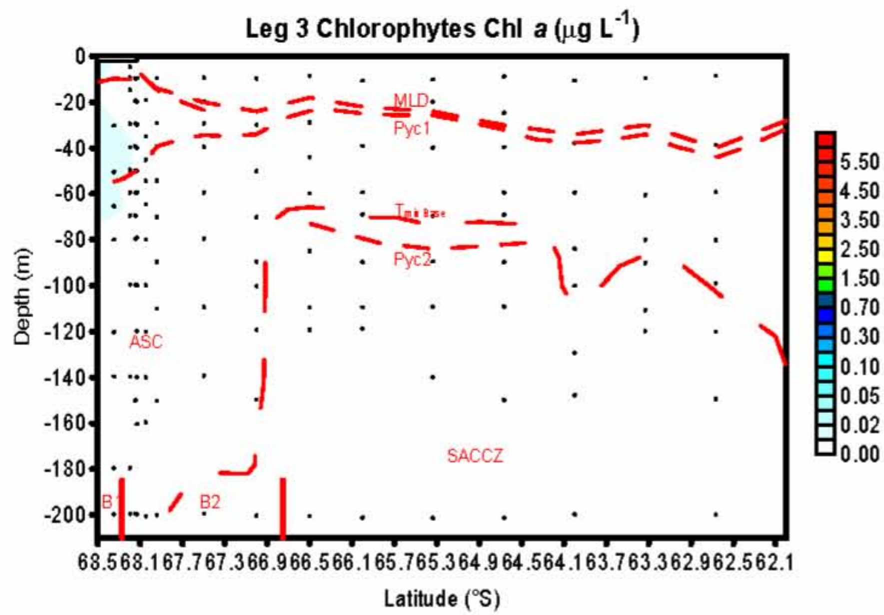


CHEMTAX analysis

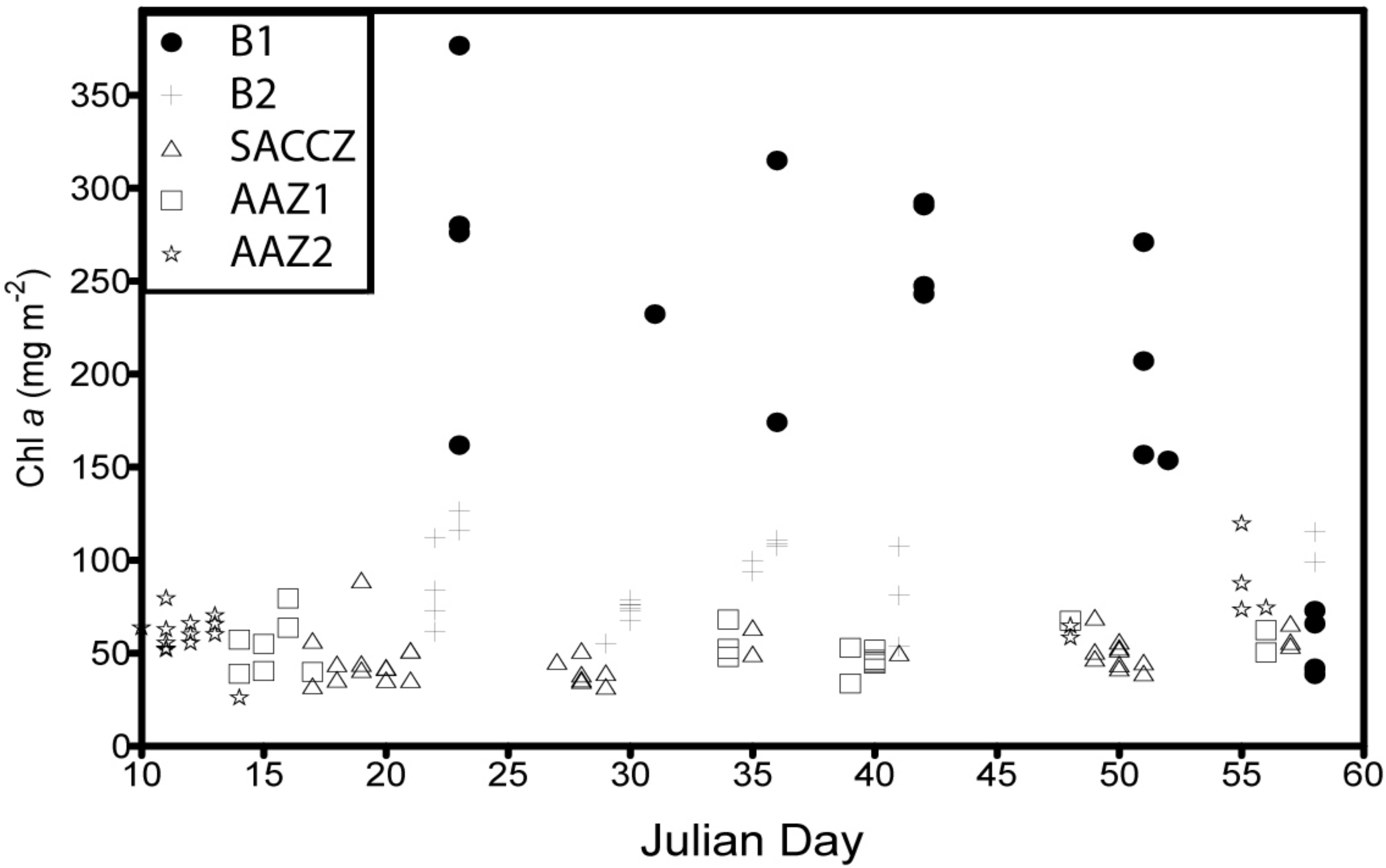


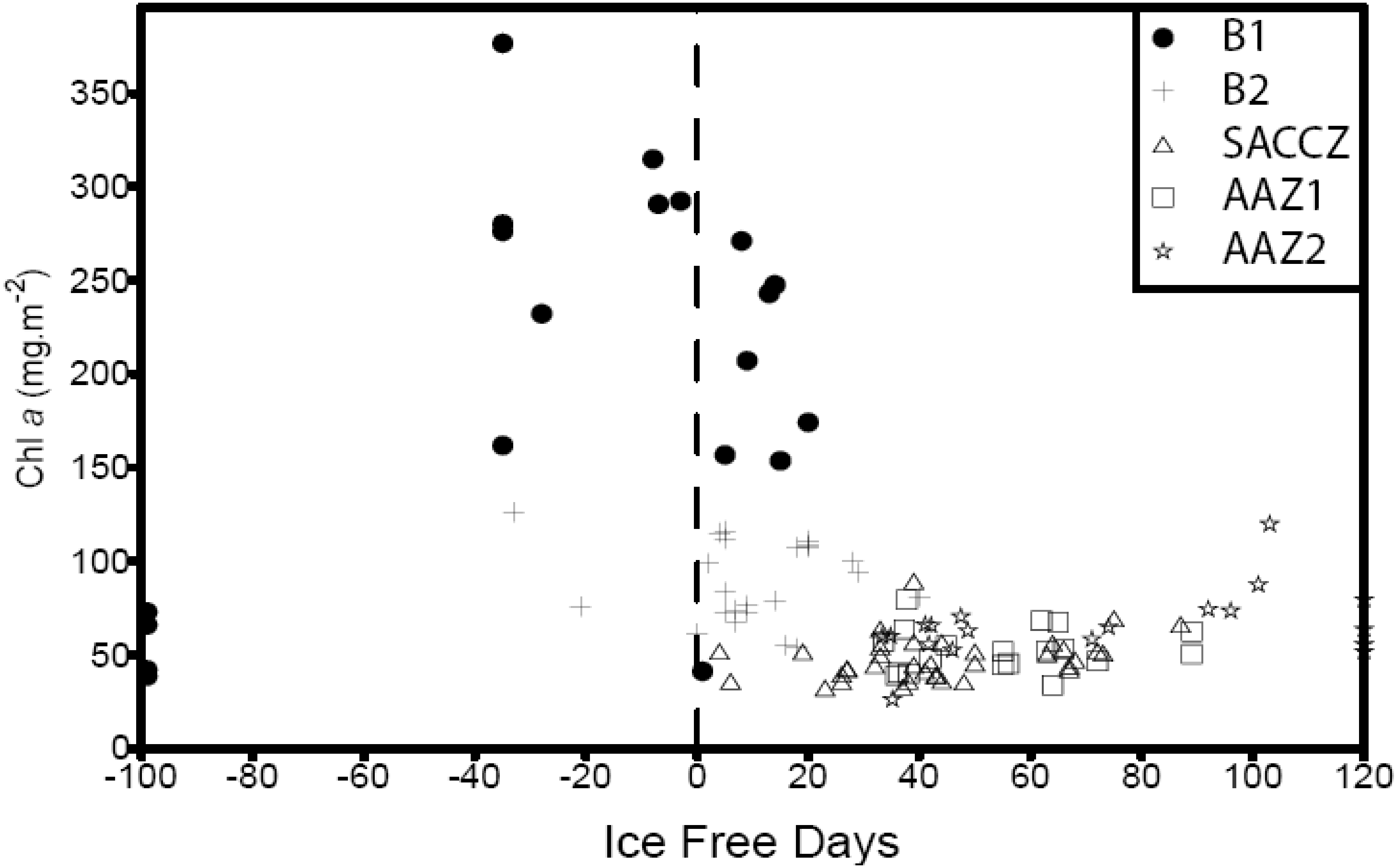




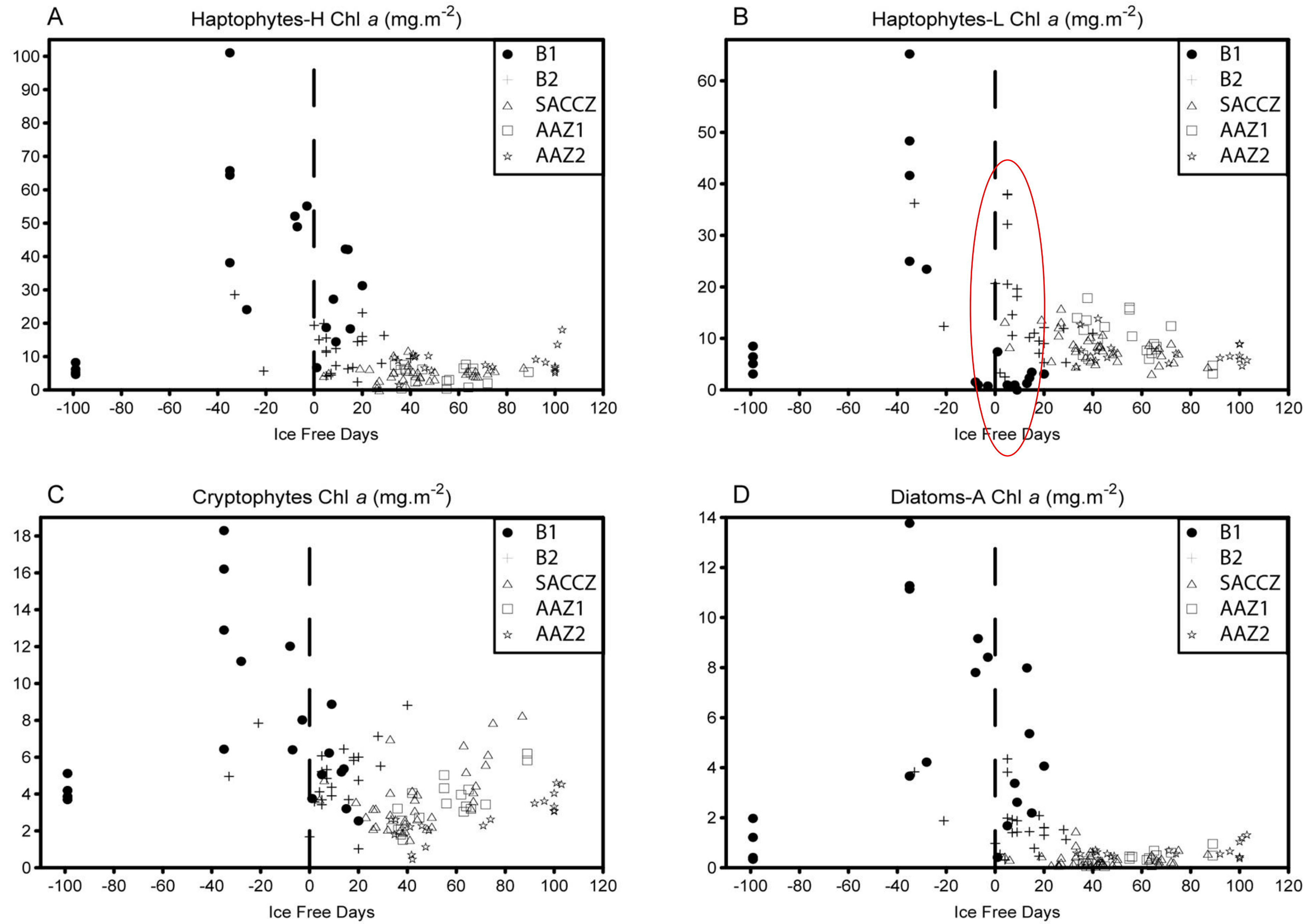


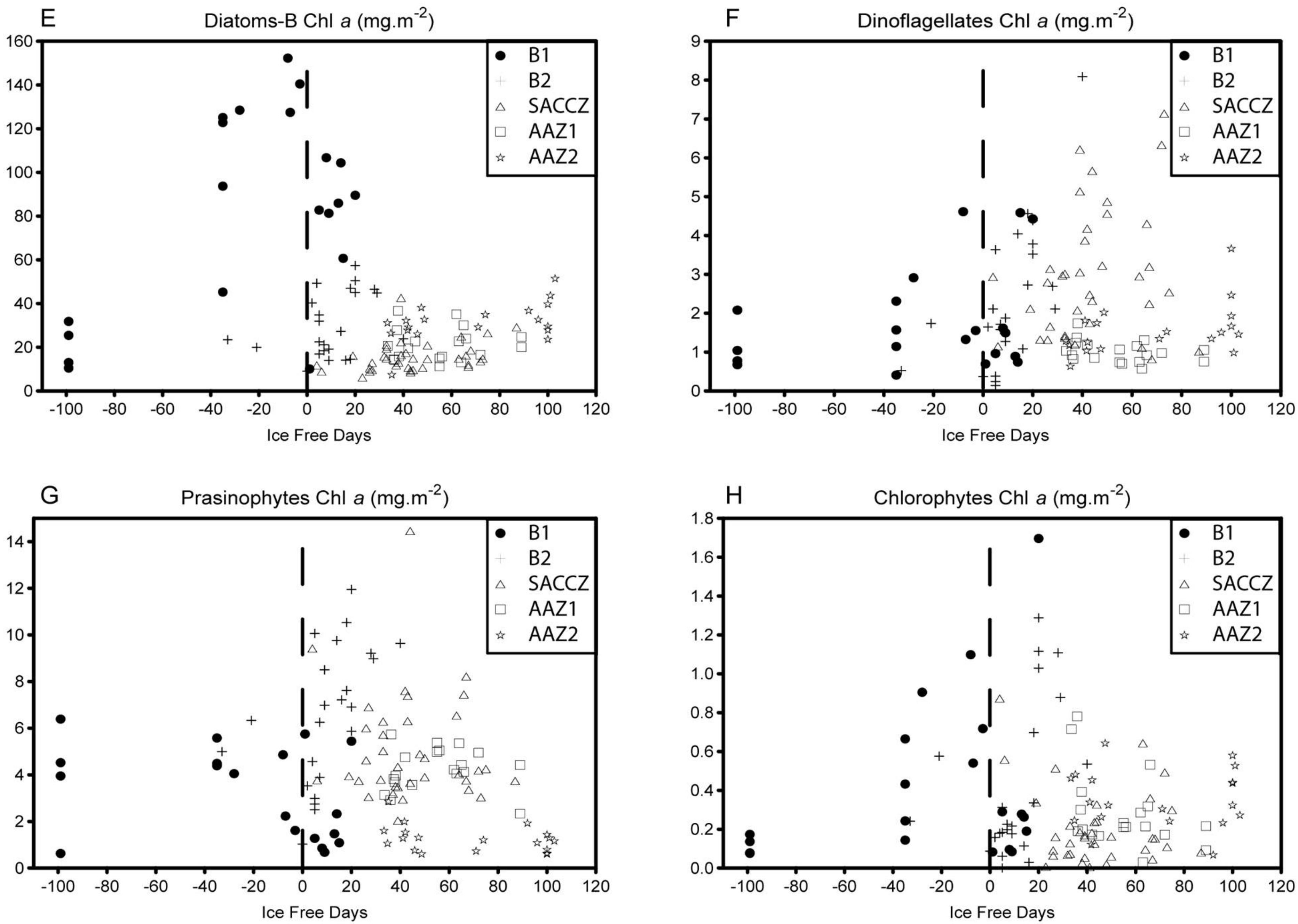
Temporal sequence



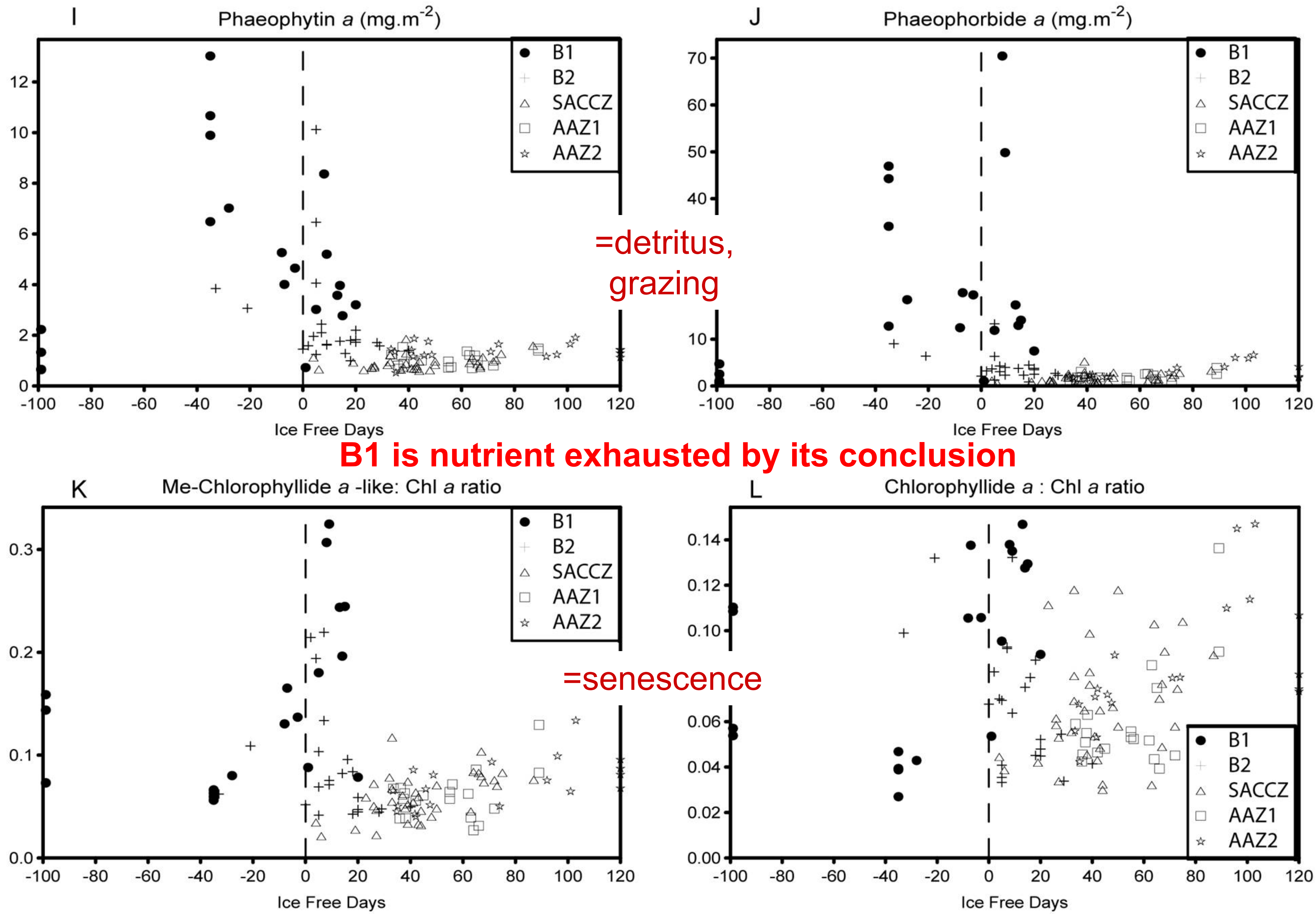


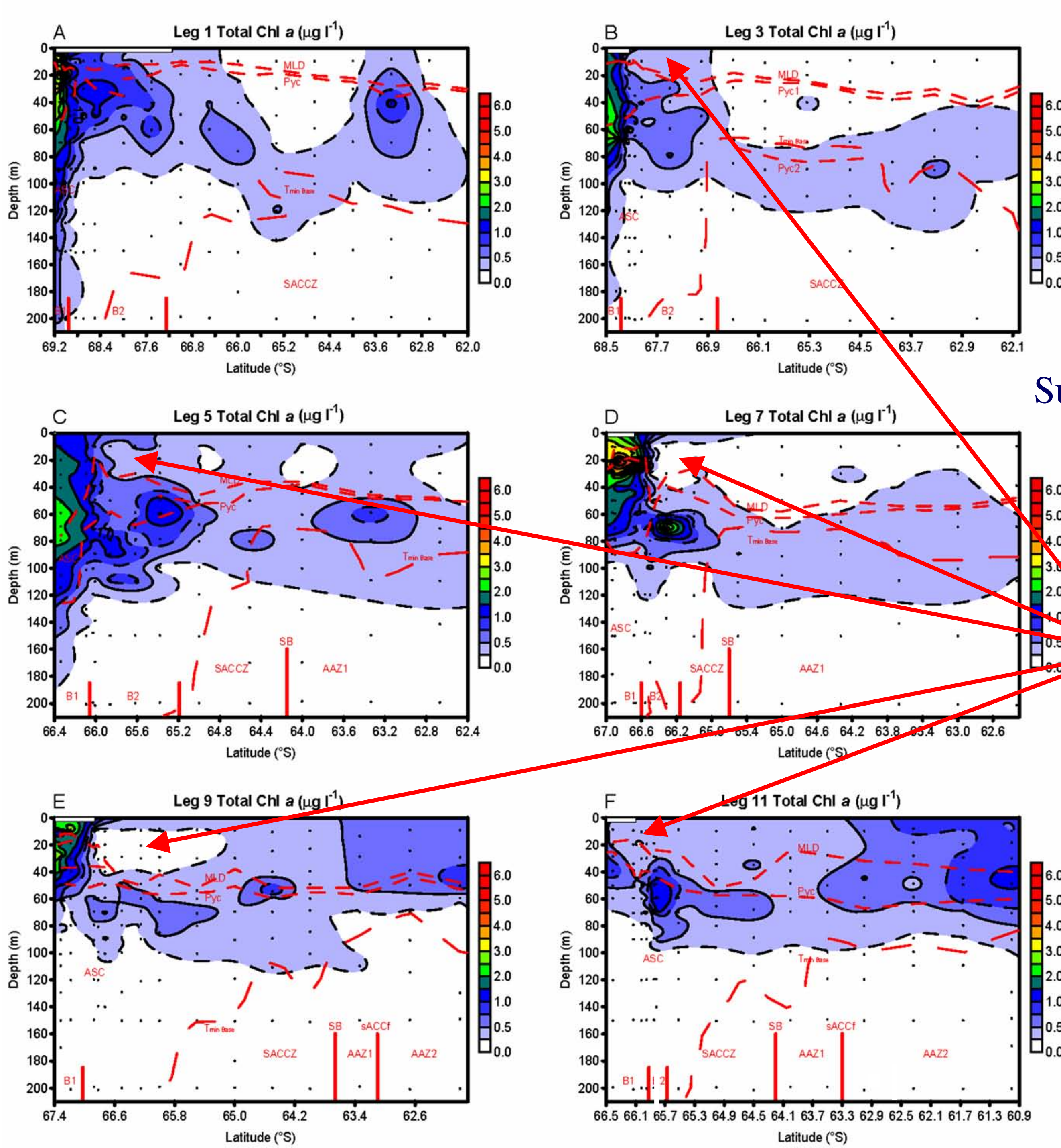
(Day ice completed melting (satellite)) – (Day sampled)


A
Haptophytes-H Chl a (mg.m-2)
B
Haptophytes-L Chl a (mg.m-2)
C
Cryptophytes Chl a (mg.m-2)
D
Diatoms-A Chl a (mg.m-2)
B1
B2
SACCZ
AAZ1
AAZ2
Ice Free Days



E
Diatoms-B Chl a (mg.m-2)
F
Dinoflagellates Chl a (mg.m-2)
G
Prasinophytes Chl a (mg.m-2)
H
Chlorophytes Chl a (mg.m-2)
B1
B2
SACCZ
AAZ1
AAZ2
Ice Free Days


I
Phaeophytin a (mg.m-2)
J
Phaeophorbide a (mg.m-2)
K
Me-Chlorophyllide a -like: Chl a ratio
L
Chlorophyllide a : Chl a ratio
B1
B2
SACCZ
AAZ1
AAZ2
Ice Free Days
=detritus, grazing
B1 is nutrient exhausted by its conclusion
=senescence



Total Chl *a* along each transect

Consistent features

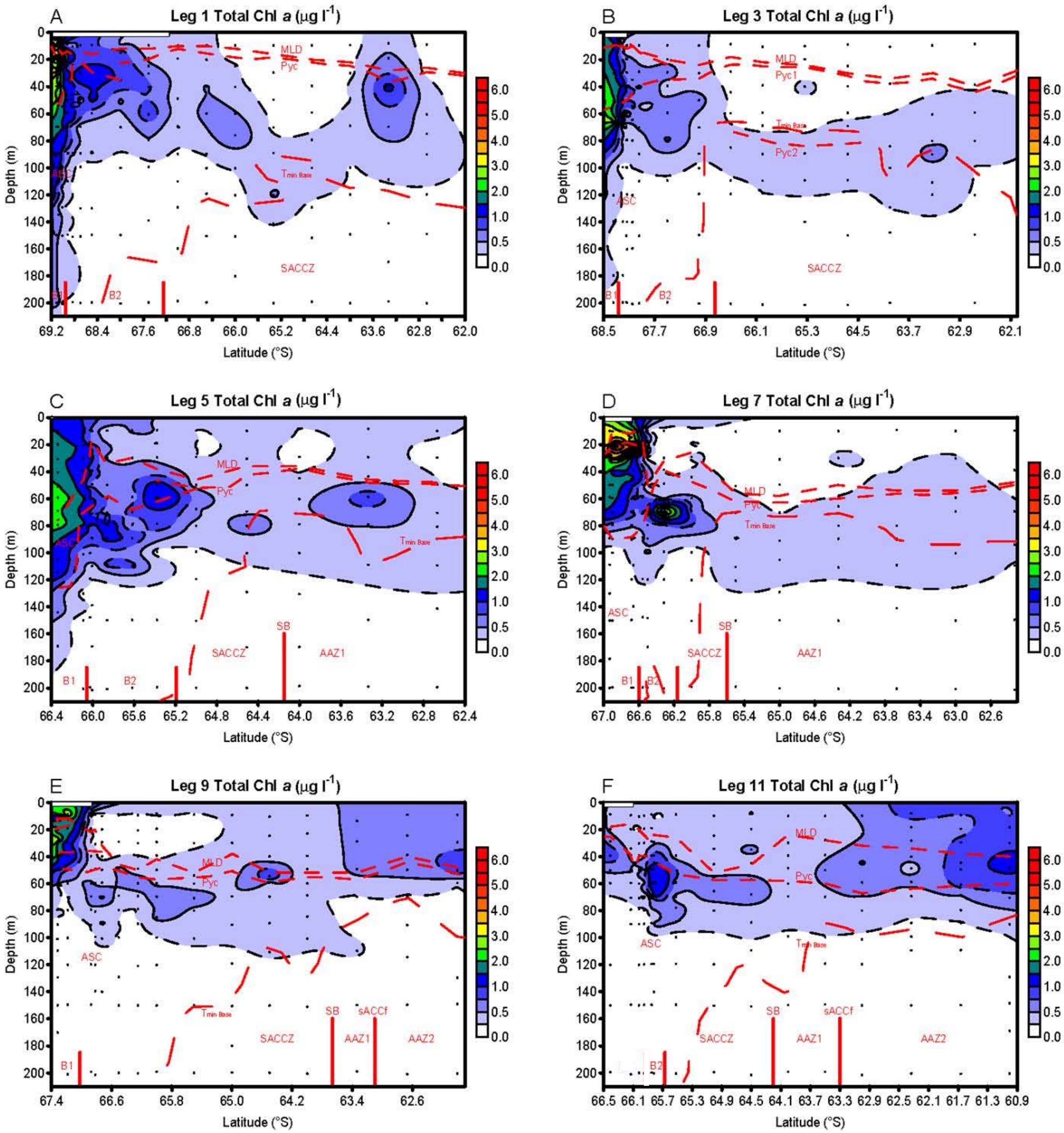
Deep bloom under ice

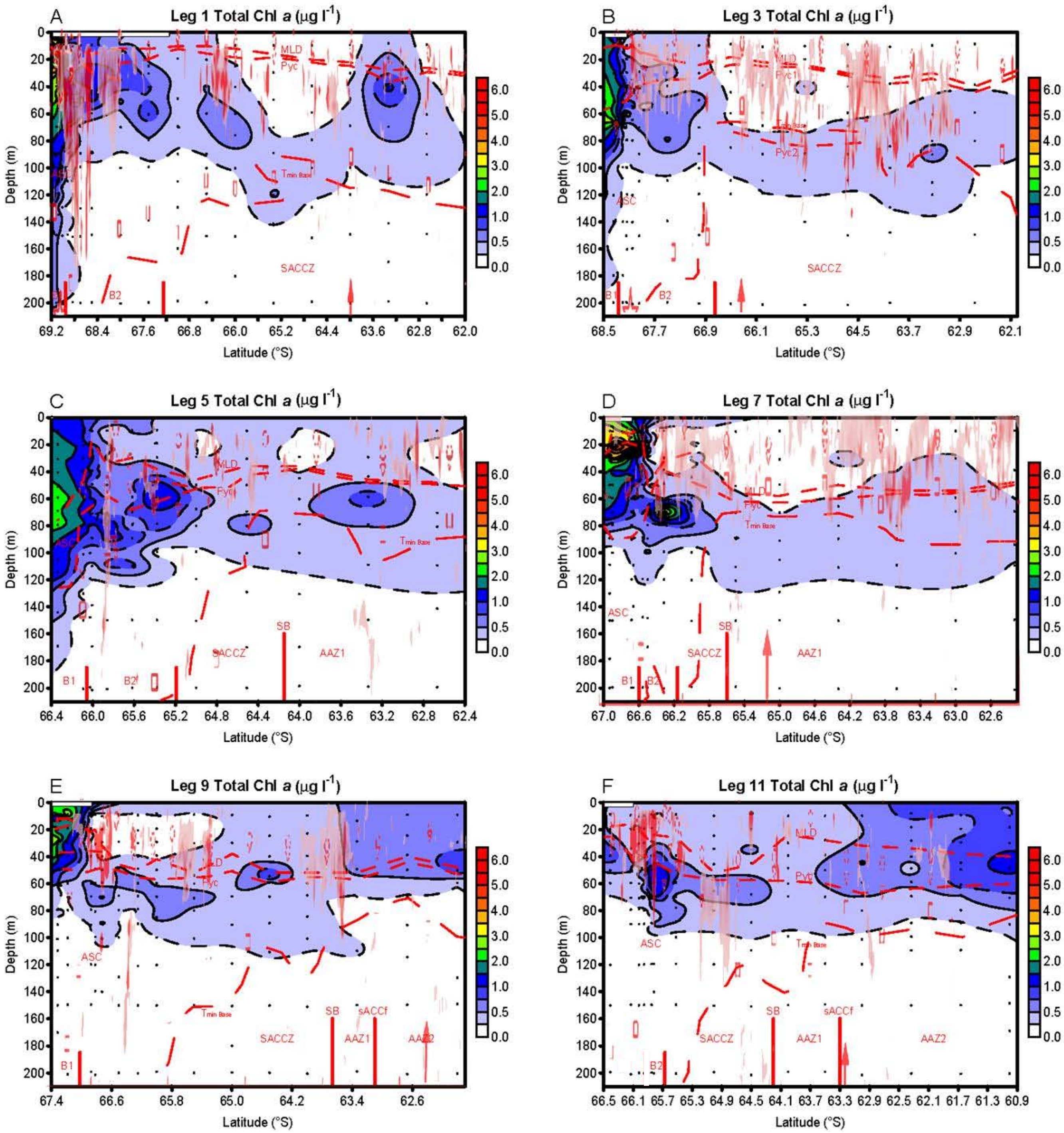
Subsurface secondary bloom

Deep chlorophyll maximum often below T_{min} layer

Hole near ice edge

Grazing


A
Leg 1 Total Chl a (μg l-1)
B
Leg 3 Total Chl a (μg l-1)
C
Leg 5 Total Chl a (μg l-1)
D
Leg 7 Total Chl a (μg l-1)
E
Leg 9 Total Chl a (μg l-1)
F
Leg 11 Total Chl a (μg l-1)
Depth (m)
Latitude (°S)
MLD
Pyc
Pyc1
Pyc2
Tmin Base
ASC
SACCZ
SB
sACCf
AAZ1
AAZ2
B1
B2
6.0
5.0
4.0
3.0
2.0
1.0
0.5
0.0



Grazing

Krill
(Jarvis et al, in press, *Deep-Sea Res.*)

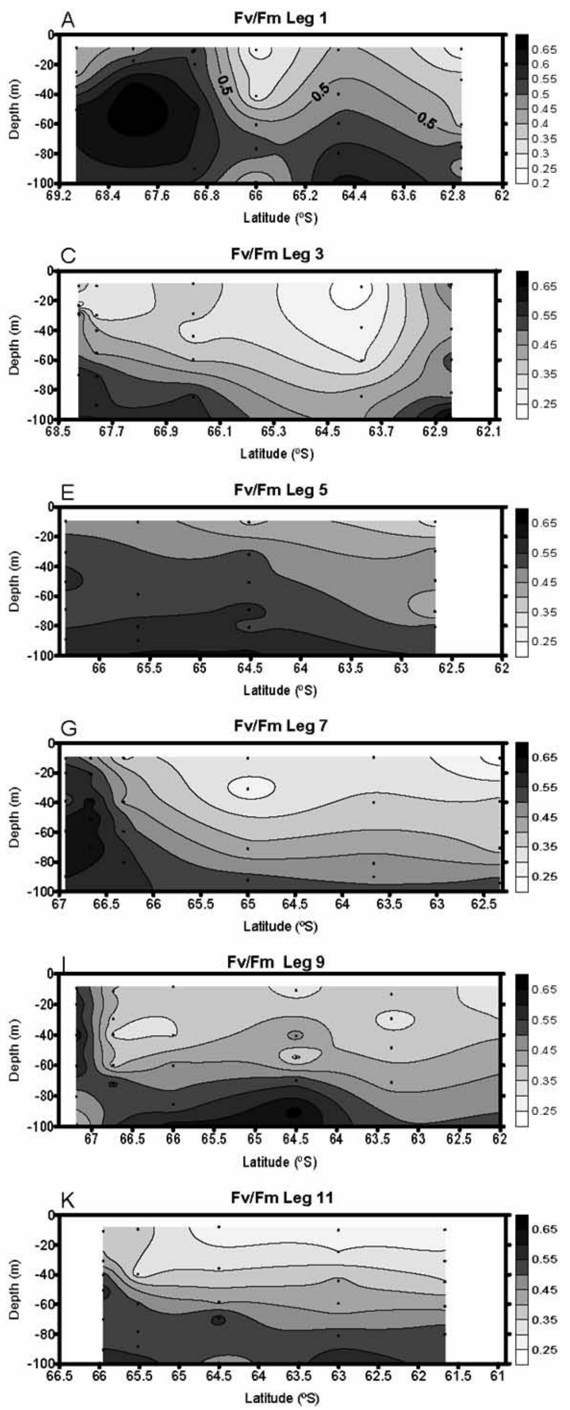
PAM Fluoro

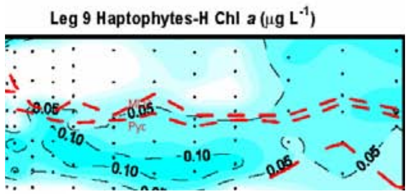
Fv/Fm

(dark adapted)

Proxy for nutrients

=Iron?

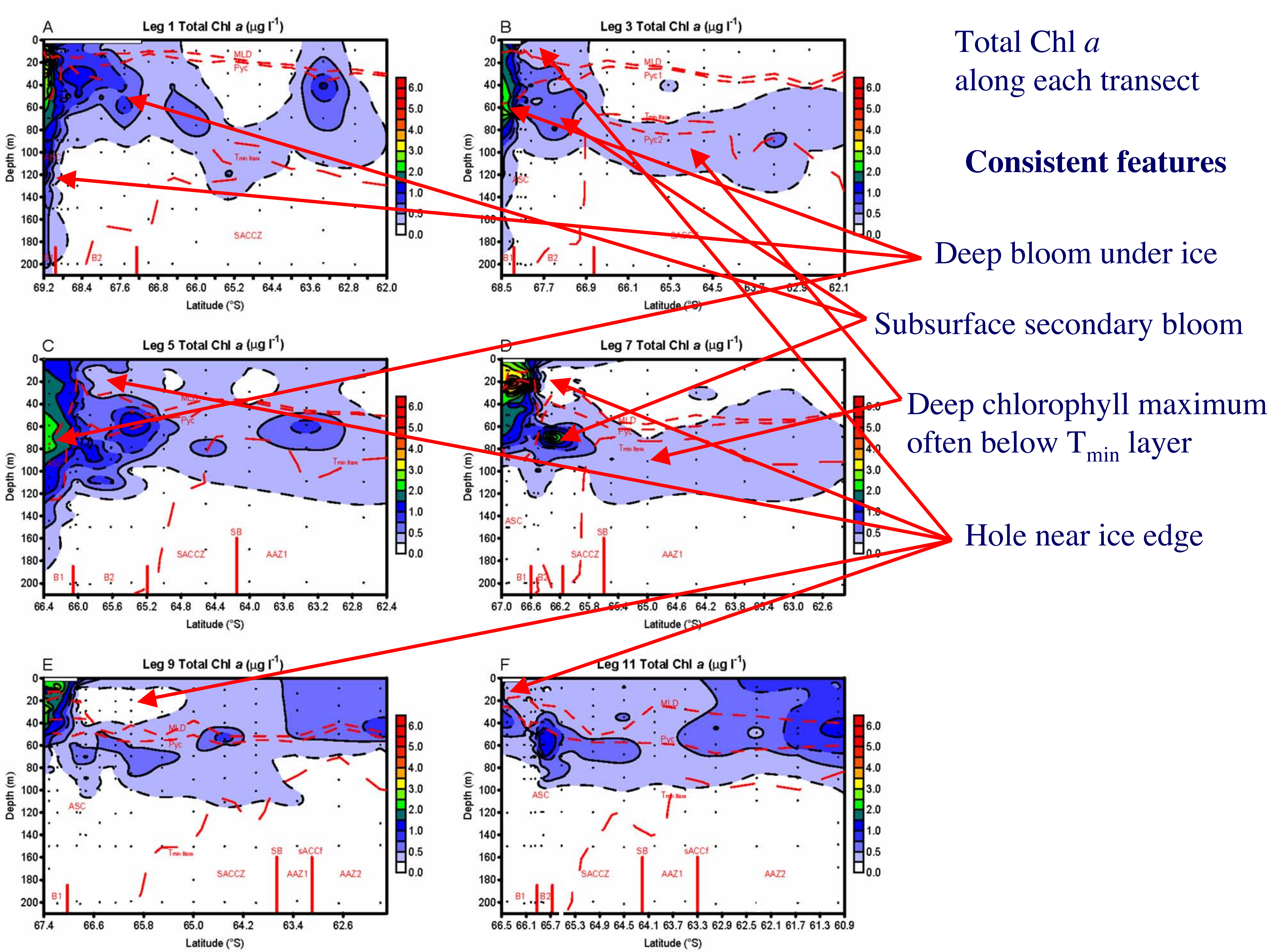




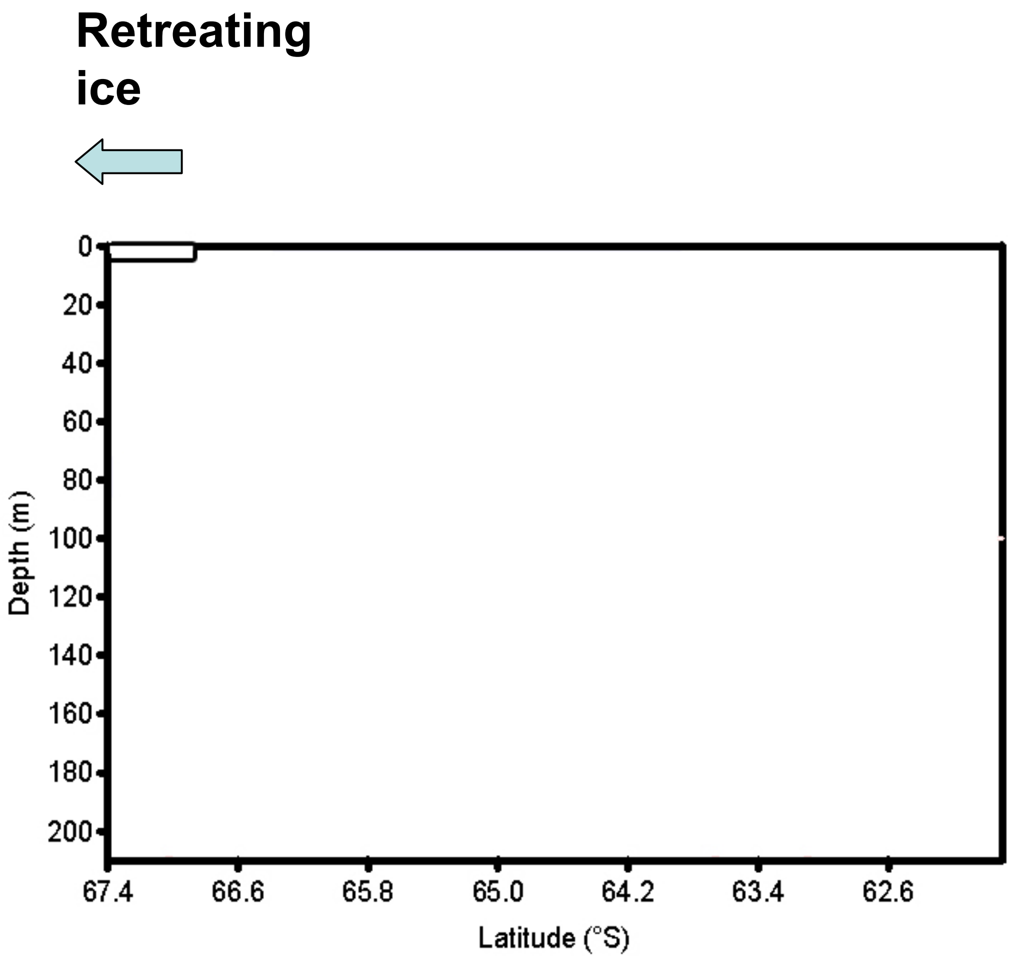
Iron controls depth of DCM

Total Chl *a*
along each transect

Consistent features

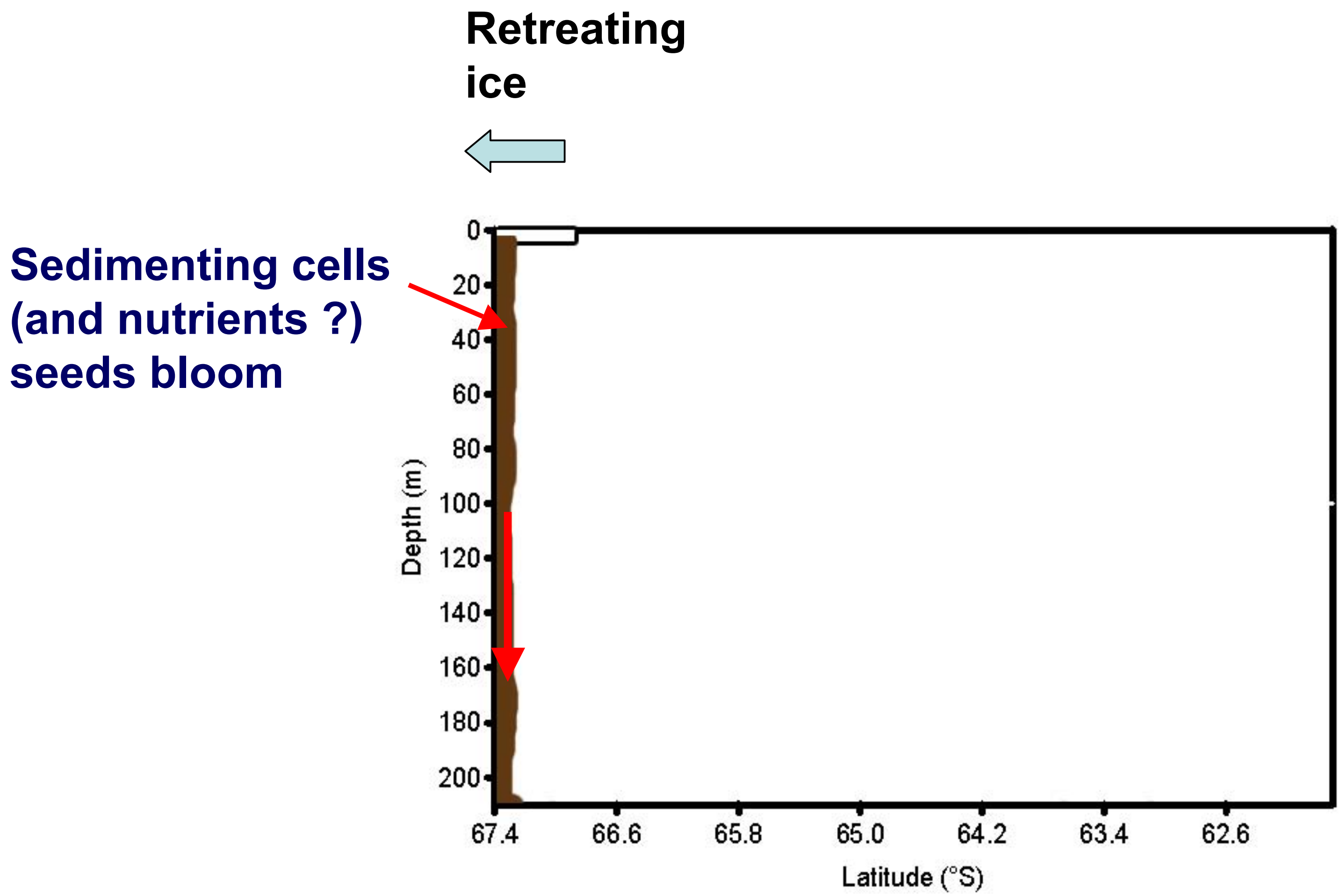


Synopsis



Retreating ice
0
20
40
60
80
100
120
140
160
180
200
Depth (m)
67.4
66.6
65.8
65.0
64.2
63.4
62.6
Latitude (°S)

Synopsis


Retreating
ice
Sedimenting cells
(and nutrients ?)
seeds bloom
0
20
40
60
80
100
120
140
160
180
200
Depth (m)
67.4
66.6
65.8
65.0
64.2
63.4
62.6
Latitude (°S)

Synopsis

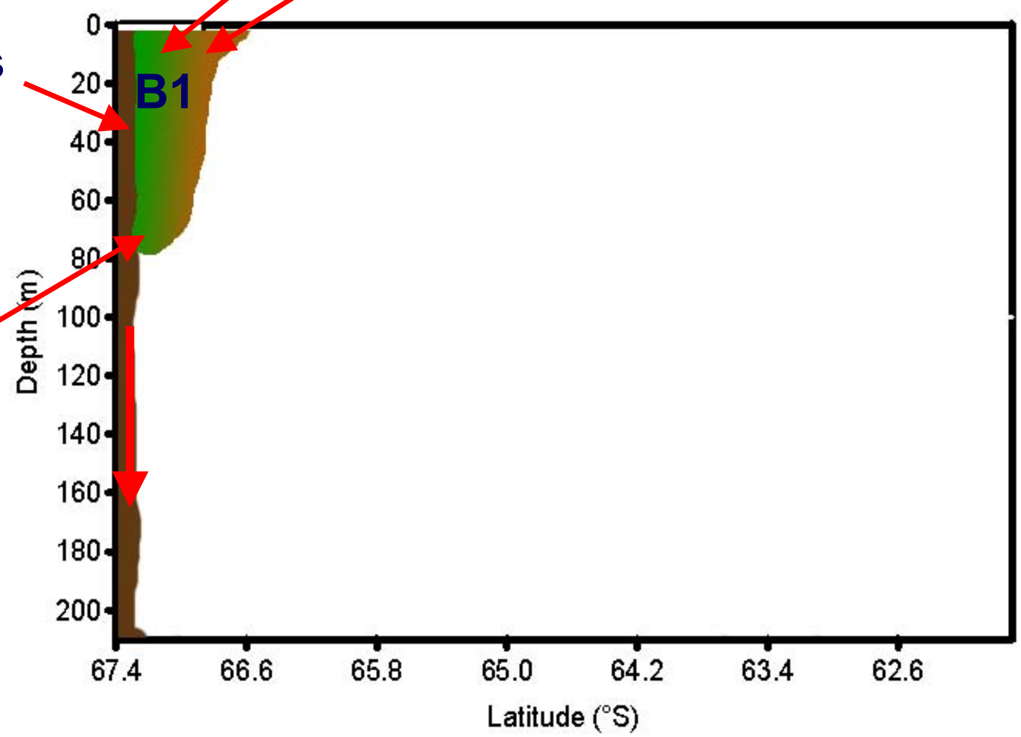
Primary bloom

Initially healthy

Soon binds all iron and senesces

Retreating ice

Sedimenting cells (and nutrients ?) seeds bloom



Light limited due to self shading

B1:
Diatoms B and A
***Phaeocystis* col or gam (H)**
Cryptophytes

Synopsis

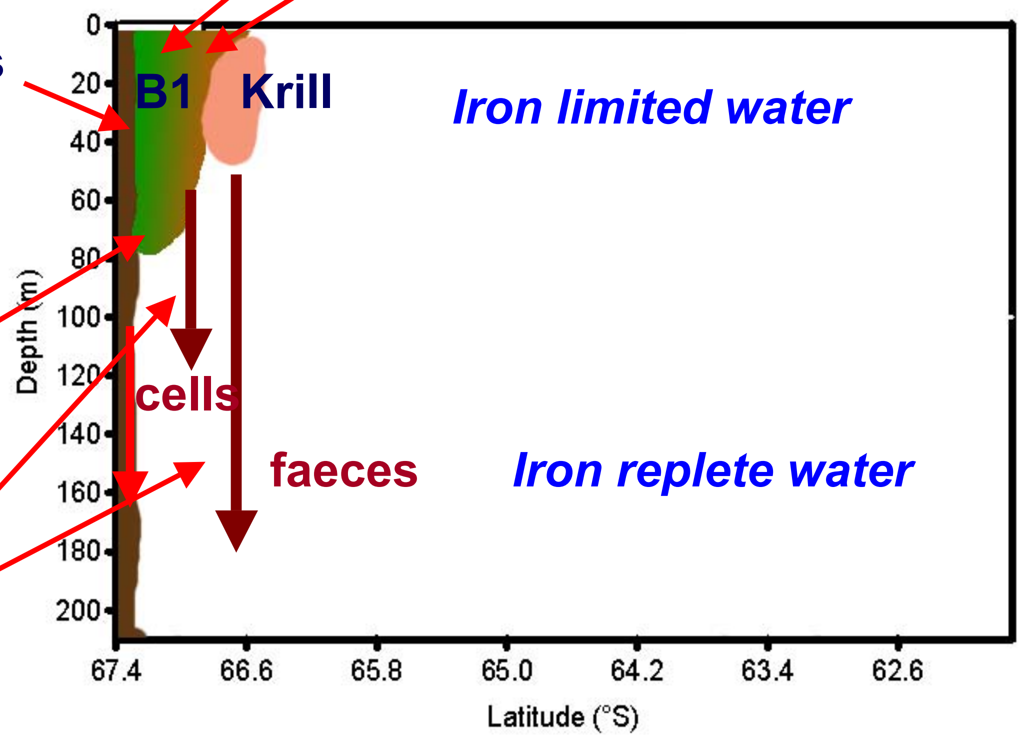
Primary bloom

Initially healthy

Soon binds all iron and senesces

Retreating ice

Sedimenting cells (and nutrients ?) seeds bloom

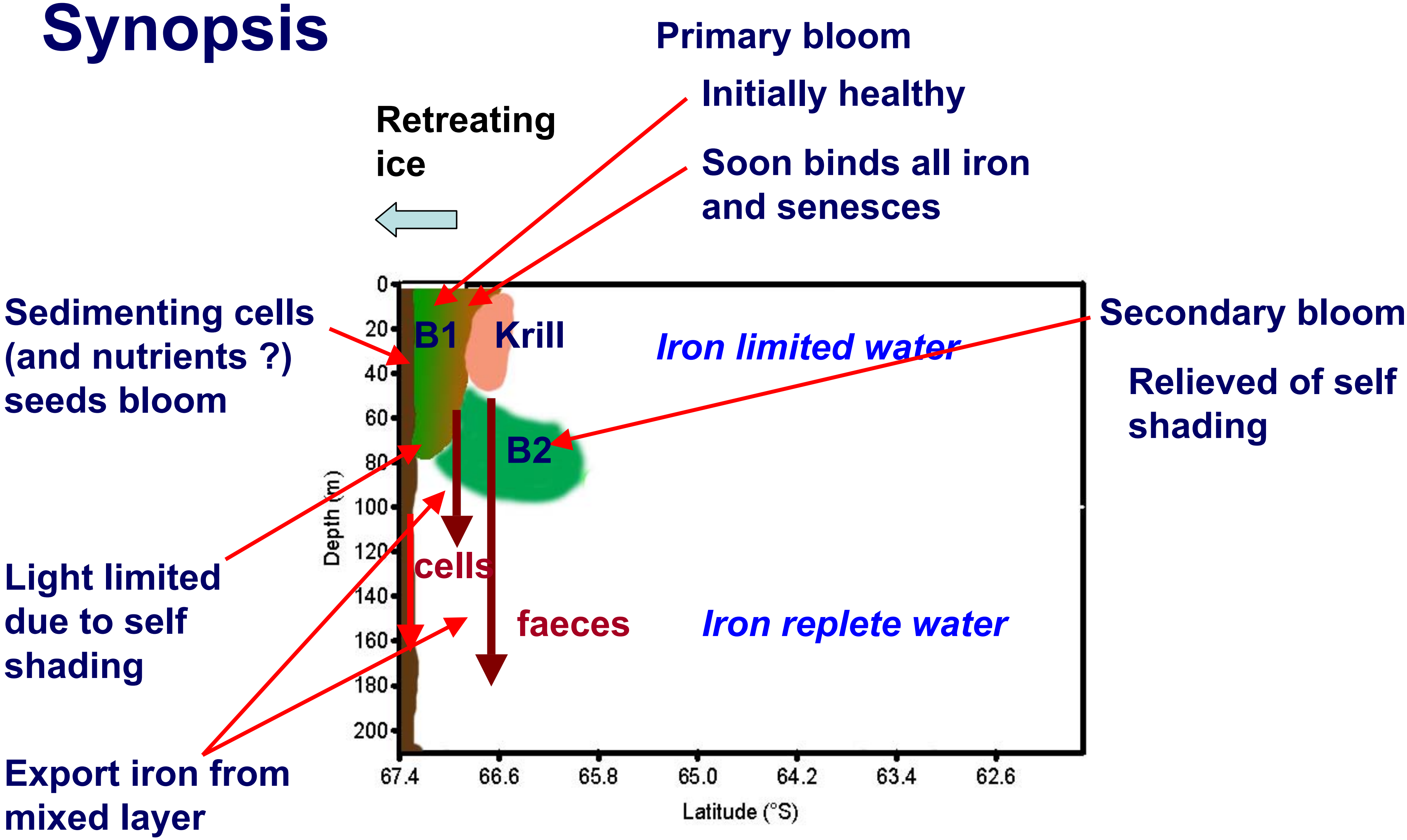


Light limited due to self shading

Export iron from mixed layer

B1:
Diatoms B and A
***Phaeocystis* col or gam (H)**
Cryptophytes

Synopsis



B1:
Diatoms B and A
Phaeocystis col or gam (H)
Cryptophytes

B2:
Phaeocystis gam.(L)
Prasinophytes

Synopsis

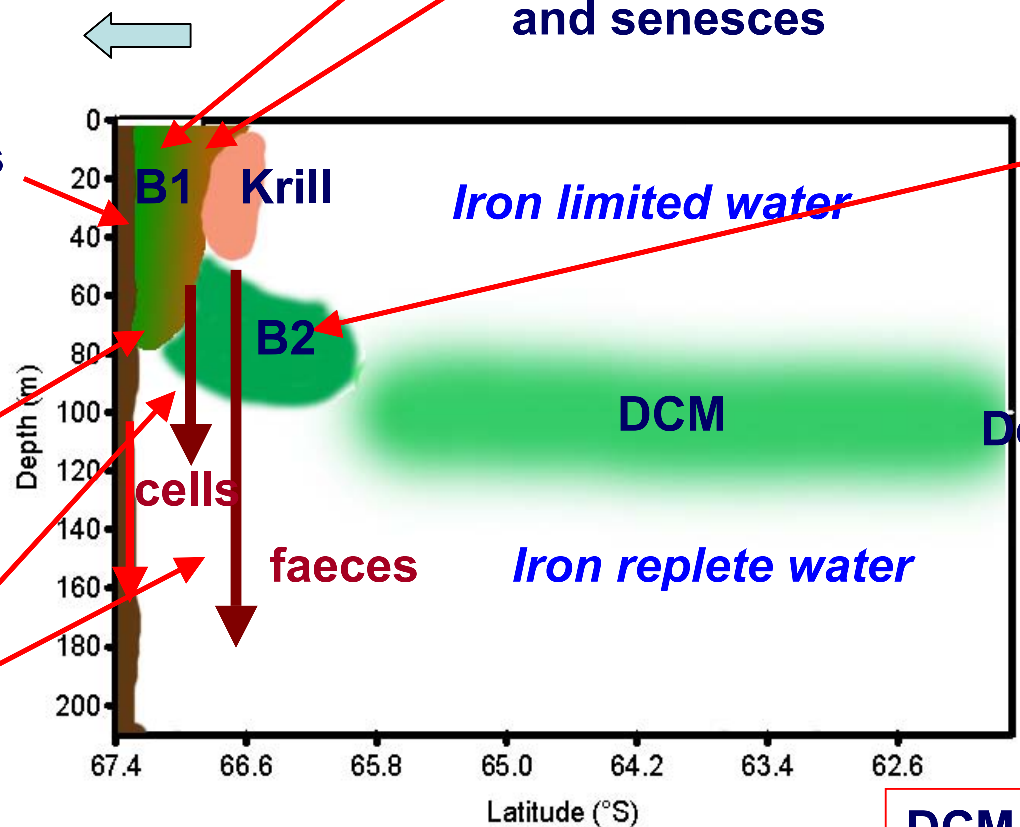
Primary bloom

Initially healthy

Soon binds all iron
and senesces

Retreating
ice

Sedimenting cells
(and nutrients ?)
seeds bloom



Secondary bloom

Relieved of self
shading

Deep Chlorophyll max

Shade community

Low productivity

Recycling

Light limited
due to self
shading

Export iron from
mixed layer

B1:
Diatoms B and A
Phaeocystis col or gam (H)
Cryptophytes

B2:
Phaeocystis gam.(L)
Prasinophytes

DCM:
Prasinophytes
Dinoflagellates
Phaeocystis gam (L)
Parmales
Small diatoms


Save the plankton !

2m. Haptophytes Type 6–8

Type	Data	n	chl c3	MV-chl c3	Mg-DVP	chl c2	chl c1	BUT	FUCO	4k-HEX	HEX	DIAD	DIAT	βε-CAR	ββ-CAR	c2-MGDG [14/18]	c2-MGDG [14/14]
HAPTO-6																	
Culture	LL	4	*0.229*		*0.018*	*0.341*			0.340	*0.125*	0.234	0.094	0.011	*0.013*	0.023	*0.099*	
"	ML	21	0.176	0.016	0.007	0.192		0.005	0.263	0.149	0.562	0.127	0.036		0.025	0.089	
"	HL	6	*0.130*	0.042	*0.022*	*0.290*			0.072	*0.029*	0.248	0.102	0.051		0.020	*0.043*	
"	Min	34	0.110	0.005	0.002	0.145	0	0	0.006	0.029	0.037	0.026	0.004	*0.013*	0.008	0.043	0
"	Max	34	0.229	0.050	0.022	0.341	0	0.008	0.722	0.313	1.507	0.316	0.169	*0.013*	0.049	0.099	0
"	**Mean**	**34**	**0.177**	**0.018**	**0.009**	**0.209**		**0.005**	**0.229**	**0.137**	**0.470**	**0.137**	**0.047**	***0.013***	**0.026**	**0.087**	
"	Std Dev	34	0.031	0.015	0.006	0.066		0.004	0.186	0.082	0.344	0.094	0.054		0.015	0.014	
Field	LL	14	0.150			*0.171*		0.016	0.224		1.342	0.099				*0.097*	
"	ML	15	0.132			0.180	0	0.015	0.186		1.138	0.100		0.007	0.013		
"	HL	42	0.155	*0.119*	0	0.157	0	0.019	0.195		1.054	0.122		0.014	0.030	*0.090*	0
"	Min	86	0.033	*0.119*	0	0.080	0	0	0	0	0.228	0.015	0	0.006	0.012	0.082	0
"	Max	86	0.300	*0.119*	0	0.230	0	0.198	0.722	0	2.066	0.196	0	0.014	0.030	0.097	0
"	**Mean**	**86**	**0.146**	***0.119***	**0**	**0.168**	**0**	**0.015**	**0.195**		**1.214**	**0.109**		**0.010**	**0.021**	**0.091**	**0**
"	Std Dev	86	0.087			0.048	0	0.028	0.172		0.595	0.046		0.004	0.010	0.008	
References (see Table p): 13, 15, 16, 18, 19, 21–24, 26–29, 31–35, 37, 40, 42–45, 47, 61, 63–65																	
HAPTO-7																	
Culture	LL	7	0.215	*0.017*	0.010	0.227	*0.009*	*0.023*	0.420	0.100	0.682	0.181	0.005	0.025	0.030	*0.091*	0.110
"	ML	15	0.210	*0.028*	0.015	0.198	*0.009*	0.009	0.436	0.215	0.543	0.267	0.015	0.017	0.037	0.097	0.081
"	HL	6	0.171	*0.030*	0.020	0.251	*0.025*	*0.013*	0.259	0.037	0.491	0.167	0.086	*0.007*	0.022	*0.085*	0.131
"	Min	29	0.060	0.008	0.003	0.094	0.009	0	0.004	0.016	0.007	0.070	0.005	0.007	0.013	0.002	0.027
"	Max	29	0.346	0.041	0.045	0.400	0.025	0.024	1.184	0.674	1.315	0.644	0.365	0.045	0.068	0.285	0.300
"	**Mean**	**29**	**0.202**	**0.026**	**0.015**	**0.216**	**0.014**	**0.011**	**0.388**	**0.151**	**0.567**	**0.201**	**0.061**	**0.020**	**0.030**	**0.094**	**0.103**
"	Std Dev	29	0.052	0.011	0.011	0.071	0.009	0.008	0.365	0.160	0.309	0.136	0.115	0.011	0.015	0.084	0.078
Field	LL	2	*0.079*			*0.144*		*0.009*	*0.125*		*1.077*						*0.134*
"	ML	0															
"	HL	4	0.171			0.190	0	0.004	0.244		0.706						0.109
"	Min	8	0.058	0	0	0.110	0	0	0.009	0	0	0	0	0	0	*0.034*	0
"	Max	8	0.296	0	0	0.422	0	0.062	0.465	0	1.281	0	0	0	0	*0.034*	0.188
"	**Mean**	**8**	**0.136**			**0.198**	**0**	**0.012**	**0.199**		**0.794**					***0.034***	**0.101**
"	Std Dev	8	0.088			0.101	0	0.021	0.168		0.386						0.073
References (see Table p): 25, 54, 61, 63, 64																	
HAPTO-8																	
Culture	LL	4	0.222	*0.010*	0.071	0.232		0.043	0.555	0.049	0.250	0.125	0.006	*0.027*	0.028	0.069	
"	ML	22	0.176	0.018	0.007	0.179		0.129	0.274	0.062	0.422	0.216	0.039	*0.024*	0.053	0.055	

- **5.4.3 Guide for quantitative chemotaxonomic interpretation of pigment data**
  - Step by step guide:
    - Examine the pigment data for specific markers for algal types (Section 5.2.1)
    - Examine available complementary data
      - *Microscopy data*:
      - *Flow cytometry and FlowCAM data*:
      - *In situ and in vivo fluorometry data*: *in situ* fluorescence profiles
      - *Environmental data*:
      - *Remote sensing data*:
      - *Productivity and grazing data*:
      - *Cluster analysis*
    - Pigment data exploration:
      - *Multiple linear regression (MLR)*
      - *Testing correlation*
      - *diatoxanthin + diadinoxanthin:Chl a*
    - CHEMTAX analysis
      - *Sub-grouping*
      - *Initial pigment:Chl a ratio and ratio limit matrices*
      - *Preliminary CHEMTAX analysis*
      - *Comprehensive CHEMTAX analysis*
      - *Publication of CHEMTAX (or ISE) estimates*

CHEMTAX uses a steepest descent algorithm

Like dropping a blind parachutist and telling him to walk downhill

The parachutist might get trapped in local minima

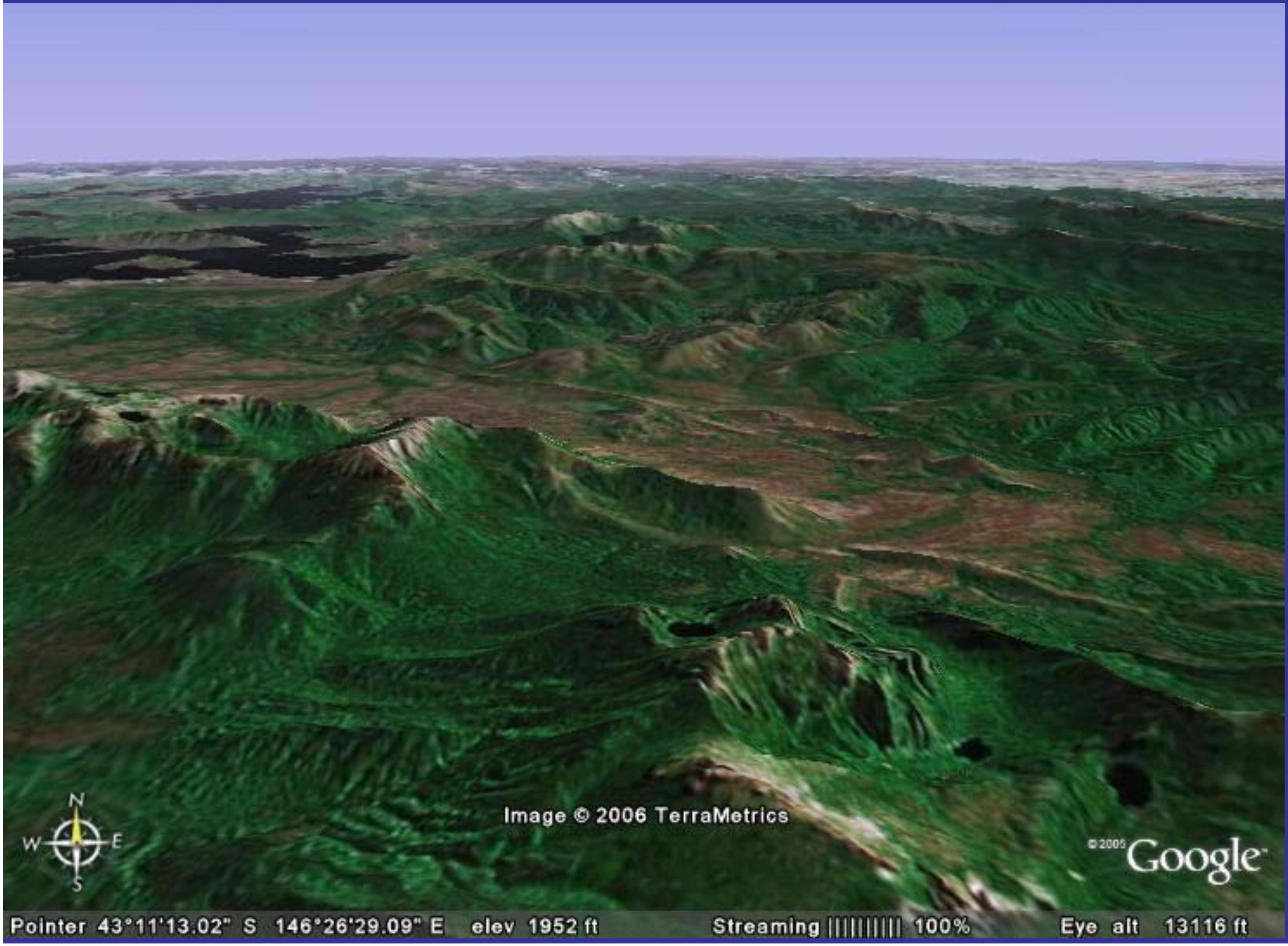



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Image © 2006 TerraMetrics
©2005 Google
Pointer 36°13'14.91" N 112°17'09.37" W elev 5548 ft
Streaming |||||||||| 100%
Eye alt 13248 ft