Phytoplankton Pigment Analysis Using HPLC

PART 2

ITCO, Training Program, INCOIS, 19-23 Mar 2018

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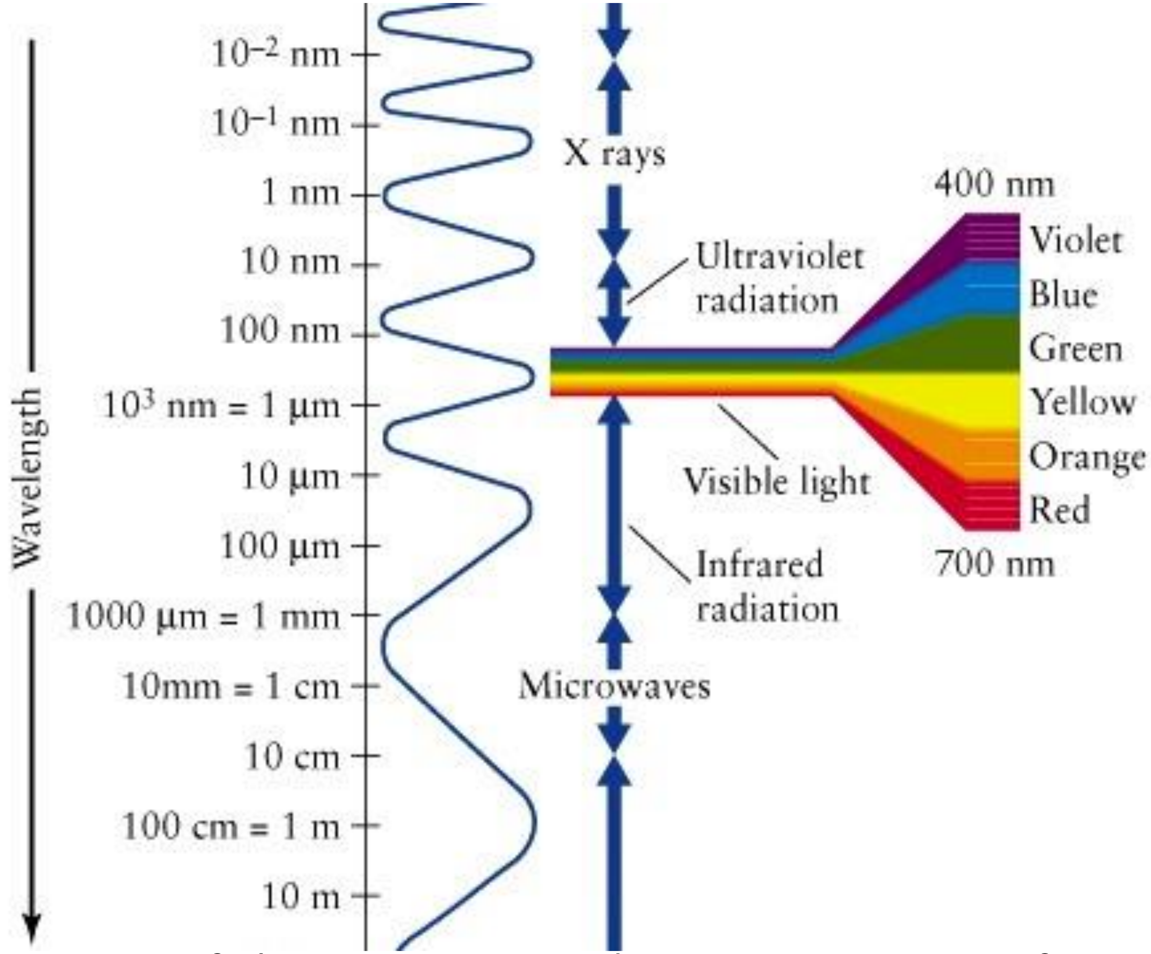
PAR = photosynthetically active radiation (visible light wavelengths)

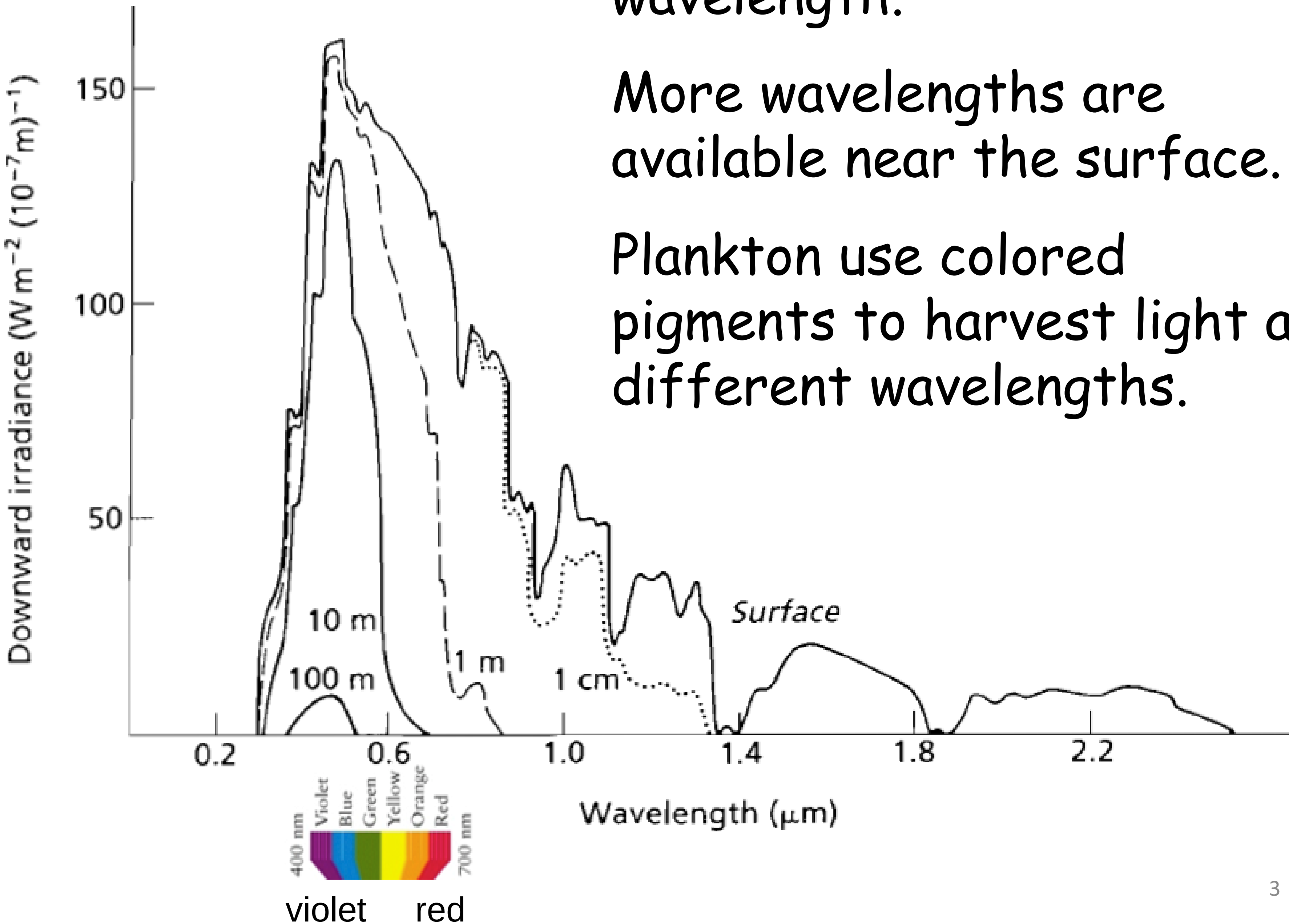


Irradiance = power of electromagnetic radiation per unit area of ocean's surface (e.g. W m^{-2}) - or -
energy per area per time (e.g. mol photons m^{-2} s^{-1}).

Attenuation varies with wavelength.

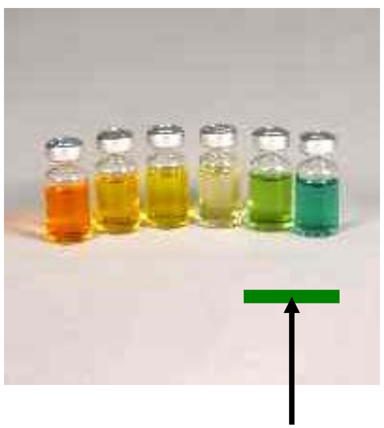
More wavelengths are available near the surface.

Plankton use colored pigments to harvest light at different wavelengths.



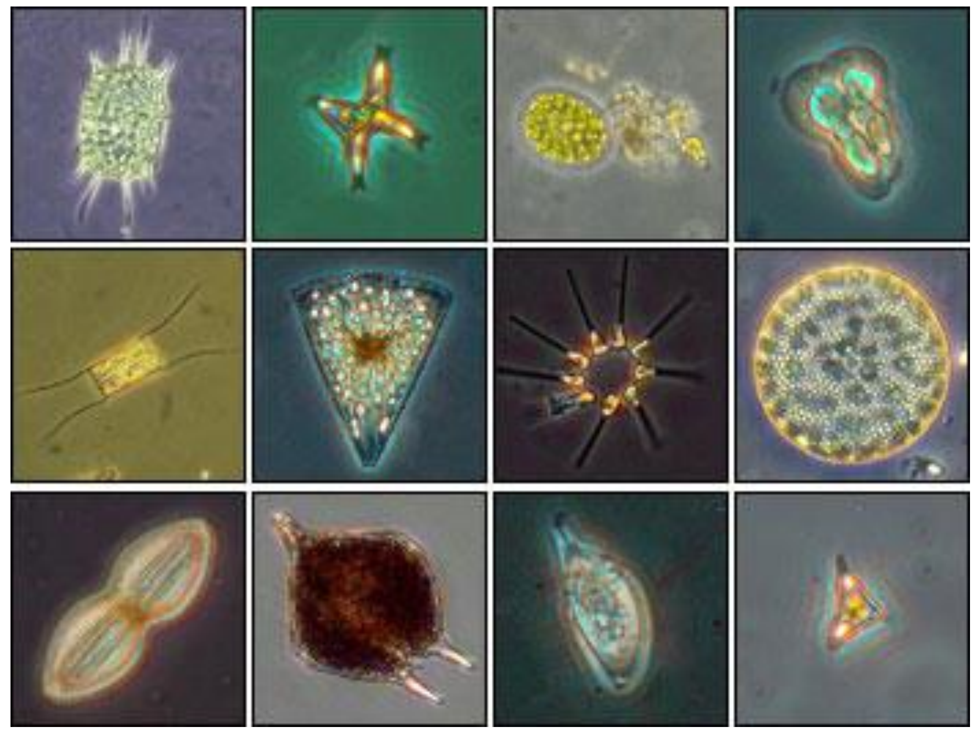
Different color pigments absorb different wavelengths of light

Pigments (colored molecules)

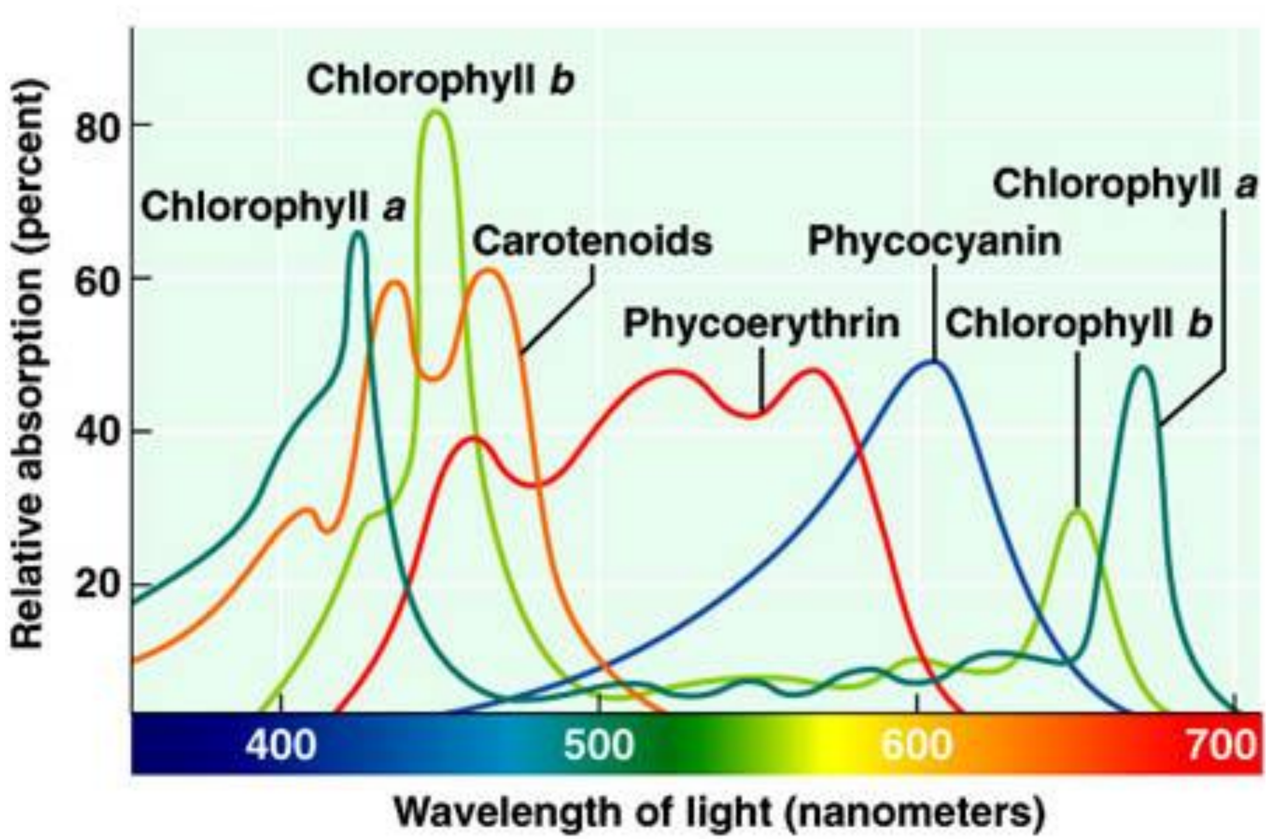


Chlorophyll*

Phytoplankton with different pigments



Phytoplankton with multiple pigments capture more wavelengths



- All phytoplankton have chlorophyll
- Coccolithophores and diatoms have carotenoids
- Cyanobacteria have phycoerythrin, phycocyanin

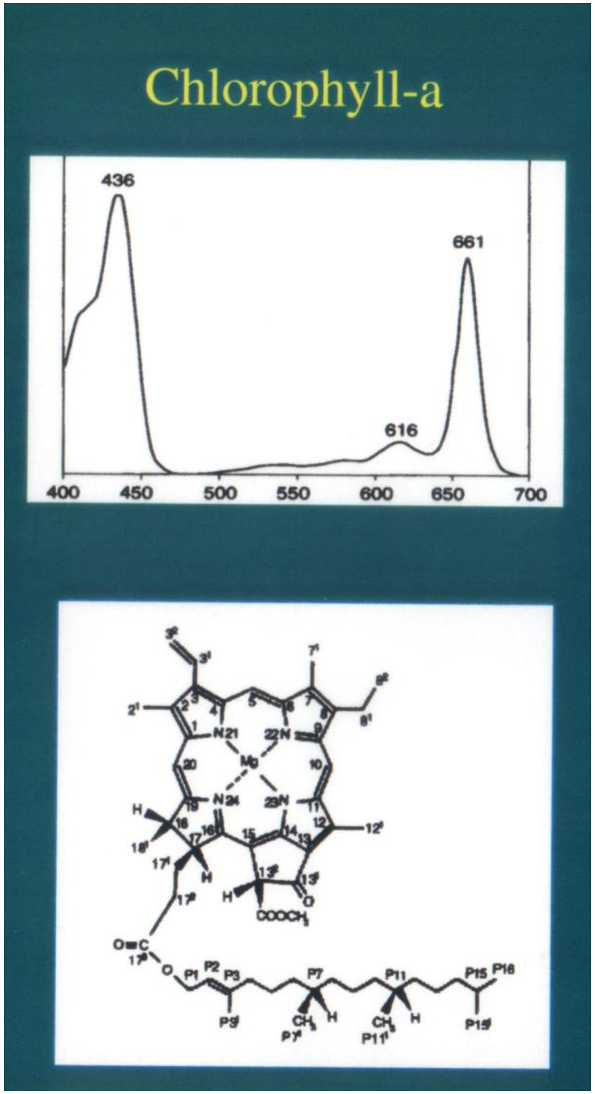
Phytoplankton pigments

- Chlorophyll-*a* (or its substitutes bacteriochlorophyll-*a* or divinyl-chlorophyll-*a*) is located in the RCs of all photosynthetic organisms.

- Three main types of accessory pigments: chlorophylls, carotenoids and biliproteins are located in the subantennae and LHCs of different taxonomic groups of algae.

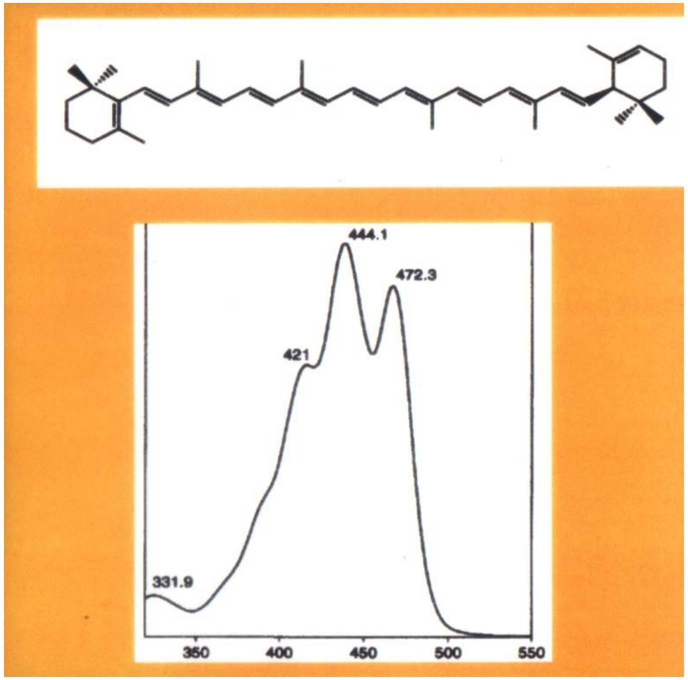
Chlorophylls

- Green coloured pigments.
- Absorb light energy in the blue and red regions of the spectrum.
- Porphyrin ring – conjugated double bonds, magnesium ion, nonpolar phytol tail.
- Three main types: *a*, *b*, and *c* (divinyl-chl-*a*, -*b*, chl-c_1, -c_2, -c_3).
- Fluoresce (máximum 680 nm).
- Photochemistry, and light-harvesting.



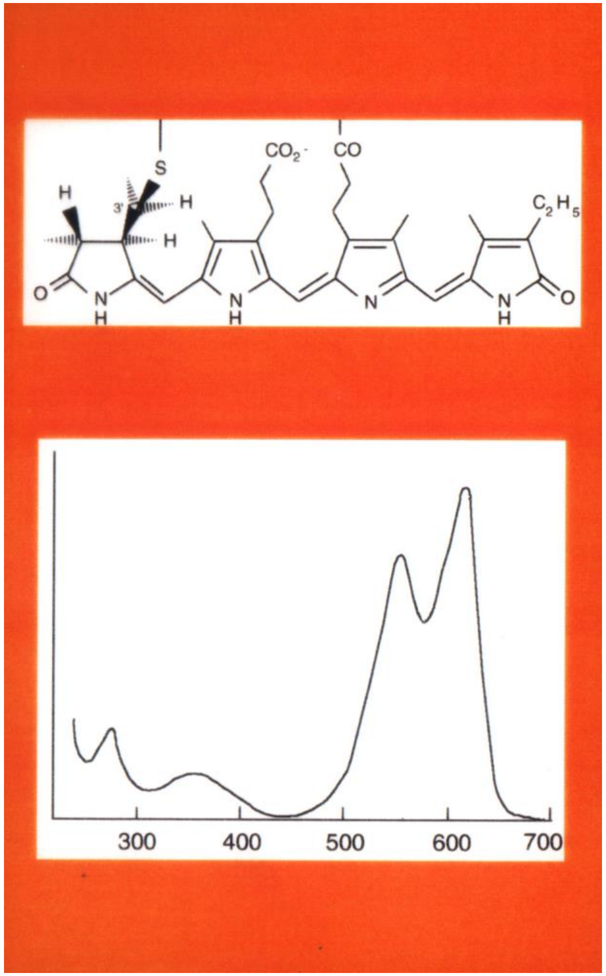
Carotenoids

- Red, orange or yellow pigments.
- Absorb light in the blue-green region.
- Conjugated hydrocarbons.
- Two main groups: carotenes (e.g., b-carotene) and xanthophylls (e.g., fucoxanthin).
- Do not fluoresce *per se*.
- Some light-harvesting, some photoprotective.



Phycobilins

- Brightly coloured pigments (red, orange, pink).
- Absorb light in the green-yellow region.
- Linear tetrapyrroles (water soluble).
- Four major types: phycocyanin, phycoerythrin, allophycocyanin, phyoerythrocyanin.
- Fluoresce (máximum 570 nm).
- Light-harvesting.



Pigment composition in phytoplankton taxa

Algal Division/Class	Common Name	Genera
Golden-brown algae (chl-*a* and *c*)		
Bacillariophyta	diatoms	210
Dinophyta	dinoflagellates	550
Chrysophyta	Golden-brown flagellates	
Chrysophyceae	chrysophytes,silicoflagellates	120
Raphydophyceae	chloromonads	4
Haptophyta	Golden-brown flagellates	
Prymnesiophyceace	coccolithophorids	50
Xanthophyta	Yellow-green algae	600
Cryptophyta*	cryptomonads	8
Eustigmatophyta	Yellow-green algae	6
Green algae (chl-*a* and –*b*)		
Chlorophyta		
Chlorophyceae	green algae	350
Prasinophyeceae	green flagellates	13
Euglenophyta	euglenoids	43
Rhodophyta (chl-*a* and biliproteins)		
Rhodophyta	red algae	3
Blue-green algae (chl-*a* and biliproteins)		
Cyanophyta	cyanobacteria	??
	prochlorophytes	3

Introduction Contd…

Pigment	Abbreviation	Groups of phytoplankton
Alloxanthin	Allo	Cryptophytes
19'-Butanoyloxyfucoxanthin	But	Chrysophytes, prymnesiophytes
Chlorophyll *a*	Chl a	Total algal biomass
Chlorophyll *b*	Chl b	Chlorophytes, prasinophytes
Chlorophyll c1	Chl c1	Diatoms,dinoflagellates, prymnesiophytes,chrysophytes
Chlorophyll c2	Chl c2	Diatoms,dinoflagellates, prymnesiophytes,chrysophytes
Chlorophyllc3	Chl c3	Prymnesiophytes,chrysophytes
Diadinoxanthin	Diad	Diatoms,dinoflagellates, prymnesiophytes,chrysophytes
Diatoxanthin	Diat	Diatoms,dinoflagellates, prymnesiophytes,chrysophytes
Divinyl chlorophyll *a*	DvCHL *a*	Prochlorophytes
Divinyl chlorophyll *b*	DvCHL *b*	Prochlorophytes
Fucoxanthin	Fuc	Diatoms, prymnesiophytes,chrysophytes
19'-Hexanoyloxyfucoxanthin	Hex	Prymnesiophytes
Lutein	Lut	Chlorophytes, prasinophytes
Peridinin	Per	Autotrophic dinoflagellates
Violaxanthin	Viol	Chlorophytes, prasinophytes
Prasinoxanthin	Pras	Prasinophytes
Zeaxanthin	Zea	Cyanobacteria, prochlorophytes

Introduction Contd…

Symbol	Pigment	Designation
	Pigment Sum	
TChla	Total chlorophyll *a*	Chl a+DVChla
Chlbc	Sum of chlorophyll b and c	$Chlb+Chlc_1+Chlc_2+Chlc_3$
PPC	Photoprotective carotenoids	Allo+Caro+Diad+Diat+Lut+Viol+Zea
PSC	Photosynthetic carotenoids	But+Fuc+Hex+Per
TPig	Total pigments	TChl *a* +Chlbc+PPC+PSC
DP	Diagnostic pigments	Allo+But+Chlb+Fuc+Hex+Per+Zea
DVChla/TChl a	Divinyl Chlorophyll *a* to Total Chlorophyll *a*	DVChl *a*/TChl *a*
$TChla_{TP}$	Total chlorophyll *a* to Total pigments	TChl *a* /Tpig
PPC_{TP}	Photoprotective carotenoids to Total pigments	PPC/ Tpig
PSC_{TP}	Photosynthetic carotenoids to Total pigments	PSC/ Tpig
DiatDP	Diatom proportion of DP	Fuc/DP
FlagDP	Flagellate proportion of DP	(Allo+But+Chlb+Hex)/DP
ProkDP	Prokaryote proportion of DP	Zea/DP

Pigment indices along with symbols and designated formulas based on and Barlow et al. (2007).

“Carotenoids + chlorophylls” as marker pigments: improving the phytoplankton community structure derived from HPLC pigment signatures. I: Discovery of new pigments

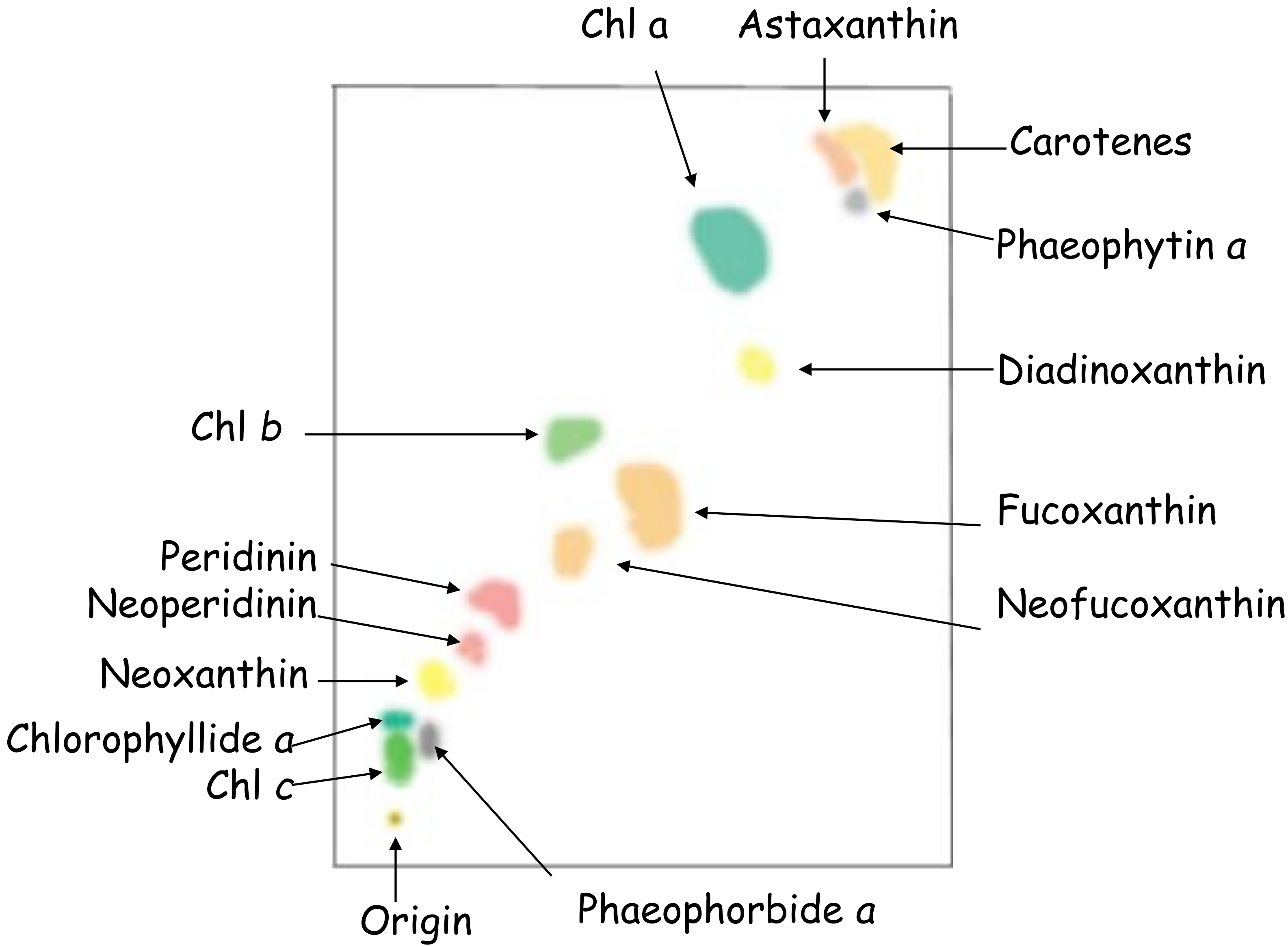
- **Divinyl chl** *a* **and** *b* **in free-living prochlorophytes e.g.** *Prochlorococcus marinus* **(Chisholm et al 1992; Goericke & Repeta 1992, 1993).**

- **Monovinyl chl** c_3 **(in addition to divinyl chl** c_3**; Jeffrey & Wright 1987; Fookes & Jeffrey 1989) in haptophytes as** *Emiliania huxleyi* **(Garrido & Zapata, 1998).**

- **Two new chl *c* pigments in *Pavlova gyrans* (Garrido & Zapata 1997) first noted by Fawley (1989).**

- **Three non-polar chl *c* pigments detected in *E. huxleyi*, *Prymnesium parvum* and *Chrysochromulina polylepis* (Chl** c_2**-MGDG; Garrido et al. 1995; Zapata et al. 1998; Garrido et al. 2000).**

TLC Jeffrey 1974



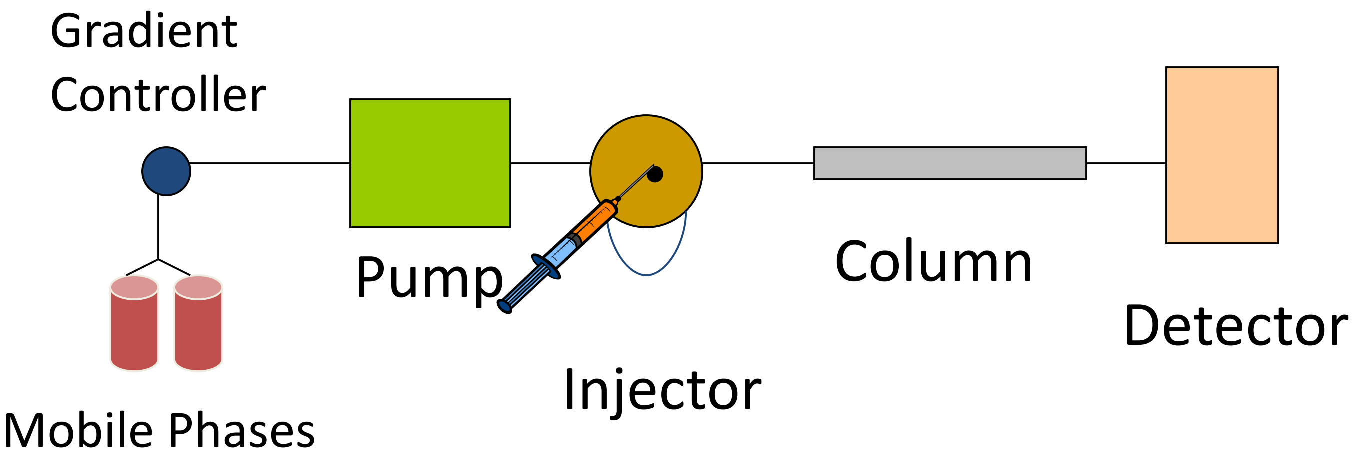
Column
s

- Solid Support - Backbone for bonded phases.
  - Usually 10µ, 5µ or 3µ silica or polymeric particles.
- Bonded Phases - Functional groups firmly linked (chemically bound) to the solid support.
  - Extremely stable
  - Reproducible
- Guard - Protects the analytical column:
  - Particles
  - Interferences
  - Prolongs the life of the analytical column
- Analytical - Performs the separation.

Bonded Phases

• C-2	Ethyl Silyl	$-Si-CH_2-CH_3$
• C-8	Octyl Silyl	$-Si-(CH_2)_7-CH_3$
• C-18	Octadecyl Silyl	$-Si-(CH_2)_{17}-CH_3$
• CN	Cyanopropyl Silyl	$-Si-(CH_2)_3-CN$

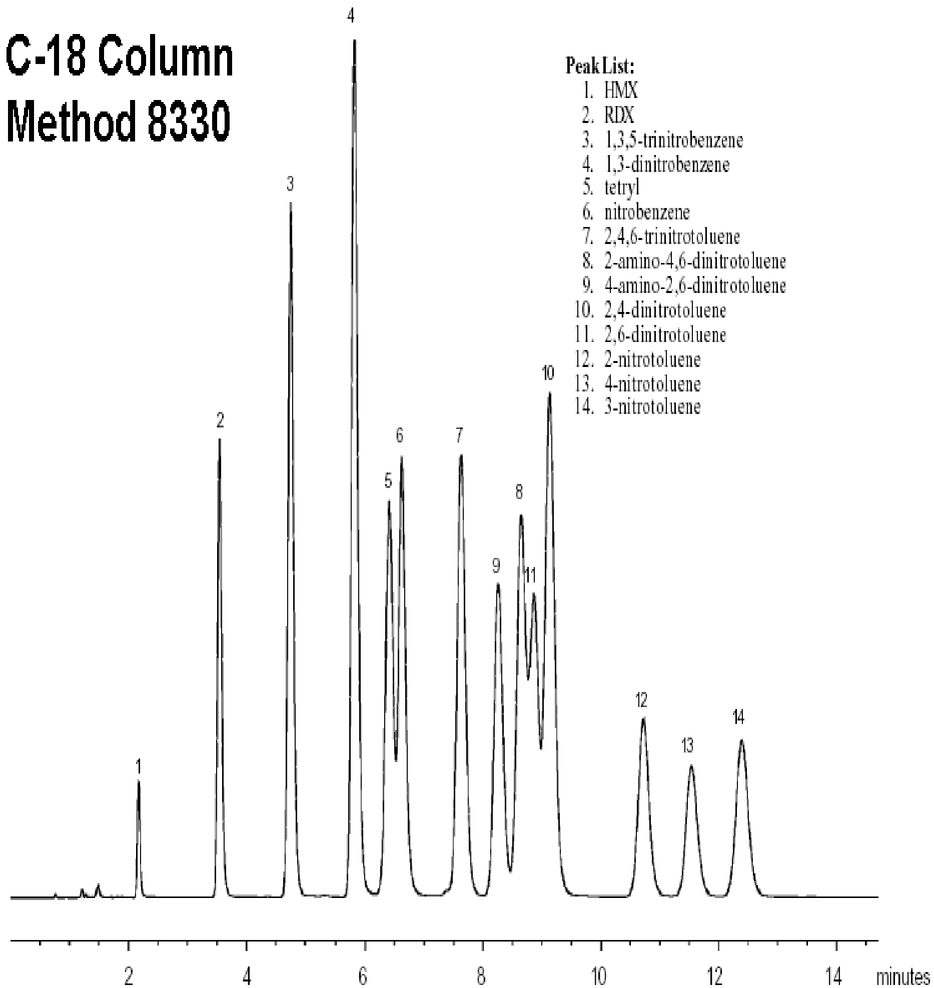
Instrumentation

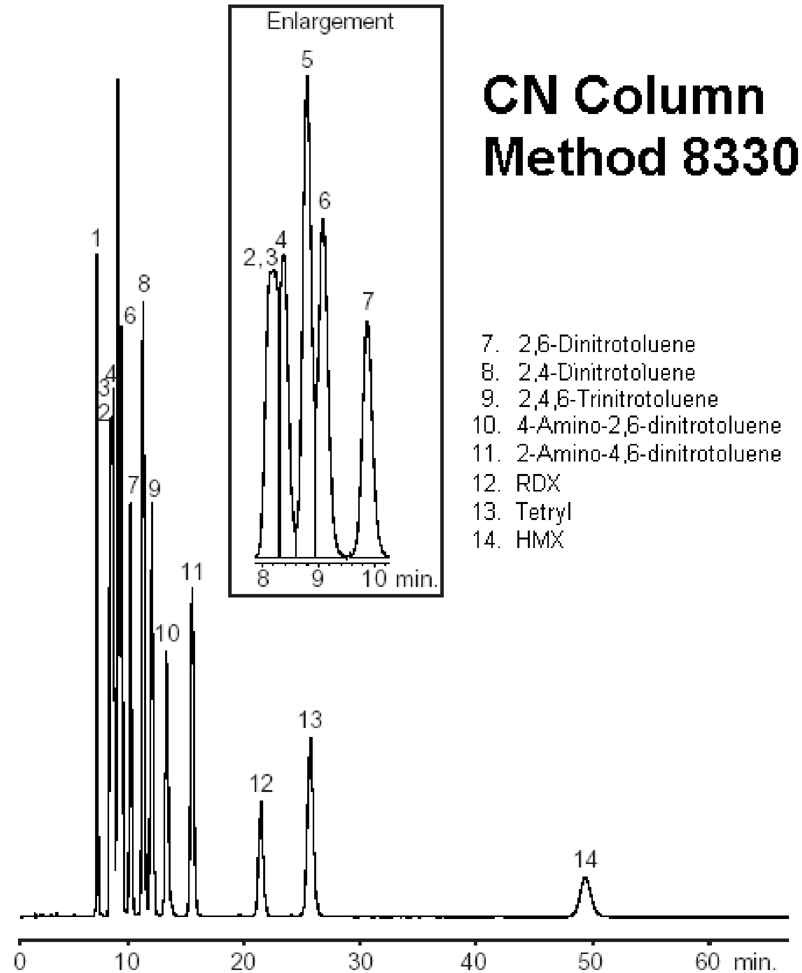


Detectors

- UV
  - Single wavelength (filter) [610, 8330]
  - Variable wavelength (monochromator) [8316, 8325]
  - Multiple wavelengths (PDA) [555]
- Fluorescence [610]
- Electrochemical [605]
- Mass Spectrometric [8325]

Chromatograms





Restek® ULTRA C-18 and CN Columns (250mm x 4.6mm, 5µ), Mobile Phase: (1:1 Methanol:Water), 1.5 mL/min.

In earlier times, we thought in terms of individual marker pigments indicating particular algal types or processes.

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

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 ●									

Jeffrey and Vesk (1997)

Major marker pigments

Ubiquitous
- Chl *a*

Unambiguous
- Alloxanthin
- Peridinin
- Prasinoxanthin

Shared
- e.g.
- 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

We can no longer talk in terms of individual marker pigments. Instead we talk of "SUITES" of pigments that may cross conventional taxonomic boundaries.

By the late 80's it became very apparent that normal interpretation of pigment data amounted to little more than guesswork.

There was an urgent need for objective computational methods for determining the phytoplankton community composition from pigment data.

Computation methods

1. Simple or multiple linear regression
2. Multiple simultaneous equations

Letelier *et al.* 1993

$[Chla]_{Prochl} = 0.91([Chl\textit{b}] - 2.5[Prasino])$

$[Chla]_{Cyano} = 2.1\{[zeax] - 0.07([Chl\textit{b}] - 2.5[Prasino])\}$

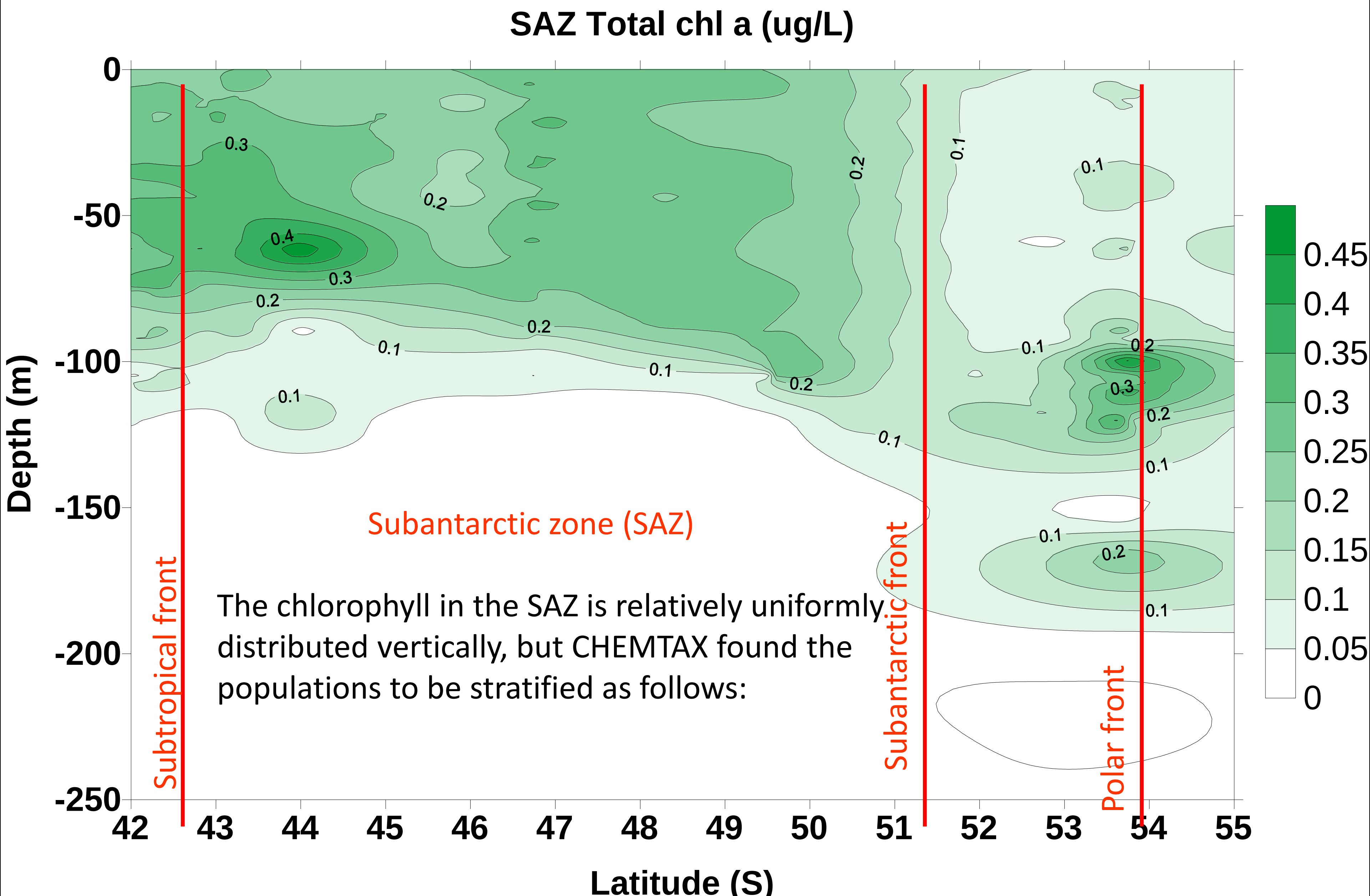
$[Chla]_{Chrys} = 0.9[19'\text{-}but]_{Chrys}$

$[Chla]_{Prym} = 1.3[19'\text{-}hex]_{Prym}$

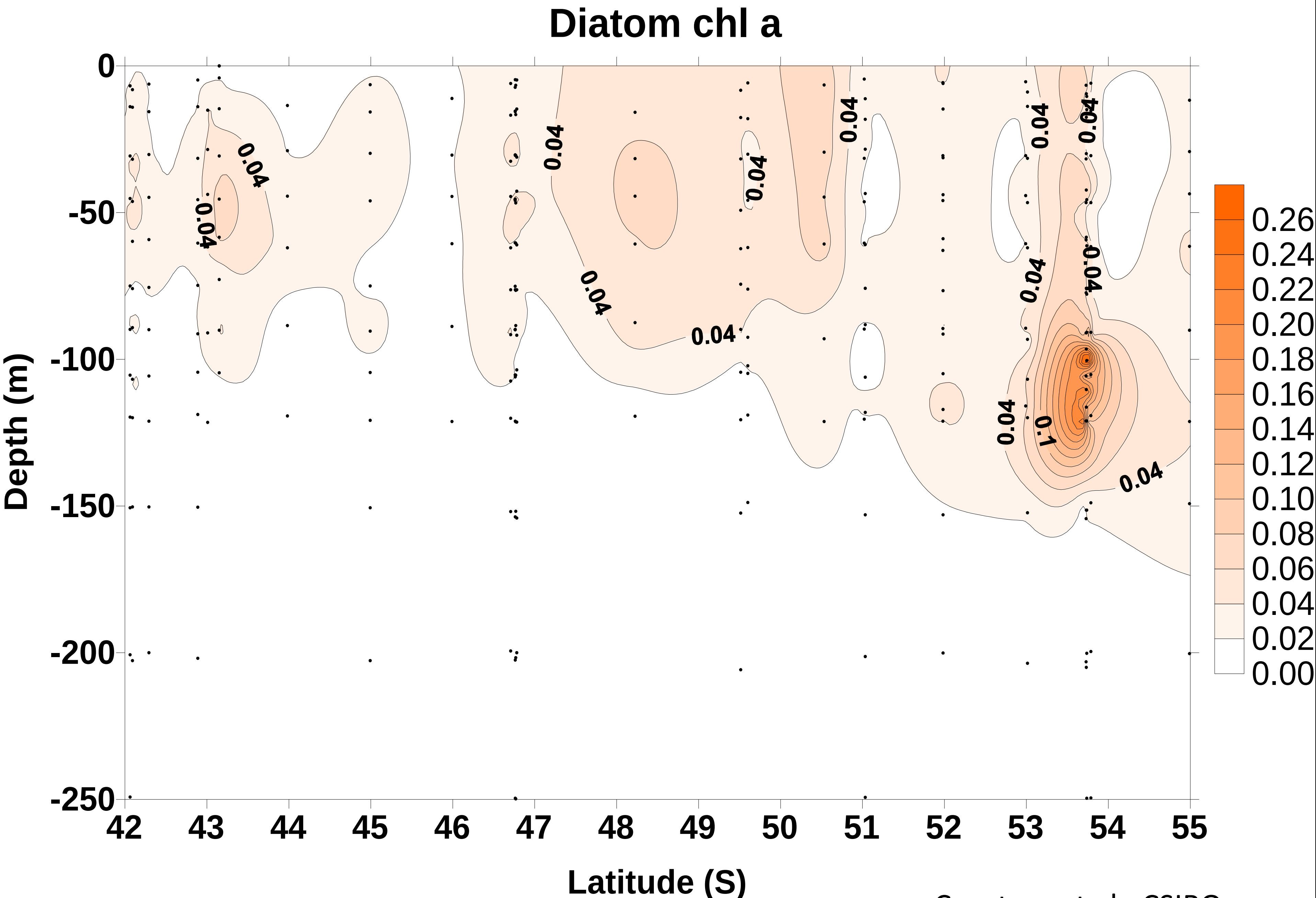
$[Chla]_{Bacill} = 0.8\{[fuco] - (0.02[19'\text{-}hex]_{Prym} + 0.14[19'\text{-}but]_{Chrys})\}$

$[Chla]_{Dino} = 1.5[perid]$

$[Chla]_{Pras} = 2.1[prasino]$



Courtesy et al., CSIRO



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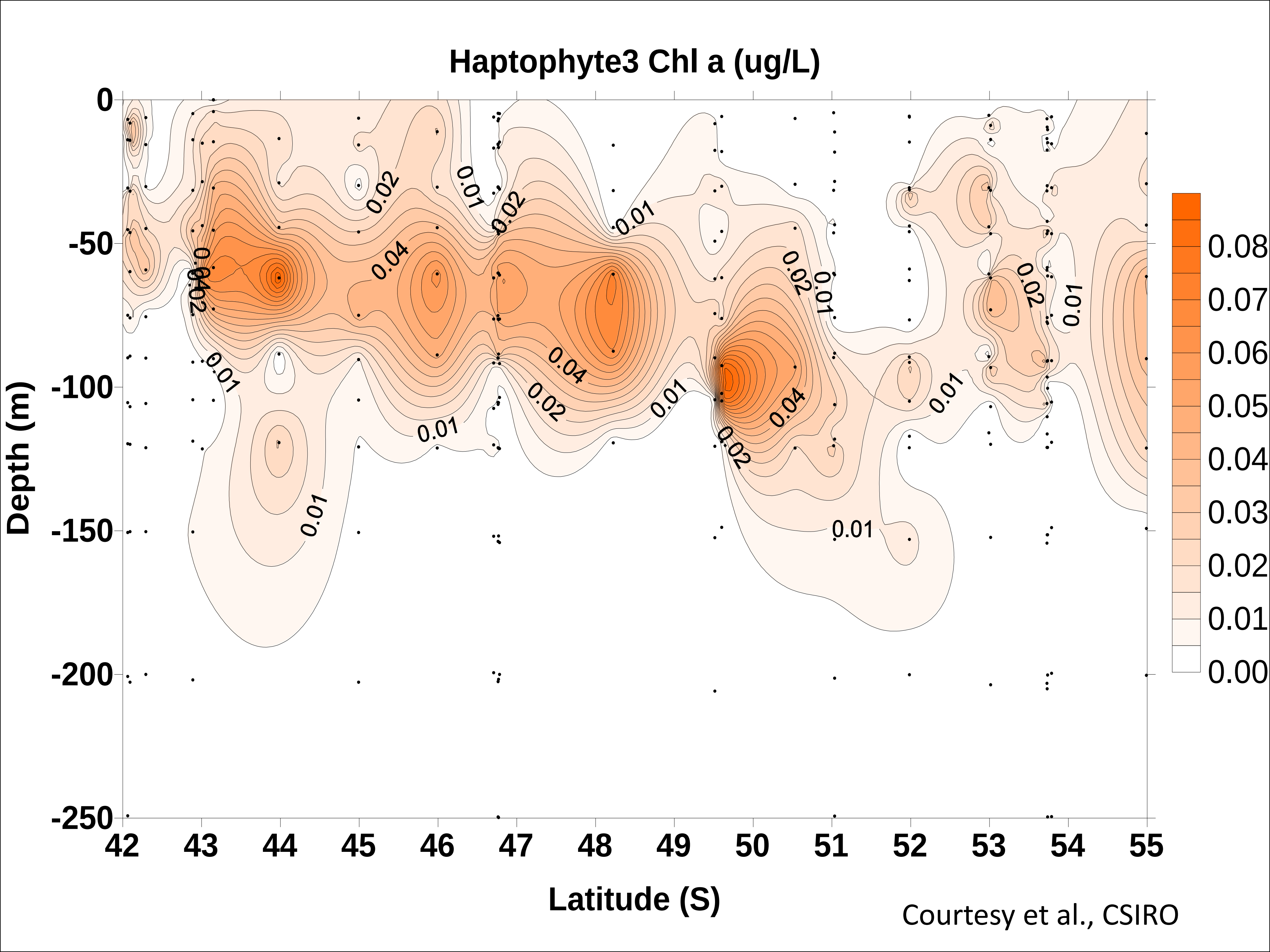
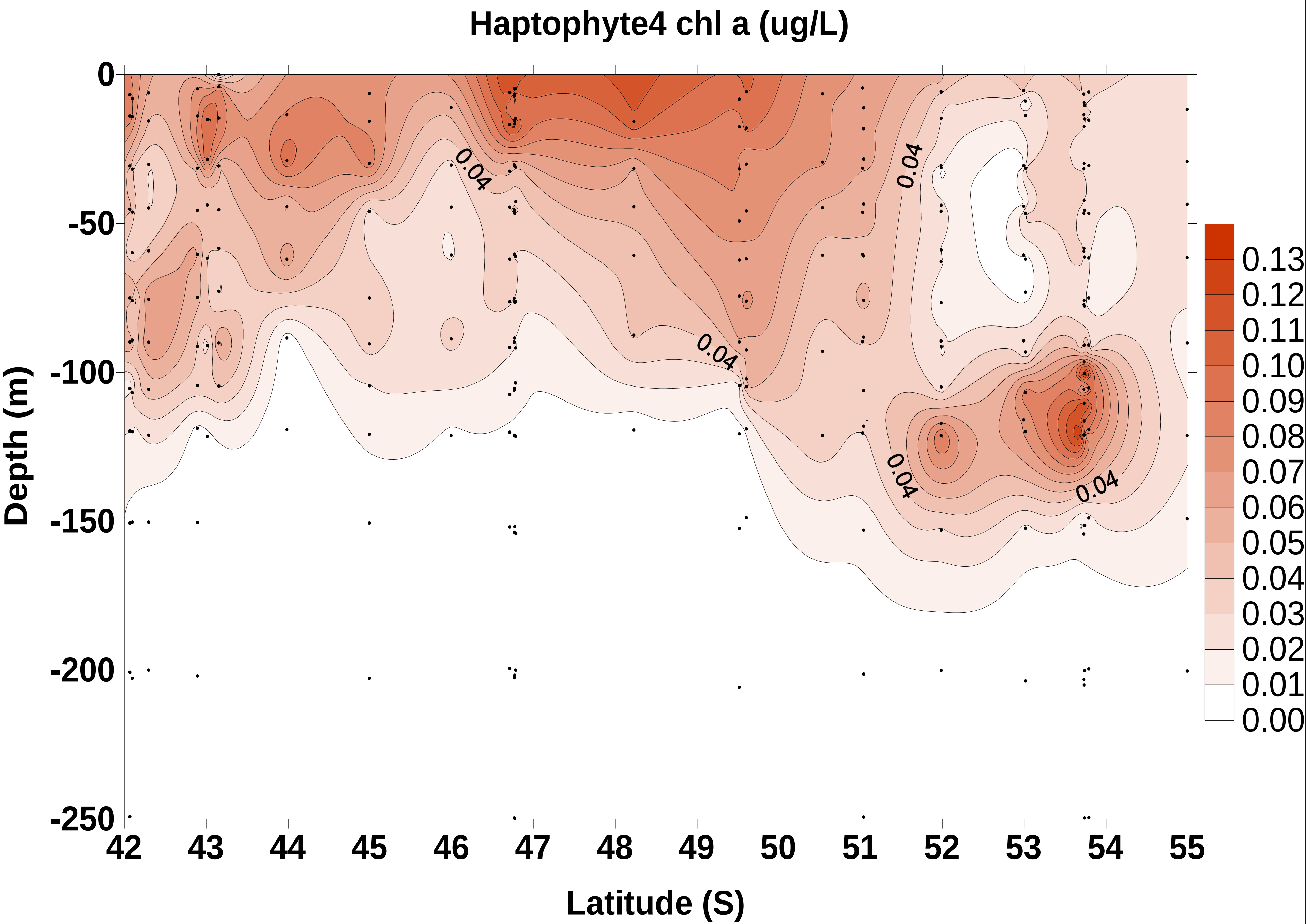
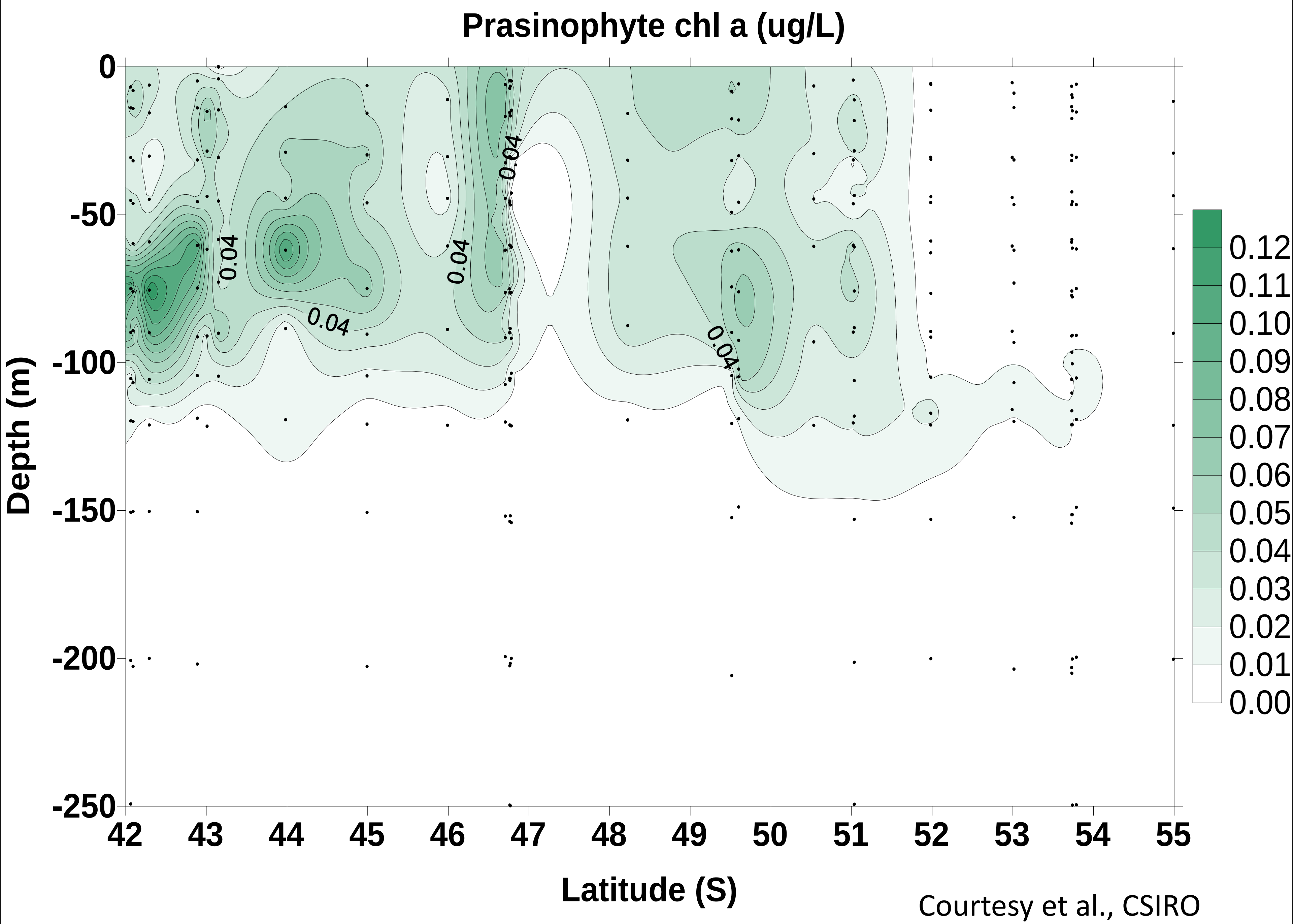
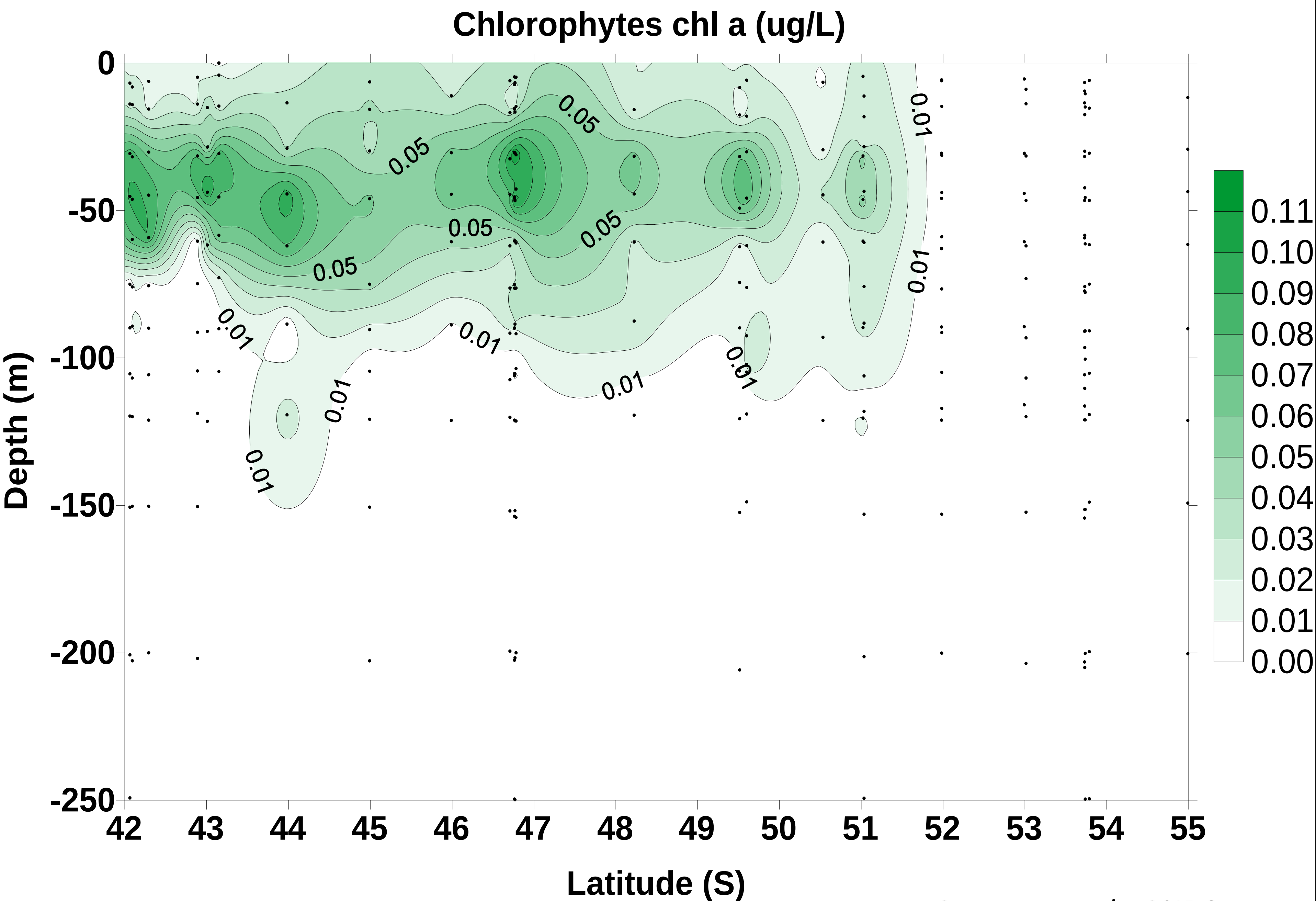

Haptophyte3 Chl a (ug/L)
0
-50
-100
-150
-200
-250
Depth (m)
42
43
44
45
46
47
48
49
50
51
52
53
54
55
Latitude (S)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.01
0.02
0.04
Courtesy et al., CSIRO

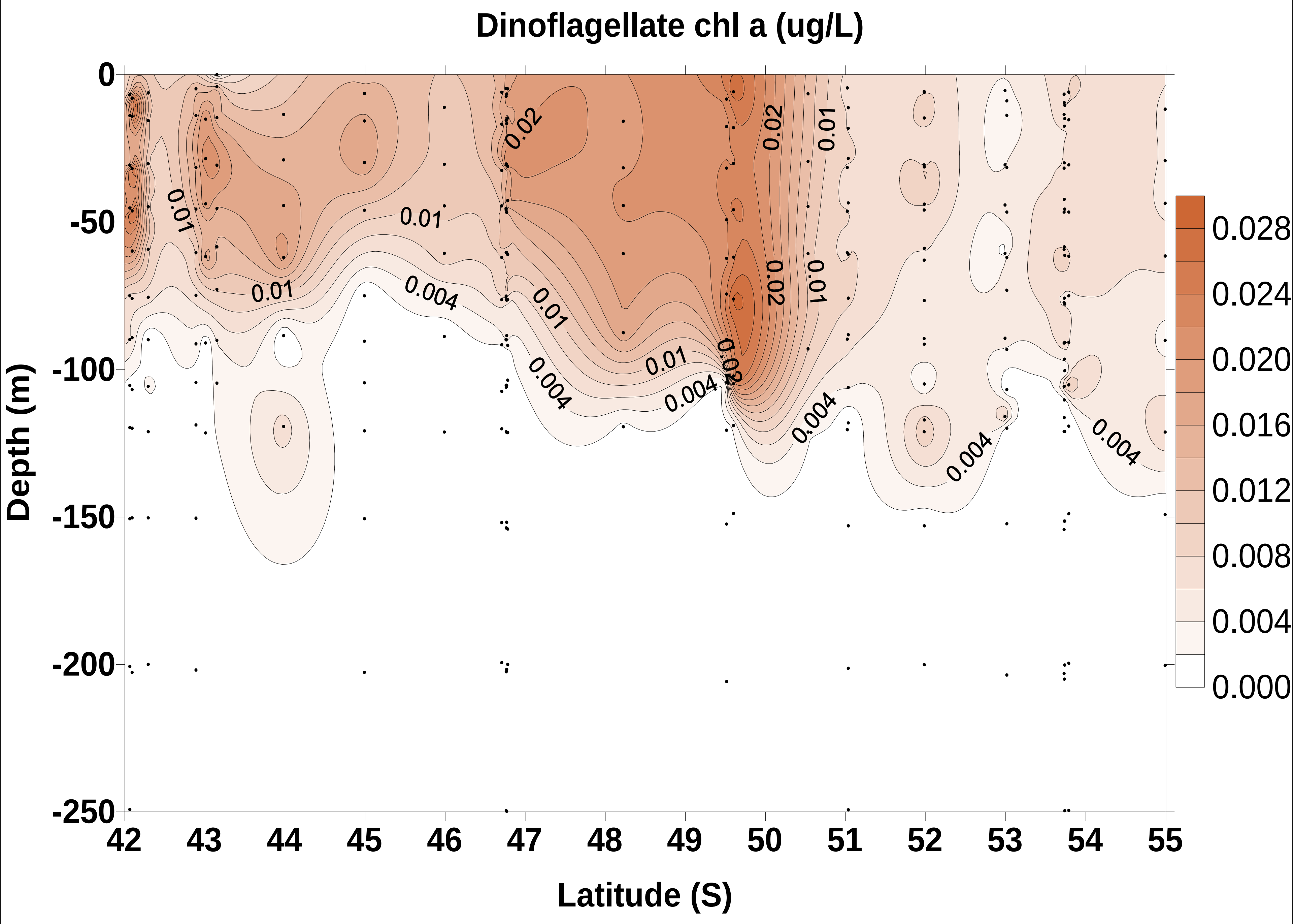


Haptophyte4 chl a (ug/L)
0
-50
-100
-150
-200
-250
Depth (m)
42
43
44
45
46
47
48
49
50
51
52
53
54
55
Latitude (S)
0.04
0.04
0.04
0.04
0.04
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00

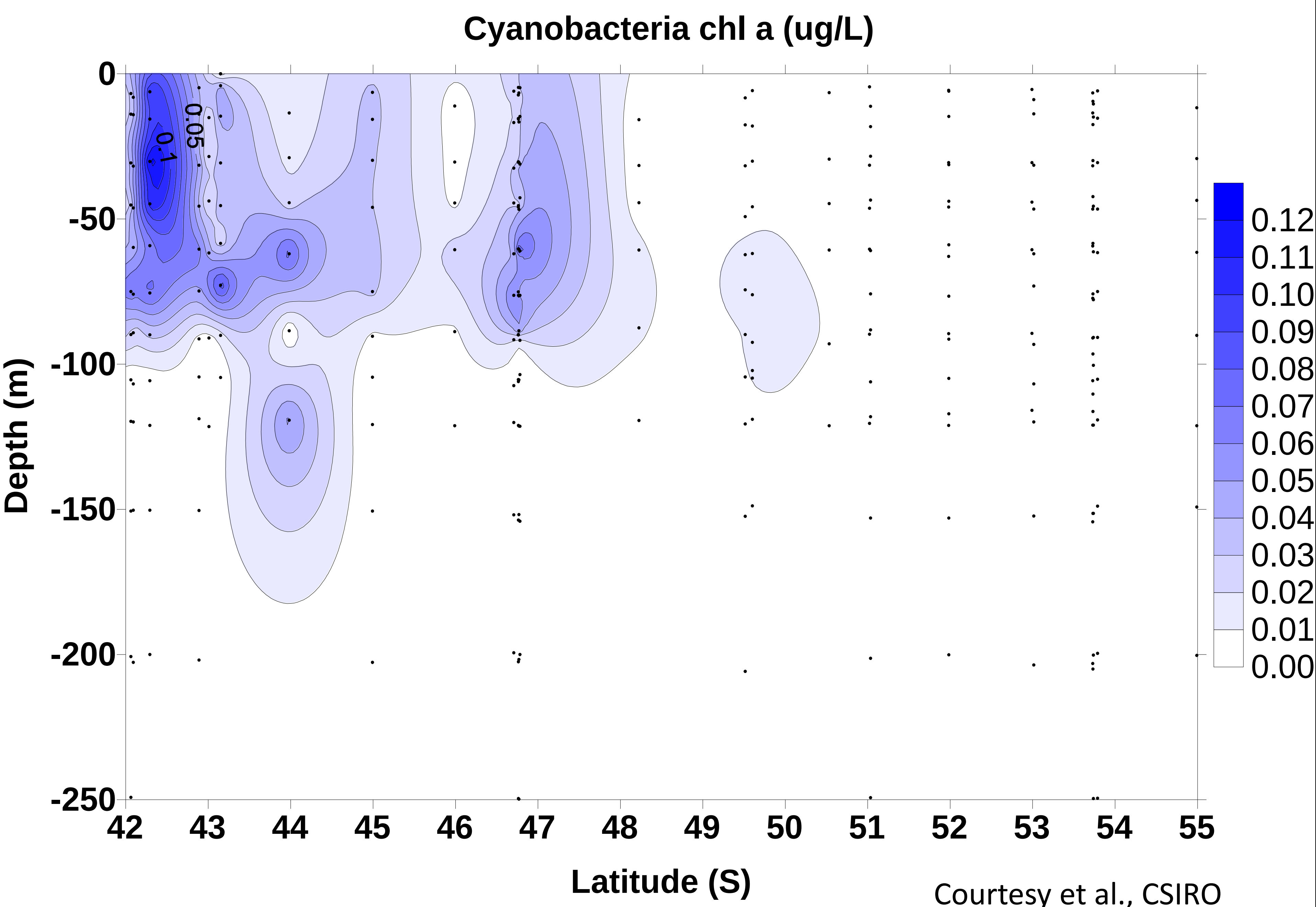

Prasinophyte chl a (ug/L)
Depth (m)
0
-50
-100
-150
-200
-250
Latitude (S)
42
43
44
45
46
47
48
49
50
51
52
53
54
55
0.04
0.04
0.04
0.04
0.04
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Courtesy et al., CSIRO

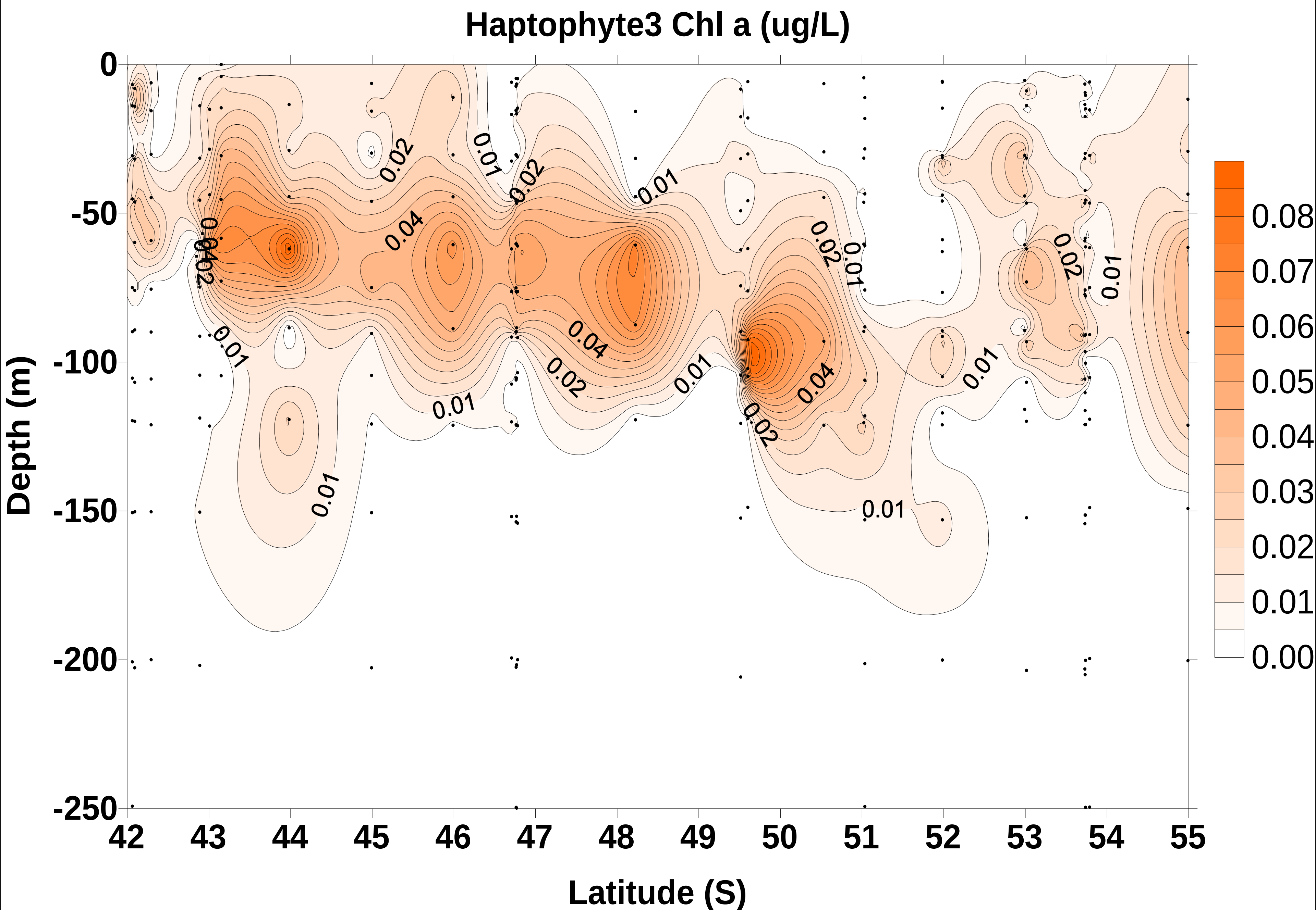


Courtesy et al., CSIRO



Dinoflagellate chl a (ug/L)
Depth (m)
Latitude (S)
0
-50
-100
-150
-200
-250
42
43
44
45
46
47
48
49
50
51
52
53
54
55
0.028
0.024
0.020
0.016
0.012
0.008
0.004
0.000
0.01
0.02
0.004

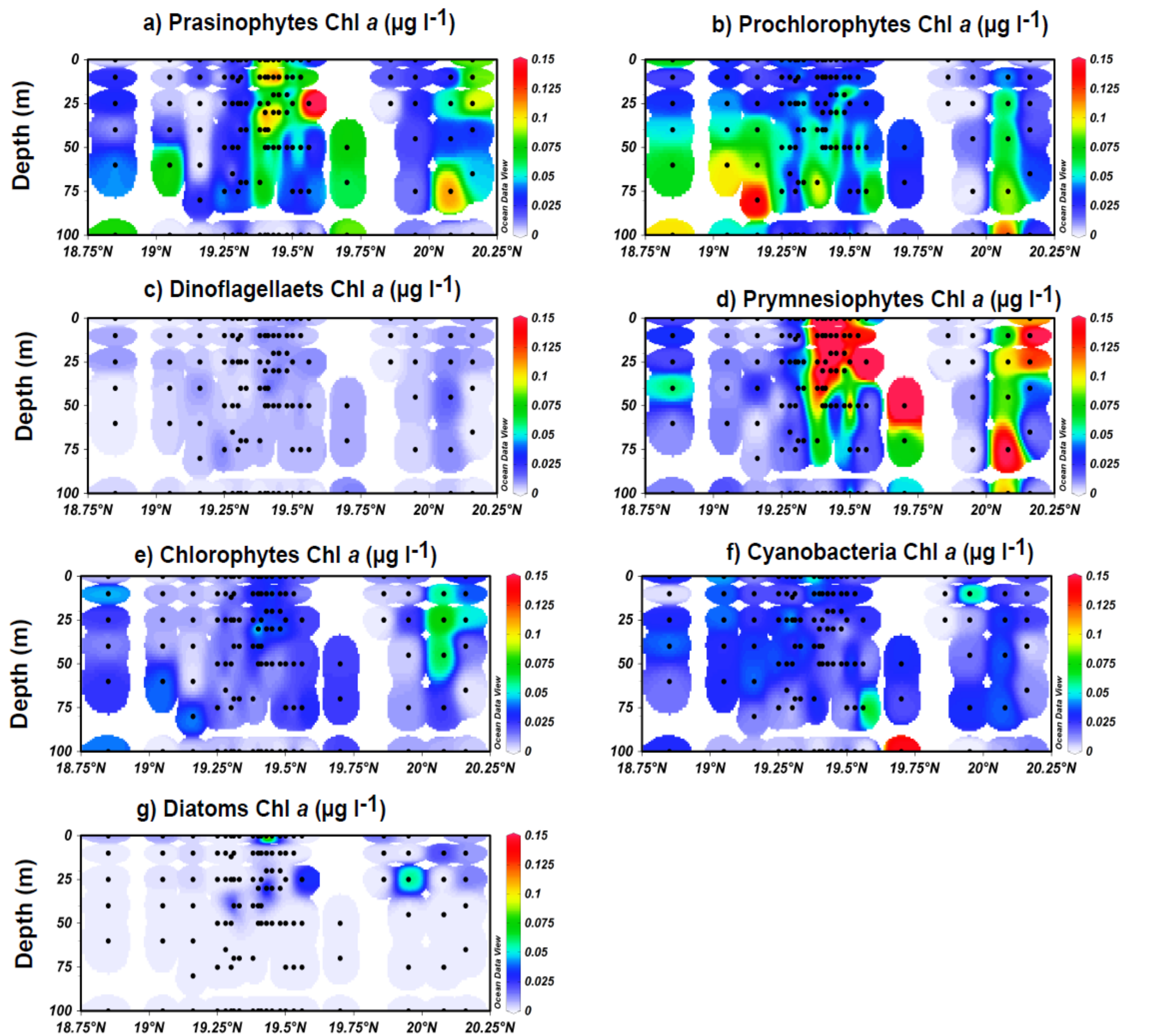

Cyanobacteria chl a (ug/L)
Depth (m)
0
-50
-100
-150
-200
-250
42
43
44
45
46
47
48
49
50
51
52
53
54
55
Latitude (S)
0.1
0.05
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Courtesy et al., CSIRO



Haptophyte3 Chl a (ug/L)
Depth (m)
0
-50
-100
-150
-200
-250
42
43
44
45
46
47
48
49
50
51
52
53
54
55
Latitude (S)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.01
0.02
0.04

Response of phytoplankton to temperature and light gradient

CHEMTAX also showed marginal build up of prasinophytes within CF1 and F1. This suggests that nanoplankton size class (e.g prymnesiophyes and prasinophytes) was efficient in utilizing the buildup of nutrients observed within the CF1 and F1.



END OF PART TWO

THANK YOU