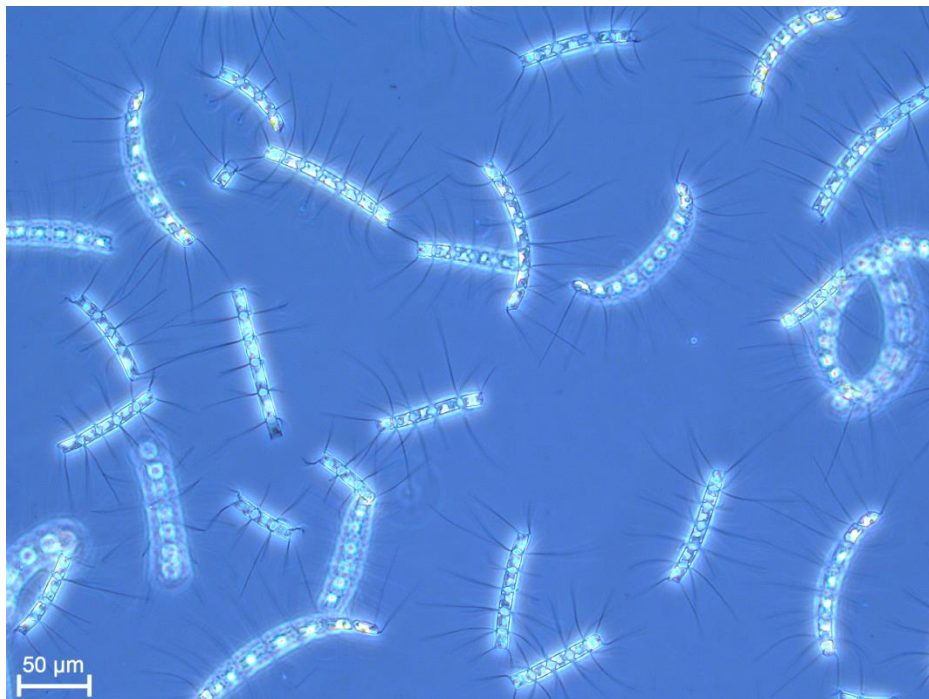


Phytoplankton and oceanography  
of the Coffs Harbour region, Eastern Australia

by

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(BSc Biology of Organisms, MSc Marine Biology)



A thesis in fulfilment of the requirements for the degree of

**Doctor of Philosophy**

Department of Biological Sciences,

Macquarie University,

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16 June 2014

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*Cover photo: Chaetoceros curvisetus (taken by L. H. Armbricht, 2012).*

**Statement of sources declaration**

I declare that this thesis is my own work and has not been submitted in any form for a higher degree to any other University or institution. This thesis contains only original material. Any additional help received during the preparation of this work has been indicated in the 'Statement of co-authorship' section. No approval from the Macquarie University Ethics Committee had to be obtained for this work. However, a research permit was granted by the Marine Parks Authority New South Wales, enabling phytoplankton sampling within the Solitary Islands Marine Park (SIMP), in which part of the sampling stations of this research were located (research permit number: SIMP 2011/002, granted on 11 May 2011, valid until 01 July 2013, Appendix 1).

*Signature:* ..... *Date:* .....

## **Statement of co-authorship**

The majority of this work has been carried out by myself, Linda Hanna Armbrrecht. All data chapters (Chapter 3 - 6) are multi-authored and the contribution of each author to each chapter has been outlined below.

## **Chapters 1 and 2**

I have carried out these two chapters myself, including the literature review and the write-up, with guidance and constructive feedback from my supervisors, Dr Leanne Armand, Dr Moninya Roughan and Prof. David Raftos. Chapter 1 and 2 provide a general introduction to, and an overview of, the sampling design and methodology of this thesis, respectively. As this thesis has been prepared according to the standards of a thesis by publication, the individual data Chapters 3 – 6 each have a separate introduction providing a specific background to the respective study, hence there may be some redundancy between chapters when compiled as a thesis.

## **Chapter 3**

I have undertaken 85% of this chapter myself. My contribution included the organisation of the majority of field campaigns, i.e. all campaigns that were conducted on *RV Circe* and the collection and processing of Conductivity-Temperature-Depth (CTD) profiles throughout all these samplings. Dr Moninya Roughan, Dr Peter Davies, Dr Tim Ingleton, Prof. Anya Waite, and Dr Vincent Rossi have organised the field campaigns and CTD data collection and processing during sampling on *RV Bombora*. Throughout all field campaigns, I undertook the sampling of phytoplankton and phytoplankton pigments, the analysis of phytoplankton by microscopy, and half of the pigment analysis at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Marine and Atmospheric Research (CMAR), Hobart, Australia. The remaining pigment samples were analysed by Dr Lesley Clementson, CMAR, Hobart, Australia, for which funding was provided by my co-supervisor Dr Moninya Roughan. I have conducted the complete pigment-optimisation in the software CHEMTAX, the statistical data analysis and the write-up of the results as a manuscript that comprises this chapter.



My co-authors have contributed 15% to this chapter. Dr Simon Wright has been particularly supportive throughout the CHEMTAX analysis and has contributed many ideas to, and constructive feedback on, the drafted paper. A/Prof. Peter Petocz suggested to apply Bland and Altman analysis to investigate the agreement between the two phytoplankton quantification techniques microscopy and CHEMTAX and has continuously provided advice in statistics. Dr Leanne Armand has provided invaluable feedback on the written work and guided the content development of the final manuscript, which was submitted to the peer-reviewed journal *Limnology and Oceanography: Methods*.

I have presented this chapter at three national conferences throughout 2013:

1. Australian Marine Science Association 2013: *Shaping the Future - Golden Jubilee Conference*, Gold Coast, 07 – 11 July 2013. Oral presentation: “Testing phytoplankton quantification estimates off Eastern Australia”.
2. Alexander von Humboldt Foundation Colloquium, Sydney, 17 – 19 October 2013. Poster presentation: “Agreement testing of two phytoplankton quantification techniques: Microscopy and CHEMTAX”.
3. Annual Higher Degree Research Conference, Department of Biological Sciences, Macquarie University, Sydney, 18 – 20 November 2013. Oral presentation: “Testing phytoplankton quantification techniques: Microscopy and CHEMTAX”. At this conference I was awarded the '2013 Best Presentation of Laboratory Based Research'.

## **Chapter 4**

I have undertaken 85% of this chapter myself. My contribution included the organisation and planning of the phytoplankton and phytoplankton pigment sampling, the sampling itself, the phytoplankton analysis by microscopy, the organisation of pigment analysis at CMAR, Hobart, Australia, the statistical data analysis, the write-up of the results and the submission of the final manuscript comprising this chapter to the journal *Continental Shelf Research* (published online on 04 December 2013).

My co-authors have contributed 15% to this work. Dr Leanne Armand and Dr Moninya Roughan have contributed to the development of the study-design, the logistics and funding involved in the sampling. Dr Leanne Armand has helped initially

with the identification of phytoplankton taxa. Dr Moninya Roughan, Dr Vincent Rossi and Dr Amandine Schaeffer have significantly contributed to the oceanographic part of the data analysis. All co-authors have given me constructive feedback during the writing process preceding the publication.

I have presented this chapter at three (national and international) conferences throughout 2012 and 2013:

1. Australian Marine Science Association (AMSA) and the New Zealand Marine Science Society: *Marine Extremes and Everything In Between*, Hobart, Australia, 01 – 05 July 2012. Poster presentation: “Phytoplankton in a biological hotspot, Solitary Islands Marine Park, Eastern Australia”.
2. Annual Higher Degree Research Conference, Department of Biological Sciences, Macquarie University, Sydney, Australia, 14 – 16 November 2012. Oral presentation: “Phytoplankton characterisation in a biological hotspot: Solitary Islands Marine Park (Eastern Australia)”.
3. Association of Limnology and Oceanography 2013 Aquatic Sciences Meeting: *Learning for the Future*, New Orleans, United States of America, 17 – 22 February 2013. Oral presentation: “Phytoplankton in a biological hotspot, Eastern Australia”.

## Chapter 5

The idea for this paper evolved during a discussion with Dr Peter Thompson at the AMSA *Golden Jubilee Conference 2013*. Post-conference, Dr Peter Thompson provided me with the CTD, nutrient, phytoplankton count and pigment datasets collected off the north-west Australian Kimberley coast during a Marine National Facility research voyage in 2010. The latter datasets form the base data for analysis of the Kimberley case study in this chapter.

Consequently, I have carried out 75% of this chapter as I have undertaken the sampling for phytoplankton and phytoplankton pigments off Coffs Harbour by myself (during a field campaign on *RV Bombora*, which was organised and funded by Dr Moninya Roughan, Dr Peter Davies, Dr Tim Ingleton and Dr Martina Doblin). I have furthermore analysed the phytoplankton via microscopy and organised the analysis of the pigment samples by Dr Lesley Clementson at CMAR, Hobart (the pigment

analysis was funded by Dr Peter Davies). I have also conducted the optimisation of the pigment data in CHEMTAX as well as the statistical analysis of all data and the write-up of the results as a paper that is currently under review with the peer-reviewed *Journal of Marine Systems*.

My co-authors have contributed 25%. Dr Simon Wright has given constructive advice regarding the CHEMTAX analysis. Dr Amandine Schaeffer has provided significant input into the oceanographic analysis of both Kimberley and Coffs Harbour datasets. A/Prof. Jorijntje Henderiks provided additional coccolithophore count data collected during the Kimberley voyage. All co-authors have made constructive comments on the drafted paper and contributed to its editing. Although not co-authoring this particular chapter, A/Prof. Peter Petocz and Mr Alexander Ferry have kindly provided advice that enabled me to conduct appropriate statistical analyses.

## Chapter 6

I have conducted 90% of this work by myself. My contribution includes the organisation of more than half of the field trips including CTD data collection (during all sampling undertaken on *RV Circe*). I have undertaken the phytoplankton sampling on all field trips as well as the phytoplankton analysis by microscopy, the statistical analysis and the write-up of the results as a manuscript that is currently under review with the *Journal of Marine Systems*.

My co-authors have contributed 10% to this chapter. Dr Amandine Schaeffer was particularly involved in the analysis of the oceanographic data and the creation of the majority of figures in the final manuscript. Dr Moninya Roughan has organised and financially supported nearly half of the field campaigns (all campaigns conducted on *RV Bombora*). She also initiated the analysis of phytoplankton data in a temperature-salinity-density continuum. Dr Leanne Armand has provided guidance and advice throughout the whole period of samplings and phytoplankton analysis. All co-authors have given me constructive feedback on the drafted paper and encouraged the development of this chapter.

## **Chapter 7**

This chapter has been researched and written by myself with constructive feedback from my supervisors, Dr Leanne Armand, Dr Moninya Roughan and Prof. David Raftos.

*Signature:* ..... *Date:* .....

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First of all I thank my three supervisors Dr Leanne Armand, Dr Moninya Roughan and Prof. David Raftos for their permanent support, guidance and encouragement throughout my whole PhD candidature. A particular thank you to Dr Penelope Ajani and Dr Tim Ingleton for their endless advice in phytoplankton identification and fieldwork matters. To Penny, thanks also for being my Australian mum, and to Tim, thanks for your unconditional help throughout many field campaigns.

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To my friends, thank you all, whether you are here in Australia and have accompanied me personally on my 3 ½ year PhD journey or at home in Germany (or in other countries) and have faithfully stayed in touch via phone, email and skype. To my family, my parents Renate and Ernst Armbrecht and my sister Kathrin Rickert, thanks for your lovely and inexhaustible support over a distance of 16,000 km and sending countless care packets to make sure that I survive in this wild world. Finally, to my better half Adam Wilkins, thank you for forcing me to have breaks – by giving me a drum kit... you must truly love me.

I thank Macquarie University for funding my research through the Higher Degree Research and the Postgraduate Research Fund. Thanks to the previous Deputy Vice Chancellor (Research) Prof. Jim Piper for awarding me an additional internal grant for writing so many grants. I thank the Australian Biological Research Study and the Australian Marine Sciences Association for granting me travel awards that enabled my participation in national and international conferences and the Linnean Society of New South Wales for awarding me a grant to support my research.

**Abstract**

The East Australian Current (EAC) is the Western Boundary Current (WBC) of the South Pacific Gyre, transporting warm water from tropical to temperate latitudes along Eastern Australia. Due to climate change, the EAC is warming and strengthening, which is expected to impact on phytoplankton abundance, distribution and composition. This thesis aims at providing the first taxonomic phytoplankton time-series survey (May 2011 - September 2012) in the tropical-temperate transition zone of Eastern Australia (~30°S, Coffs Harbour). An interdisciplinary approach of oceanography, microscopy, phytoplankton pigment analysis through CHEMTAX and statistics was used. By applying this approach, the phytoplankton community was estimated and investigated under contrasting oceanographic conditions, along cross-shelf gradients of environmental variables and throughout one annual cycle. The influence of the EAC on the shelf-scale and seasonal phytoplankton variability was examined in detail. Microscopy analysis revealed the abundance of 137 microphytoplankton taxa within 74 genera, including diatoms, dinoflagellates, silicoflagellates and *Trichodesmium erythraeum*. In addition, CHEMTAX determined the presence of pico- and nanophytoplankton, including cyanobacteria, cryptophytes, euglenophytes, haptophytes, pelagophytes and prasinophytes. Both microscopy and CHEMTAX revealed diatoms as being the most abundant phytoplankton taxon off Coffs Harbour. Shelf-scale phytoplankton abundance, distribution and composition were found to be driven by the highly variable oceanographic environment, and, on a seasonal scale, by the combination of the EAC and intrinsic seasonal cycles. Upwelling triggered two diatom blooms during December 2011 (*Pseudo-nitzschia* spp.) and September 2012 (*Leptocylindrus danicus*). This thesis has provided baseline information on interactions between oceanography and phytoplankton dynamics at ~30°S, Eastern Australia. The results from this survey are a key reference for future studies investigating changes in phytoplankton communities along the east Australian coast as a consequence of the strengthening EAC. Furthermore, the findings of this thesis find applicability in globally changing subtropical WBC systems. All of these systems are currently undergoing long-term changes, particularly in tropicalisation evidenced by poleward species shifts.

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No Tables included.

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**List of Abbreviations**

ADCP	Acoustic Doppler Current Profiler	CH100	IMOS mooring at the 100 m isobath at Coffs Harbour
AIC	Akaike's Information Criterion	Chl <i>a</i>	Chlorophyll <i>a</i>
Allo	Alloxanthin	Chl <i>b</i>	Chlorophyll <i>b</i>
AMSA	Australian Marine Science Association	Chl <i>c</i> <sub>1</sub>	Chlorophyll <i>c</i> <sub>1</sub>
ANOSIM	Analysis of Similarity	Chl <i>c</i> <sub>2</sub>	Chlorophyll <i>c</i> <sub>2</sub>
ANOVA	Analysis of Variance	Chl <i>c</i> <sub>3</sub>	Chlorophyll <i>c</i> <sub>3</sub>
AODN	Australian Ocean Data Network	CPR	Continuous Plankton Recorder
AVHRR	Advanced Very High Resolution Radiometer	CMAR	CSIRO Marine and Atmospheric Research
B-Line	Northern sampling transect within this thesis	CSIRO	Commonwealth Scientific and Industrial Research Organisation
BOM	Bureau of Meteorology	CTD	Conductivity-Temperature-Depth
But-fuco	19'Butanoyloxyfucoxanthin	DCM	Depth of the Chl <i>a</i> maximum
CCA	Canonical Correspondence Analysis	DF	Degrees of Freedom
CHEMTAX	CHEMical TAXonomy	DIN	Dissolved inorganic nitrogen
CH-Line	Southern sampling transect within this thesis	DistLM	Distance-based Linear Model
CH070	IMOS mooring at the 70 m isobath at Coffs Harbour	DV Chl <i>a</i>	Divinyl chlorophyll <i>a</i>
		DV Chl <i>b</i>	Divinyl chlorophyll <i>b</i>

## List of Abbreviations

EAC	East Australian Current	Lut	Lutein
ENSO	El Niño Southern Oscillation	MQ	Macquarie University
FOV	Field-of-views	Neo	Neoxanthin
$f_{\text{micro}}$	Fraction of micro-phytoplankton to the total Chl <i>a</i> biomass	nMDS	non-metric Multidimensional Scaling
$f_{\text{nano}}$	Fraction of nano-phytoplankton to the total Chl <i>a</i> biomass	NMSC	National Marine Science Centre, Coffs Harbour
$f_{\text{pico}}$	Fraction of pico-phytoplankton to the total Chl <i>a</i> biomass	NOAA	National Oceanic and Atmospheric Administration
Fuco	Fucoxanthin	NSW	New South Wales
GHRST	Group for High Resolution Sea Surface Temperature	MDS	non-metric Multidimensional Scaling
Hex-fuco	19'Hexanoyloxyfucoxanthin	MLD	Mixed layer depth
HPLC	High-Performance Liquid Chromatography	MPA	Marine Parks Authority
IMOS	Integrated Marine Observing System	OC	Oceanographic condition
IDH	Intermediate Disturbance Hypothesis	OEH	Office of Environment and Heritage
IOD	Indian Ocean Dipole events	Perid	Peridinin
IPO	Interdecadal Pacific Oscillation	PH	Port Hacking
ITF	Indonesian Throughflow	Pras	Prasinoxanthin
LC	Leeuwin Current	Pseudo-F	Test statistic
		PSU	Practical Salinity Units
		RSS	Residual Sum of Squares
		RV	Research Vessel
		$R^2$	Coefficient of determination

SAM	Southern Annular Mode	TChl <i>a</i>	Total chlorophyll <i>a</i>
SEC	South Equatorial Current	TChl <i>a</i> <sub>micro</sub>	Total Chl <i>a</i> biomass associated with micro-phytoplankton
SEM	Scanning Electron Microscopy		
SI	Solitary Island (Chapter 2)	TChl <i>a</i> <sub>nano</sub>	Total Chl <i>a</i> biomass associated with nano-phytoplankton
SI	Station ID (Chapter 4)		
SIMP	Solitary Islands Marine Park	TChl <i>a</i> <sub>pico</sub>	Total Chl <i>a</i> biomass associated with pico-phytoplankton
SIMR	Solitary Islands Marine Reserve		
SIMS	Sydney Institute of Marine Science	TEM	Transmission Electron Microscopy
SOI	Southern Oscillation Index	T-S	Temperature-salinity
SS	Solitary coastal sampling station between B- and CH-Line	UNSW	University of New South Wales
		Viola	Violaxanthin
SST	Sea surface temperature	WBC	Western Boundary Current
SSS	Sea surface salinity	WoRMS	World Register of Marine Sciences
S:V	Surface : volume	Zea	Zeaxanthin

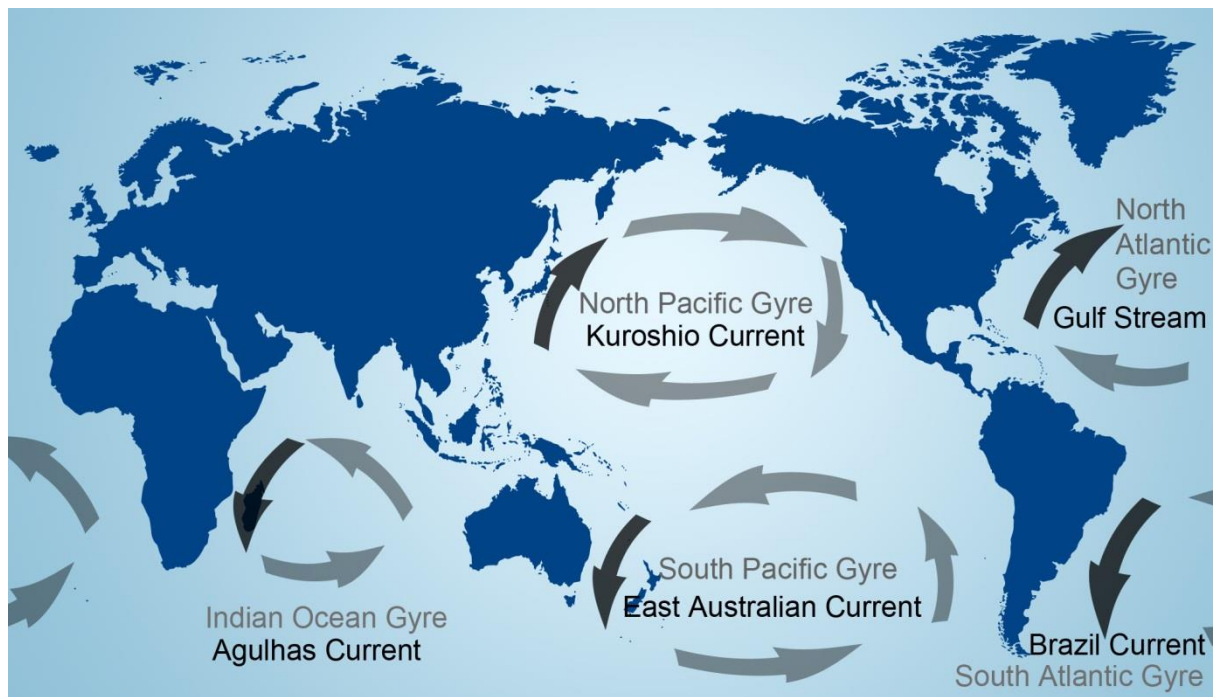
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## 1 General Introduction

### ***1.1 Western Boundary Currents as hotspots of global temperature warming***

The global ocean is currently undergoing a variety of modifications in its physical and chemical properties due to climate change. These changes are expected to severely impact on worldwide marine ecosystems. Examples of modifications in the oceanic environment include altered ocean circulation patterns, oceanic and sea surface temperature (SST) warming, sea level rise, ocean acidification and enhanced stratification, which in turn has the potential to reduce nutrient exchange from below the nutricline to oligotrophic surface waters via vertical mixing (Behrenfeld et al. 2006, Doney et al. 2012, IPCC 2013). Within this context, subtropical Western Boundary Currents (WBCs), including the Northern Hemisphere Kuroshio Current and Gulf Stream, and the Southern Hemisphere Brazil, Agulhas and East Australian Current (summarised in Fig.1), are hotspots of worldwide SST warming (Wu et al. 2012). These WBCs have been warming two to three times faster than the global mean ocean surface over the last century, which is associated with a poleward shift and/or intensification of these currents (Wu et al. 2012).

Specifically, the warming of the Gulf Stream, Kuroshio and Brazil Current is sourced in a poleward shift of the current axes caused by a poleward shift of the mid-latitude zero wind-stress curl line (Wu et al. 2012). This is the mid-latitude 'line' at which the curl of wind-stress acting on the sea surface (thus influencing ocean circulation) is zero (Wu et al. 2012). In contrast, the warming of the East Australian Current (EAC) and Agulhas Current appears to be caused by their intensification related to a climate change-induced increased surface wind-stress curl. The extensions of the EAC and Agulhas Current are not related to the zero wind-stress curl line but the meridional extent of the Australian and South African coasts (Wu et al. 2012). Wu et al. (2012) further reported that all subtropical ocean gyres in the Southern Hemisphere have accelerated due to an increased wind-stress curl. Using an earlier alternative approach, Cai et al. (2005) noted that the EAC axis had shifted as a result of a committing southward shift of the South Pacific Gyre (see section 1.2).



**Figure 1. Global Western Boundary Currents.** World map showing the simplified flow direction of the five subtropical Western Boundary Currents (WBCs, black arrows and font) that have been shown to experience an above-global average rate in sea surface temperature warming (Wu et al. 2012). The simplified flow directions of the gyres associated with each WBC are indicated by light grey arrows and font.

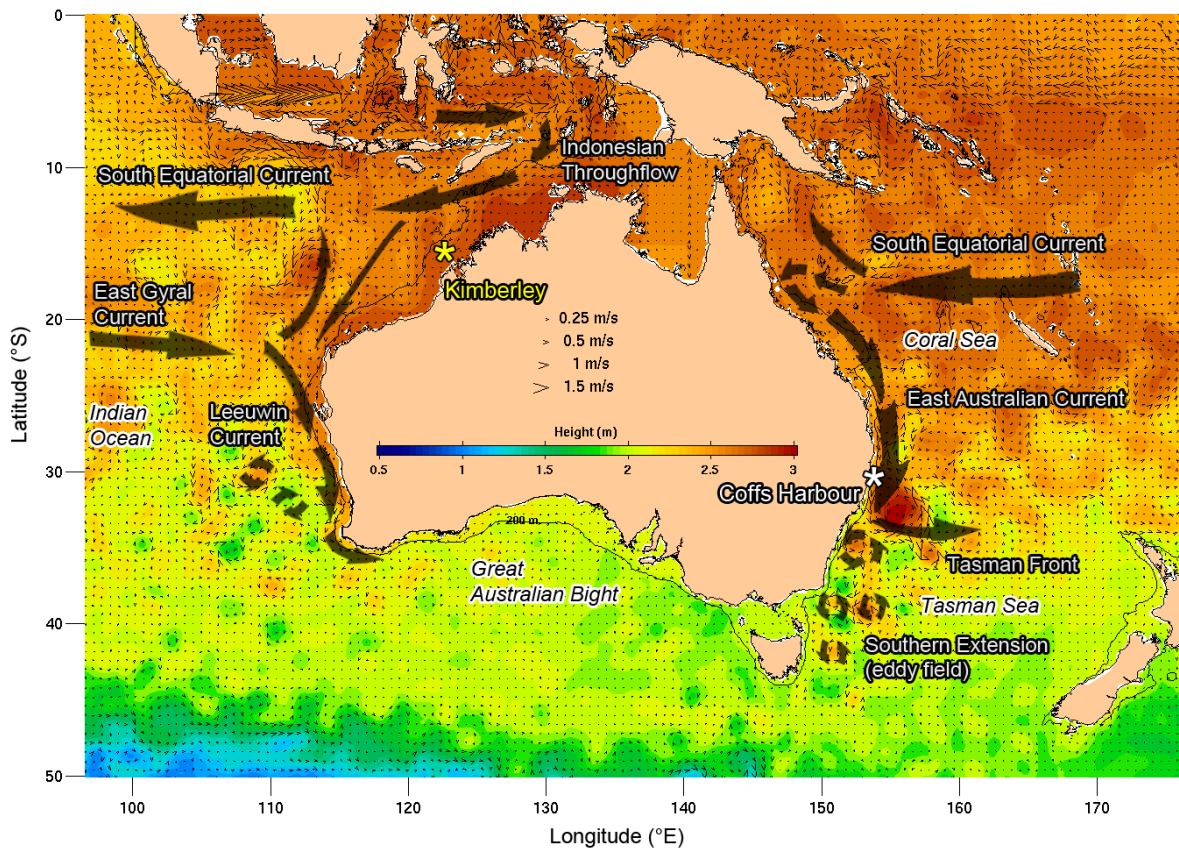
## **1.2 The East Australian Current - exemplifying a strengthening Western Boundary Current**

Within the last two decades, considerable effort has been made to investigate the current state of the EAC (shown in Fig. 2) and the extent, effects and causes of its progressive strengthening. Observational data from  $\sim 43^{\circ}\text{S}$ ,  $148^{\circ}\text{E}$  (Maria Island Station, Tasmania) between 1944 - 2002 are consistent with the EAC warming trends reported by Wu et al. (2012) (section 1.1). An increase in SST of  $2.28^{\circ}\text{C century}^{-1}$ , and sea surface salinity (SSS) of  $0.34 \text{ psu century}^{-1}$ , have been reported (Ridgway 2007). These values correspond to a strengthening of the EAC and its poleward shift of approximately 350 km (Ridgway 2007). Data from *in situ* observations and atmospheric re-analysis (Hill et al. 2008), as well as climate models (Cai et al. 2005, Cai 2006), have shown that these changes in the EAC's physical parameters result from a long-term spin-up of the South Pacific Gyre caused by increased winds. Increased atmospheric  $\text{CO}_2$  levels associated with a shift of the Southern Annular Mode (SAM; a measure of the atmospheric pressure difference between the mid-latitudes and Antarctic; Feng et al. 2009) towards positive phases and Antarctic



ozone depletion have led to maximum change in the wind-stress curl at 48°S (Cai et al. 2005, Cai 2006). Consequently, circumpolar winds have strengthened while latitudinal winds have weakened (Cai et al. 2005, Cai 2006). As a result, southward water advection within the South Pacific Gyre has accelerated, leading to EAC intensification expressed within the Tasman Sea (Fig. 2) by the highest SST warming rate reported in the Southern Hemisphere (Cai et al. 2005, Cai 2006). A recent investigation into increases in the poleward extension of the EAC revealed that transport within eddies has increased over the past 30 years downstream of the zone where the EAC typically separates from the coast (~31°S - 32°S; Cetina-Heredia et al. 2014).

The poleward advance of the EAC has major implications for sea level rise (Cai et al. 2005), especially in the context of ongoing anthropogenically-driven atmospheric CO<sub>2</sub> enrichment and ozone depletion during climate change (IPCC 2013). The strengthening of the oligotrophic EAC and continuing trends towards enhanced stratification concur with a shortage in nutrients off the east Australian coast. This is evidenced in a long-term silicate decline of ~2 and 5.8  $\mu\text{M century}^{-1}$  that has been detected at 34°S and 43°S, respectively, over the last 30 years (Thompson et al. 2009).



**Figure 2. Major currents including East Australian and Leeuwin Current influencing the oceanography along the east and west Australian coast.** The currents (schematically indicated by black arrows and labelled in grey-black font) are overlaid onto a map of geostrophic velocity (derived from altimeter sea level for 25 January 2012, including mean dynamic height relative to 200 m, *IMOS Ocean Current website*: <http://oceancurrent.imos.org.au/uv/2012/20120125.html>). The width of the arrows corresponds to the relative strength of the currents. The Southern Extension of the East Australian Current is indicated as an eddy field. Adjacent seas are indicated in italic black-white font. The primary sampling location of this PhD research (Coffs Harbour, ~30°S, 153°E) is indicated by the white asterisk, the sampling location included in a spatial comparative study within Chapter 5 of this thesis (Kimberley region, ~15°S, 122°E) is indicated by the yellow asterisk.

### 1.3 The Leeuwin Current – a changing Eastern Boundary Current

Although the focus of this PhD thesis is on the phytoplankton dynamics associated with the EAC, an overview will also be given about the general oceanography (this section) and current phytoplankton research (sections 1.9 and 1.11) along the west Australian coast. This approach is intended to provide a broad context towards Chapter 5, a comparative study of cross-shelf phytoplankton variability off Eastern and Western Australia.

Climate change-induced modifications in physical properties have been observed in the Leeuwin Current (LC), an Eastern Boundary Current transporting warm water from  $\sim 22^{\circ}\text{S}$  poleward along the west Australian coast (Fig. 2). The LC is driven by a meridional pressure gradient over the Indian Ocean, with this pressure gradient weakening due to reduced trade winds over the tropical Pacific (Feng et al. 2009, Feng & Weller 2010). As a consequence, the LC's water transport is decreasing in the long-term (10 - 30% reduction in the LC's transport between the 1970s and 1990s; Feng et al. 2009, Feng & Weller 2010). A considerable increase in temperature of  $0.034^{\circ}\text{C year}^{-1}$  and a slight increase in salinity of  $0.003 \text{ psu year}^{-1}$  have been detected in the upper 50 m at  $\sim 32^{\circ}\text{S}$ ,  $115.4^{\circ}\text{E}$  (Rottnest Island Station, Western Australia) since the 1950's (Pearce & Feng 2007). Furthermore, an increase in coastal sea levels of  $1.54 \text{ mm year}^{-1}$  over the 20<sup>th</sup> century has been determined at  $\sim 32^{\circ}\text{S}$ ,  $115.4^{\circ}\text{E}$  (Feng 2004). The warming of the LC has been suggested to be enhanced by an increase in Indian Ocean Dipole events (the Indian Ocean El Niño Southern Oscillation) and positive SAM trends (Cai et al. 2009, Feng et al. 2009). A reduction in westerly winds, winter storms and rainfall above south-west Australia has further been shown to contribute to increasing solar radiation and SST warming (Bates et al. 2008). Caputi et al. (2009) have reported that the warming of the LC itself is most noticeable during autumn and winter causing a delay in seasonal cycles of SSTs by up to 20 days. The salinity increase has been suggested to result from a decrease in the Indonesian Throughflow (Fig. 2) and LC (both transporting relatively fresh water), an increase in evaporation, and a reduction in rainfall along the west Australian coast (Feng et al. 2009). Physical modelling studies from the west Australian coast that incorporate increasing greenhouse gas emissions generally predict a further warming and weakening in the LC in combination with increasing salinity and a shallowing thermocline (Feng 2004, Feng et al. 2009).

#### **1.4 Present-day variability of the East Australian and Leeuwin Current**

In order to provide a concise overview and comparison of the EAC and LC including their temporal variation from seasonal to multi-annual, the major characteristics of both currents are summarised in Table 1. A visual representation of the EAC and LC and their connective currents as described in Table 1 is shown in Fig. 2.

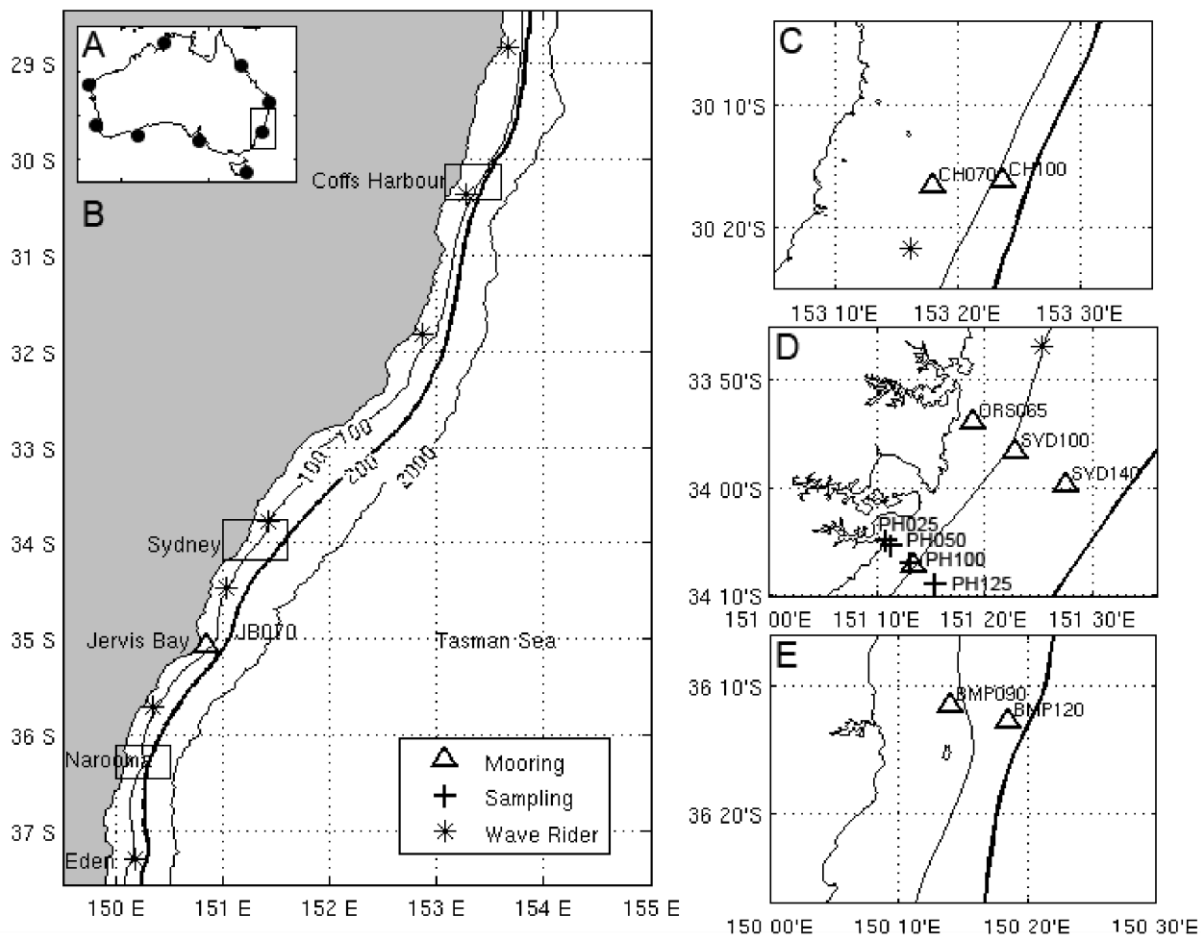
**Table 1. Characteristics of the Leeuwin Current (LC, Western Australia) and East Australian Current (EAC, Eastern Australia).** See Figure 2 for a graphical illustration of the LC and EAC and their connective currents. SST and SSS = Sea surface temperature and salinity, respectively.

Characteristic	Leeuwin Current (LC)	East Australian Current (EAC)
Type	Eastern Boundary Current, transporting warm water from tropical to temperate latitudes of Western Australia.	Western Boundary Current, transporting warm water from tropical to temperate latitudes of Eastern Australia.
Position	Surface current.	Surface current.
Driving force	Meridional pressure gradient (in southeast Indian Ocean) generated by the Indonesian Throughflow (ITF) in combination with thermohaline forcing (Cresswell & Golding 1980, Godfrey & Ridgway 1985, McCreary et al. 1986, Feng et al. 2009).	Forms as southward component of the bifurcating South Equatorial Current (SEC; Church 1987, Ridgway & Dunn 2003). Comprises the western boundary of the South Pacific subtropical gyre and connecting the Pacific and Indian Ocean gyres (Boland & Church 1981, Speich et al. 2002).
Path	Along the continental shelf (~200 m isobath). From ~22°S, 114°E around south-west Australia into the Great Australian Bight continuing as the South Australian Current at ~35°S (Godfrey & Ridgway 1985, Ridgway 2004).	Along the continental shelf (~200 m isobath). From ~15 - 20°S to separation point at ~32°S*, bifurcation into eastward directed Tasman Front and the southward proceeding EAC Extension (Godfrey et al. 1980, Tilburg et al. 2001).
Eddies	Strongest eddy kinetic energy level among worldwide Eastern Boundary Currents (Cresswell & Griffin 2004, Feng et al. 2005).	The Southern Extension is comprised of a highly energetic eddy field travelling southward along the shelf (Nilsson & Cresswell 1981, Ridgway & Dunn 2003, Mata et al. 2006).
Southward velocity	Slow in summer ( $0.1 \text{ m s}^{-1}$ ), fast in winter ( $0.54 \text{ m s}^{-1}$ ) (east of 110°E; Feng 2003). Cresswell & Peterson (1993) reported eastward winter maxima of $\sim 1 \text{ m s}^{-1}$ at ~35°S, 117°E (south of Western Australia).	Slow ( $0.2 \text{ m s}^{-1}$ ) north of 23°S, accelerating ( $2 \text{ m s}^{-1}$ ) between 30°S and 32°S, declining steadily south of 32°S (Cresswell & Domingues 2001, Roughan & Middleton 2002, Ridgway & Dunn 2003).
Seasonal variation in transport	2 - 3 Sv (1 Sverdrup = $10^6 \text{ m}^3 \text{ s}^{-1}$ ) in summer to 5 Sv in winter (east of 110°E; Feng 2003, Feng et al. 2009).	7 Sv in winter to 16 Sv in summer (at 28°S; Ridgway & Godfrey 1997).
Inter-annual, decadal and multi-decadal variation	Inter-annual: Primarily El Niño Southern Oscillation (ENSO)-driven phenomena in the tropical Pacific (Feng 2003, Feng et al. 2009). La Niña (positive phases of the Southern Oscillation, SOI, index): strong LC, above average SST, deep anomalies in the thermocline, high inshore sea surface level. El Niño (negative SOI phase): vice versa.  Pacific Decadal Oscillation, Indian Ocean Dipole events and the Southern Annular Mode also influence the LC inter-annually (Feng et al. 2009).	Inter-annual: Primarily El Niño Southern Oscillation (ENSO)-driven (Holbrook et al. 2011). La Niña (positive phases of the Southern Oscillation, SOI, index): Strong SEC and EAC, above-average SST, high sea surface level (Feng 2004, Holbrook et al. 2011). El Niño (negative SOI phase): vice versa.  Decadal: Occurs as a response to the Interdecadal Pacific Oscillation (IPO; Holbrook et al. 2011).  Multi-decadal: Primarily as a response to extra-tropical climate modes, i.e. the Southern Annular Mode and the Pacific South American Mode (Holbrook et al. 2011). Also as response to the IPO (Holbrook et al. 2011).
Long-term trends	Increase in SST, SSS, sea level rise, weakening current, shallowing thermocline (Feng 2004, Pearce & Feng 2007, Feng et al. 2009, Caputi et al. 2009).	Increase in SST, SSS, sea level rise, strengthening current (Ridgway 2007, Hill et al. 2008, Cai et al. 2005, 2006). Increased stratification expected but not yet determined (Thompson et al. 2009).

\*New evidence shows the EAC separated from the coast between 30.7°S and 32.4°S 50% of the time between 1980 and 2010, with separation possible anywhere between 28°S and 38°S (Cetina-Heredia et al. 2014).

### ***1.5 Measuring the oceanography around Australia: Integrated Marine Observing System***

The Integrated Marine Observing System (IMOS) was established since 2007 by the Australian government to comprehensively monitor Australia's coasts and open ocean (<http://imos.org.au/about.html>). Within the IMOS framework, moored instruments that permanently measure physical, chemical and biological (fluorescence) properties are located along the coastline of Australia. Moorings are deployed at nine National Reference Stations and additional regional sites (Fig. 3A, B). They provide an extensive infrastructure to monitor currents, shelf waters, their interaction and their association with productivity (<http://imos.org.au/anmn.html>). Along the coast of South-Eastern Australia, the New South Wales node of IMOS (NSW-IMOS) has been built focussing particularly on the EAC, its variability, eddy shedding, separation and influence on biological properties (Roughan et al. 2010, 2013). As part of the NSW node, oceanographic property moorings are in permanent operation off Coffs Harbour (~30°S; Fig. 3C), Sydney (~34°S; Fig. 3D) and Narooma (~36°S; Fig. 3E). The oldest hydrological observations are available from three stations off Sydney, namely PH050, PH100 and ORS065 (Fig. 3D). These stations have been maintained since 1942, 1953 and 1989, respectively, preceding IMOS activities (Roughan et al. 2010). Samples for chlorophyll *a* (Chl *a*, the major pigment of phytoplankton), phytoplankton and zooplankton have been continuously collected at PH100 since 1953 (Roughan et al. 2010). The remaining oceanographic moorings off Sydney, Narooma and Coffs Harbour were established between 2008 and 2011. Off Coffs Harbour, two moorings positioned at the 70 m and 100 m isobaths (CH070 and CH100, respectively) have been in operation since 2010 (Fig. 3; Roughan et al. 2013).

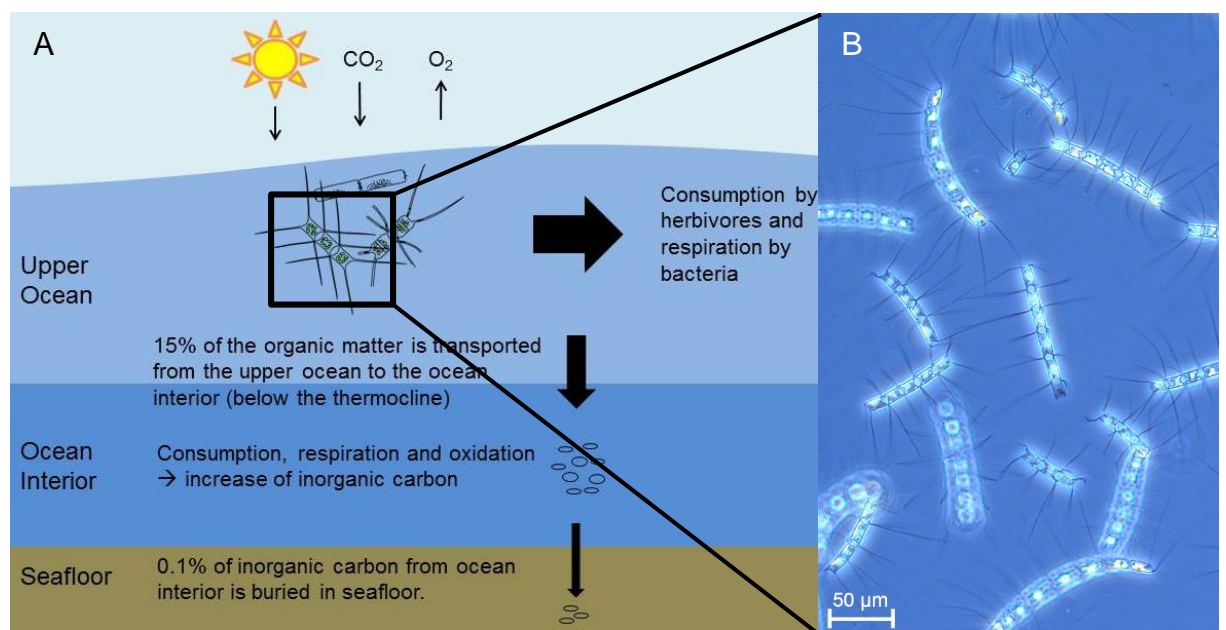


**Figure 3. Monitoring stations of the Integrated Marine Observing System (IMOS) along the south-east Australian coast.** A) Outline of Australia showing the location of nine National Reference Stations (black dots) at which IMOS has established permanent moorings since 2007. The black box indicates part of the NSW coast expanded in B. B) Map showing the locations of deployed moorings (triangles), hydrographic sampling locations (crosses) and wave-rider buoys (asterisks) along the NSW coast. The 100, 200 and 2000 m isobaths are shown as black lines. Subpanels (right) show expansions of the observation sites at C) Coffs Harbour, D) Sydney and E) Narooma. (*Figure adapted from: Roughan et al. 2013, figure 1*).

### 1.6 Global importance of phytoplankton

Marine phytoplankton are unicellular microalgae (Fig. 4) that are indispensable for the sustainability of our planet. Phytoplankton are at the base of the marine food web. They are of vital nutritional value to higher trophic organisms and are ultimately responsible for the functioning of the entire oceanic ecosystem. Phytoplankton have diversified substantially over evolutionary time-scales and currently ~25,000 eukaryotic species of phytoplankton are morphologically described (Falkowski et al.

2004). This estimate is most likely only a fraction (~10%) of the true number of species existing (Mann & Droop 1996, Mora et al. 2011). Phytoplankton find a wide range of applications in science due to their high diversity and quality as environmental indicators. These applications include paleoceanography and paleothermometry (Baumann et al. 2005, Haley et al. 2005, Giuliani et al. 2006), forensics (Cox 2012) and biofuel production (Mata et al. 2010). Although phytoplankton contribute only 0.2% to the plant biomass worldwide, they conduct nearly half (46.2%) of the global annual net primary production (Field et al. 1998, Falkowski et al. 1998). By photosynthesising and exporting carbon to the deep ocean via the biological carbon pump, phytoplankton link the atmospheric and oceanic carbon cycle (Fig. 4). In addition to carbon, many other elements are crucial to the survival of phytoplankton, such as nitrogen, phosphorous, silica and iron. Thus, phytoplankton play a key role in global biogeochemical cycles, making the study of these microorganisms imperative, especially with the impacts of climate change underway.



**Figure 4. The role of phytoplankton in the carbon cycle.** A) Simplified schematic of the biological carbon pump, which works against a gradient of inorganic  $\text{CO}_2$  with depth. The schematic is redrawn based on Falkowski and Oliver 2007, figure 2, who reproduced a figure provided by John Delaney, University of Washington USA. B) Insert showing the diatom *Chaetoceros curvisetus* (class Bacillariophyceae) as an example phytoplankton species. Scale bar = 50  $\mu\text{m}$ .

### **1.7 Climate change impacts on phytoplankton**

Projected long-term changes in phytoplankton dynamics include an earlier timing of annual phytoplankton blooms (peak production periods), disruptions of food web structures and seasonal succession patterns, poleward range expansions of warm-water species and a higher frequency of harmful algal blooms (Hays et al. 2005, Hallegraeff 2010, Winder et al. 2012). Recent investigations suggest that such alterations at the primary producer level have already started. However, clear global trends are not yet discernible. For example, a few studies have reported an earlier onset of phytoplankton spring blooms (Weyhenmeyer 2001, Ajani et al. 2011) while retardations have also been shown (Wiltshire & Manly 2004, Wiltshire et al. 2008). Declines in global net primary production (between 1996 and 2006; Behrenfeld et al. 2006) and phytoplankton biomass (over the last century; Boyce et al. 2010) have been reported, as well as an increase in primary production (over the last six decades; Chavez et al. 2011). A trend towards smaller phytoplankton body size in a warming ocean has also been documented (Daufresne et al. 2009, Morán et al. 2010), although the global relevance of this trend is still under debate (Gardner et al. 2011).

### **1.8 Long-term implications of the strengthening EAC for phytoplankton**

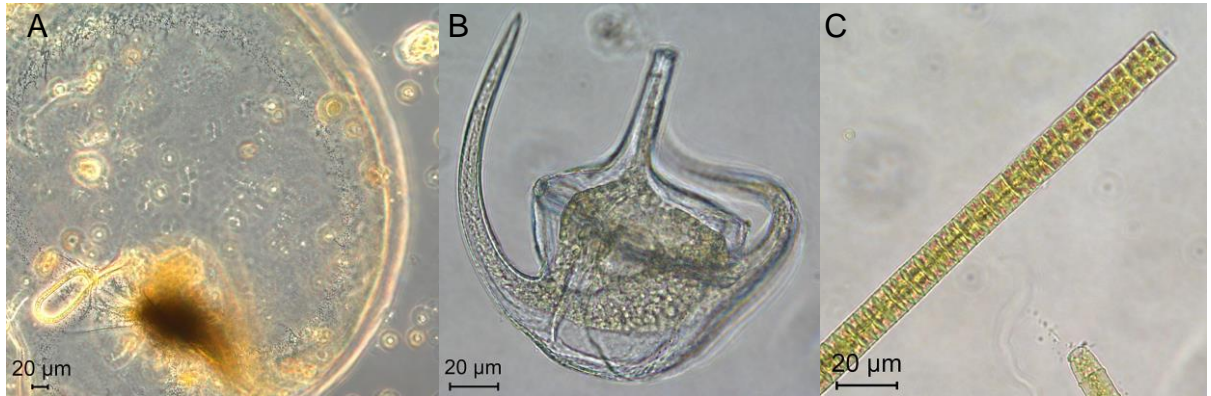
Climate change-induced shifts in phytoplankton distribution and seasonal abundance appear to have begun along the east Australian coast, principally linked to the strengthening EAC.

The red-tide dinoflagellate *Noctiluca scintillans* (Fig. 5A) and several species belonging to the dinoflagellate genus *Ceratium* (Fig. 5B) have broadened their range southward as a result of the enhanced poleward penetration of the EAC (Hallegraeff et al. 2008, Buchanan et al. 2014). *Noctiluca scintillans* has recently been recorded within an eddy as far south as the Southern Ocean (McLeod et al. 2012). Dinoflagellates are not the only phytoplankton group affected. Frequent blooms of the tropical cyanobacterium *Trichodesmium erythraeum* (Fig. 5C) at ~34°S (Port Hacking, Eastern Australia) also indicate a strong EAC influence during spring and summer (Ajani et al. 2001, 2014).

One study, compiling phytoplankton bloom records along the east Australian coast from two consecutive decades (1990 – 1999 and 2000 – 2009) found an increase in



the frequency as well as a shift towards an earlier onset of phytoplankton blooms from January to October (Ajani et al. 2011). This finding is consistent with a study by Thompson et al. (2009), who predicted that enhanced SSTs would have a direct effect on phytoplankton by lengthening the growing season. Regardless of an increased growing season, no changes in the dominant bloom species were found in the decadal analysis of Ajani et al. (2011), in which *Noctiluca scintillans* and *Trichodesmium erythraeum* were the most abundant phytoplankton taxa.



**Figure 5. Phytoplankton taxa that have been found to migrate poleward due to the southward advance of the EAC.** A) *Noctiluca scintillans* (Dinophyceae), B) *Ceratium concilians* (Dinophyceae), C) *Trichodesmium erythraeum* (Cyanophyceae). Scale bars = 20 µm.

Thompson et al (2009) also showed that the long-term silicate decline identified along the east Australian coast (section 1.2) coincided with a 50% decrease in spring bloom growth rate at ~43°S, Maria Island Station, Tasmania (from 1997 to 2007). As a consequence of this silicate decline, diatoms have been predicted to considerably decrease in abundance along the east Australian coast in the future, as they depend on silicic acid to form their robust shells (frustules) (Thompson et al. 2009). However, a study by Ajani et al. (2014) reported an increase in the diatom:dinoflagellate ratio at ~34°S (Port Hacking) over one decade (1998 to 2009) while overall phytoplankton abundance decreased. Previous studies have shown phytoplankton dynamics to be highly influenced by inter- and multi-annual variations in oceanographic parameters along the Australian east coast (primarily due to El Niño Southern Oscillations, ENSO; Lee et al. 2001a, Ajani et al. 2011, 2014). Therefore, the findings of Ajani et al. (2014) are not necessarily contradictory to the predictions by Thompson et al. (2009), and demonstrate our need for more long-term research to identify clear future trends in total abundance and proportions of diatoms and dinoflagellates.

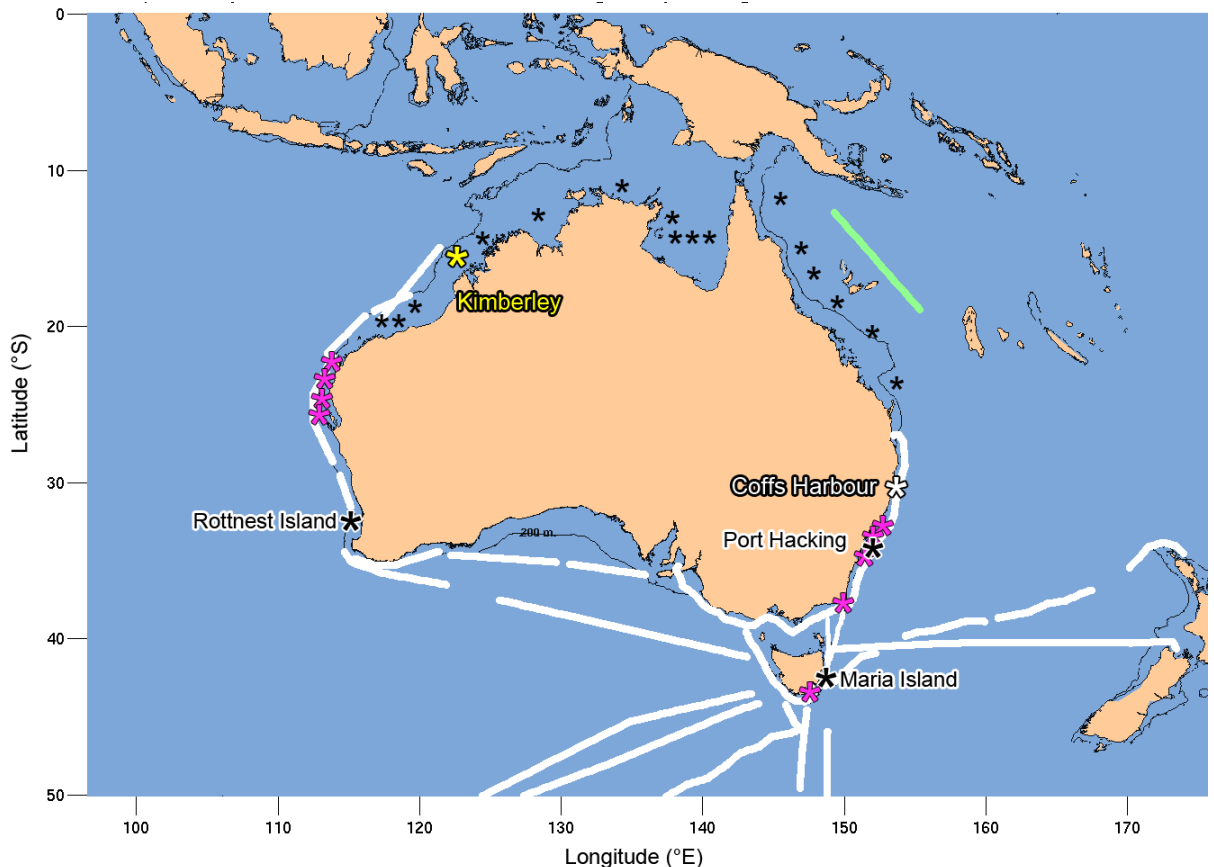
### ***1.9 Long-term implications of the weakening LC for phytoplankton***

Physical modelling studies from the west Australian coast are consistent with global predictions of a shallowing thermocline impeding vertical nutrient flux (Feng et al. 2009, Gordon et al. 2010, Chamberlain et al. 2012). Severe impacts on phytoplankton dynamics in the LC system are expected to result from these changes (Caputi et al. 2009, Feng et al. 2009). Nevertheless, clear trends towards enhanced stratification and a decline in phytoplankton abundance based on a long-term (60 year) record from ~32°S (Rottnest Island Station, Western Australia) are not yet distinguishable (Thompson et al. 2009). However, evidence of biological and economical damage has already been found at higher trophic levels. For example, western rock lobsters have been shown to decrease in maturity size and increasingly migrate from shallow to deep waters over the past 40 years (Sainte-Marie et al. 2010). Undoubtedly, such development and habitat shifts in higher marine organisms call for more observations that allow us to understand environmental changes and their biological responses (Thompson et al. 2009). With phytoplankton comprising the base of all higher trophic interactions and marine ecosystem function, research aimed at the specific responses of phytoplankton to oceanographic conditions on regionally and temporally large- and small-scales is crucial.

### ***1.10 History of phytoplankton research along the east Australian coast***

Historic and long-term phytoplankton data along the east Australian coast has been primarily collected downstream of the EAC separation zone, at ~34°S (Port Hacking, Fig. 6; Grant & Kerr 1970, Hallegraeff & Reid 1986, Ajani et al. 2001, 2014; and references therein). Sporadic studies have addressed broader regional areas. For example Wood (1954), Crosby & Wood (1958, 1959) and Wood et al. (1959) have completed extensive studies to broadly define the biogeography of two major taxa of phytoplankton (diatoms and dinoflagellates) around Australia. Further taxonomic phytoplankton information exists from locations between 32°S and 35°S (Lee et al. 2001a, b, Lee et al. 2007) and from ~38°S (Bax et al. 2001; Fig. 6). A single study has provided a detailed species list including nano- (2 – 20 µm) and microphytoplankton (20 – 200 µm), as well as quantitative data (cell counts and Chl a) from the north Australian coast between ~10°S and ~22°S (Hallegraeff & Jeffrey 1981; Fig. 6). Since 2008, phytoplankton abundance, biovolume and pigment data is collected at the nine National Reference Stations within the IMOS framework (see

section 1.5, Fig. 3A; Lynch et al. 2011). In addition, since 2009, modern phytoplankton data is being collected along several sea routes around Australia with the Continuous Plankton Recorder (CPR; Fig. 6). The CPR is a robust device towed behind opportunistically available ships at about seven metres depth that filters plankton onto a 270  $\mu\text{m}$  mesh band of silk and preserves it inside the instrument (Richardson et al. 2006, <http://imos.org.au/australiancontinuousplanktonr3.html>).



**Figure 6. Map of historic and currently occupied phytoplankton sampling locations along the Australian coast.** Large white and yellow asterisks indicate sampling locations within this thesis (Coffs Harbour,  $\sim 30^\circ\text{S}$ ,  $153^\circ\text{E}$ , and the Kimberley region,  $\sim 15^\circ\text{S}$ ,  $122^\circ\text{E}$ , respectively). Large black asterisks show long-term sampling locations Port Hacking ( $\sim 34^\circ\text{S}$ , Eastern Australia), Maria Island ( $\sim 43^\circ\text{S}$ , Tasmania) and Rottnest Island ( $\sim 32^\circ\text{S}$ , Western Australia). Large pink asterisks indicate additional regions where taxonomic phytoplankton research has been conducted sporadically (see section 1.10). Small black asterisks indicate approximate sampling stations within a survey along Northern Australia (Hallegraeff & Jeffrey 1981). White lines indicate the present routes along which phytoplankton data has been collected by the Continuous Plankton Recorder (CPR) between June 2009 and April 2012 (adapted from <http://imos.org.au/australiancontinuousplanktonr3.html>). The green line indicates a proposed route for CPR data collection off North-Eastern Australia.

Early studies from ~34°S (Port Hacking, Eastern Australia) have shown that the timing of the annual phytoplankton spring bloom matches observations from the Northern Hemisphere (Dakin & Colefax 1933, 1940). Dakin & Colefax (1933, 1940), Humphrey (1963), Newell (1966), Grant & Kerr (1970), as well as more recent investigations by Hallegraeff (1981), Hallegraeff & Reid (1986) and Ajani et al. (2001), have shown that phytoplankton abundances are generally low compared to the Northern Hemisphere, with minima during austral winter and maxima (~5 µg Chl *a* L<sup>-1</sup>) during austral spring and summer. Upwelling of cold, nutrient-rich (phosphate, nitrate and silicate), bottom water into the euphotic zone during spring and summer has been shown to be responsible for such Chl *a* maxima, or phytoplankton blooms, that usually last for 2 – 6 weeks (Hallegraeff & Reid 1986, Hallegraeff & Jeffrey 1993, Ajani et al. 2001). Generally, upwelling can be driven by different mechanisms, including wind, EAC encroachment onto the continental shelf, EAC acceleration resulting from a narrowing continental shelf (at ~31°S) and EAC separation (at ~32°S; Roughan & Middleton 2002). However, at Port Hacking, the combination of wind- and current-driven upwelling, both of which are at maximum in summer (Rossi et al. 2014), has been shown to control the onset of the annual spring bloom (Hallegraeff & Jeffrey 1993, Ajani et al. 2001, Pritchard et al. 2003). As such, the upwelling-induced phytoplankton blooms along the Australian east coast differ from blooms in temperate regions of the Northern Hemisphere, which are the result of climatically-induced increased vertical mixing, temperature and light during spring (Hallegraeff & Jeffrey 1993).

Taxonomic phytoplankton research conducted at Port Hacking has shown that species succession patterns mirror the seasonal compositional cycle recognised in temperate regions worldwide. For example, small diatoms (*Asterionellopsis*, *Chaetoceros*, *Leptocylindrus*, *Skeletonema*, *Thalassiosira*) have been found to dominate short-lived spring and summer blooms in the initial phase, followed by large diatoms (*Detonula*, *Rhizosolenia*, *Stephanopyxis*) and dinoflagellates (*Ceratium*, *Protoperidinium*; Dakin & Colefax 1933, 1940, Hallegraeff & Reid 1986). Such a seasonal sequence from small diatoms to large diatoms to dinoflagellates is consistent with previous investigations from other temperate regions (Margalef 1978, Sommer 1985, Wyatt 2014).

The importance of small-sized phytoplankton (pico- and nanophytoplankton within the size-ranges of 0.2 – 2 µm and 2 – 20 µm, respectively) in contributing to the

phytoplankton community off Eastern Australia has been recognised only recently. Initially, Hallegraeff (1981) demonstrated that planktonic nanoflagellates, including coccolithophores and green flagellates, contribute 50 – 80% to the total Chl *a* throughout the year, and only 10 – 20% during the short-lived spring and summer blooms of diatoms. Despite their high abundance, little is currently known about the composition, distribution and preferred environmental conditions of the small-sized taxa occurring along Australia's coasts. Thompson et al. (2011a) have shown that picoplankton decrease in abundance with increasing latitude along the east and west Australian coast (between 27.5°S and 34.5°S), while nano- and microphytoplankton increase. The authors also reported a rapid decrease in microphytoplankton with distance from the coast, while pico- and nanophytoplankton increase. In particular, the ubiquitous picoplanktonic cyanobacterium *Synechococcus* has been shown to predominate in the offshore phytoplankton community, comprising 60% of the total Chl *a* based on a 14-year average (Thompson et al. 2011a). Considering the projected success of pico- and nanophytoplankton as climate change selects towards a smaller phytoplankton size (Rodríguez et al. 2006, Falkowski & Oliver 2007, Morán et al. 2010, Hallegraeff 2010), the study of pico- and nanophytoplankton is of crucial importance.

### **1.11 Overview of the phytoplankton dynamics off the west Australian coast**

Along the west Australian coast, phytoplankton research has mainly been conducted at ~32°S (Rottnest Island Station, Perth, Fremantle; Wood 1954, 1964a, b, Thompson & Waite 2003, Thompson et al. 2007), between ~22°S – 27°S (Gascoyne region; Hanson 2004, Hanson et al. 2005a, b, 2007) and ~15°S (Kimberley coast; (Thompson & Bonham 2011) (Fig. 6).

From these studies, it is known that phytoplankton biomass is generally very low, with Chl *a* concentration being on average only half the concentration of the east coast (Thompson et al. 2011a). A lack of the annual spring bloom has also been reported and derives from the seasonal progression of the warm oligotrophic LC, which strengthens during winter and reduces vertical mixing (Thompson et al. 2011a). The LC creates large-scale downwelling while travelling south along the shelf break (Pearce & Griffiths 1991, Smith et al. 1991, Waite et al. 2007). Such downwelling has been shown to restrict phytoplankton growth, biomass and

productivity in the euphotic zone (Hanson et al. 2005a, Thompson et al. 2009, 2011a). Generally, nitrogen has been identified as the limiting nutrient in the LC system (Hanson et al. 2007). This has been demonstrated by the relatively high amount of regenerative, ammonium based production, and nitrate concentrations being, on average, 15% that of east coast concentrations (Hanson et al. 2007). However, annual shelf-scale phytoplankton blooms have been observed between 22°S and 34°S during winter as a result of the seasonally enhanced Indonesian Throughflow (ITF; Thompson et al. 2011b), which feeds in to the LC at ~22°S (Fig. 2). The ITF has been shown to inject nitrogen into deep waters of the LC and after various mixing processes this nitrogen supply enables phytoplankton to bloom downstream (Thompson et al. 2011b). In addition, sporadic nutrient input promoting phytoplankton productivity has also been found to be caused by wind-driven upwelling resulting from counter currents (inshore of the LC) present during summer, and by coastal nutrient enrichment at ~26°S (Hanson et al. 2005a, b, Woo et al. 2006).

The distinction of offshore waters dominated by the oligotrophic LC from inshore-waters characterised by relatively high nutrient accessibility due to sporadic wind-driven upwelling are reflected in phytoplankton composition and distribution. Picoplankton, including the cyanobacteria *Prochlorococcus* and *Synechococcus*, haptophytes and nanoplanktonic cryptophytes have been shown to dominate the phytoplankton community in oligotrophic offshore regions, while diatoms have been found to occur mainly in nutrient-rich inshore waters (Hanson et al. 2007, Thompson et al. 2011a). Dinoflagellates seem to be relatively rare and equally composed of photoautotrophic and heterotrophic forms. Heterotrophic dinoflagellates have been found mainly offshore where they nutritionally benefit from the high abundance of picoplankton (Hanson et al. 2007). Little is known about the preferences of small and rare phytoplankton groups (e.g. prasinophytes) for specific environmental conditions that might drive their distributional patterns along the west Australian coast (as along the east Australian coast).

## ***1.12 Knowledge gaps in phytoplankton research along the east Australian coast***

### ***1.12.1 Research gaps on a large spatial (latitudinal) scale***

Phytoplankton research along the east Australian coast dates back to the 1930's, and some investigations have covered extended coastal and offshore areas. However, these studies are either numerically limited, focussed on areas south of the EAC separation zone (~34°S), or had priorities other than the coastal small-scale resolution of phytoplankton communities (section 1.10). Consequently, a clear lack of detailed taxonomic phytoplankton abundance and compositional data exists for about 1000 km upstream of the EAC separation point, spanning the tropical-temperate transition zone of Eastern Australia. Baseline phytoplankton research is particularly important in this region because the coastal oceanography within this transition zone is likely to be highly impacted by the anticipated strengthening EAC (Ridgway 2007, Hill et al. 2008). The lack of knowledge regarding natural variations in latitudinal phytoplankton distribution patterns along the east Australian tropical-temperate coast will complicate studies aimed at determining potential long-term range expansions of species within the context of a strengthening EAC.

### ***1.12.2 Research gaps on a small spatial (shelf) scale***

It is known from previous investigations at the east coast of Australia (mainly at ~34°S, Port Hacking) that oceanographic conditions related to the EAC are strong drivers of phytoplankton dynamics (section 1.10). However, little is known about the interactions between local oceanography and fine-scale cross-shelf phytoplankton community structures. Open questions include: (i) How do rapidly changing and highly differing oceanographic conditions, including encroachments of the EAC, influence the cross-shelf phytoplankton community structure along the Australian east coast? (ii) Which specific environmental variables associated with different oceanographic regimes are of major importance for explaining the variability in phytoplankton composition? Considering that the east Australian tropical-temperate transition zone is influenced by the main flow of the EAC and is exposed to frequent current-, wind-, and topographically-induced upwelling (Roughan & Middleton 2002, Schaeffer et al. 2013, Rossi et al. 2014), this region provides an excellent location for

investigating shelf-scale interactions of oceanographic forcing mechanisms and phytoplankton dynamics.

### ***1.12.3 Research gaps regarding pico- and nanophytoplankton and their environmental preferences***

Little is known about composition and distribution patterns in pico- and nanophytoplankton occurring along the east Australian coast, and even less about the preferences of these small-sized microalgae for certain environmental conditions. Pico- and nanophytoplankton have long been overlooked using traditional identification techniques, most of which are biased towards larger species. Their increased recognition has only come with the application of molecular and pigment analyses (see section 2.2; Moreira & López-García 2002, Jeffrey et al. 2011, Simon et al. 2009). Since the 1980's, it was known that small-sized phytoplankton are highly abundant in the world's ocean including the east Australian coast, and that, despite their size, these tiny cells make a globally significant contribution to ocean biomass and primary production (Hallegraeff 1981, Azam et al. 1983, Fogg 1986). Subsequent research has focussed on the major groups, such as cyanobacteria (Rost 2004, Paerl & Paul 2012) and coccolithophores (Balch 2004, Read et al. 2013) while information on the ecology and distribution patterns of less abundant groups (e.g. prasinophytes and cryptophytes) has remained sparse (Thomsen & Buck 1998, Zingone et al. 1999, Kristiansen 2000). A better understanding of the oceanographic driving forces of small-sized phytoplankton dynamics is vital to understand the functioning of Australian marine ecosystems, to improve ecosystem models, and to assess the role of pico- and nanophytoplankton in biogeochemical cycles. Such investigations are crucial at present and in the future, considering the predicted increase in abundance of these small-sized phytoplankton (Bopp 2005, Morán et al. 2010).



### **1.13 Aims and structure of this thesis**

The three major aims of this thesis address the main outstanding gaps in phytoplankton research along the East Australian coast. Those three aims are to:

1. Provide the first detailed taxonomic phytoplankton survey in the east Australian tropical-temperate transition zone upstream of the EAC separation point as a reference for future research.
2. Include pico-, nano- and microphytoplankton in the survey by applying microscopy and pigment analyses; and to determine strengths and weaknesses of both phytoplankton quantification techniques (Chapter 3).
3. Investigate the responses of individual phytoplankton taxa to different oceanographic conditions on a local (upwelling/downwelling, Chapter 4), regional (Eastern/Western Australia, Chapter 5) and temporal scale (within one year, Chapter 6).

The study design, instrumentation and methodology to pursue the three major aims of this thesis are described in Chapter 2.

It should be noted that as this phytoplankton survey is restricted to approximately one year of sampling, predictions cannot be made with regard to potential phytoplankton responses to climate change. However, baseline research as provided by this thesis is crucial to assess the natural variability in phytoplankton community structure, and the physical and chemical driving forces behind it. This thesis serves as a point of comparison for prospective studies, and will improve our understanding of interactions between phytoplankton and their oceanographic environment, in the present and in the future.

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## **2 Study design, instrumentation and methodology**

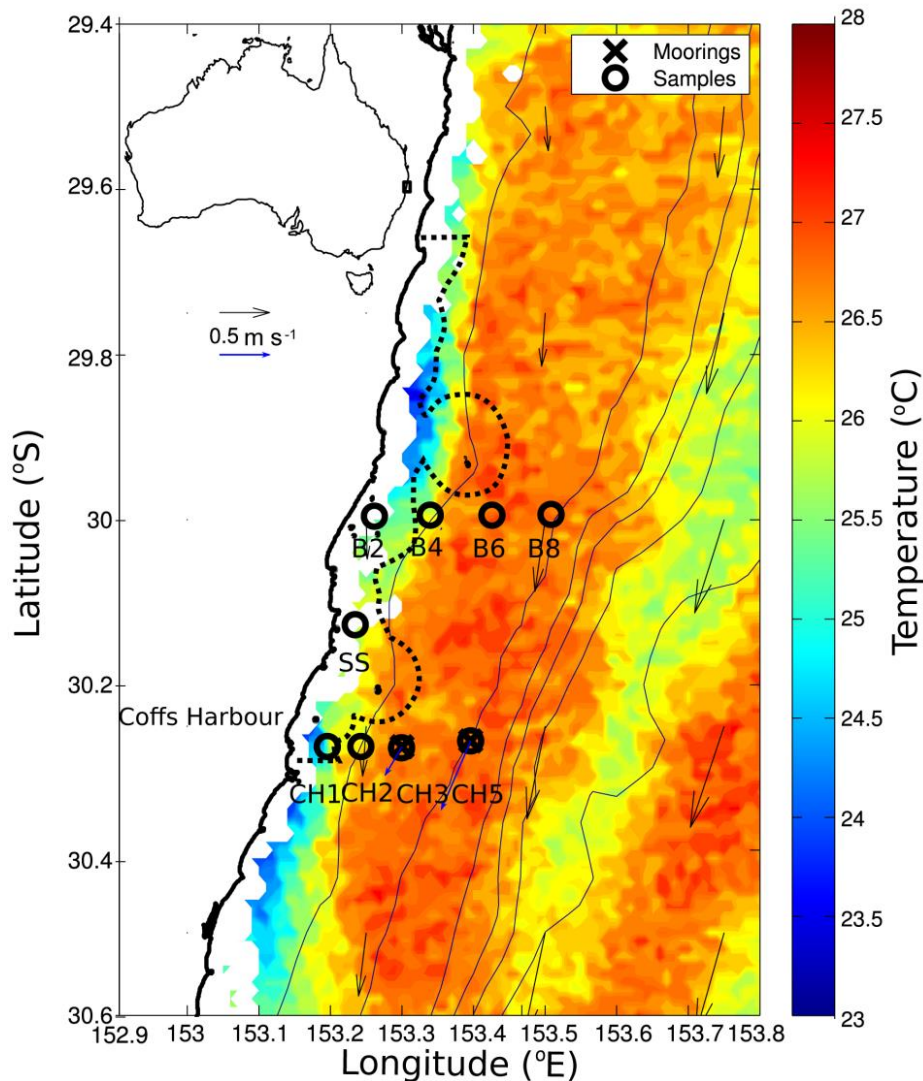
This chapter describes background information on the study design, instrumentation and methodology to pursue the three major aims of this thesis. Hence, in some cases the following descriptions represent a justification of the methodology adopted, rather than a detailed description of methods and materials. The methodology specific to each chapter is described in that chapter.

### ***2.1 Aim 1: Providing the first taxonomic phytoplankton survey in the east Australian tropical-temperate transition zone upstream of the EAC separation point as a reference for future research.***

#### ***2.1.1 Study location: Coffs Harbour***

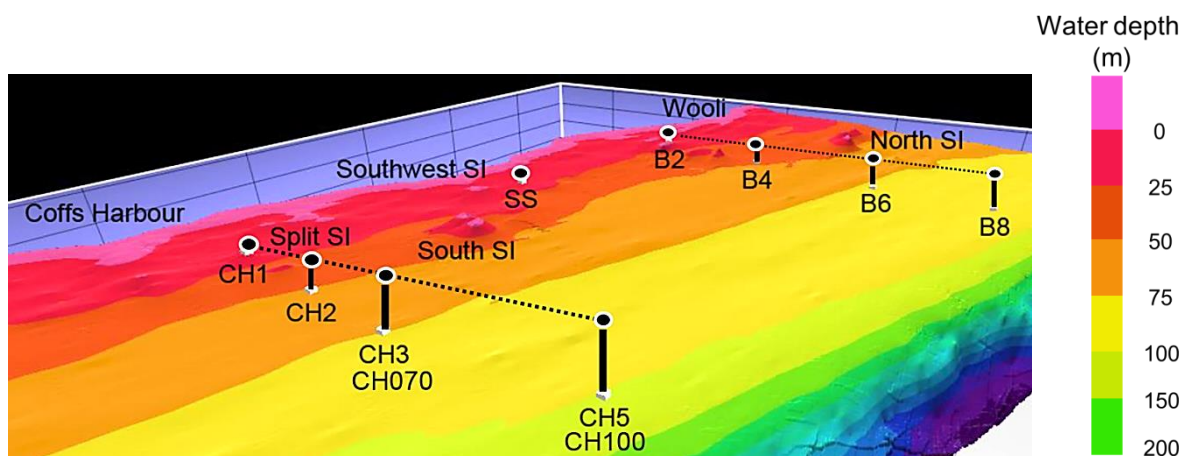
The primary study site for this Aim was located in the Coffs Harbour region (30°S, 153°E) at the southernmost delimitation of the Solitary Islands Marine Park (SIMP; Fig. 1). This region is regarded as a biological hotspot where the SIMP was established to protect the unique biodiversity between the temperate-tropical provinces (NSW MPA 2008, 2009). The SIMP accommodates the southernmost extent of coral and the northernmost extent of kelp along the coast of Eastern Australia (Harriott et al. 1994, Zann 2000, NSW MPA 2008, 2009). Being located just upstream of the EAC's typical separation point, the Coffs Harbour region is usually exposed to the influence of the main flow of the EAC. Therefore, it is expected that a high proportion of both temperate and tropical phytoplankton species will be found within this study. The continental shelf in the Coffs Harbour region is narrow (~30 km) and seasonally enhanced wind- and current-driven upwelling, as well as topographically-induced upwelling, is a common feature (Roughan & Middleton 2002). Several coastal and offshore reefs and eight small islands fringed by shallow rocky reefs contribute to an irregular bathymetry (Fig. 2; NSW MPA 2008). The combination of this complex topography and frequent EAC intrusions cause highly variable oceanographic conditions in the area associated with rapid temperature changes (NSW MPA 2008, Malcolm et al. 2011, Schaeffer et al. 2013). This thesis investigated whether shelf-scale physical processes affect the phytoplankton community. It is hypothesised that frequent and pronounced upwelling will enhance diatom growth where turbulent water occurs near the coast. As diatoms are highly

efficient primary producers (Uitz et al. 2010) the increased abundance of this phytoplankton group might be of importance for local biogeochemical cycles in the Coffs Harbour region.



**Figure 1. Sampling locations in the Coffs Harbour region (~30°S, 153°E).** Map showing the two cross-shelf sampling transects B-Line (north) and CH-Line (south) and one coastal intermediate station (SS) off Coffs Harbour. Black circles indicate sampling locations, crossed circles indicate the two IMOS moorings CH070 and CH100. The thick black lines represents the contour of the coastline while thin black lines indicate the 50, 100, 250, 500, 1000, 2000 m isobaths. The dashed black line represents the offshore delimitation of Solitary Islands Marine Park. The sampling locations are overlaid on an image showing sea surface temperature (Group for High Resolution Sea Surface Temperature from National Oceanic and Atmospheric Administration (NOAA) Advanced Very High Resolution Radiometer products) and geostrophic current velocity vectors from altimetry (NOAA, provided by CSIRO, black arrows) and from the IMOS moorings (blue arrows) on 25 January 2012. *Figure created in collaboration with A. Schaeffer, University of New South Wales, and adapted from Chapter 6 of this thesis, figure 1.*





**Figure 2. Bottom topography of the Coffs Harbour region (~30°S, 153°E).** Map showing the bottom topography including North, Southwest, Split and South Solitary Island (SI). Overlaid are the sampling stations along the two cross-shelf transects CH-Line and B-Line and the solitary sampling station SS near Southwest SI. Stations CH3 and CH5 are also the sites of the two IMOS moorings CH070 and CH100, respectively. (Map created in collaboration with A/Prof. Ian Goodwin and Miss Rhaelene Freeman, Department of Environment and Geography, Macquarie University.)

### 2.1.2 Sampling design

The sampling design for this phytoplankton survey built on the established IMOS infrastructure at Coffs Harbour and stations were chosen to include the moorings CH070 and CH100 (Figs. 1, 2; section 1.5). Sampling was undertaken along a Northern and a Southern transect (hereafter B-line and CH-Line, respectively) about 30 km apart. An intermediate coastal station (SS) was included to create an alongshore-transect (Figs. 1, 2). The terminology used here is consistent with previous oceanographic work that has been conducted in the Coffs Harbour region within the IMOS context. The CH-Line and the B-Line extended 26 and 28 km off the coast, respectively. Phytoplankton sampling stations, numbered in ascending order (B2 and CH1, B4 and CH2, B6 and CH3, B8 and CH5) were located at the 25, 50, 70 and 100 m isobaths across the continental shelf (Figs. 1, 2). Both the CH and the B-line extend from the coastal SIMP into the adjacent Solitary Islands Marine Reserve (east of the 50 m isobath). Sampling was undertaken monthly during the morning (usually between 9 am and 2 pm, with variations depending on the weather) from offshore to inshore. The CH- and the B-Line were sampled separately on two days

(usually consecutive, depending on the weather) and SS was sampled on the same day as the B-Line.

### **2.1.3 Hydrographic sampling**

Two different *Research Vessels* (RV) were used depending on availability and concurrent field surveys employing the same vessels. *RV Bombora* (Fig. 3A) was provided by the NSW Office of Environment and Heritage, and *RV Circe* (Fig. 3B) was provided by the National Marine Science Centre (NMSC, Coffs Harbour).

*RV Bombora* was equipped with an A-Frame via which a water sampling carousel (SBE32, Sea-Bird Electronics, Inc., USA) with 12 x 5 L Niskin-bottles (General Oceanics, USA), an SBE 911*plus* (Sea-Bird Electronics, USA) Conductivity-Temperature-Depth (CTD) profiler and an ECO FLNTU fluorescence sensor (Wetlabs, USA) were automatically operated (Fig. 3C). After a three minute surface soak, the CTD was lowered until about 3 - 5 m above the seafloor. Real-time fluorescence profiles during downward casts enabled the detection of the depth of the Chl *a* maximum (DCM). Samples were taken during the upward casts at the DCM and in 20 m intervals depending on the bottom depth, except for one 30 m interval at CH5 between 60 m and 90 m depth. Additional samples were collected at the inshore stations at 10 m depth. Surface samples were collected in a 10 L plastic bucket. After collection, 2 L water samples were immediately fixed with 6 mL Lugol's acid solution (100 g KI + 50 g I<sub>2</sub> in 1 L H<sub>2</sub>O + 100 mL glacial acetic acid; Sournia, 1978) for subsequent laboratory-based identification and enumeration of phytoplankton by microscopy (section 2.1.4). For phytoplankton pigment analysis 2 L of water sample was filtered on board under dark conditions onto 25 mm GF/F filter papers (Whatman, UK) and frozen in liquid nitrogen until further processing (at CSIRO Hobart, Tasmania, section 2.1.5). Sampling on *RV Bombora* was undertaken during May, June, August, September 2011 and September 2012.

On *RV Circe* sampling was undertaken manually by attaching a CTD at the end of a high-strength rope. Individual 5 L Niskin bottles were attached to the rope (Fig. 3D) in the same fixed intervals as described above. After a three minute surface soak, the equipment was lowered into the water using an electrical winch. Depending on availability due to concurrent IMOS work, three different CTDs were used throughout this study. During July 2011 a SBE25 Sealogger (Sea-Bird Electronics, USA) fitted

with an AquaTracka III fluorescence sensor (Chelsea Technologies Ltd., UK) was employed. During October 2011 and in all following months (except during September 2012 when sampling was done on *RV Bombora*) a SBE19+ SEACAT Profiler (Sea-Bird Electronics, USA) equipped with a ECO FLNTU fluorescence sensor (Wetlabs, USA) was used. For further information on the maintenance and calibration of these instruments see section 2.1.6. The 2 L water samples were fixed immediately for subsequent analysis of phytoplankton by microscopy as on the *Bombora*. The *Circe* was much smaller (7.3 m) and less stable than the *Bombora* (11.6 m). So, water samples for pigment analysis were kept in 10 L carboys under dark conditions and filtered at the NMSC laboratories four hours after sampling.



**Figure 3. Research Vessels and sampling equipment.** A) *RV Bombora* provided by the New South Wales Office of Environment and Heritage, Sydney, and B) *RV Circe* provided by the National Marine Science Centre, Coffs Harbour, to undertake the field campaigns in the Coffs Harbour region. C) A-frame and sampling rosette including Niskin bottles and CTD on *RV Bombora*. D) Niskin bottle manually attached to a rope on *RV Circe*.

### **2.1.4 *Microphytoplankton sample preparation and analysis***

The 2 L samples collected on board *RV Bombora* and *RV Circe* were transported to the laboratories of the Sydney Institute of Marine Sciences (SIMS, Chowder Bay, NSW) and concentrated by sedimentation. During the sedimentation procedure, the 2 L samples were settled for 48 hrs and siphoned twice, to achieve a final concentrated sample volume of 100 mL. Depending on the cell density, a 3 mL subsample of the 100 mL sample was counted, or the 100 mL sample was further concentrated within an Utermöhl sediment cylinder mounted onto a 3 mL counting chamber (HydroBios, Germany). Identification and enumeration under an inverted microscope (Leica DMI 3000B) followed the protocols of Utermöhl (1958). At least 400 cells per sample were counted at 200x. Taxonomic guides used included Dakin & Colefax (1940), Wood (1954, 1961a, b), Crosby & Wood (1958, 1959), Wood et al. (1959), Tomas (1997), Hallegraeff et al. (2010) as well as studies by Hallegraeff & Reid (1986), Ajani et al. (2001), Gómez et al. (2008) and Stidolph et al. (2012). To facilitate the identification of thecate dinoflagellates, a Calcofluor White Stain (Sigma Aldrich, USA) solution (was added to each sample 30 minutes prior to counting, at a final concentration of 20  $\mu\text{g mL}^{-1}$  (Fritz & Triemer 1985). Species and genus names currently accepted as defined by the World Register of Marine Sciences (WoRMS, <http://www.marinespecies.org/>) are used throughout this thesis.

### **2.1.5 *Pigment analyses***

Pigment analyses were conducted at CSIRO, Hobart (Tasmania), following internationally standardised protocols (Clementson 2010, Hooker et al. 2010). After thawing, pigments were extracted in 3 mL of 100% acetone, vortexed (~30 sec), sonicated in an ice-water bath under darkened conditions (~15 min) and then kept in the dark at 4 °C for approximately 15 hrs. Subsequently, a 90:10 acetone:water mixture was prepared, sonicated in an ice-water bath (~15 min), quantitatively transferred to a clean centrifuge, centrifuged to remove remaining filter paper, and filtered through a 0.2  $\mu\text{m}$  membrane filter (Whatman). High-Performance Liquid Chromatography (HPLC) was conducted using a Waters-Alliance system (a 2695XE separations module with column heater and refrigerated autosampler and a 2996 photo-diode array detector). Each sample was mixed with a buffer solution (90:10 28 mM tetrabutyl ammonium acetate, pH 6.5 : methanol) prior to injection. Pigments were separated using a Zorbax Eclipse XDB-C8 stainless steel 150 mm x 4.6 mm ID

column with 3.5  $\mu\text{m}$  particle size (Agilent Technologies, USA) and a binary gradient elution procedure. The flow rate was 1.1  $\text{mL min}^{-1}$  and the column temperature was 55°C. The separated pigments were detected at 436 nm, and identified and quantified against standard spectra using Waters Empower software. Pigment standards of chlorophyll *a*, chlorophyll *b*, and  $\beta\beta$ -carotene were obtained from Sigma Aldrich, USA while all other pigment standards were obtained from DHI, Denmark. A total of 29 standards were used, of which all but antheraxanthin, myxoxanthophyll and lycopene are listed in Clementson (2010).

### **2.1.6 Conductivity-Temperature-Depth profiles**

Throughout this thesis, environmental data recorded during Conductivity-Temperature-Depth (CTD) profiles was used to determine the oceanographic conditions at the time of sampling. Raw data recorded by the conductivity, temperature, pressure and fluorescence sensors were converted to salinity (in practical salinity units, psu, derived from conductivity), temperature (in °C) and depth (in m, derived from pressure) using Sea-Bird software (Sea-Bird Electronics, USA). To ensure standardisation of the data collected within this survey, processing of CTD and fluorescence profiles was consistently conducted using the Seabird SBE data processing software (Sea-Bird Electronics, Inc., USA) following IMOS CTD processing protocols (<http://imos.org.au/anmndocuments.html>). However, individual casts were carefully examined as three different CTD and fluorescence instruments were used throughout this survey (see section 2.1.3). CTD sensors were calibrated in approximately annual intervals at Seabird-Electronic, Inc., USA, or at CSIRO, Hobart. Factory calibrations prior to 2011 indicated a mean  $\pm$  drift in the CT-cells at 0.001°C year<sup>-1</sup> for temperature and 0.002 psu month<sup>-1</sup> for salinity. The AquaTracka III fluorometer on the SBE25 was calibrated quarterly against a spinach extraction solution (Sigma Aldrich, USA) in acetone (as per instruction by the manufacturer Chelsea, USA) whereas the FLNTU fluorometers on the SBE911*plus* and the SBE19+ were calibrated against a *Thalassiosira rotula* culture (in 2008 for the SBE19+ and in 2010 for the SBE911*plus*) at Wetlabs, USA. Based on regression analyses between fluorescence recorded during CTD casts and Chl *a* concentrations derived from simultaneously collected pigment samples, fluorescence values recorded by the FLNTU sensor on *RV Bombora* were determined to be on average 0.07  $\mu\text{g Chl a L}^{-1}$  lower than the FLNTU recordings on *RV Circe* (no HPLC data was

collected during CTD casts using the AquaTracka III sensor). This offset between different sensors has no effect on the data in this thesis because fluorescence recordings were only included in the data analysis in Chapters 4 and 5, both of which were based on samples collected on *RV Bombora* using the same FLNTU sensor.

### ***2.2 Aim 2: To include pico-, nano- and microphytoplankton in the survey by applying microscopy and pigment analyses; and to determine strengths and weaknesses of both phytoplankton quantification techniques (Chapter 3).***

Broad-scale techniques, such as remote sensing (Blondeau-Patissier et al. 2014) and molecular techniques (Ryneckson & Palenik 2011), have revolutionised the assessment of phytoplankton research in recent years and will be acknowledged here. However, this thesis applies only microscopy and phytoplankton pigment analysis to investigate phytoplankton community structures from pico- to microphytoplankton. A description of the methodology for microscopy and pigment (HPLC) analysis was provided above (sections 2.1.4 and 2.1.5). This section gives further background information on the two techniques, which is crucial for the interpretation of their results.

Microscopy is a traditionally and commonly used worldwide to identify phytoplankton to species level based on morphology. Different techniques include light, scanning and transmission electron microscopy (SEM/TEM). Phytoplankton species identification by light microscopy is usually restricted to microphytoplankton, due to its relatively low resolution. However, sample preparation for light microscopy is easy because live and fixed phytoplankton in water samples can be analysed directly in specialised counting chambers (Lund et al. 1958). SEM and TEM have high resolution and are therefore better suited to identify pico- and nanophytoplankton. However, sample preparation is more laborious compared to light microscopy and SEM/TEM microscopes are both expensive and less readily available. Disadvantages of all microscopy techniques include their bias towards larger cells, the frequent loss of fragile species in fixatives, their reliance on the expertise of the analyst and poor counting statistics (Lund et al. 1958, Sournia 1978, and described in detail in Chapter 3).



Pigment analyses are based on the observation that individual phytoplankton taxa can be distinguished based on their taxon-specific pigment composition. Over evolutionary time-scales, a sequence of endosymbiosis events occurred in the emergence of modern phytoplankton. In these events microalgae were engulfed, incorporated as membrane-bound organelles (plastids) and partially integrated into the hosts nuclear genome, leading to the development of differently pigmented phytoplankton taxa (Falkowski et al. 2004, Simon et al. 2009, Jeffrey et al. 2011). The first taxa belonged to the Archeplastida, which developed after a heterotrophic host had engulfed a photosynthetic cyanobacterium 1.5 billion years ago (Simon et al. 2009). This group includes the phyla Glaucophyta, Rhodophyta and Chloroplastida (Simon et al. 2009, and references therein). Ultimately, the three major phytoplankton classes of the contemporary ocean developed through secondary endosymbiosis by a common heterotrophic protist engulfing an early rhodophyte. The resulting diatoms, dinoflagellates, and haptophytes (including coccolithophores), contained fucoxanthin, peridinin and 19'hexanoyloxyfucoxanthin as their main pigments, respectively (Simon et al. 2009).

High-Performance Liquid Chromatography (HPLC) is a common method to detect pigments in water samples. It enables a qualitative estimate of the phytoplankton taxa present (see section 2.1.5). However, some pigments occur in several taxa (shared pigments), in which case the taxon-specific pigment ratio is used for identification. To enable quantitative assessment of phytoplankton taxa in water samples based on such ratios, complementary computer software has been developed such as CHEMTAX (Mackey et al. 1996), which will be applied in this thesis. There are some difficulties associated with CHEMTAX analyses. For example, a pre-selection of phytoplankton taxa to be included in the analysis has to be made by the analyst. This firstly restricts and secondly biases the analysis towards the initial taxon selection. The presence of shared pigments and pigments retained in heterotrophic taxa that were abundant in the water sample might also complicate and confuse the analysis. To ensure the most appropriate initial taxon selection and to account for the presence of heterotrophs in the samples, a simultaneous examination of the sample by microscopy is unavoidable.

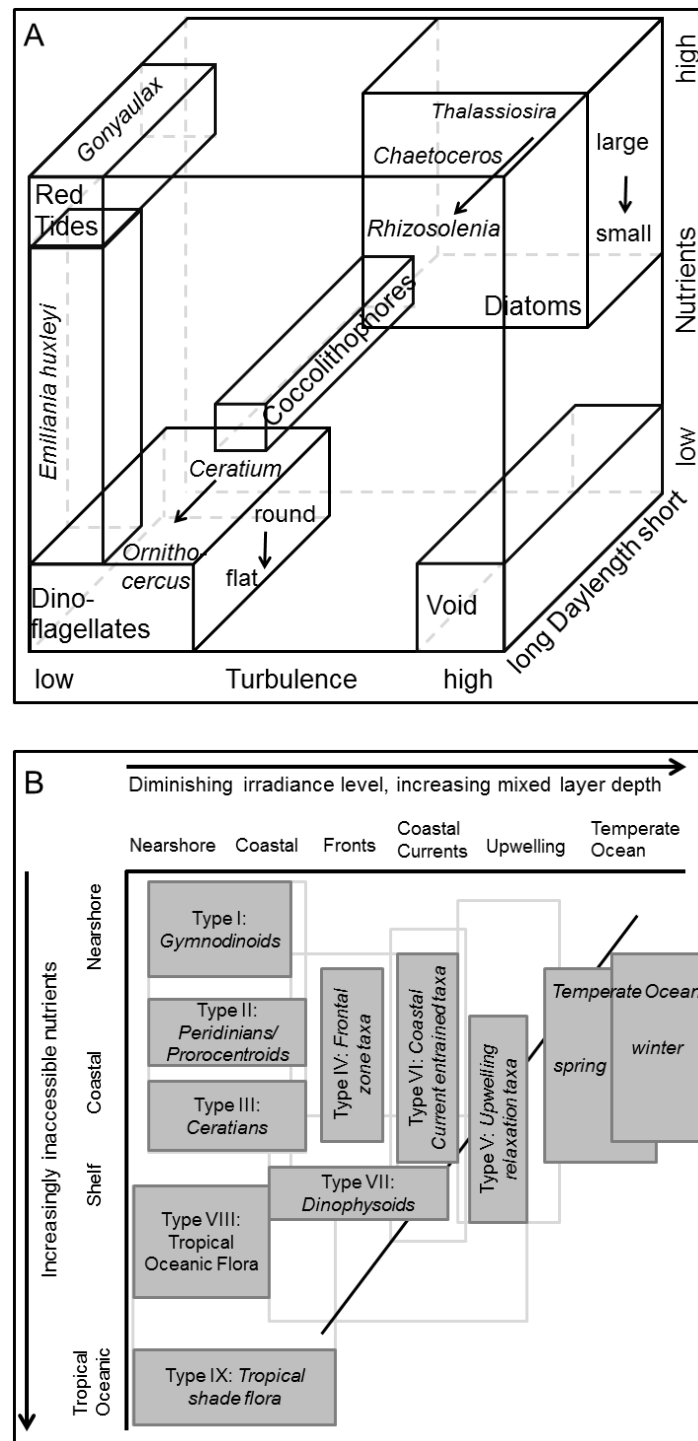
It is evident that both microscopy and pigment/CHEMTAX analyses each have their advantages and disadvantages. In order to comprehensively characterise phytoplankton communities, the results of each technique need to be carefully

assessed and compared. Thus, a comparative study highlighting the strengths and weaknesses of both techniques is included as the first data chapter of this thesis (Chapter 3). The interaction of oceanographic conditions and individual phytoplankton taxa, including pico- to microphytoplankton determined by both microscopy and pigment analyses, is addressed in Chapters 4 – 6.

### ***2.3 Aim 3: To determine the responses of individual phytoplankton taxa to different oceanographic conditions on a local (upwelling/downwelling, Chapter 4), regional (Eastern/Western Australia, Chapter 5) and temporal scale (within about 1 year, Chapter 6).***

Phytoplankton are free-floating (planktos = Greek for drift or wander), sensitive organisms with high turnover rates. As a result, they can be used as indicators for distinct water masses and environmental conditions. Conceptual models have demonstrated that different phytoplankton morphotypes occur along defined gradients of turbulence (vertical mixing) and nutrient availability (Fig. 4A, B; Margalef 1978, Smayda & Reynolds 2001, Wyatt 2014). For example, tough and relatively large diatoms have been shown to prefer vertically mixed regions, such as coastal, upwelling and surf regions (Fig. 4A; Margalef 1978, Richardson et al. 1983, Kiørboe 1993). Coccolithophore blooms occur under a wide range of nutrient concentrations in stratified waters during periods of elongated irradiance (Fig. 4A; Balch 2004). Dinoflagellates prefer calm, warm (stratified) and oligotrophic waters (Margalef 1978, Smayda & Reynolds 2001). They occur along cross-shelf gradients characterised by turbulence, nutrient availability and irradiance, and can bloom if exposed to high nutrients (such as in estuaries or eutrophic areas; Margalef 1978, Smayda & Reynolds 2001; Fig 4B). In a seasonal context, such knowledge has greatly improved the predictability of highly sporadic and stochastic harmful algal blooms (Burkholder et al. 2006).





**Figure 4. Conceptual models of phytoplankton preferences for environmental conditions.** A) Schematic showing a combination of Margalef's two- and Balch's three-dimensional Mandala (*redrawn based on Margalef, 1978, figure 2 and Balch et al. 2004, figure 9*). Phytoplankton life-forms (morphotypes) are placed in a three-dimensional space defined by nutrient concentration, turbulence and day-length. B) Schematic showing nine dinoflagellate life-forms (morpho-types) occurring along cross-shore gradients of decreasing nutrients, reduced mixing, and deepened euphotic zone. Light grey boxes indicate overlapping habitats. Diagonal black line indicates successive patterns depicted in Margalef, 1978 (*redrawn based on Smayda and Reynolds, 2001, figures 4 and 5*).

Not much is known about the phytoplankton responses to specific oceanographic conditions and environmental variables occurring along gradients across the shelf. Such knowledge may be of particular importance along the east Australian coast with its high EAC exposure. Thus, the shelf-scale oceanographic environment and responses of individual phytoplankton taxa in the Coffs Harbour region were investigated in this thesis. Phytoplankton variability (i.e. abundance, distribution and composition) were examined under contrasting oceanographic conditions, specifically, an EAC-driven upwelling and a wind-driven downwelling event (Chapter 4). The natural variability in phytoplankton community structure under rapidly (within 10 days) changing oceanographic conditions was determined and specific taxa associated with upwelling or downwelling identified. Chapter 5 was designed to address the importance of stratification and nutrient accessibility in determining the cross-shelf phytoplankton distribution, composition and size structure off Australia's coast. The phytoplankton community of two regions characterised by distinct topographic and oceanographic conditions, the Kimberley region (Western Australia, ~16°S, 126°E, shelf width of ~200 km), and the Coffs Harbour region (shelf width ~30 km), were contrasted. Parallels were investigated between phytoplankton preferences for certain environmental conditions that occur along cross-shelf gradients in both regions. This chapter provided a key step forward in comparing the phytoplankton community composition (pico-, nano-, and microphytoplankton) from two different coasts of Australia. Chapter 6 was based on the microscopically determined phytoplankton data collected throughout a nearly complete annual cycle (2011/2012) and aims at elucidating baseline seasonal patterns in phytoplankton abundance and composition in the Coffs Harbour region and the influence of the EAC on any such patterns.

All data chapters within this thesis used a statistical approach to reveal correlations and associations of environmental variables and phytoplankton dynamics. In Chapters 4 – 6, the statistical approach was undertaken with the explicit purpose of moving away from purely descriptive phytoplankton assessments and to provide a rigorous, statistics-based analysis of interactions between oceanographic forcing mechanisms and phytoplankton dynamics. Statistical software used in this thesis were Minitab Version 16 (2010), PRIMER Version 6.1.12 including the add on PERMANOVA Version 1.0.2 (Clarke & Gorley 2006) and PAST (Hammer et al. 2001).

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## Chapter 3

A new approach to testing the agreement of two phytoplankton quantification techniques: Microscopy and CHEMTAX

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**Key words:** HPLC; Bland and Altman analysis; Optimisation ratios; Diatoms, Dinoflagellates; Coffs Harbour.

**Abbreviations:** MF = multiplying factor; MLD = mixed layer depth; Chl *a* = chlorophyll *a*; DCM = depth of the deep Chl *a* maximum; HPLC = High-Performance Liquid Chromatography.



**Abstract**

Strengths of numerical relationships between phytoplankton abundance estimates made by microscopy and CHEMTAX have often been tested using regression analysis. To specifically test agreement, the Bland and Altman technique is commonly used in the medical literature and applied here to phytoplankton analysis for the first time (simultaneously with regression). Our analyses are based on a sample set collected off Coffs Harbour (~30°S), Eastern Australia, where diatoms are the dominant phytoplankton group during productive upwelling periods and a focus taxon of our investigation. While comparing abundance estimates of individual phytoplankton pigment-types derived from microscopy and CHEMTAX we specifically aim at identifying the lowest taxonomic level (i.e. inter- or intra-class level) at which phytoplankton abundance estimates made by both quantification techniques agree. Microscopy and CHEMTAX confirmed that diatoms were the most abundant phytoplankton class off Coffs Harbour. Microscopy tended to overestimate abundances of individual phytoplankton taxa relative to CHEMTAX. Best/poor agreement was found within dinoflagellates (class-level)/individual diatom pigment-types (intra-class level). We attributed the poor agreement between the diatom abundance estimates to classification errors of microscopically determined taxa into pigment-types. Our results suggest that intra-specific pigment composition is likely to be more variable than generally assumed, invalidating the use of microscopy and CHEMTAX interchangeably beyond the class level. We conclude that regression and Bland and Altman analyses are suited to resolve imbalances between phytoplankton abundance estimates made by microscopy and CHEMTAX. More research aimed at determining the magnitude of intra-specific pigment variation is crucial if we are to comprehensively picture natural phytoplankton assemblages.

**1 Introduction**

Techniques to rapidly identify and quantify phytoplankton have been a matter of discussion in the past with regard to their advantages and disadvantages. Ultimately, the approach of choice usually depends on the aim of the investigation, for example, to detect particular ecologically significant species or to assess phytoplankton communities over large spatial scales. Equally, minimising costs and analysis-time,

technical experience and equipment accessibility can all be additional or competing concerns in technique selection.

Traditionally, light microscopy has been widely applied for the identification and enumeration of phytoplankton to species level. However, due to the limited resolution of this technique, only the microphytoplankton size class (20 - 200  $\mu\text{m}$ ) is well distinguished. For some phytoplankton genera, species-specific morphological characteristics are extremely fine-structured, making determination to species level difficult or impossible (e.g. species of the genera *Prorocentrum* spp. (dinoflagellate) or *Pseudo-nitzschia* spp. (diatom); Hoppenrath et al. 2013, Ajani et al. 2013, respectively). Additionally, fragile phytoplankton taxa (such as naked flagellates) are often difficult to distinguish or are lost completely as they are poorly preserved even in samples that contain the best fixatives. High-resolution scanning or transmission electron microscopy (SEM/TEM) has been used to identify small-sized (i.e. pico- and nanoplankton, 0.2 - 2 and 2 - 20  $\mu\text{m}$ , respectively), or morphologically fine-structured phytoplankton to species level. However, SEM and TEM are costly. In general, the main disadvantage of all three microscopy techniques lies in their time-consuming nature and the reliance on the taxonomic experience of the analyst. Also microscopy analyses are selective for large robust cells and suffer from inherent poor counting precision unless huge numbers of cells are counted (Lund et al. 1958, Sournia 1978).

Pigment analysis provides an approach to investigate phytoplankton community structure on broader spatial and temporal scales. While chlorophyll *a* (Chl *a*) serves as a proxy for phytoplankton biomass, the composition of so-called biomarker pigments in a given sample resolves qualitative and quantitative information about the sampled phytoplankton community (Jeffrey et al. 2005, Jeffrey & Vesk 2005). Some biomarker pigments are restricted to specific algal classes, such as prasinoxanthin and peridinin in prasinophytes and dinoflagellates, respectively, while others are less specific, e.g. fucoxanthin in diatoms, haptophytes, raphidophytes and some dinoflagellates (Jeffrey et al. 2005). High-Performance Liquid Chromatography (HPLC) is commonly applied to analyse pigment samples harvested from phytoplankton cultures or field samples (Van Heukelem & Thomas 2001, Clementson 2010, Hooker et al. 2010). Complementary computer software has been developed to optimise pigment data and assign it to particular microalgal classes, such as CHEMTAX (Mackey et al. 1996, Wright & Jeffrey 2006). CHEMTAX is a matrix factorisation program in which biomass (in Chl *a* units) of algal classes is estimated from concentrations of marker pigments

determined by HPLC analyses of water samples (Mackey et al. 1996). A set of predetermined marker pigment:Chl *a* ratios (characteristic for each algal class, normally derived from literature values) is optimised through multiple iterations in CHEMTAX, based on a given HPLC dataset. However, pigment-based identification techniques are generally restricted to phytoplankton determination down to class level (with a few exceptions such as *Prochlorococcus marinus*, the only microalgal species containing divinyl Chl *a* and *b*; Jeffrey et al. 2005, Jeffrey & Vesk 2005). Furthermore, pigment data can be misleading as it might suggest the presence of pigmented species that have been ingested by heterotrophic or mixotrophic phytoplankton prior to sampling. Unless simultaneous microscopic analyses are made to establish the relative abundance of the heterotrophic/mixotrophic community against the phytoplankton source, the interpretation might be deceptive.

Several comparisons between CHEMTAX and microscopy have generally found good correlations between the techniques, despite sampling and processing errors involved in both techniques (reviewed in Higgins et al. 2011). However, a statistically significant correlation does not guarantee good agreement between techniques. The regression coefficient  $r$  measures the strength of a linear relationship between two variables ( $x$ ,  $y$ ) of the form  $y = a + bx$ , where  $a$  is the offset and  $b$  is the slope. It does not measure the agreement between the variables: specifically, it does not test whether the slope ( $b$ ) equals 1, whether there are systematic offsets ( $a$ ), or whether there are non-linearities in the relationship. A better comparison of techniques is the Bland and Altman plot in which, for each data point, the difference between two estimates is plotted against their mean (Bland & Altman 1986). This technique allows biases and non-linearities to be better identified, and it is the predominant technique for comparing methods in the field of medicine (reviewed in Zaki et al. 2012).

In this study, our aims were to provide clear evidence of any differences or similarities between abundance estimates derived from the two major phytoplankton quantification techniques: Light microscopy and CHEMTAX. Abundance estimates made by both techniques were critically compared using both regression and agreement analysis. We determined the level of taxonomic discrimination achievable from pigment data and compared pigment-derived abundance estimates of three diatom-types (i.e. within-class) with abundance estimates derived from microscopy (where phytoplankton species were classified into pigment-types based on the literature). We expected good correlations and agreement between abundance estimates of phytoplankton taxa

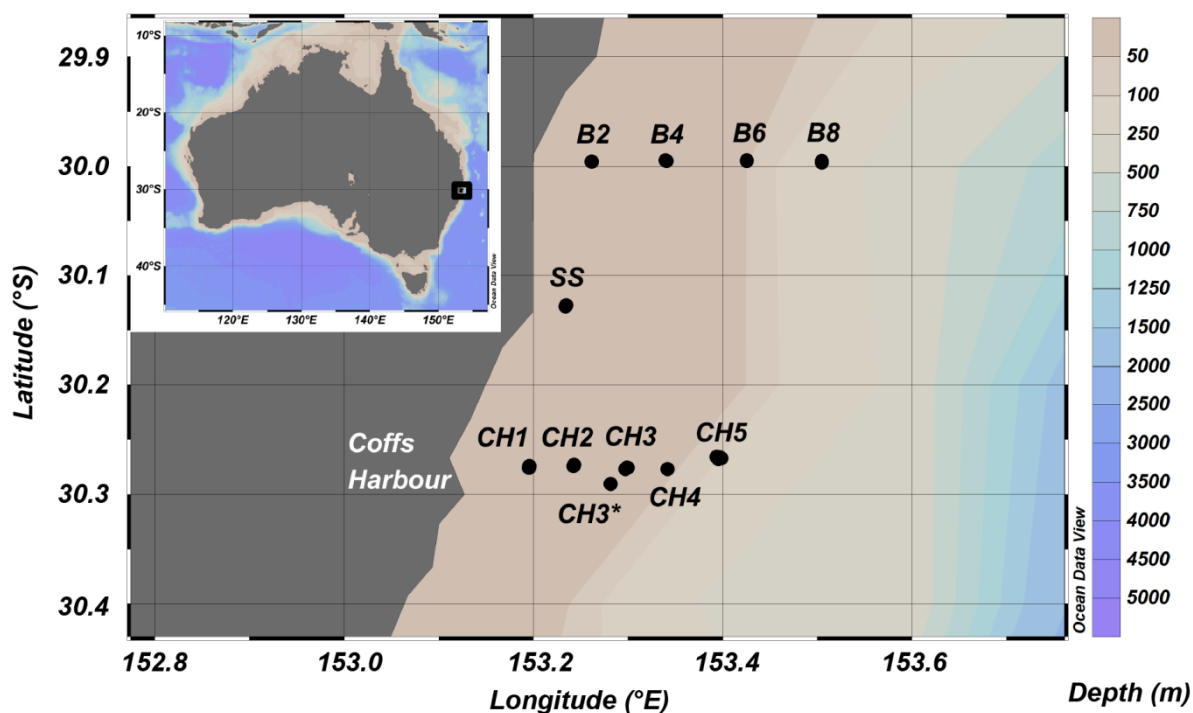
containing unambiguous pigment markers, such as peridinin in dinoflagellates. On the other hand, we expected that intra-class discrimination of diatoms, which contain the commonly shared pigment fucoxanthin, would be associated with weak correlations and poor agreement.

## 2 Methods

### 2.1 Study location and equipment

Water sampling for subsequent phytoplankton and pigment analysis was undertaken along a Northern and a Southern cross-shelf transect (henceforth B- and CH-Line, respectively) and one coastal intermediate station (SS) off Coffs Harbour (~30°S), Eastern Australia, between May 2011 and September 2012 (Fig. 1, Table 1). The terminology used for all sampling locations (B-, CH-Line and SS) is consistent with previous phytoplankton research conducted off Coffs Harbour (Armbrecht et al. 2014). Along the B- and CH-Line, four sampling stations were located above the 25, 50, 70 and 100 m isobaths (Fig. 1). Both the B- and the CH-Line extend 28 and 26 km in an inshore-offshore direction, respectively (Fig.1).

Sampling was either undertaken on the *RV Bombora* (provided by the NSW Office of Environment and Heritage, OEH) or the *RV Circe* (provided by the National Marine Science Centre, NMSC, Coffs Harbour). The vessel and equipment used for each sampling and the exact sampling station coordinates are given in Table 1. On *RV Bombora*, an A-frame was used to deploy an automatic SBE32 carousel water sampler (Sea-Bird Electronics, Inc., USA) with 12 x 5 L Niskin-bottles (General Oceanics, USA). The carousel was fitted with a SBE 911*plus* Conductivity-Temperature-Depth (CTD) profiler (Seabird Electronics, Inc., USA) and an ECO FLNTU fluorescence sensor (Wetlabs, Inc., USA). Samples were taken at the depth of the Chl *a* maximum as recorded in real-time by the fluorescence sensor. Surface samples were collected in a 10 L plastic bucket. On *RV Circe*, water samples from 20 m depth were taken manually with a Niskin bottle (5 L capacity, General Oceanics, USA) attached to a rope and lowered into the water. Surface water was collected in a 10 L plastic bucket. Physical parameters were recorded using an SBE19*plus* CTD (Seabird Electronics, Inc., USA) that was attached to the end of the rope. Post-processing of CTD profiles was conducted using the Seabird SBE Data Processing software (Sea-Bird Electronics,



**Figure 1. Sampling locations in the Coffs Harbour region, Eastern Australia.** Map of Australia (upper left) and expansion of the Coffs Harbour region showing the sampling sites (B-Line, CH-Line and SS, see Table 1 for sampling details). Land and coastline contour are shown in dark grey (colour bar settings adapted from default configurations in Ocean Data View; Schlitzer, R., Ocean Data View, <http://odv.awi.de>, 2013).

**Table 1. Sampling dates and locations.** Coordinates are given in decimal degrees. Also listed are the *Research vessel* (RV) and the Conductivity-Temperature-Depth (CTD) profiler used during each sampling. Asterisks indicate depths at which duplicate HPLC samples for validation purposes were collected. On 27 May 2011, sampling at CH3\* was undertaken under poor weather conditions, therefore the position slightly deviated from usual sampling location (CH3; see Fig. 1).

Date	Station ID	Latitude (S)	Longitude (E)	Sample depths (m)	RV, CTD
27 May 2011	CH3*	30.291	153.282	0*, 40*	<i>RV Bombora</i> , SBE911 <i>plus</i> (including an ECO FLNTU fluorescence sensor)
28 May 2011	CH1	30.276	153.195	0, 21	
	CH2	30.274	153.243	0*, 25*	
	CH3	30.276	153.300	0*, 20*	
7 June 2011	CH5	30.266	153.393	0*, 40*, 60	<i>RV Circe</i> , SBE19+ SEACAT Profiler
	CH1	30.276	153.196	0*, 12*, 26*	
	CH3	30.277	153.297	0*, 30*	
	CH5	30.267	153.399	0*, 35*	
6 December 2011	B2	29.996	153.262	0, 20	<i>RV Bombora</i> , SBE911 <i>plus</i> (including an ECO FLNTU fluorescence sensor)
	B4	29.995	153.340	0, 20	
	B6	29.995	153.426	0, 20	
	SS	30.127	153.235	0, 20	
7 December 2011	CH1	30.274	153.195	0*, 20*	<i>RV Bombora</i> , SBE911 <i>plus</i> (including an ECO FLNTU fluorescence sensor)
	CH2	30.274	153.242	0*, 20*	
	CH3	30.276	153.299	0*, 20*	
	CH5	30.266	153.397	0*, 20*	
24 January 2012	CH1	30.274	153.195	0, 20	<i>RV Bombora</i> , SBE911 <i>plus</i> (including an ECO FLNTU fluorescence sensor)
	CH2	30.274	153.242	0, 20	
	CH3	30.276	153.299	0, 20	
	CH5	30.266	153.397	0, 20	
27 February 2012	B2	29.996	153.262	0, 20	<i>RV Bombora</i> , SBE911 <i>plus</i> (including an ECO FLNTU fluorescence sensor)
	B4	29.995	153.340	0, 20	
	B6	29.995	153.426	0, 20	
	B8	29.995	153.505	0, 20	
	SS	30.127	153.235	0, 20	
28 February 2012	CH1	30.274	153.195	0, 20	<i>RV Bombora</i> , SBE911 <i>plus</i> (including an ECO FLNTU fluorescence sensor)
	CH2	30.274	153.242	0, 20	
	CH3	30.276	153.299	0, 20	
	CH5	30.266	153.397	0, 20	
11 September 2012	B2	29.996	153.262	0, 8	<i>RV Bombora</i> , SBE911 <i>plus</i> (including an ECO FLNTU fluorescence sensor)
	B4	29.995	153.341	0, 20	
	B6	29.995	153.425	0, 12	
	B8	29.997	153.505	0, 12	
	SS	30.128	153.234	0, 8	
12 September 2012	CH1	30.274	153.196	0, 12	<i>RV Bombora</i> , SBE911 <i>plus</i> (including an ECO FLNTU fluorescence sensor)
	CH2	30.273	153.243	0, 8	
	CH3	30.275	153.299	0, 20	
	CH5	30.268	153.396	0, 24	

## **2.2 Sample preparation and analysis**

### **2.2.1 Microphytoplankton abundance and composition**

Immediately after collection, 2 L of seawater were preserved in plastic containers through the addition of 6 mL of Lugol's acid solution and returned to the laboratory for concentration by sedimentation (48 hrs). Identification was made under an inverted microscope (Leica DMI 3000B) at the lowest taxonomic level possible by an expert analyst using appropriate taxonomic guides (Dakin & Colefax 1940, Crosby & Wood 1958, 1959, Wood et al. 1959, Wood 1961a, b, Tomas 1997, Gómez et al. 2008, Hallegraeff et al. 2010, Stidolph et al. 2012). The lower cell size limit that could be determined was 10  $\mu\text{m}$ . Enumeration followed Utermöhl (1958) with a minimum of 400 cells counted per sample at 200x magnification. To help identify thecate dinoflagellates, a Calcofluor White Stain (Sigma Aldrich, USA) solution was added to each 3 mL sample 30 minutes prior to counting, at a final concentration of 20  $\mu\text{g mL}^{-1}$  (Fritz & Triemer 1985). In the event that the genus of a diatom or dinoflagellate could not be determined (e.g. due to overlying particles or degradation of distinct morphological structures), it was classified as an "undefined centric or pennate diatom" or "undefined dinoflagellate". All dinoflagellates belonging to the genera *Alexandrium*, *Gonyaulax*, or *Heterocapsa* were grouped in a complex. Distinct *Gymnodinium* spp. and *Gyrodinium* spp. >20  $\mu\text{m}$  were included in the counts. Smaller individuals were difficult to distinguish from each other and from a large number of small unidentifiable flagellates, and were therefore excluded from the counts. To quantify the abundance of the filamentous cyanobacterium *Trichodesmium erythraeum*, each filament was measured via an eyepiece micrometer. Cell numbers were calculated by dividing the filament length through the average cell length (7.5  $\mu\text{m}$ ; standard deviation = 1.9  $\mu\text{m}$ ), which was determined prior to counting by measuring a single cell's length from 10 random filaments of a randomly picked sample.

### **2.2.2 HPLC**

A single sample was prepared for HPLC analyses at most sampling locations (in order to enable the broadest possible temporal and spatial comparison while minimising costs). Duplicate samples were prepared at all sampling locations during May and June 2011 and along the CH-Line in December 2011 to determine the natural variability between samples taken at the same depths.

On board *RV Bombora*, water samples were directly filtered, whereas on board *RV Circe*, water from each sampling location was transferred into 10 L carboys and stored in the dark until further processing at the laboratories of the NMSC (~4 hrs after sampling). Water samples of 2 L were filtered onto pre-combusted 25 mm GF/F filter papers (Whatman Ltd, UK) and frozen in liquid nitrogen until further analysis (at the laboratories of the Commonwealth Scientific and Industrial Research Organisation, CSIRO, Hobart, Tasmania). After acetone-based pigment extraction (15 - 18 hrs) HPLC analysis followed Clementson (2010) using a Waters Alliance® system. Pigments were separated using a C<sub>8</sub> column and a binary gradient system with a column temperature of 55°C, detected at 436 nm and identified by retention time and absorption spectrum from a photo-diode array detector. Concentrations of pigments were determined from commercially available international standards (Sigma Aldrich, USA, or DHI, Denmark).

### **2.2.3 CHEMTAX**

Algal classes included in the CHEMTAX analysis were selected based on pigments detected, the regional literature (Jeffrey et al. 1975, Hallegraeff 1981, Hallegraeff & Jeffrey 1981, Hallegraeff & Reid 1986) and microscopy data. This approach led to the selection of twelve algal classes: Chrysophytes, cryptophytes, cyanobacteria-1, -2, -4, diatoms-1, -2, dinoflagellates-1, euglenophytes, haptophytes-6, -8 and prasinophytes-3. Example taxa of these twelve algal classes and their characteristic pigments are summarised in Table 2, and a detailed definition of each class is given in Higgins et al. (2011). Several trial runs in CHEMTAX with our initial diatom selection were inconclusive and led to the addition of a 13<sup>th</sup> algal class, namely diatoms-4, detected in previous work by Stauber & Jeffrey (1988), containing the shared pigments Chl *c*<sub>2</sub> and fucoxanthin but lacking Chl *c*<sub>1</sub> or Chl *c*<sub>3</sub> (the characteristic pigments of diatoms-1 and -2, respectively, Table 2).



**Table 2. List of 13 algal classes included in the CHEMTAX analysis.** Example taxa and characteristic pigments of each algal class following Higgins et al. (2011) are also included.

Algal class	Example taxa	Characteristic pigments
Chrysophytes	"equatorial species" following Mackey et al. 1996	19'Butanoyloxyfucoxanthin, Fucoxanthin
Cryptophytes	<i>Chroomonas salina</i>	Alloxanthin
Cyanobacteria-1	<i>Trichodesmium</i> sp.	Zeaxanthin
Cyanobacteria-2	<i>Synechococcus</i> sp.	Zeaxanthin
Cyanobacteria-4	<i>Prochlorococcus marinus</i>	Chl <i>b</i> , Zeaxanthin
Diatoms-1	<i>Chaetoceros didymus</i>	Chl <i>c</i> <sub>1</sub> , Chl <i>c</i> <sub>2</sub> , Fucoxanthin
Diatoms-2	<i>Leptocylindrus</i> sp., <i>Pseudo-nitzschia</i> sp., <i>Rhizosolenia setigera</i>	Chl <i>c</i> <sub>3</sub> , Chl <i>c</i> <sub>2</sub> , Fucoxanthin
Diatoms-4	<i>Ceratoneis closterium</i> (previously <i>Nitzschia closterium</i> ) following Stauber & Jeffrey 1988	Chl <i>c</i> <sub>2</sub> , Fucoxanthin (lacking Chl <i>c</i> <sub>3</sub> , Chl <i>c</i> <sub>1</sub> )
Dinoflagellates-1	<i>Amphidinium carterae</i>	Peridinin
Euglenophytes	<i>Euglena gracilis</i> , <i>Eutreptiella gymnastica</i>	Chl <i>b</i> , Neoxanthin, Zeaxanthin
Haptophytes-6	<i>Emiliania huxleyi</i> , <i>Gephyrocapsa oceanica</i>	19'Hexanoyloxyfucoxanthin
Haptophytes-8	<i>Phaeocystis pouchetii</i>	19'Hexanoyloxyfucoxanthin
Prasinophytes-3	<i>Micromonas pusilla</i> , <i>Pycnococcus provasolii</i>	Prasinoxanthin

Prior to CHEMTAX analyses, an initial biomarker pigment:Chl *a* ratio matrix and a ratio limit matrix were created (for definitions of terminology see Mackey et al. 1996) following S. Wright (unpublished) (Supplementary Material Table 1A, B). Biomarker pigment:Chl *a* ratios for each selected algal class were based on the geometric means of minimum and maximum values of pigment ratios derived from cultures given in Higgins et al. (2011). These ratios were assumed to be within their naturally varying range (hereafter we refer to ratios between the minimum and maximum values given in Higgins et al. (2011) as being in a reasonable range). By calculating a multiplying factor (MF) following S. Wright (unpublished), we set a limit for the maximum percent change that could be made to the initial ratios while CHEMTAX completed the iterations. In some cases our calculated MFs exceeded 1000%, however, the highest % change reached throughout our analysis was 338% while it remained below 100% in most instances. Ratios of diatoms-4 were based on geometric means of absolute

minimum and maximum values given for any of the two diatom groups diatoms-1 or -2 (field or culture values) in Higgins et al. (2011). In the case of fucoxanthin, we additionally included the fucoxanthin:Chl *a* ratio of the diatom *Ceratoneis closterium* (Stauber & Jeffrey 1988). Higgins et al. (2011) did not provide an initial ratio or ratio limit for chrysophytes and so we based these values on ratios given in Mackey et al. (1996) for “equatorial” chrysophytes and allowed a 500% limit of change.

To ensure that CHEMTAX would find the best global solution for optimised pigment ratios, we created 60 randomised copies of the initial ratio matrix as multiple starting points for the iteration process of the program (Wright et al. 2009). Initially, CHEMTAX was run on the complete HPLC dataset ( $n = 107$ ), aiming at optimising the initial ratio matrix developed from literature values for our Coffs Harbour pigment data. We anticipated an optimisation procedure following Latasa (2007), using the output ratios of one run as input ratios for a consecutive run until the pigment ratios stabilise. However, with consecutive runs the ratios became increasingly unreasonable, therefore, we used the output ratios of the first run on the complete dataset as the initial input ratios for the following runs on subsets of the data.

In the following runs, data was binned according to sampling month and, in the case of February 2012, also according to mixed layer depth (MLD = 13.5 m) into surface ( $n = 9$ ) and 20 m depth ( $n = 8$ ) samples (in all other months, MLD exceeded the sampling depth). MLD was calculated for each CTD cast as the minimum depth at which either: temperature < temperature (10 m) - 0.4°C, or: salinity > salinity (10 m) + 0.03 psu (Condie & Dunn 2006). Casts that were not deep enough to calculate a MLD were removed from the analysis. Up to six successive CHEMTAX runs were conducted on the binned data until an increase in unreasonable ratios was found. We used the same ratios (optimised initial ratio matrix) and 60 randomised copies for the first binned runs to enable an equal starting point for each bin. Absolute concentrations of Chl *a* ( $\mu\text{g L}^{-1}$ ) assigned to each algal class per sample from the best solutions of each most reasonable run (i.e. the last run, which delivered the most reasonable ratios while approximating their stabilisation) were used in the subsequent statistical analyses. All ratio matrices (initial, optimised for Coffs Harbour and final for each subset), including their classification as reasonable/unreasonable, and the ratio limit matrix are given Supplementary Material Table 1.

## 2.3 Statistical analyses

Prior to regression and Bland and Altman analyses, phytoplankton taxa that were determined by microscopy (i.e. diatoms and dinoflagellates, silicoflagellates and *Trichodesmium erythraeum*) were classified into pigment-types based on Jeffrey et al. (1975), Stauber & Jeffrey (1988), Johnsen & Sakshaug (1993), Schlüter et al. (2000), Brotas & Plante-Cuny (2003), Örnólfssdóttir et al. (2003), Irigoien et al. (2004), Llewellyn et al. (2005), Rodríguez et al. (2006) and Higgins et al. (2011). Thus diatoms were grouped into diatoms-1, -2, -4 (see section 2.2.3) and dinoflagellates were grouped into dinoflagellates-1 (containing peridinin) and -2 (containing fucoxanthin), where the second type showed a negligible abundance and was therefore excluded from the CHEMTAX analyses. Silicoflagellates were classified as chrysophytes and *T. erythraeum* as cyanobacteria-1. In order to establish a comparable quantity for the phytoplankton abundance estimates made through microscopy and CHEMTAX (initially in cells L<sup>-1</sup> and µg Chl a L<sup>-1</sup>, respectively), we converted cell numbers determined by microscopy for each pigment-type (per sample) to Chl a concentrations by calculating:

Conversion factor = CHEMTAX estimate (µg Chl a L<sup>-1</sup>) / Cell number (cells L<sup>-1</sup>).

The conversion factors of each pigment-type from all samples (excluding samples in which abundances were zero and led to errors) were averaged, thus obtaining one conversion factor each for diatoms-1, -2, -4, the sum of the three diatom-types (hereafter total diatoms), dinoflagellates-1, cyanobacteria/*Trichodesmium erythraeum*, chrysophytes/silicoflagellates and the sum of each of these phytoplankton pigment-groups (hereafter total comparable taxa). These averaged conversion factors were used as a final taxon-specific conversion factor from cell numbers to Chl a:

TChl a of counted species/genus = Abundance of species/genus (cells L<sup>-1</sup>) x taxon-specific conversion factor.

### 2.3.1 Regression analyses

Regression analyses using Minitab 16 Statistical Software (2010) were conducted to investigate the numerical relationships of: (i) pigment concentrations determined in replicate HPLC samples to validate the accuracy/natural variability of HPLC samples; and (ii) TChl a of individual phytoplankton pigment-types determined by microscopy

and CHEMTAX. In particular, we analysed diatoms-1, -2, -4, total diatoms, dinoflagellates-1, chrysophytes/silicoflagellates and cyanobacteria-1/*Trichodesmium erythraeum* and TChl *a* of comparable taxa derived from microscopy (by conversion) and CHEMTAX. The fit of our data to the regression model was determined based on  $R^2$ , however, we also checked the adjusted  $R^2$  (adjusted for the number of predictors in the model, here one, i.e. expected to be similar to or equal to  $R^2$ ) to ensure robustness of our model.

### **2.3.2 Bland and Altman analyses**

The agreement of individual phytoplankton abundance estimates made by microscopy and CHEMTAX was tested using Bland and Altman analyses (Bland & Altman 1986). In this technique the differences of abundance estimates per sample ( $\mu\text{g Chl } a \text{ L}^{-1}$ ) against their average are plotted for each phytoplankton pigment-type. As standard deviations increased with increasing Chl *a* concentrations (see section 3.3.2), we chose to use a regression plot based on absolute Chl *a* concentrations in combination with the display of the 95% prediction interval as suggested by Dewitte et al. (2002).

## **3 Results**

### **3.1 Total phytoplankton abundance determined by microscopy**

Total phytoplankton abundances ranged between 850 cells  $\text{L}^{-1}$  (CH3 40 m, 27 May 2011) and  $1.9 \times 10^6$  cells  $\text{L}^{-1}$  (CH1 20 m, 07 December 2011), with highest abundances generally found inshore (regardless of season) (Supplementary Material Table 2). Diatoms dominated the microphytoplankton community across all sampling times, particularly at the inshore stations, with on average (across all samples) 78% of the total counted phytoplankton (Table 3). When microscopic diatom counts were allocated into pigment-based species type-1, -2 and -4, diatoms-2 contributed on average 58% to the microscopically determined phytoplankton community while diatoms-1 and -4 were less abundant (Table 3). Dinoflagellates-1, -2, silicoflagellates and *T. erythraeum* each contributed 2 - 12%, to the total counted phytoplankton (Table 3). Converted Chl *a* concentrations derived for each taxon including their respective conversion factors are given in Supplementary Material Table 3. The classification of individual taxa into pigment types is summarised in Supplementary Material Table 4. A detailed

description of abundances of individual taxa found in each sample exceeds the purpose of this study but can be accessed at <http://catalogue.aodn.org.au/geonetwork/srv/en/metadata.show?uuid=f7502841-a2c7-4437-b557-20ef89e754e0>.

**Table 3. Averaged phytoplankton abundance.** Relative abundance estimates (average and standard deviation (SD) across all samples) of phytoplankton taxa determined by microscopy (cell counts) and CHEMTAX (pigment analysis). Note that within the group “chrysophytes/silicoflagellates” and “cyanobacteria-1/*Trichodesmium erythraeum*” abundance of chrysophytes and cyanobacteria-1 was estimated by CHEMTAX, while silicoflagellates and *T. erythraeum* were counted during microscopy.

Relative abundance (%)	Microscopy		CHEMTAX	
	Average	SD	Average	SD
Chrysophytes/silicoflagellates	1	2	4	4
Cryptophytes	-	-	3	5
Cyanobacteria-1/ <i>T. erythraeum</i>	12	22	15	12
Cyanobacteria-2	-	-	13	16
Cyanobacteria-4	-	-	2	5
Diatoms-1	14	12	9	16
Diatoms-2	58	28	12	7
Diatoms-4	6	7	17	15
Dinoflagellates-1	8	8	2	3
Dinoflagellates-2	2	2	-	-
Euglenophytes-1	-	-	3	4
Haptophytes-6	-	-	5	3
Haptophytes-8	-	-	10	6
Prasinophytes-3	-	-	6	8

### 3.2 Phytoplankton composition estimate made by CHEMTAX

TChl *a* concentrations ranged between 0.11  $\mu\text{g L}^{-1}$  (CH5 20 m, 28 February 2012) and 4.49  $\mu\text{g L}^{-1}$  (CH1 20 m, 07 December 2011) and were generally higher inshore station than offshore (regardless of season) (Supplementary Material Table 5). TChl *a* and the contribution of algal taxa to absolute TChl *a* ( $\mu\text{g L}^{-1}$ ) in each sample derived from the best solutions for each bin are given in Supplementary Material Table 5. Diatoms contributed the largest part to TChl *a*, especially at the inshore stations, consistent with microscopy results. On average (all samples), CHEMTAX assigned 38% of the total

Chl *a* to diatoms (diatoms-1 = 9%, diatoms-2 = 12%, diatoms-4 = 17%), while each of the other phytoplankton taxa were assigned less than 15% of TChl *a* (Table 3). These proportions of diatoms (type-1, -2 and -4), dinoflagellates-1 and cyanobacteria-1 from our technique optimising CHEMTAX ratios deviated from microscopically determined proportions.

### **3.3 Statistical evaluation of microscopy- and CHEMTAX-derived Chl *a* estimates**

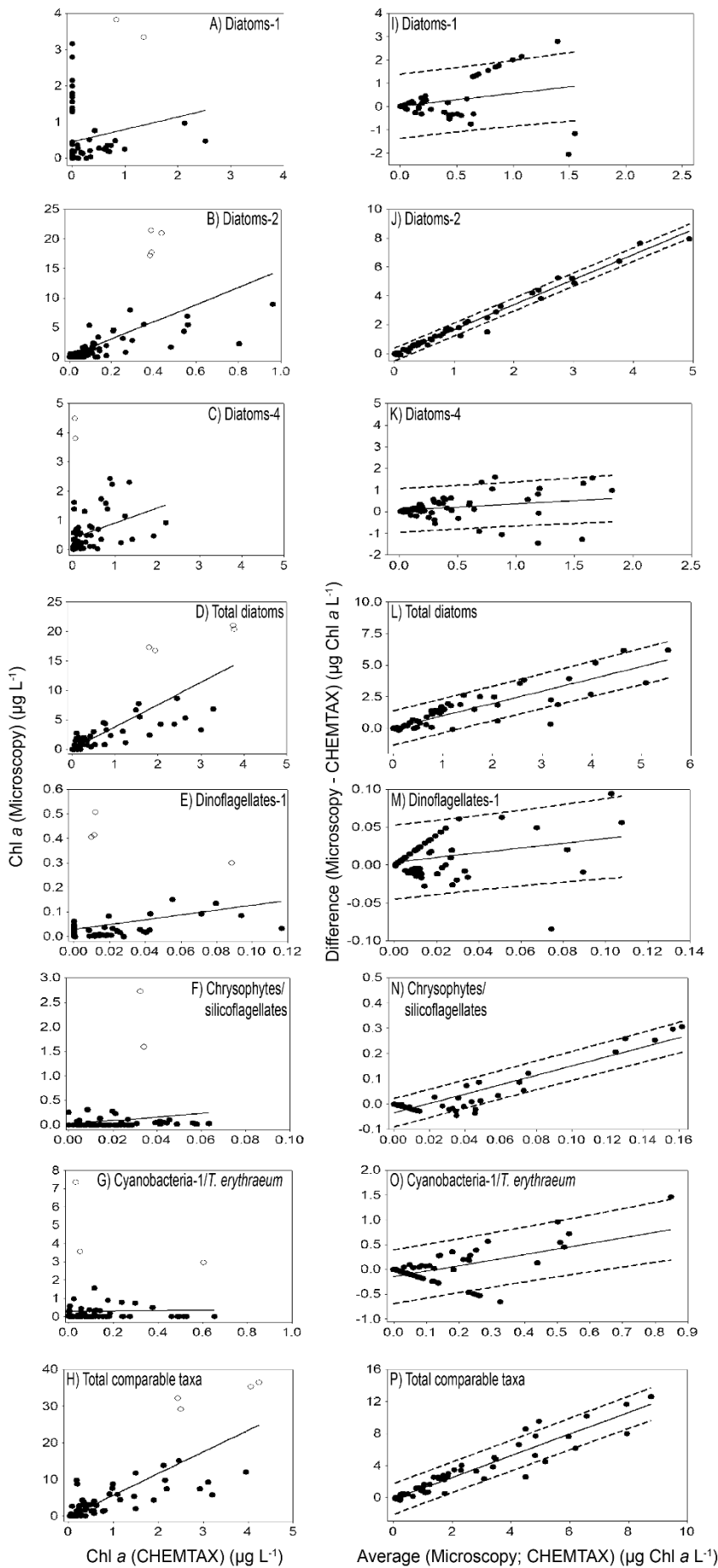
Both regression and Bland and Altman analyses showed incongruences between Chl *a* estimates made for individual phytoplankton pigment-types derived from microscopy and CHEMTAX. Imbalances between abundance estimates made by the two methods were more pronounced when a high taxonomic discrimination was anticipated, i.e. correlation and agreement was poorer for individual pigment-types within the diatom class than on a class level for total diatoms, dinoflagellates, chrysophytes/silicoflagellates and cyanobacteria-1/*Trichodesmium erythraeum*.

#### **3.3.1 Regression analysis of Chl *a* estimates derived from microscopy and CHEMTAX**

Pigment concentrations determined in replicate HPLC samples collected for validation purposes (Table 1) were significantly positively correlated (all  $R^2$  between 0.992 and 1, average  $R^2 = 0.997$ , standard deviation = 0.002).

Separate regression analyses of Chl *a* estimates of individual phytoplankton pigment-types derived from microscopy and CHEMTAX revealed weak positive correlations for diatoms-1, -2 and -4 ( $R^2 < 0.36$ , Table 4, Fig. 2A-C). A stronger positive correlation was found for total diatoms ( $R^2 = 0.65$ , Table 4, Fig. 2D). Chl *a* estimates for dinoflagellates-1, chrysophytes/silicoflagellates and cyanobacteria-1/*Trichodesmium erythraeum* were weakly positively correlated (all  $R^2 < 0.06$ ; Table 4, Fig. 2E-G). Total Chl *a* concentrations of comparable taxa derived from microscopy and CHEMTAX were significantly positively correlated ( $R^2 = 0.64$ , Table 4, Fig. 2H). Frequently, microscopy or CHEMTAX made an abundance estimate in a sample while the other technique did not (Fig. 2A-G). Samples for which such differences between microscopy and CHEMTAX abundance estimates were largest (hereafter outlier samples) were

indicated as black open circles in Fig. 2A-H. A few taxa showed particularly high abundances in these outlier samples and included *Asterionellopsis glacialis* within diatoms-1 (in two samples, Fig. 2A), *Leptocylindrus danicus* within diatoms-2 (in four samples, Fig. 2B, ultimately also highly influencing total diatom abundances, Fig. 2D), *Ceratoneis closterium/Nitzschia longissima* within diatoms-4 (in two samples, Fig. 2C), *Scrippsiella trochoidea* and cf. *Oxytoxum variabile* within dinoflagellates-1 (each in two samples, Fig. 2E), *Dictyocha fibula* within chrysophytes/silicoflagellates (in two samples, Fig. 2F) and *T. erythraeum* in cyanobacteria-1/*T. erythraeum* (in three samples Fig. 2G).



**Figure 2. Regression and Bland and Altman plots of microscopy- and CHEMTAX-derived phytoplankton abundance estimates.** The left panel displays regressions (property vs. property), the right panel displays Bland and Altman plots (average of properties vs. difference between properties) of abundance estimates made by both techniques for each sample. Black lines indicate regression lines (A-P). Open circles (A-H) indicate outlier samples that were removed in the Bland and Altman analyses. Black dashed lines indicate the 95% prediction interval (I-P). Values of test statistics and regression equations are given in Table 4.



**Table 4. Summary of statistical results.** Values of test statistics (S,  $R^2$  and  $R^2$  (adj.) =  $R^2$  adjusted for the number of predictors in the model, here one) and regression equations of regression analyses and agreement tests following Bland and Altman (1986) for abundance estimates of individual phytoplankton pigment-types and total chlorophyll *a* (TChl *a*) for comparable taxa (i.e. diatoms-1, -2, -4, dinoflagellates-1, chrysophytes/silicoflagellates and cyanobacteria-1/*Trichodesmium erythraeum*) derived from microscopy (by conversion) and CHEMTAX. For corresponding regression and Bland and Altman plots see Figure 2.

Taxon	Regression analysis				Bland and Altman analysis			
	S	$R^2$ (%)	$R^2$ (adj.) (%)	Regression equation	S	$R^2$ (%)	$R^2$ (adj.) (%)	Regression equation
Diatoms-1	0.8739	3.1	1.8	Diatoms-1 (Microscopy) = 0.4498 + 0.3440 Diatoms-1 (CHEMTAX)	0.6862	8.6	7.3	Difference = 0.01594 + 0.5546 Average
Diatoms-2	3.6028	36.1	35.3	Diatoms-2 (Microscopy) = 0.1867 + 14.51 Diatoms-2 (CHEMTAX)	0.2224	98.6	98.6	Difference = - 0.06318 + 1.733 Average
Diatoms-4	0.7872	7.8	6.6	Diatoms-4 (Microscopy) = 0.4013 + 0.5122 Diatoms-4 (CHEMTAX)	0.5022	6.7	5.5	Difference = 0.05636 + 0.3037 Average
Total diatoms	2.5964	65	64.5	Total diatoms (Microscopy) = 0.0521 + 3.773 Total diatoms (CHEMTAX)	0.6746	79.4	79.1	Difference = 0.02035 + 0.9752 Average
Dinoflagellates-1	0.0888	6.6	5.4	Dinoflagellates-1 (Microscopy) = 0.0287 + 0.985 Dinoflagellates-1 (CHEMTAX)	0.0244	8.1	6.8	Difference = 0.003937 + 0.3107 Average
Chrysophytes/silicoflagellates	0.3570	2.2	0.9	Silicoflagellates (Microscopy) = 0.01278 + 3.761 Chrysophytes (CHEMTAX)	0.0279	85.7	85.5	Difference = - 0.03456 + 1.859 Average
Cyanobacteria-1/ <i>T. erythraeum</i>	0.9964	0	0	<i>T. erythraeum</i> (Microscopy) = 0.2900 + 0.1057 Cyanobacteria-1 (CHEMTAX)	0.2702	30.2	29.3	Difference = - 0.1482 + 1.121 Average
TChl <i>a</i> comparable taxa	4.6616	63.7	63.2	TChl <i>a</i> (Microscopy) = - 0.1735 + 5.894 TChl <i>a</i> (CHEMTAX)	0.9525	90.7	90.6	Difference = - 0.1432 + 1.348 Average

### 3.3.2 Bland and Altman analysis of individual phytoplankton pigment-type estimates determined by microscopy and CHEMTAX

The outlier samples identified in 3.3.1 impacted strongly on the average and difference Chl *a* values in the Bland and Altman analyses (data not shown) and were therefore excluded. Differences increased with average Chl *a* concentrations in all phytoplankton pigment-types (indicated by a linear arrangement of data points in all plots, i.e. one quantification technique measured an abundance of a phytoplankton pigment-type while the other did not) (Fig. 2I-P). Regression lines towards positive differences (x-axes) and averages (y-axes) showed that microscopically derived Chl *a* was overestimated in each phytoplankton pigment-type and TChl *a* of all comparable taxa relative to the CHEMTAX estimate (Fig. 2I-P). In some samples Chl *a* assigned to diatoms-1, diatoms-4, total diatoms, dinoflagellates-1, chrysophytes/silicoflagellates

and cyanobacteria-1/*Trichodesmium erythraeum* were also underestimated by microscopy relative to CHEMTAX (indicated by a linear arrangement of some data points towards increasingly negative differences) (Fig. 2I, K-O).

The limits of agreement (indicated by 95% prediction intervals in the Bland and Altman plots) allowed the quantification of over-/underestimates in individual phytoplankton pigment-types made by microscopy relative to CHEMTAX. Best agreement was found in dinoflagellates-1 as indicated by the nearly horizontal regression line close to zero difference (y-axis) and limits of agreement of  $\pm 0.05 \mu\text{g Chl } a \text{ L}^{-1}$  (Fig. 2M). Chrysophytes/silicoflagellates, cyanobacteria-1/*Trichodesmium erythraeum*, diatoms-1 and -4 followed in descending order (limits of agreement of about  $\pm 0.06$ ,  $\pm 0.5$ , and  $\pm 1 \mu\text{g Chl } a \text{ L}^{-1}$  (for both diatoms-1 and -4), respectively; Fig. 2I, K, N, O). Relatively poor agreement was found in diatoms-2 (indicated by the steep slope of the regression line, Fig. 2J), which impacted on the slope response in total diatoms (Fig. 2L) and TChl *a* of comparable taxa (Fig. 2P). Limits of agreement were at about  $\pm 0.5$ ,  $\pm 1.5$  and  $\pm 2 \mu\text{g Chl } a \text{ L}^{-1}$  for diatoms-2, total diatoms and TChl *a* of comparable taxa, respectively (Fig. 2J, L, P).

## 4 Discussion

### 4.1 Comparison of phytoplankton abundance estimates made by microscopy and CHEMTAX

Both microscopy and CHEMTAX showed that diatoms were the dominant phytoplankton taxon in the Coffs Harbour region during the study period, followed by the cyanobacterium *Trichodesmium erythraeum*. Other taxa (cyanobacteria-2 and -4, chrysophytes, cryptophytes, dinoflagellates, euglenophytes, haptophytes and prasinophytes) were less abundant. Microscopy tended to overestimate abundances of comparable phytoplankton taxa relative to CHEMTAX. Best correlation and agreement was found between abundance estimates made on a class-level for dinoflagellates and cyanobacteria-1/*T. erythraeum*. Poor correlation and agreement was found between abundance estimates for diatoms made by microscopy and CHEMTAX, with microscopy estimating total diatom abundance to be about double that of the diatom estimate by CHEMTAX (averaged across all samples). The large divergence in abundance estimates made by microscopy and CHEMTAX for total

diatoms seemed to derive from highly differing abundance estimates made for individual pigment-types on an intra-class level. For example, while microscopy highly overestimated diatoms-2 relative to CHEMTAX and determined them to be the dominant pigment-type, CHEMTAX found diatoms-4 to be dominant. Such divergences between the diatom abundance estimates made by microscopy and CHEMTAX illustrated that the two techniques did not agree beyond the class level.

#### ***4.2 Regression versus Bland and Altman analyses to test the agreement between phytoplankton abundance estimates***

Our study showed the usefulness of both regression and Bland and Altman analyses in comparing and resolving biases of microscopy and CHEMTAX. Regression analysis defined the strength of numerical relationships of phytoplankton pigment-types derived from both methods and identified outlier samples, i.e. samples in which abundance estimates from the two phytoplankton quantification techniques differed considerably. Bland and Altman analysis highlighted the numerical relationships identified by regression analysis. In particular, the general overestimate of phytoplankton abundance made by microscopy relative to CHEMTAX was clearly reflected by the mostly positive data points in the Bland and Altman plots (whereas high agreement would be indicated by randomly distributed data points around the zero-difference line; Bland & Altman 1986, Dewitte et al. 2002). These results are consistent with previous investigations, in which microscopy has been shown to overestimate diatom biomass relative to CHEMTAX (Schlüter & Møhlenberg 2003, Havskum et al. 2004, Vidussi et al. 2004). However, Bland and Altman plots also visualised that the divergences in abundance estimates increased with the biomass in our samples. This pattern might be important to consider when quantifying phytoplankton by microscopy or CHEMTAX during bloom periods.

The Bland and Altman technique is commonly applied in the medical field, where successive models of medical instruments are usually manufactured to measure the same quantity. In our study, we had to establish a comparable basis prior to our analysis by conversion of microscopically determined abundances to Chl *a* biomass. Firstly, such a conversion makes the Bland and Altman technique laborious when applied to phytoplankton analysis, and secondly, it adds a level of noise to the data as species-specific Chl *a* content varies highly (Higgins et al. 2011). We have repeated

the Bland and Altman analysis based on relative abundances and biomass (cells L<sup>-1</sup> for microscopy versus µg Chl a L<sup>-1</sup> for CHEMTAX), however, this variation reduced the agreement between abundance estimates made by the two methods (data not shown). Plotting the 95% prediction interval, or limits of agreement, around the regression line in the Bland and Altman plots enabled the quantification of over-/underestimates between abundance estimates made by microscopy and CHEMTAX. Generally, the quantification of such ranges might be of great benefit for the interpretation and accuracy estimates of microscopy and pigment data (acknowledging that these might vary regionally). However, our samples covered a broad temporal range and many different species within the different phytoplankton taxa tested. We therefore suggest that future investigations should consider investigating ranges of over-/underestimates between microscopy and CHEMTAX by applying the Bland and Altman technique based on abundance estimates from monocultures. The Chl a content of individual species can be more reliably estimated and Chl a conversion factors can be attained species-specifically, which inevitably will help to improve the agreement between abundance estimates made by microscopy and CHEMTAX.

### ***4.3 Classification errors of microscopically determined diatom taxa into pigment-types***

We are aware that various sources of errors, including sampling and analytical errors (Lund et al. 1958, Sournia 1978, Hooker et al. 2010, Latasa 2014) as well as omissions (Twomey et al. 2007, Higgins et al. 2011) and the conversion of cell numbers into Chl a units (Breton et al. 2000, Higgins et al. 2011) may have comprised a source of error influencing phytoplankton abundance estimates made by microscopy and CHEMTAX. However, such errors are well known and have been shown to influence phytoplankton abundance estimates made by the two quantification techniques previously. Therefore, we focus on discussing classification errors in this section, which have been the predominant source of imbalances between microscopy and CHEMTAX estimates in our study.

Generally, classification errors can result from the misidentification of phytoplankton taxa during microscopy or CHEMTAX analysis. During microscopy analyses, overlying particles, compromised positioning or degradation of cells might cause misidentification. During CHEMTAX analyses, the distribution of commonly shared

pigments in a sample to multiple taxa whilst keeping the pigment:Chl *a* ratio of each of the respective taxa within a reasonable range is particularly challenging. It has been reported before that abundances of diatoms and haptophytes (estimated from pigments such as fucoxanthin and 19'-hexanoyloxyfucoxanthin, respectively) involve errors as other algal classes, such as chrysophytes and dinoflagellates, also contribute to these carotenoid pools (Rodriguez et al. 2002). On the other hand, abundances of taxa that contain unambiguous pigment markers, such as peridinin in dinoflagellates-1, can be estimated much more precisely from the concentrations of such biomarkers in a water sample (this study, Rodriguez et al. 2002).

On a class level, we suggest that senescence might be an important factor to consider when comparing abundance estimates made by microscopy and CHEMTAX. For example, identification and examination of outlier samples, in which microscopy determined much higher abundances than CHEMTAX, revealed the elevated abundance of a few specific phytoplankton taxa counted by microscopy (detailed in 3.3.1). Provided that these taxa were correctly identified, the non-detection of elevated Chl *a* in the respective samples by CHEMTAX suggests that the counted taxa might have entered senescence prior to sampling. The latter seems likely, as phytoplankton containing cellular material were counted as live cells which might have enhanced the cell counts. Simultaneously, elevated concentrations of the degradation pigments chlorophyllide *a*, phaeophytin *a*, and phaeophorbide *a* were detected in the respective samples (data not shown).

On an intra-class level (i.e. diatoms), both statistical approaches demonstrated pronounced deviations between microscopy and CHEMTAX estimates. We attribute the poor agreement within diatoms-1, -2, -4 to classification errors of counted diatom taxa into pigment-types. For example, diatoms-1 and -2 were mainly under-estimated by CHEMTAX while diatoms-4 were both over- and underestimated depending on the sample (or vice versa). It should be noted that many samples did not contain Chl *c*<sub>1</sub> (or only trace amounts), the biomarker pigment for diatoms-1, i.e. CHEMTAX determined diatoms-1 as not being abundant in significant amounts (this was not the case in diatom-2 as nearly all samples contained Chl *c*<sub>3</sub>). We therefore believe that many diatoms were misclassified as diatoms-1 as they seemed to lack the biomarker pigment Chl *c*<sub>1</sub> and should have been classified as diatoms-4. We have tried to re-classify questionable diatom-1 (*Asterionellopsis glacialis*, *Lauderia annulata*/*Thalassiosira* spp., *Chaetoceros* spp.) and diatom-2 species (*Leptocylindrus*

*danicus*) as diatoms-4 and repeated the agreement tests without any improvement (data not shown).

We suggest that classification of diatoms into pigment-types based on microscopic identification and reference to published pigment data for each species was unreliable. We tried to improve the correlations by removing any species from the phytoplankton pigment-types that were identified by microscopy when CHEMTAX estimated their abundance as zero. The latter approach left only a few species that could be considered “true” diatoms-1, -2, -4, dinoflagellates-1, chrysophytes/silicoflagellates and cyanobacteria-1/*Trichodesmium erythraeum*. These taxa are indicated in bold font in Supplementary Table 4. However, many of the excluded taxa have been shown to contain the pigments they were classified upon previously (see references given in section 2.3). Such evidence versus the poor agreement and weak correlation between microscopy and CHEMTAX abundance estimates for diatom-types found in this study indicate the high uncertainty associated with the classification of microscopically determined diatom species into pigment-types. Intra-specific variation in pigment composition has been shown before (Stauber & Jeffrey 1988). In order to improve the agreement between abundance estimates of individual diatom pigment-types made by microscopy and CHEMTAX, it would have been necessary to determine the exact pigment composition of every single counted species to be able to classify them correctly (as an alternative approach to basing the classification on the literature). Such an approach has been used successfully (Laza-Martinez et al. 2007) but is very labour intensive for a local study. Nevertheless, we will need more studies aimed at resolving species-specific pigment composition to improve the analysis of natural phytoplankton assemblages.

## Conclusions

Our comparison of CHEMTAX and microscopy derived from the fact that there is no gold standard technique that can give a definite analysis of phytoplankton communities, and this problem also limits our interpretation of the results. Using regression and Bland and Altman analyses, we were able to show that comparisons of phytoplankton abundance estimates made by microscopy and CHEMTAX correlate and agree well on a class level. Beyond the class-level, classification errors of microscopically determined diatoms into pigment-types resulted in poor agreement

between abundance estimates made by microscopy and CHEMTAX. In order to overcome the classification errors of individual phytoplankton taxa identified here, we suggest the following potential solutions: (i) The quantification of senescent material during both microscopy and CHEMTAX analyses (admitting that this will be easier during microscopy, for example by using dyes such as SYTOX<sup>®</sup> green for the staining of dead cells, while attribution of senescent and dead cells is beyond the present capability of CHEMTAX). (ii) More investigations aimed at resolving variation in species-specific pigment composition in phytoplankton may help to conform abundance estimates made by microscopy and CHEMTAX beyond the class level.

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**Supplementary Material Table 1. Initial and optimised biomarker pigment:Chl *a* ratios.** The initial ratio matrix (A) and the ratio limit matrix (B) were created following S. Wright (unpublished; based on culture values from table 6.2 in Higgins et al. 2011). For diatoms-4, we additionally considered the fucoxanthin:Chl *a* ratio of *Ceratoneis closterium* given in Stauber and Jeffrey (1988). Ratios for chrysophytes were taken from Mackey et al. (1996). C) Optimised ratios for complete Coffs Harbour sample set (n = 107) after one CHEMTAX run. D - J) Optimised ratios for each sampling month (May, June, December 2011, January, February, September 2012) after one to six consecutive CHEMTAX runs following Latasa (2007) using ratio matrix C as starting ratio. I) Mixed layer depth during February 2012 equalled 13.5 m, therefore ratios were optimised separately for 0 m and 20 m samples. Ratios below/exceeding the reasonable literature range are indicated by italic/bold font, respectively (this excludes chrysophytes as no ranges were given in Higgins et al. 2011). For pigment abbreviations see Jeffrey et al. (2005).

Class / Pigment	Chl <i>c</i> <sub>3</sub>	Chl <i>c</i> <sub>2</sub>	Chl <i>c</i> <sub>1</sub>	Perid	But-fuco	Fuco	Neo	Pras	Hex-fuco	Allo	Zea	Lut	DV Chl <i>b</i>	Chl <i>b</i>	Chl <i>a</i>
<b>A) Initial input ratio matrix following S. Wright (unpublished)</b>															
Chrysophytes	0	0	0	0	0.366	0.976	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0.169	0	0	0	0	0	0	0	0.359	0	0	0	0	1
Cyanobacteria-1	0	0	0	0	0	0	0	0	0	0	0.059	0	0	0	1
Cyanobacteria-2	0	0	0	0	0	0	0	0	0	0	0.362	0	0	0	1
Cyanobacteria-4	0	0	0	0	0	0	0	0	0	0	0.224	0	0.495	0	1
Diatoms-1	0	0.061	0.017	0	0	0.571	0	0	0	0	0	0	0	0	1
Diatoms-2	0.0654	0.221	0	0	0	1.025	0	0	0	0	0	0	0	0	1
Diatoms-4	0	0.067	0	0	0	0.597	0	0	0	0	0	0	0	0	1
Dinoflagellates-1	0	0.226	0	0.541	0	0	0	0	0	0	0	0	0	0	1
Euglenophytes-1	0	0	0	0	0	0	0.066	0	0	0	0.014	0.01	0	0.393	1
Haptophytes-6	0.1587	0.222	0	0	0.006	0.066	0	0	0.236	0	0	0	0	0	1
Haptophytes-8	0.0818	0.146	0	0	0.108	0.124	0	0	0.597	0	0	0	0	0	1
Prasinophytes-3	0	0	0	0	0	0	0.051	0.145	0	0	0.084	0.013	0	0.368	1
<b>B) Ratio limits matrix following S. Wright (unpublished)</b>															
Chrysophytes	500	500	500	500	500	500	500	500	500	500	500	500	500	500	100
Cryptophytes	500	338	500	500	500	500	500	500	500	220	500	500	500	500	100
Cyanobacteria-1	500	500	500	500	500	500	500	500	500	500	1469	500	500	500	100
Cyanobacteria-2	500	500	500	500	500	500	500	500	500	500	476	500	500	500	100
Cyanobacteria-4	500	500	500	500	500	500	500	500	500	500	447	500	707	500	100
Diatoms-1	500	508	338	500	500	299	500	500	500	500	500	500	500	500	100
Diatoms-2	409	170	500	500	500	124	500	500	500	500	500	500	500	500	100
Diatoms-4	500	559	500	500	500	519	500	500	500	500	500	500	500	500	100
Dinoflagellates-1	500	251	500	190	500	500	500	500	500	500	500	500	500	500	100
Euglenophytes-1	500	500	500	500	500	500	208	500	500	500	721	524	500	210	100
Haptophytes-6	144	153	500	500	126	1097	500	500	638	500	500	500	500	500	100
Haptophytes-8	390	198	500	500	252	1130	500	500	239	500	500	500	500	500	100
Prasinophytes-3	500	500	500	500	500	500	281	606	500	500	420	1342	500	281	100
<b>C) Optimised ratio matrix after one CHEMTAX run on complete Coffs Harbour sample set (n = 107)</b>															
Chrysophytes	0	0	0	0	0.614	0.368	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0.122	0	0	0	0	0	0	0	0.263	0	0	0	0	1
Cyanobacteria-1	0	0	0	0	0	0	0	0	0	0	0.045	0	0	0	1
Cyanobacteria-2	0	0	0	0	0	0	0	0	0	0	0.678	0	0	0	1
Cyanobacteria-4	0	0	0	0	0	0	0	0	0	0	0.148	0	0.332	0	1
Diatoms-1	0	0.156	<b>0.098</b>	0	0	0.353	0	0	0	0	0	0	0	0	1
Diatoms-2	0.0504	0.262	0	0	0	0.52	0	0	0	0	0	0	0	0	1
Diatoms-4	0	0.072	0	0	0	0.767	0	0	0	0	0	0	0	0	1
Dinoflagellates-1	0	0.15	0	0.398	0	0	0	0	0	0	0	0	0	0	1
Euglenophytes-1	0	0	0	0	0	0	0.062	0	0	0	0.01	0.009	0	0.814	1
Haptophytes-6	<b>1.0282</b>	<b>0.65</b>	0	0	<b>0.026</b>	0.239	0	0	0.309	0	0	0	0	0	1
Haptophytes-8	0.0939	0.185	0	0	0.143	0.101	0	0	0.725	0	0	0	0	0	1
Prasinophytes-3	0	0	0	0	0	0	0.043	0.151	0	0	0.101	0.015	0	0.425	1

Supplementary Material Table 1. Continued.

Class / Pigment	Chl <i>c</i> <sub>3</sub>	Chl <i>c</i> <sub>2</sub>	Chl <i>c</i> <sub>1</sub>	Perid	But-fuco	Fuco	Neo	Pras	Hex-fuco	Allo	Zea	Lut	DV Chl <i>b</i>	Chl <i>b</i>	Chl <i>a</i>
D) Optimised ratio matrix May (n = 24) after one CHEMTAX run															
Chrysophytes	0	0	0	0	0.7868	0.0913	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0.1488	0	0	0	0	0	0	0	0.242	0	0	0	0	1
Cyanobacteria-1	0	0	0	0	0	0	0	0	0	0	0.0428	0	0	0	1
Cyanobacteria-2	0	0	0	0	0	0	0	0	0	0	0.5524	0	0	0	1
Cyanobacteria-4	0	0	0	0	0	0	0	0	0	0	0.1474	0	0.2735	0	1
Diatoms-1	0	0.1362	<b>0.0885</b>	0	0	0.2426	0	0	0	0	0	0	0	0	1
Diatoms-2	0.0546	<b>0.4004</b>	0	0	0	0.1911	0	0	0	0	0	0	0	0	1
Diatoms-4	0	0.0667	0	0	0	1.0035	0	0	0	0	0	0	0	0	1
Dinoflagellates-1	0	0.1493	0	0.34	0	0	0	0	0	0	0	0	0	0	1
Euglenophytes-1	0	0	0	0	0	0	0.0506	0	0	0	0.0113	0.0124	0	<b>1.1406</b>	1
Haptophytes-6	<b>1.4623</b>	<b>0.756</b>	0	0	<b>0.0302</b>	0.1835	0	0	0.4089	0	0	0	0	0	1
Haptophytes-8	0.1266	0.1326	0	0	0.1025	0.1088	0	0	1.0478	0	0	0	0	0	1
Prasinophytes-3	0	0	0	0	0	0	0.04	0.0725	0	0	0.1193	0.0465	0	0.3855	1
E) Optimised ratio matrix June (n = 16) after two consecutive CHEMTAX runs															
Chrysophytes	0	0	0	0	0.6956	0.4543	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0.1312	0	0	0	0	0	0	0	0.1626	0	0	0	0	1
Cyanobacteria-1	0	0	0	0	0	0	0	0	0	0	0.0415	0	0	0	1
Cyanobacteria-2	0	0	0	0	0	0	0	0	0	0	0.8409	0	0	0	1
Cyanobacteria-4	0	0	0	0	0	0	0	0	0	0	0.1231	0	0.3706	0	1
Diatoms-1	0	0.1008	<b>0.1152</b>	0	0	0.5615	0	0	0	0	0	0	0	0	1
Diatoms-2	0.0552	0.2939	0	0	0	0.5466	0	0	0	0	0	0	0	0	1
Diatoms-4	0	0.0656	0	0	0	0.568	0	0	0	0	0	0	0	0	1
Dinoflagellates-1	0	0.1475	0	0.4485	0	0	0	0	0	0	0	0	0	0	1
Euglenophytes-1	0	0	0	0	0	0	0.0363	0	0	0	0.0168	<b>0.1078</b>	0	0.3261	1
Haptophytes-6	<b>1.3981</b>	0.3362	0	0	<b>0.0305</b>	0.2596	0	0	0.3332	0	0	0	0	0	1
Haptophytes-8	0.2537	<b>0.4152</b>	0	0	<b>0.6433</b>	0.1529	0	0	1.2146	0	0	0	0	0	1
Prasinophytes-3	0	0	0	0	0	0	0.0825	0.2011	0	0	0.0757	0.0095	0	0.7744	1
F) Optimised ratio matrix December (n = 24) after three consecutive CHEMTAX runs															
Chrysophytes	0	0	0	0	0.8004	0.1712	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0.1777	0	0	0	0	0	0	0	0.3339	0	0	0	0	1
Cyanobacteria-1	0	0	0	0	0	0	0	0	0	0	0.0021	0	0	0	1
Cyanobacteria-2	0	0	0	0	0	0	0	0	0	0	1.5815	0	0	0	1
Cyanobacteria-4	0	0	0	0	0	0	0	0	0	0	0.2455	0	0.1432	0	1
Diatoms-1	0	0.0774	<b>0.1599</b>	0	0	0.2373	0	0	0	0	0	0	0	0	1
Diatoms-2	0.0766	<b>0.4719</b>	0	0	0	0.154	0	0	0	0	0	0	0	0	1
Diatoms-4	0	0.0778	0	0	0	0.841	0	0	0	0	0	0	0	0	1
Dinoflagellates-1	0	0.2133	0	0.391	0	0	0	0	0	0	0	0	0	0	1
Euglenophytes-1	0	0	0	0	0	0	0.0161	0	0	0	0.008	0.0074	0	4.2915	1
Haptophytes-6	<b>1.5113</b>	<b>0.4955</b>	0	0	<b>0.0218</b>	0.1426	0	0	0.1772	0	0	0	0	0	1
Haptophytes-8	0.0687	0.1114	0	0	0.095	0.0548	0	0	1.014	0	0	0	0	0	1
Prasinophytes-3	0	0	0	0	0	0	0.0577	0.107	0	0	0.0599	0.0017	0	0.3434	1
G) Optimised ratio matrix January (n = 8) after one CHEMTAX run															
Chrysophytes	0	0	0	0	0.5348	0.3069	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0.1228	0	0	0	0	0	0	0	0.1899	0	0	0	0	1
Cyanobacteria-1	0	0	0	0	0	0	0	0	0	0	0.0324	0	0	0	1
Cyanobacteria-2	0	0	0	0	0	0	0	0	0	0	0.5775	0	0	0	1
Cyanobacteria-4	0	0	0	0	0	0	0	0	0	0	0.1438	0	0.219	0	1
Diatoms-1	0	0.1939	<b>0.0939</b>	0	0	0.4379	0	0	0	0	0	0	0	0	1
Diatoms-2	0.0085	0.3666	0	0	0	0.2559	0	0	0	0	0	0	0	0	1
Diatoms-4	0	0.0946	0	0	0	0.822	0	0	0	0	0	0	0	0	1
Dinoflagellates-1	0	0.1904	0	0.5373	0	0	0	0	0	0	0	0	0	0	1
Euglenophytes-1	0	0	0	0	0	0	0.0211	0	0	0	0.0084	0.0057	0	<b>0.9953</b>	1
Haptophytes-6	<b>0.9743</b>	<b>0.4887</b>	0	0	<b>0.0312</b>	0.3145	0	0	0.321	0	0	0	0	0	1
Haptophytes-8	0.0129	0.1961	0	0	0.1438	0.0795	0	0	0.9338	0	0	0	0	0	1
Prasinophytes-3	0	0	0	0	0	0	0.0539	0.1048	0	0	0.0817	0.0007	0	0.3726	1
H) Optimised ratio matrix February 0 m (n = 9) after one CHEMTAX run															
Chrysophytes	0	0	0	0	0.4896	0.2718	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0.0834	0	0	0	0	0	0	0	0.2903	0	0	0	0	1
Cyanobacteria-1	0	0	0	0	0	0	0	0	0	0	0.0562	0	0	0	1
Cyanobacteria-2	0	0	0	0	0	0	0	0	0	0	0.6237	0	0	0	1
Cyanobacteria-4	0	0	0	0	0	0	0	0	0	0	0.1922	0	0.3923	0	1
Diatoms-1	0	0.2525	<b>0.131</b>	0	0	0.4252	0	0	0	0	0	0	0	0	1
Diatoms-2	0.049	0.2924	0	0	0	0.5908	0	0	0	0	0	0	0	0	1
Diatoms-4	0	0.0528	0	0	0	0.8915	0	0	0	0	0	0	0	0	1
Dinoflagellates-1	0	0.14	0	0.4968	0	0	0	0	0	0	0	0	0	0	1
Euglenophytes-1	0	0	0	0	0	0	0.0502	0	0	0	0.0075	0.0091	0	<b>0.9926</b>	1
Haptophytes-6	<b>1.3117</b>	<b>0.5701</b>	0	0	<b>0.0264</b>	0.2073	0	0	0.2803	0	0	0	0	0	1
Haptophytes-8	0.0212	<b>0.3097</b>	0	0	0.0283	0.0755	0	0	0.4953	0	0	0	0	0	1
Prasinophytes-3	0	0	0	0	0	0	0.0516	0.1787	0	0	0.1007	0.0201	0	0.3071	1

## Supplementary Material Table 1. Continued.

Class / Pigment	Chl <i>c</i> <sub>3</sub>	Chl <i>c</i> <sub>2</sub>	Chl <i>c</i> <sub>1</sub>	Perid	But-fuco	Fuco	Neo	Pras	Hex-fuco	Allo	Zea	Lut	DV Chl <i>b</i>	Chl <i>b</i>	Chl <i>a</i>
I) Optimised ratio matrix February 20 m (n = 8) after one CHEMTAX run															
Chrysophytes	0	0	0	0	0.803	0.4239	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0.1386	0	0	0	0	0	0	0	0.1759	0	0	0	0	1
Cyanobacteria-1	0	0	0	0	0	0	0	0	0	0	0.0392	0	0	0	1
Cyanobacteria-2	0	0	0	0	0	0	0	0	0	0	0.6164	0	0	0	1
Cyanobacteria-4	0	0	0	0	0	0	0	0	0	0	0.1653	0	0.293	0	1
Diatoms-1	0	0.1217	<b>0.1262</b>	0	0	0.3513	0	0	0	0	0	0	0	0	1
Diatoms-2	0.0097	0.2435	0	0	0	0.3952	0	0	0	0	0	0	0	0	1
Diatoms-4	0	0.0864	0	0	0	0.6081	0	0	0	0	0	0	0	0	1
Dinoflagellates-1	0	0.042	0	0.3177	0	0	0	0	0	0	0	0	0	0	1
Euglenophytes-1	0	0	0	0	0	0	0.0881	0	0	0	0.0115	0.0089	0	0.1479	1
Haptophytes-6	<b>1.3423</b>	<b>0.7141</b>	0	0	<b>0.0274</b>	0.2866	0	0	0.2891	0	0	0	0	0	1
Haptophytes-8	0.0174	<b>0.4304</b>	0	0	0.1115	0.1093	0	0	0.7477	0	0	0	0	0	1
Prasinophytes-3	0	0	0	0	0	0	0.0162	0.1152	0	0	0.0866	0.0017	0	0.6094	1
J) Optimised ratio matrix September (n = 18) after 6 consecutive CHEMTAX runs															
Chrysophytes	0	0	0	0	0.7845	0.3167	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0.2208	0	0	0	0	0	0	0	0.2149	0	0	0	0	1
Cyanobacteria-1	0	0	0	0	0	0	0	0	0	0	0.0007	0	0	0	1
Cyanobacteria-2	0	0	0	0	0	0	0	0	0	0	0.3948	0	0	0	1
Cyanobacteria-4	0	0	0	0	0	0	0	0	0	0	0.1065	0	0.2953	0	1
Diatoms-1	0	0.2093	<b>0.2338</b>	0	0	0.3265	0	0	0	0	0	0	0	0	1
Diatoms-2	0.0515	<b>0.6635</b>	0	0	0	0.4129	0	0	0	0	0	0	0	0	1
Diatoms-4	0	0.0386	0	0	0	0.5924	0	0	0	0	0	0	0	0	1
Dinoflagellates-1	0	0.3418	0	0.5624	0	0	0	0	0	0	0	0	0	0	1
Euglenophytes-1	0	0	0	0	0	0	0.013	0	0	0	0.0022	0.0324	0	<b>1.5273</b>	1
Haptophytes-6	<b>2.1176</b>	<b>1.2531</b>	0	0	<b>0.0123</b>	0.2848	0	0	0.1328	0	0	0	0	0	1
Haptophytes-8	0.1586	0.1781	0	0	0.0492	0.1752	0	0	0.6634	0	0	0	0	0	1
Prasinophytes-3	0	0	0	0	0	0	0.1399	0.4323	0	0	0.0024	0.008	0	0.3708	1



**Supplementary Material Table 2. Phytoplankton abundance (cells L<sup>-1</sup>) determined by microscopy.** For classification of diatoms into type-1, -2 and -4, and dinoflagellates into types-1 and -2 see Supplementary Material Table 4.

Date	Station	Depth (m)	Diatoms-1	Diatoms-2	Diatoms-4	Dino-flagellates-1	Dino-flagellates-2	Silico-flagellates	<i>Trichodesmium erythraeum</i>	Total abundance
27 May 2011	CH3*	0	648	919	250	439	71	31	0	2,359
	CH3*	40	288	282	87	139	35	19	0	850
28 May 2011	CH1	0	919	5,513	1,368	1,103	20	82	0	9,005
	CH1	20	562	2,195	1,205	602	112	31	0	4,707
	CH2	0	1,144	2,627	1,239	640	27	150	0	5,827
	CH2	25	1,021	2,062	1,144	705	123	71	0	5,126
	CH3	0	592	1,726	664	470	71	51	0	3,574
	CH3	20	301	812	521	459	36	36	0	2,165
	CH4	0	187	408	149	309	85	35	0	1,173
	CH4	20	272	478	110	230	75	72	0	1,236
	CH5	0	684	1,164	327	439	102	112	0	2,828
	CH5	40	467	928	216	578	158	53	0	2,398
	CH5	60	204	670	167	408	82	41	0	1,572
06 Jun 2011	CH1	0	1,368	5,534	1,940	898	265	388	0	10,394
	CH1	12	1,011	1,858	960	194	92	470	0	4,584
	CH1	26	660	1,497	354	238	20	116	0	2,886
	CH3	0	1,676	4,572	1,676	1,076	228	455	0	9,683
	CH3	30	3,247	4,778	919	939	347	204	0	10,435
	CH5	0	400	898	176	327	37	135	0	1,973
	CH5	35	783	1,334	361	483	306	54	0	3,322
06 Dec 2011	B2	0	6,499	132,031	3,078	3,420	684	0	0	145,713
	B2	20	5,445	388,667	4,765	3,403	681	0	0	402,962
	B4	0	5,228	30,712	1,348	2,001	327	0	204	39,820
	B4	20	13,443	56,496	1,532	4,424	1,191	170	2,212	79,469
	B6	0	15,656	48,669	3,403	58,198	7,147	340	13,954	147,367
	B6	20	490	3,496	670	1,307	376	0	98	6,436
	SS	0	5,445	98,018	1,134	4,538	1,815	0	0	110,951
	SS	20	6,898	155,739	1,089	5,445	1,815	0	0	170,986
07 Dec 2011	CH1	0	16,336	1,850,084	8,168	2,723	1,361	0	0	1,878,672
	CH1	20	34,034	1,892,286	6,807	6,807	2,723	0	0	1,942,656
	CH2	0	12,252	1,568,283	10,210	0	4,084	0	116,396	1,711,226
	CH2	20	12,252	1,519,274	14,294	4,084	2,042	0	0	1,551,947
	CH3	0	6,466	145,665	1,702	3,403	340	0	5,786	163,363
	CH3	20	9,454	199,004	1,418	3,782	945	0	0	214,603
	CH5	0	70,110	155,195	26,546	71,471	14,294	4,084	139,539	481,240
	CH5	20	46,967	112,652	9,529	57,177	16,677	2,382	287,587	532,971
24 Jan 2012	CH1	0	13,205	25,185	7,760	4,492	2,450	0	30,631	83,723
	CH1	20	2,042	5,951	1,809	2,917	438	0	4,026	17,182
	CH2	0	5,462	27,057	766	3,420	1,123	0	61,823	99,651
	CH2	20	7,419	43,087	1,497	3,335	885	0	17,630	73,854
	CH3	0	48,703	97,405	3,369	2,859	817	408	14,805	168,366
	CH3	20	61,465	122,522	4,492	4,084	817	0	38,390	231,771
	CH5	0	1,429	4,656	449	2,675	245	0	22,401	31,856
	CH5	20	1,480	9,189	434	1,072	281	0	11,155	23,611
27 Feb 2012	B2	0	98,183	174,501	2,888	11,963	2,475	0	29,290	319,300
	B2	20	17,798	53,237	2,810	468	0	0	0	74,313
	B4	0	59,423	60,036	3,267	8,577	1,838	0	19,808	152,948
	B4	20	45,265	80,320	8,168	2,723	2,042	0	12,593	151,111
	B6	0	218	1,899	293	1,048	238	0	5,030	8,726
	B6	20	367	1,796	666	753	145	0	4,770	8,496
	B8	0	102	1,028	177	1,075	395	0	12,171	14,948
	B8	20	51	408	138	1,327	240	5	3,094	5,263
28 Feb 2012	CH1	0	117,757	473,071	22,462	6,126	681	0	0	620,098
	CH1	20	134,774	247,313	13,160	0	0	0	0	395,247
	CH2	0	111,291	77,938	1,702	4,765	1,702	0	7,147	204,544
	CH2	20	63,099	74,534	3,063	3,063	408	0	10,823	154,990
	CH3	0	75,555	63,099	4,084	2,042	817	0	35,123	180,720
	CH3	20	972	2,957	507	901	127	0	13,168	18,632
	CH5	0	82	750	168	965	225	5	5,187	7,382
	CH5	20	92	434	102	965	174	0	3,885	5,651

**Supplementary Material Table 2. Continued.**

Date	Station	Depth (m)	Diatoms-1	Diatoms-2	Diatoms-4	Dino- flagellates-1	Dino- flagellates-2	Silico- flagellates	<i>Trichodesmium erythraeum</i>	Total abundance
11 Sep	B2	0	7,487	206,586	4,424	19,059	340	0	0	237,897
2012	B2	8	6,807	481,240	2,042	3,403	681	0	0	494,173
	B4	0	0	67,923	145	5,214	434	0	0	73,716
	B4	20	123	37,982	245	858	82	0	0	39,289
	B6	0	0	300,860	681	11,572	4,084	0	0	317,196
	B6	12	1,225	277,308	204	3,267	1,225	0	0	283,230
	B8	0	54,795	152,472	13,614	6,807	1,702	0	0	229,389
	B8	12	98,018	202,161	5,445	3,403	0	0	0	309,028
	SS	0	10,210	492,811	4,084	12,933	0	0	0	520,038
	SS	8	6,126	384,583	1,361	2,042	681	0	0	394,793
12 Sep	CH1	0	9,529	407,046	2,042	42,202	2,042	0	0	462,861
2012	CH1	12	17,102	613,637	2,736	684	1,368	0	0	635,528
	CH2	0	8,849	699,057	2,042	21,101	0	0	0	731,049
	CH2	8	8,849	784,822	681	6,126	2,042	0	0	802,520
	CH3	0	12,661	55,850	3,982	12,967	1,532	0	0	86,991
	CH3	20	694	25,444	449	1,470	327	0	0	28,384
	CH5	0	4,492	68,340	1,497	3,676	1,089	0	0	79,095
	CH5	24	26,682	70,382	9,393	681	1,225	0	0	108,364

**Supplementary Material Table 3. Microscopy-derived total chlorophyll a (TChl a).** Converted TChl a based on phytoplankton abundance determined by microscopy (see Supplementary Material Table 1) and phytoplankton pigment-type specific conversion factors. For classification of diatoms into type-1, -2 and -4, and dinoflagellates type-1 see Supplementary Material Table 4.

Date	Station	Depth (m)	Diatoms-1	Diatoms-2	Diatoms-4	Total diatoms	Dino-flagellates-1	Silico-flagellates	<i>Trichodesmium erythraeum</i>	TChl a comparable taxa
27 May 2011	CH3*	0	0.01844	0.01043	0.04244	0.01977	0.00312	0.02050	0.00000	0.04309
	CH3*	40	0.00820	0.00320	0.01477	0.00715	0.00099	0.01249	0.00000	0.01535
28 May 2011	CH1	0	0.02614	0.06258	0.23213	0.08486	0.00783	0.05467	0.00000	0.16928
	CH1	20	0.01598	0.02492	0.20441	0.04309	0.00428	0.02050	0.00000	0.08656
	CH2	0	0.03253	0.02982	0.21019	0.05450	0.00454	0.10023	0.00000	0.10926
	CH2	25	0.02905	0.02341	0.19402	0.04598	0.00500	0.04784	0.00000	0.09426
	CH3	0	0.01685	0.01959	0.11260	0.03243	0.00334	0.03417	0.00000	0.06598
	CH3	20	0.00857	0.00921	0.08835	0.01777	0.00326	0.02392	0.00000	0.04011
	CH4	0	0.00531	0.00464	0.02524	0.00809	0.00220	0.02343	0.00000	0.02050
	CH4	20	0.00773	0.00542	0.01874	0.00935	0.00163	0.04796	0.00000	0.02188
	CH5	0	0.01946	0.01321	0.05543	0.02366	0.00312	0.07517	0.00000	0.05136
	CH5	40	0.01328	0.01053	0.03663	0.01752	0.00410	0.03515	0.00000	0.04221
06 Jun 2011	CH5	60	0.00581	0.00760	0.02841	0.01133	0.00290	0.02734	0.00000	0.02809
	CH1	0	0.03892	0.06281	0.32914	0.09618	0.00638	0.25969	0.00000	0.19082
	CH1	12	0.02876	0.02109	0.16284	0.04165	0.00138	0.31436	0.00000	0.08464
	CH1	26	0.01878	0.01700	0.06005	0.02732	0.00169	0.07745	0.00000	0.05399
	CH3	0	0.04768	0.05190	0.28433	0.08620	0.00764	0.30465	0.00000	0.17814
	CH3	30	0.09237	0.05424	0.15591	0.09729	0.00667	0.13668	0.00000	0.19006
	CH5	0	0.01139	0.01020	0.02980	0.01604	0.00232	0.09021	0.00000	0.03647
06 Dec 2011	CH5	35	0.02227	0.01514	0.06121	0.02695	0.00343	0.03645	0.00000	0.05681
	B2	0	0.18489	1.49866	0.52231	1.54043	0.02429	0.00000	0.00000	2.73240
	B2	20	0.15492	4.41169	0.80842	4.33902	0.02417	0.00000	0.00000	7.57912
	B4	0	0.14872	0.34861	0.22867	0.40562	0.01421	0.00000	0.00522	0.74406
	B4	20	0.38245	0.64128	0.25985	0.77747	0.03143	0.11390	0.05654	1.47479
	B6	0	0.44539	0.55243	0.57744	0.73674	0.41336	0.22779	0.35663	2.64179
	B6	20	0.01394	0.03968	0.11364	0.05065	0.00928	0.00000	0.00251	0.11419
07 Dec 2011	SS	0	0.15492	1.11258	0.19248	1.13782	0.03223	0.00000	0.00000	2.05615
	SS	20	0.19623	1.76777	0.18478	1.78102	0.03868	0.00000	0.00000	3.18725
	CH1	0	0.46476	20.99997	1.38586	20.39189	0.01934	0.00000	0.00000	35.36924
	CH1	20	0.96824	21.47900	1.15488	21.02868	0.04835	0.00000	0.00000	36.54907
	CH2	0	0.34857	17.80130	1.73232	17.30423	0.00000	0.00000	2.97480	32.16318
	CH2	20	0.34857	17.24501	2.42525	16.81554	0.02901	0.00000	0.00000	29.20078
	CH3	0	0.18397	1.65342	0.28872	1.67341	0.02417	0.00000	0.14787	3.07140
24 Jan 2012	CH3	20	0.26896	2.25886	0.24060	2.28304	0.02686	0.00000	0.00000	4.02539
	CH5	0	1.99457	1.76159	4.50403	2.73965	0.50764	2.73353	3.56628	8.79743
	CH5	20	1.33617	1.27870	1.61683	1.84001	0.40611	1.59456	7.35001	9.72718
	CH1	0	0.37568	0.28587	1.31656	0.50202	0.03191	0.00000	0.78284	1.53121
	CH1	20	0.05809	0.06755	0.30687	0.10662	0.02072	0.00000	0.10289	0.31548
	CH2	0	0.15540	0.30712	0.12992	0.36208	0.02429	0.00000	1.58004	1.85631
	CH2	20	0.21108	0.48907	0.25407	0.56570	0.02369	0.00000	0.45057	1.37476
27 Feb 2012	CH3	0	1.38555	1.10563	0.57167	1.62602	0.02031	0.27335	0.37837	3.15669
	CH3	20	1.74864	1.39073	0.76222	2.05030	0.02901	0.00000	0.98116	4.35126
	CH5	0	0.04067	0.05285	0.07622	0.07108	0.01900	0.00000	0.57252	0.59556
	CH5	20	0.04212	0.10430	0.07362	0.12079	0.00761	0.00000	0.28508	0.43955
	B2	0	2.79323	1.98073	0.48995	2.99769	0.08497	0.00000	0.74858	5.96910
	B2	20	0.50633	0.60428	0.47679	0.80328	0.00333	0.00000	0.00000	1.40008
	B4	0	1.69055	0.68146	0.55434	1.33502	0.06092	0.00000	0.50624	2.84698
28 Feb 2012	B4	20	1.28776	0.91170	1.38586	1.45498	0.01934	0.00000	0.32183	2.80851
	B6	0	0.00620	0.02156	0.04966	0.02621	0.00745	0.00000	0.12856	0.15992
	B6	20	0.01044	0.02038	0.11303	0.03077	0.00535	0.00000	0.12190	0.15735
	B8	0	0.00290	0.01167	0.03003	0.01422	0.00764	0.00000	0.31105	0.27418
	B8	20	0.00145	0.00464	0.02339	0.00650	0.00943	0.00342	0.07907	0.09464
	CH1	0	3.35011	5.36975	3.81110	6.67142	0.04351	0.00000	0.00000	11.67005
	CH1	20	3.83423	2.80721	2.23277	4.29953	0.00000	0.00000	0.00000	7.44661
28 Feb 2012	CH2	0	3.16614	0.88466	0.28872	2.07695	0.03384	0.00000	0.18266	3.82162
	CH2	20	1.79512	0.84603	0.51970	1.53050	0.02176	0.00000	0.27660	2.91238
	CH3	0	2.14949	0.71622	0.69293	1.55272	0.01450	0.00000	0.89766	3.38945
	CH3	20	0.02764	0.03357	0.08602	0.04826	0.00640	0.00000	0.33653	0.34864
	CH5	0	0.00232	0.00852	0.02858	0.01088	0.00685	0.00342	0.13256	0.13485
	CH5	20	0.00261	0.00493	0.01732	0.00683	0.00685	0.00000	0.09929	0.10320

**Supplementary Material Table 3. Continued.**

Date	Station	Depth (m)	Diatoms- 1	Diatoms- 2	Diatoms- 4	Total diatoms	Dino- flagellates-1	Silico- flagellates	<i>Trichodesmium</i> <i>erythraeum</i>	TChl <i>a</i> comparable taxa
11 Sep	B2	0	0.21301	2.34492	0.75067	2.37683	0.13537	0.00000	0.00000	4.47566
2012	B2	8	0.19365	5.46246	0.34646	5.33121	0.02417	0.00000	0.00000	9.29757
	B4	0	0.00000	0.77098	0.02457	0.74045	0.03703	0.00000	0.00000	1.38065
	B4	20	0.00349	0.43113	0.04158	0.41717	0.00609	0.00000	0.00000	0.73868
	B6	0	0.00000	3.41501	0.11549	3.28018	0.08219	0.00000	0.00000	5.89915
	B6	12	0.03486	3.14768	0.03465	3.03213	0.02321	0.00000	0.00000	5.31308
	B8	0	1.55887	1.73068	2.30976	2.40275	0.04835	0.00000	0.00000	4.28971
	B8	12	2.78853	2.29470	0.92390	3.32460	0.02417	0.00000	0.00000	5.82220
	SS	0	0.29047	5.59381	0.69293	5.51633	0.09186	0.00000	0.00000	9.79772
	SS	8	0.17428	4.36534	0.23098	4.26497	0.01450	0.00000	0.00000	7.42523
12 Sep	CH1	0	0.27111	4.62030	0.34646	4.55375	0.29975	0.00000	0.00000	8.68201
2012	CH1	12	0.48655	6.96528	0.46427	6.89099	0.00486	0.00000	0.00000	11.94781
	CH2	0	0.25174	7.93487	0.34646	7.72286	0.14987	0.00000	0.00000	13.77323
	CH2	8	0.25174	8.90837	0.11549	8.64101	0.04351	0.00000	0.00000	15.08130
	CH3	0	0.36019	0.63394	0.67560	0.78858	0.09210	0.00000	0.00000	1.61008
	CH3	20	0.01975	0.28881	0.07622	0.28922	0.01044	0.00000	0.00000	0.52861
	CH5	0	0.12781	0.77572	0.25407	0.80857	0.02611	0.00000	0.00000	1.46966
	CH5	24	0.75910	0.79890	1.59373	1.15806	0.00483	0.00000	0.00000	2.01853
Conversion factors			2.84E-05	1.14E-05	1.70E-04	1.09E-05	7.10E-06	6.69E-04	2.56E-05	1.88E-05

**Supplementary Material Table 4. Classification of microscopically determined phytoplankton taxa into pigment-types.** Classification was based on literature (see text section 2.3). Bold font indicates phytoplankton taxa that were left within a pigment-type after taxa estimated as non-abundant by CHEMTAX were excluded, i.e. species “truly” belonging to the assigned pigment-type (see text section 4.3).

Pigment-type	Genus/species	Pigment-type	Genus/species
Diatoms-1	<i>Amphora</i> spp.	Dino- flagellates -1	cf. <i>Alexandrium</i> / <i>Gonyaulax</i> / <i>Heterocapsa</i> spp.
	<b>Anaulus minutus</b>		<i>Ceratium arietinum</i>
	<i>Asterionellopsis glacialis</i>		<i>Ceratium candelabrum</i>
	<i>Asteromphalus flabellatus</i>		<i>Ceratium carriense</i>
	<i>Asteromphalus</i> spp.		<b><i>Ceratium extensum</i></b>
	<i>Bacteriastrum elongatum</i>		<i>Ceratium furca</i>
	<i>Bacteriastrum furcatum/delicatulum</i>		<i>Ceratium fusus</i>
	<i>Bacteriastrum hyalinum</i>		<i>Ceratium lineatum</i>
	<i>Bacteriastrum</i> spp.		<b><i>Ceratium massiliense</i></b>
	<i>Cerataulina pelagica</i>		<i>Ceratium pentagonum</i>
	<i>Chaetoceros atlanticus</i>		<i>Ceratium ranipes</i>
	<i>Chaetoceros compressus</i>		<i>Ceratium symmetricum</i>
	<i>Chaetoceros coronatus</i>		<b><i>Ceratium trichoceros</i></b>
	<i>Chaetoceros curvisetus</i>		<i>Ceratium tripos</i>
	<i>Chaetoceros lorenzianus</i>		<i>Ceratium</i> spp.
	<i>Chaetoceros peruvianus</i>		<i>Ceratocorys horrida</i>
	<i>Chaetoceros socialis</i>		<b><i>Dinophysis acuminata</i></b>
	<i>Chaetoceros</i> spp. Hyalochaete		<i>Dinophysis caudata</i>
	<i>Chaetoceros</i> spp. Phaeoceros		<b><i>Dinophysis dens</i></b>
	<i>Climacodium frauenfeldianum</i>		<i>Dinophysis hastata</i>
	<i>Corethron pennatum</i>		<i>Dinophysis schuettii</i>
	<i>Corethron</i> spp.		<i>Dinophysis</i> spp.
	<i>Coscinodiscus</i> spp.		<b><i>Dissodinium pseudolunula</i></b>
	<i>Cyclotella</i> spp.		<i>Gymnodinium</i> spp.
	<i>Detonula pumila</i>		<b><i>Noctiluca scintillans</i></b>
	<i>Ditylum brightwellii</i>		<i>Ornithocercus magnificus</i>
	<i>Eucampia cornuta</i>		<b><i>Ornithocercus thumii</i></b>
	<i>Eucampia zodiacus</i>		<i>Ornithocercus quadratus</i>
	<b><i>Grammatophora oceanica</i></b>		<i>Ornithocercus</i> spp.
	cf. <i>Gyrosigma balticum</i>		<i>Oxytoxum compressum</i>
	<i>Helicotheca tamesis</i>		<i>Oxytoxum constrictum</i>
	<i>Hemiaulus hauckii</i>		<i>Oxytoxum diploconus</i>
	<i>Hemiaulus membranaceus</i>		<i>Oxytoxum laticeps</i>
	<i>Lauderia annulata</i> / <i>Thalassiosira</i> spp.		<b><i>Oxytoxum milneri</i></b>
	<i>Odontella aurita</i>		<i>Oxytoxum scolopax</i>
	<i>Odontella sinensis</i>		<i>Oxytoxum tessellatum</i>
	<i>Paralia sulcata</i>		cf. <i>Oxytoxum turbo</i>
	<i>Planktoniella sol</i>		cf. <i>Oxytoxum variabile</i>
	<i>Pleurosigma</i> spp.		<i>Oxytoxum</i> spp.
	<i>Skeletonema</i> sp.		<i>Phalacroma rotundatum</i>
	<i>Surirella fastuosa</i>		<i>Phalacroma</i> spp.
	<i>Synedra</i> spp.		<i>Podolampas elegans</i>
	<i>Triceratium dubium</i>		<b><i>Podolampas bipes</i></b>
	<i>Triceratium obtusum</i>		<i>Podolampas palmipes</i>
	<i>Triceratium</i> spp.		<i>Podolampas spinifera</i>
	<i>Trigonium alternans</i>		<i>Podolampas</i> spp.
	Undefined centric		<i>Pronoctiluca</i> spp.
Diatoms-2	<b><i>Climacosphenia moniligera</i></b>		<i>Prorocentrum cordatum</i>
	<i>Dactyliosolen fragilissimus</i>		<i>Prorocentrum dentatum</i>
	<i>Entomoneis</i> sp.		<b><i>Prorocentrum lima</i></b>
	<b><i>Guinardia delicatula</i></b>		<i>Prorocentrum micans</i>
	<i>Guinardia flaccida</i>		<i>Prorocentrum rostratum</i>
	<i>Guinardia striata</i>		<i>Prorocentrum triestinum</i>
	<i>Leptocylindrus danicus</i>		<i>Prorocentrum</i> spp.
	<i>Leptocylindrus mediterraneus</i>		<i>Schuetliella mitra</i>
	<b><i>Mastogloia rostrata</i></b>		<i>Scrippsiella trochoidea</i>
	<b><i>Neodenticula seminae</i></b>		cf. <i>Torodinium robustum</i>
	<i>Proboscia alata</i>		<i>Warnowia polyphemus</i>
	<i>Pseudo-nitzschia</i> spp.		<i>Dinoflagellate</i> undefined
	cf. <i>Pseudo-nitzschia subcurvata</i>	Dino- flagellates-2	<i>Gyrodinium</i> spp.
	<b><i>Rhabdonema adriaticum</i></b>		<i>Karlodinium</i> spp.
	<i>Rhizosolenia</i> spp.		<i>Protoperidinium bipes</i>
Diatoms-4	<i>Thalassionema bacillare/nitzschoides/frauenfeldii</i>		<i>Protoperidinium elegans</i>
	Undefined pennate <40µm	Chrysophytes/ silico- flagellates	<i>Protoperidinium</i> spp.
	Undefined pennate >40µm		<b><i>Dictyocha fibula</i></b>
	<i>Ceratoneis closterium/Nitzschia longissima</i>	Cyano- bacteria-1	<i>Dictyocha octonaria</i>
	<i>Diploneis</i> spp.		<i>Dictyocha speculum</i>
	<b><i>Meuniera membranacea</i></b>		<i>Dictyocha</i> spp.
	<i>Nitzschia/Lioloma/Thalassiothrix</i> spp.		<i>Trichodesmium erythraeum</i>
	<b><i>Trachyneis aspera</i></b>		
	<i>Navicula</i> spp.		

**Supplementary Material Table 5. Phytoplankton abundance determined by CHEMTAX.** Absolute concentrations of chlorophyll *a* (Chl *a*,  $\mu\text{g L}^{-1}$ ) assigned to each of the 13 algal classes per sample by CHEMTAX from the best solutions (= lowest pigment content unexplained by the CHEMTAX solution, as root mean square) of each most reasonable run (see text sections 2.2.3 and 3.2 for further details). Average where  $n = 2$  (see Table 1).

Date	Station	Depth (m)	Chryso-phytes	Crypto-phytes	Cyano-bacteria-1	Cyano-bacteria-2	Cyano-bacteria-4	Diatoms-1	Diatoms-2	Diatoms-4	Dino-flagellates-1	Eugleno-phytes	Hapto-phytes-6	Hapto-phytes-8	Prasino-phytes-3	Total Chl <i>a</i>
27 May 2011	CH3*	0	0.04906	0.00000	0.00208	0.08880	0.02825	0.00000	0.04425	0.00155	0.00000	0.01327	0.02532	0.07069	0.07310	0.39637
	CH3*	40	0.05781	0.00000	0.00077	0.08048	0.03251	0.00000	0.04175	0.00550	0.00000	0.01427	0.03454	0.07850	0.07382	0.41996
28 May 2011	CH1	0	0.04267	0.04820	0.06079	0.10688	0.00000	0.00000	0.06128	0.06349	0.01281	0.00553	0.02866	0.07659	0.16405	0.67095
	CH1	20	0.04118	0.05023	0.07538	0.09110	0.00000	0.00001	0.05672	0.07399	0.02077	0.01616	0.03311	0.07530	0.19471	0.72868
	CH2	0	0.04551	0.02668	0.01415	0.10348	0.03040	0.00000	0.05357	0.03760	0.01482	0.00223	0.02881	0.07247	0.16832	0.59805
	CH2	25	0.03935	0.02078	0.02188	0.08888	0.04068	0.00000	0.06856	0.03463	0.01125	0.00580	0.02759	0.06638	0.16958	0.59537
	CH3	0	0.04435	0.00001	0.03153	0.06596	0.02762	0.00000	0.03096	0.00261	0.00000	0.02311	0.02334	0.07405	0.00000	0.32355
	CH3	20	0.04168	0.00000	0.09640	0.05489	0.02851	0.00000	0.00000	0.00795	0.00000	0.02298	0.03923	0.06998	0.00365	0.36527
	CH4	0	0.03085	0.00003	0.05211	0.08416	0.03163	0.00000	0.03191	0.00000	0.00001	0.03002	0.02374	0.05548	0.00079	0.34073
	CH4	20	0.02396	0.00003	0.08859	0.04698	0.01854	0.00000	0.04560	0.00068	0.00001	0.02511	0.00871	0.05299	0.00000	0.31120
	CH5	0	0.04133	0.00000	0.03401	0.08450	0.00000	0.00000	0.03659	0.01201	0.01431	0.02556	0.03042	0.07166	0.02263	0.37302
	CH5	40	0.05611	0.00000	0.01919	0.07644	0.00000	0.00000	0.03476	0.01452	0.01428	0.02513	0.03738	0.08760	0.05785	0.42326
	CH5	60	0.06334	0.00000	0.00858	0.04984	0.04488	0.00000	0.01946	0.01729	0.01772	0.01939	0.04761	0.08542	0.05616	0.42969
06 Jun 2011	CH1	0	0.00010	0.13779	0.09156	0.03998	0.00000	0.00000	0.13464	0.05152	0.01588	0.07879	0.04802	0.11873	0.11714	0.83415
	CH1	12	0.00868	0.13778	0.15385	0.03929	0.00000	0.00001	0.12334	0.06958	0.01161	0.04706	0.06905	0.13480	0.15417	0.94921
	CH1	26	0.00404	0.07388	0.05840	0.02258	0.00000	0.00000	0.05853	0.05896	0.00864	0.02055	0.03077	0.07583	0.08637	0.49854
	CH3	0	0.00856	0.09392	0.11673	0.02782	0.00000	0.00001	0.04311	0.04454	0.01352	0.07001	0.05371	0.11230	0.07002	0.65427
	CH3	30	0.01419	0.06792	0.13379	0.01452	0.00000	0.00001	0.06059	0.06621	0.01156	0.05646	0.05987	0.10142	0.06710	0.65365
	CH5	0	0.00492	0.04258	0.04162	0.05500	0.00000	0.00000	0.00668	0.00720	0.01159	0.08288	0.01962	0.05981	0.03578	0.36769
	CH5	35	0.00892	0.02970	0.04436	0.04110	0.00000	0.00000	0.00794	0.01052	0.00002	0.06937	0.01822	0.05385	0.03616	0.32017
06 Dec 2011	B2	0	0.02352	0.01570	0.05045	0.02748	0.06973	0.00000	0.11391	0.15961	0.02409	0.00402	0.02491	0.05834	0.09029	0.66205
	B2	20	0.02114	0.01222	0.17998	0.01732	0.00000	0.16876	0.20594	0.39155	0.00000	0.00635	0.04301	0.05076	0.10933	1.20635
	B4	0	0.02660	0.00000	0.01076	0.02076	0.08739	0.00000	0.08697	0.09019	0.02591	0.00549	0.02505	0.06207	0.05529	0.49648
	B4	20	0.02689	0.00000	0.00783	0.02724	0.06070	0.00000	0.09350	0.09324	0.02161	0.00334	0.02919	0.05861	0.07895	0.50111
	B6	0	0.02117	0.00000	0.00217	0.03478	0.08480	0.00000	0.04029	0.00768	0.01141	0.00464	0.01200	0.05061	0.00000	0.26955
	B6	20	0.02063	0.00000	0.04868	0.04017	0.05883	0.00000	0.03256	0.00897	0.01429	0.00505	0.01033	0.04374	0.00000	0.28325
	SS	0	0.02170	0.00001	0.15028	0.04139	0.00001	0.00003	0.09466	0.12795	0.00000	0.00563	0.01834	0.05281	0.01896	0.53176
	SS	20	0.02052	0.00000	0.18000	0.03278	0.00000	0.00000	0.10898	0.18405	0.00000	0.00846	0.01159	0.04666	0.00000	0.59306
07 Dec 2011	CH1	0	0.00395	0.08375	0.24378	0.00433	0.00000	2.52001	0.43457	0.81570	0.03933	0.01357	0.08308	0.00906	0.10346	4.35459
	CH1	20	0.01554	0.05871	0.48733	0.00000	0.00000	2.12696	0.38589	1.23372	0.00002	0.01042	0.08107	0.00432	0.08227	4.48626
	CH2	0	0.00691	0.02572	0.60182	0.01687	0.00000	0.73094	0.38875	0.66835	0.02800	0.00804	0.08086	0.02229	0.04758	2.62612
	CH2	20	0.01049	0.02205	0.52492	0.00432	0.00000	0.66398	0.38111	0.88106	0.03713	0.00438	0.10239	0.01255	0.03706	2.68146
	CH3	0	0.01531	0.00000	0.07122	0.02098	0.02059	0.04207	0.06303	0.10870	0.00850	0.00682	0.01638	0.04819	0.00000	0.42179
	CH3	20	0.02721	0.01739	0.08010	0.01684	0.01607	0.11248	0.11731	0.19066	0.04260	0.00618	0.03254	0.07438	0.07099	0.80475
	CH5	0	0.03253	0.00000	0.05065	0.04647	0.02697	0.00000	0.07240	0.03126	0.01182	0.00975	0.01455	0.06880	0.00000	0.36522
	CH5	20	0.03409	0.00000	0.03046	0.05108	0.03571	0.00000	0.08048	0.02334	0.00965	0.00966	0.01438	0.07444	0.00000	0.36328
24 Jan 2012	CH1	0	0.00379	0.12088	0.23742	0.07199	0.00001	0.00003	0.17472	0.27212	0.11649	0.04174	0.06302	0.04056	0.07860	1.22137
	CH1	20	0.02516	0.00000	0.05412	0.08209	0.00000	0.00000	0.00000	0.07715	0.02445	0.00009	0.07958	0.08959	0.15305	0.58528
	CH2	0	0.01697	0.03790	0.11542	0.14902	0.00000	0.00000	0.04051	0.04736	0.00000	0.04833	0.03024	0.03914	0.00000	0.52490
	CH2	20	0.01805	0.02552	0.05438	0.10442	0.00000	0.00000	0.05239	0.05979	0.00000	0.01605	0.05117	0.05876	0.08471	0.52524
	CH3	0	0.01979	0.00000	0.09205	0.12601	0.03513	0.00000	0.14452	0.17559	0.00000	0.01678	0.05257	0.02369	0.02860	0.71472
	CH3	20	0.01614	0.00000	0.02287	0.12133	0.00000	0.00000	0.14069	0.12404	0.00000	0.03545	0.04272	0.02490	0.00000	0.52813
	CH5	0	0.01342	0.00000	0.00383	0.17269	0.00000	0.00000	0.03276	0.00242	0.00000	0.00000	0.01112	0.02179	0.00000	0.25803
	CH5	20	0.01196	0.00000	0.00000	0.16786	0.00000	0.00004	0.03741	0.00003	0.00000	0.00000	0.00000	0.02276	0.00000	0.24007
27 Feb 2012	B2	0	0.01622	0.08448	0.29695	0.00005	0.00000	0.00005	0.17386	0.32509	0.09391	0.03569	0.06214	0.02335	0.07351	1.18530
	B2	20	0.02098	0.11065	0.00000	0.00203	0.00000	0.33149	0.00568	0.41410	0.00000	0.00000	0.04312	0.02022	0.08445	1.03270
	B4	0	0.00985	0.00000	0.37439	0.06214	0.00000	0.00000	0.10657	0.11976	0.00000	0.00000	0.02510	0.01232	0.00000	0.71012
	B4	20	0.00720	0.00000	0.14038	0.08471	0.00000	0.00000	0.05838	0.02640	0.00000	0.00000	0.01422	0.03229	0.00000	0.36357
	B6	0	0.01629	0.00002	0.06531	0.08483	0.00000	0.00000	0.02302	0.00000	0.00001	0.00000	0.00000	0.01531	0.00000	0.20479
	B6	20	0.00513	0.00014	0.04936	0.12563	0.00000	0.00000	0.01484	0.00000	0.00001	0.00000	0.00000	0.02834	0.00000	0.22344
	B8	0	0.02077	0.00001	0.11509	0.08203	0.00000	0.00002	0.02035	0.00083	0.00000	0.00000	0.00000	0.02058	0.00000	0.25969
	B8	20	0.00513	0.00000	0.04158	0.08441	0.00000	0.00000	0.00000	0.00046	0.00000	0.00000	0.00000	0.03054	0.00000	0.16213
28 Feb 2012	CH1	0	0.01544	0.00002	0.00000	0.00000	0.00000	1.34564	0.09344	0.04206	0.00001	0.03066	0.13782	0.00076	0.03420	1.70005
	CH1	20	0.02754	0.05090	0.10808	0.00000	0.00000	0.83689	0.29830	0.91925	0.00074	0.06364	0.16764	0.00059	0.11350	2.58707
	CH2	0	0.01590	0.00000	0.18449	0.05109	0.00000	0.00000	0.06649	0.07108	0.00000	0.02810	0.01809	0.02336	0.00000	0.45860
	CH2	20	0.01209	0.00000	0.00207	0.06895	0.00000	0.00000	0.26528	0.08446	0.00000	0.05498	0.02642	0.03802	0.06158	0.61385
	CH3	0	0.00000	0.00000	0.17577	0.05080	0.00000	0.00000	0.06504	0.06845	0.04042	0.00000	0.01307	0.01980	0.00000	0.43335
	CH3	20	0.00940	0.00005	0.12233	0.10861	0.00000	0.00005	0.02081	0.00413	0.00000	0.00000	0.00000	0.04094	0.00000	0.30634
	CH5	0	0.01416	0.00000	0.11545	0.06590	0.00000	0.00000	0.04324	0.00000	0.00000	0.00000	0.01807	0.01406	0.00000	0.28688
	CH5	20	0.00227	0.00000	0.00066	0.06901	0.00000	0.00000	0.00000	0.00410	0.01832	0.00000	0.00000	0.01888	0.00000	0.11325

**Supplementary Material Table 5. Continued.**

Date	Station	Depth (m)	Chryso- phytes	Crypto- phytes	Cyano- bacteria- 1	Cyano- bacteria- 2	Cyano- bacteria- 4	Diatoms- 1	Diatoms- 2	Diatoms- 4	Dino- flagellates- 1	Eugleno- phytes	Hapto- phytes-6	Hapto- phytes-8	Prasino- phytes-3	Total Chl a
11 Sep	B2	0	0.00429	0.06149	0.17121	0.01628	0.00001	0.33508	0.11203	0.44555	0.07956	0.11983	0.03492	0.20486	0.11308	1.69819
2012	B2	8	0.01340	0.00000	0.45927	0.00000	0.00000	0.67579	0.55938	1.40646	0.00000	0.07037	0.09450	0.14094	0.06751	3.48762
	B4	0	0.02051	0.00002	0.13362	0.01449	0.00001	0.12060	0.04990	0.17498	0.01713	0.03211	0.01894	0.06515	0.02201	0.66948
	B4	20	0.02879	0.01745	0.10867	0.00000	0.00000	0.09038	0.09037	0.25008	0.00000	0.03528	0.02580	0.08434	0.03848	0.76964
	B6	0	0.00506	0.00000	0.27283	0.00000	0.00000	0.26553	0.13715	0.38999	0.01946	0.02343	0.04997	0.04386	0.00859	1.21587
	B6	12	0.00396	0.11600	0.27445	0.00000	0.00000	0.34486	0.25413	0.57783	0.00000	0.01980	0.04088	0.04984	0.00000	1.68174
	B8	0	0.01545	0.02672	0.06978	0.02907	0.00000	0.00000	0.47990	1.33094	0.00000	0.02473	0.05548	0.06643	0.01205	2.11055
	B8	12	0.01873	0.00000	0.16899	0.00000	0.00000	0.00000	0.80233	2.20250	0.00000	0.01255	0.18942	0.03756	0.01856	3.45064
	SS	0	0.00988	0.04078	0.51500	0.00000	0.00000	0.63422	0.35256	0.59171	0.04285	0.11432	0.04586	0.20728	0.10429	2.65875
	SS	8	0.02228	0.20844	0.52692	0.00000	0.00000	0.70068	0.54175	1.13648	0.00000	0.11406	0.11341	0.29278	0.14017	3.79696
12 Sep	CH1	0	0.00596	0.00000	0.16399	0.00000	0.00000	0.50922	0.20944	0.00882	0.08846	0.04827	0.04339	0.03088	0.00000	1.10843
2012	CH1	12	0.01149	0.00000	0.65240	0.00000	0.00000	0.81626	0.55672	1.91426	0.00000	0.02999	0.15374	0.02574	0.02772	4.18832
	CH2	0	0.00805	0.00002	0.49501	0.00000	0.00000	0.60748	0.28697	0.65996	0.05536	0.04196	0.05605	0.03620	0.01653	2.26357
	CH2	8	0.01148	0.05845	0.00000	0.00000	0.00000	0.99612	0.96102	0.48625	0.00000	0.03589	0.10261	0.05542	0.01662	2.72386
	CH3	0	0.01088	0.00000	0.04661	0.02250	0.00000	0.09411	0.03061	0.10966	0.07159	0.01503	0.01214	0.05567	0.00000	0.46880
	CH3	20	0.02259	0.12339	0.04482	0.00887	0.00000	0.03920	0.01101	0.07647	0.00000	0.02379	0.01041	0.05332	0.03455	0.44842
	CH5	0	0.01290	0.00000	0.00034	0.02451	0.00000	0.19580	0.03161	0.29870	0.00848	0.01533	0.02417	0.04483	0.00000	0.65666
	CH5	24	0.01041	0.05511	0.23983	0.00000	0.00000	0.42480	0.04136	0.77930	0.00000	0.00000	0.08364	0.01319	0.01940	1.66703

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## **Chapter 4**

Phytoplankton composition under contrasting oceanographic conditions: Upwelling and downwelling (Eastern Australia)

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## Research papers

## Phytoplankton composition under contrasting oceanographic conditions: Upwelling and downwelling (Eastern Australia)



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## ABSTRACT

Phytoplankton abundance and distribution along the east coast of Australia are driven primarily by the southward flowing East Australian Current (EAC), which transports tropical water masses to temperate latitudes. The Solitary Islands Marine Park (SIMP, ~30°S) is located north of the EAC separation point (~32°S) in this tropical–temperate transition zone. In this study, we describe the oceanographic context (wind, current and nutrient load) during a wind-driven downwelling and a current-driven upwelling event, both sampled in austral winter only ten days apart. We investigate the effect of these contrasting oceanographic conditions on phytoplankton abundance, composition and distribution along a cross-shelf transect. During downwelling we find a cross-shelf transition in microphytoplankton composition from an offshore- to an inshore-community associated with nutrient gradients (nitrate and silicate). Strong vertical mixing leads to increased occurrences of benthic diatoms in near-shore surface waters. During upwelling conditions, elevated nutrient availability results in maximum microphytoplankton abundances (mainly oceanic diatoms) and increased species richness on the mid-shelf. An increase in dinoflagellates and silicoflagellates (mid-shelf) and the appearance of tropical phytoplankton (especially picoplankton and dinoflagellates, offshore) signals a strong impact of the EAC across all shelf communities. Nanoplankton are a major part of the winter phytoplankton community during both oceanographic regimes (~40–50% of Tchl *a*). Our findings provide evidence of EAC-driven, nutrient-rich, slope water intrusion in the SIMP as expressed by cross-shelf phytoplankton variability. We suggest that rapid (~weekly) changes in phytoplankton composition along the east Australian coast are likely to be enhanced by the climate change-induced warming/strengthening of the EAC.

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## 1. Introduction

In the light of climate change and associated worldwide changing oceanographic regimes, the study of phytoplankton as primary producers within the marine realm is crucial. Phytoplankton (photosynthetic microalgae) are responsible for ~50% of the global annual net primary production and link the atmospheric and the oceanic carbon cycle via the biological carbon pump (Field et al., 1998; Falkowski and Oliver, 2007; Falkowski and Raven, 2007). Equally, at the base of the marine food web, phytoplankton are of

indispensable nutritional value to higher trophic organisms, and their abundance and distribution patterns ultimately affect the sustainability of all marine life. As a result of global warming, shifts in phytoplankton peak production periods, species range expansions and alterations in community structures are expected (Hallegraeff, 2010). These adaptations might disrupt established food-web interactions, impact primary productivity and influence biogeochemical cycles.

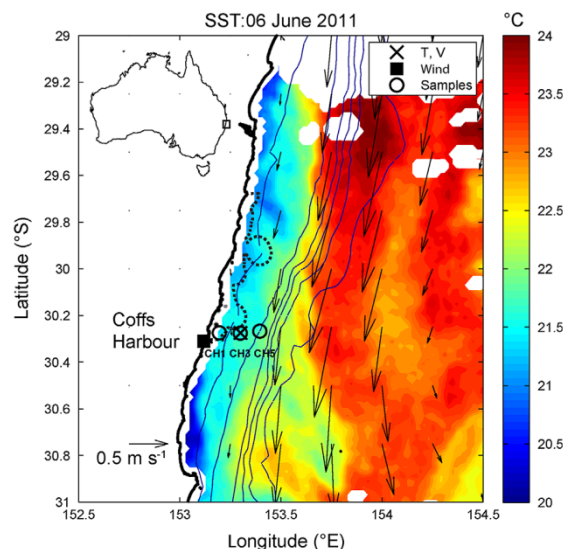
Subtropical western boundary currents (WBCs), which transport warm tropical water to the mid-latitudes, are hotspots of climate change-induced sea surface temperature (SST) warming (Wu et al., 2012). These authors reported a rapid increase in SSTs of WBCs, at a rate 2–3 times faster than the global mean ocean surface warming, and associated with a poleward shift and/or intensification of these currents. As a consequence changes in coastal physical forcing mechanisms and biological properties

Abbreviations: SIMP, Solitary Islands Marine Park; IMOS, Integrated Marine Observing System; OC, Oceanographic condition (upwelling and downwelling); SI, Station ID (CH1, CH3 and CH5)

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**Fig. 1.** Sampling location. Insert showing sea surface temperature (GHRSSST from NOAA AVHRR products) and geostrophic current velocity vectors from altimetry (NOAA) of 06 June 2011. The location of the sampling sites (CH1, CH3 and CH5, circles), the BOM station (black square) measuring wind and the permanent IMOS mooring station (crossed circle) measuring temperature (T) and velocity (V) are shown. Contours of the coastline and the 50, 100, 250, 500, 1000, 2000 m isobaths are shown as black lines, while the offshore delimitation of Solitary Islands Marine Park is indicated by the black dashed line.

have been observed, such as in the Agulhas (Lutjeharms, 2006), Kuroshio (Chen et al., 2006) and East Australian Current (Thompson et al., 2009). In oligotrophic WBC systems, productivity is enhanced in regions of upwelling of cold, nutrient-rich, bottom water in the euphotic zone; thus, local oceanography and phytoplankton productivity are tightly linked (e.g. Probyn et al., 1994; Lutjeharms and De Ruijter, 1996; Thompson et al., 2009, 2011).

Along the Australian east coast, phytoplankton abundance and distribution are driven primarily by the southward flowing East Australian Current (EAC). The EAC is the WBC of the South Pacific sub-tropical gyre and flows (with up to  $2 \text{ m s}^{-1}$ ) from its formation point ( $\sim 10\text{--}15^\circ\text{S}$ ) along the continental shelf break, until it separates from the coast ( $\sim 32^\circ\text{S}$ ) (Godfrey et al., 1980; Ridgway and Dunn, 2003). At the separation point the current bifurcates into the eastward flowing Tasman Front and a southward directed component; the latter being comprised of a sequence of quasi-permanent eddies travelling along the shelf and a residual southward EAC flow reaching down to Tasmania (Fig. 1). The EAC's influence appears to have increased poleward due to climate change over the past 60 years (Ridgway, 2007; Hill et al., 2008), while seasonal and decadal variability has also been documented (Ridgway and Godfrey, 1997; Ridgway and Hill, 2009). EAC strengthening together with increasing insolation and topographically induced vertical mixing has led to the promotion of nutrient enhanced phytoplankton blooms in spring and summer (Hallegraeff and Reid, 1986; Ajani et al., 2001; Thompson et al., 2011; and references therein). However, sporadic nutrient enrichment favoring primary production on the east Australian continental shelf has also been reported, especially in the EAC separation zone, and may be by wind or current-driven (Oke and Middleton, 2001; Roughan and Middleton, 2002, 2004, Rossi et al., in preparation).

Phytoplankton research along the east coast of Australia has been conducted sporadically since the 1930s (e.g. from Dakin and Colefax, 1933, 1940; to Wood, 1961a, b, 1964a, b) and was centralised at the long term sampling station Port Hacking, Sydney

( $\sim 34^\circ\text{S}$ ), since 1960 (Ajani et al., 2001, in press; and references therein). More recent investigations have addressed broader regional areas from Port Stephens ( $32^\circ\text{S}$ ) to Jervis Bay ( $35^\circ\text{S}$ ) (Hallegraeff and Jeffrey, 1993; Lee et al., 2001, 2007) and Lakes Entrance ( $38^\circ\text{S}$ ) (Bax et al., 2001). Tropical phytoplankton communities have been identified and described around northern Australia (between  $\sim 10$  and  $25^\circ\text{S}$ ) by Hallegraeff and Jeffrey (1984). Ultimately though, there is a lack of detailed taxonomic information regarding phytoplankton composition and distribution for  $\sim 1000 \text{ km}$  along the east Australian coastline north of Sydney.

This study is undertaken in the Solitary Islands Marine Park (SIMP) located north of the EAC separation point in the tropical–temperate transition zone of Eastern Australia (Fig. 1  $\sim 30^\circ\text{S}$ ). The bathymetry of the region is complex, including numerous coastal and offshore reefs, as well as eight islands fringed by shallow rocky reefs (NSW MPA, 2008). Due to the irregular topography combined with episodic encroachment of the EAC, cold bottom water intrusions and northward flowing counter currents, small-scale variations of the circulation and water temperature changes are typical in the area (Malcolm et al., 2011). The impact of such temperature gradients (generally spanning  $16\text{--}27.5^\circ\text{C}$ ) and variations in the shelf circulation is not known but it is thought that they may strongly affect the distribution of planktonic organisms (Boland and Church, 1981; Roughan and Middleton, 2004; Roughan et al., 2011).

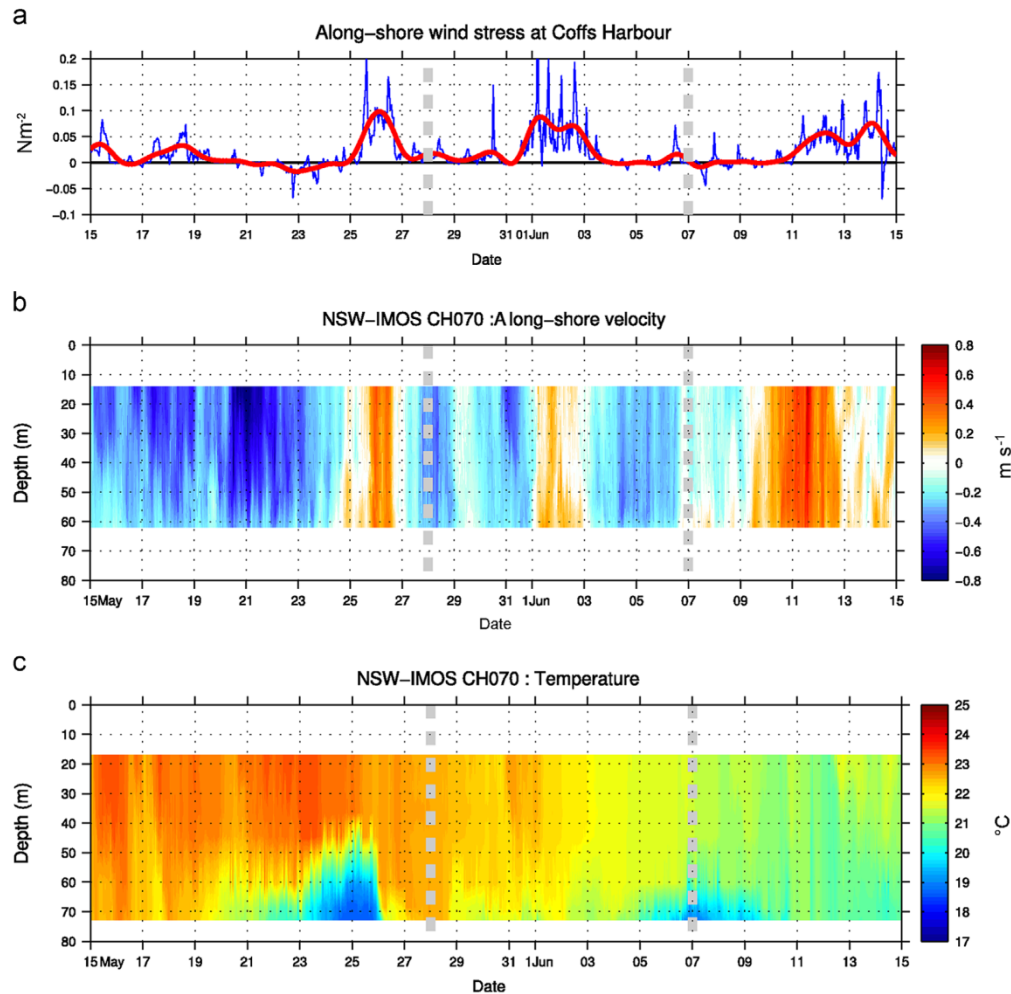
In this study we compare the phytoplankton composition and distribution in the SIMP under two contrasting oceanographic conditions: (1) a wind-driven downwelling event and (2) a current-driven upwelling event. Both scenarios closely followed each other during a 12-day field campaign in the SIMP during the austral winter of 2011, thereby providing a unique opportunity to investigate the phytoplankton response to rapidly changing oceanographic conditions during the same season. We describe the general oceanographic background, including nutrient concentrations (nitrate and silicate), and analyse the associated biological response through a detailed taxonomic phytoplankton survey.

## 2. Methods

### 2.1. Sampling design and instrumentation

Sampling was undertaken at the 25, 70 and 100 m isobaths in a cross-shelf transect off the east coast of Australia on 28 May and 07 June, 2011 (Fig. 1). The most inshore located station (CH1) is located within the SIMP, whereas the other two stations (CH3 – mid-shelf and CH5 – offshore) are located in the adjacent Solitary Islands Marine Reserve. Station CH5, is most influenced by the intrusion of the EAC onto the shelf. This cross-shelf transect, henceforth identified as the CH-line, extends about 26 km off Coffs Harbour, Australia.

Station CH3 is also the site of a long-term in-situ oceanographic mooring (CH070) which is part of the NSW Integrated Marine Observing System (IMOS). The mooring is instrumented with seven temperature loggers at 8 m intervals through the water column from the bottom to within approximately 15 m of the sea surface, while a bottom Acoustic Doppler Current Profiler (ADCP) measures the current velocity again at 8 m intervals in the vertical (Roughan et al., 2010; Schaeffer et al., 2013). Data from this mooring as well as hourly land-based wind observations (Australian Bureau of Meteorology, BOM, Fig. 2) were analysed to describe the oceanographic context. The wind stress was calculated from the 10 m wind velocity and time series were low-pass filtered following Wood et al. (2012). Both current and wind data are



**Fig. 2.** Wind and current observations. (a) Raw and low-pass filtered along-shelf wind stress observations (blue and red lines respectively) ( $\text{N m}^{-2}$ ), (b) along-shelf current velocity ( $\text{m s}^{-1}$ ) and (c) temperature ( $^{\circ}\text{C}$ ) from the CH070 mooring between 15 May–15 June 2011. The grey dashed lines indicate the dates of the two sampling cruises discussed in this paper. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rotated to an along/across-shelf coordinate system, positive values being northward/eastward, respectively (Schaeffer et al., 2013).

## 2.2. Hydrographic sampling

Sampling was undertaken on the RV *Bombora* (NSW Office of Environment and Heritage vessel) by the deployment an automatic SBE32 rosette water sampler (Sea-Bird Electronics, Inc., USA) equipped with  $12 \times 5$  L Niskin-bottles (General Oceanics, USA). The rosette was fitted with a SBE 911plus Conductivity–Temperature–Depth profiler (CTD) (Seabird Electronics, Inc., USA) and an ECO FLNTU fluorescence sensor (Wetlabs, Inc., USA).

At each station, the CTD was lowered to within 5 m of the seabed after a 3 min surface soak. Water samples were taken at 20–30 m intervals throughout the water column. In addition, surface samples were collected in a 10 L plastic bucket. The CTD profiles were visualised in real time, allowing for an adaptive sampling strategy to capture the depth of the deep Chl *a* maximum. As CTD casts lasted for a maximum of 20 min and boat

drift with the mean current was negligible, we assumed that the CTD and rosette sampled the same water and thus we refer to the start times and coordinates only.

Data post-processing was conducted using the Seabird SBEData Processing software (Sea-Bird Electronics, Inc., USA) following IMOS CTD processing protocols (<http://imos.org.au/anmndocuments.html>).

## 2.3. Sample preparation and analysis

Preliminary sample preparations were conducted at sea within 30 min of sample collection.

### 2.3.1. Nutrients (dissolved nitrate and silicate)

About 1 L of Niskin water from each sampling location was used to triple rinse a syringe, a  $0.45 \mu\text{m}$  filter and three 10 mL sample tubes. The tubes were filled 75% with seawater filtered through the syringe filter, labelled, frozen and sent for analysis packed in dry ice (at the Commonwealth Scientific and Industrial



Table 1

Pigments, SCOR abbreviations and phytoplankton classes associated according to Jeffrey et al. (2005).

Pigment	SCOR abbreviation	Taxonomic distribution
<i>Chlorophyll a</i>	Chl a	All photosynthetic algae and higher plants
<i>Divinyl chlorophyll a</i>	DV Chl a	<i>Prochlorococcus</i> spp.
<i>Divinyl chlorophyll b</i>	DV Chl b	<i>Prochlorococcus</i> spp.
<i>Alloxanthin</i>	Allo	Cryptomonads, some dinoflagellates with endosymbionts
<i>19'-Butanoyloxy-fucoxanthin</i>	But-fuco	Chrysophytes, some prymnesiophytes, some dinoflagellates with endosymbionts
<i>Fucoxanthin</i>	Fuco	Diatoms, prymnesiophytes, brown seaweeds, raphidophytes, some dinoflagellates with endosymbionts
<i>19'-Hexanoyloxy-fucoxanthin</i>	Hex-fuco	Prymnesiophytes, some dinoflagellates with endosymbionts
<i>Lutein</i>	Lut	Red seaweeds, green algae, higher plants
<i>Neoxanthin</i>	Neo	Green algae, euglenophytes, higher plants (9'-cis isomer); brown seaweeds, chrysophytes ( <i>all-trans</i> )
<i>Peridinin</i>	Perid	Photosynthetic dinoflagellates, except those containing endosymbionts of other algal classes
<i>Prasinolaxanthin</i>	Pras	Prasinophytes (e.g. Micromonadophyceae)
<i>Violaxanthin</i>	Viola	Higher plants, green algae, eustigmatophytes, brown seaweeds
<i>Zeaxanthin</i> (photoprotective pigment)	Zea	Prochlorophytes, cyanobacteria (coccoid), green algae, most chrysophytes, raphidophytes

Pigment names in italic font indicate biomarker pigments used to calculate phytoplankton size classes derived from HPLC following Uitz et al. (2006) and Ras et al. (2008).

Research Organization, CSIRO, Floreat, Western Australia). The concentrations, of nitrate + nitrite (hereafter nitrate) and silicate, were determined by colorimetric methods using a Lachat 8000 autoanalyser, following the WOCE protocol (Gordon et al., 1993).

### 2.3.2. Total chlorophyll a (TChl a)

For total Chl *a* analysis, 1 L samples were filtered onto Whatman GF/F filters (while protected from sunlight) and stored frozen ( $-20^{\circ}\text{C}$ ) until extraction in 8 mL 90% acetone within 3 days of collection. All samples were analysed for Chl *a* with a TD700 Turner fluorometer after overnight extraction at  $-20^{\circ}\text{C}$  without grinding. Samples were acidified with 10% HCl to correct for phaeopigments, and Chl *a* concentrations were calculated according to Parsons et al. (1984).

### 2.3.3. High-performance liquid chromatography (HPLC)

Duplicate samples for HPLC analyses were prepared from water collected at 0 and 20 m and/or the Chl *a* maximum depth at all stations to enable spatial comparisons for different depths and locations. Two liters were filtered (as in Section 2.3.2) onto pre-combusted 25 mm GF/F filter papers (Whatman Ltd., UK) and stored in liquid nitrogen until further analysis (at CSIRO Hobart, Tasmania). HPLC analysis followed a modified version of the Van Heukelem and Thomas (2001) technique. The method is based on acetone pigment extraction for 15–18 h, pigment separation using a  $\text{C}_8$  column and a binary gradient system with an elevated column temperature ( $55^{\circ}\text{C}$ ) (for further details see Clementson, 2010). Pigments were identified by retention time and absorption spectrum from a photo-diode array (PDA) detector and concentrations of pigments were determined from commercial and international standards (Sigma Aldrich Missouri, DHI Denmark). A Waters Alliance<sup>®</sup> HPLC system was used and separated pigments were detected at 436 nm using Waters Empower software.

A first approximation of the relative percentage of micro-, nano-, and picoplankton ( $>20\text{ }\mu\text{m}$ ,  $2\text{--}20\text{ }\mu\text{m}$ ,  $<2\text{ }\mu\text{m}$ , respectively) was derived from HPLC data following a linear regression model (Uitz et al., 2006; Ras et al., 2008). The model uses the sum of seven weighted diagnostic pigments (Table 1) to calculate fractions of each size class with respect to total phytoplankton biomass (TChl *a*). We used microscopic analysis to verify species attributions from HPLC, therefore reducing the risks of mis-interpreting pigment associations (e.g. the same pigment can occur in several phytoplankton taxa and/or not all species are limited to the indicated size range, see Uitz et al. (2006)).

### 2.3.4. Microphytoplankton ( $>20\text{ }\mu\text{m}$ ) abundance

At each sampling location 2 L of water were fixed in opaque plastic containers with 6 mL of Lugol's acid solution for later concentration by sedimentation (48 h), identification and enumeration under an inverted microscope. A minimum of 400 cells were counted per sample at  $200\times$  magnification following Utermöhl (1958), and identification was made at the lowest taxonomic level possible by an analyst with advanced taxonomic expertise. Taxonomic guides used included Dakin and Colefax (1940), Wood (1954), Crosby and Wood (1958, 1959), Wood et al. (1959), Wood (1961a, b), Tomas, 1997 and Hallegraeff et al., 2010. Additional literature authored by Hallegraeff and Jeffrey, 1984, Ajani et al. (2001) and Stidolph et al. (2012) were also referred to.

When the genus could not be determined (e.g. due to overlying particles in the sample or morphological degradation) diatoms were classified as “undefined centric” or “undefined pennate”. The lower size limit that could be determined was  $\sim 10\text{ }\mu\text{m}$ . Dinoflagellates were determined to genus level in most cases except for a few distinct species. To facilitate identification of thecate dinoflagellates  $100\text{ }\mu\text{L}$  of a Calcofluor White Stain working solution ( $20\text{ }\mu\text{g mL}^{-1}$ ) was added to each 3 mL sample 30 min prior to counting (Fritz and Triemer, 1985). All dinoflagellates belonging to the genera *Alexandrium*/*Gonyaulax*/*Heterocapsa*/*Scripsiella* spp. were grouped in a complex. Distinct *Gymnodinium* spp. and *Gyrodinium* spp.  $>20\text{ }\mu\text{m}$  were included in the counts. Smaller individuals were difficult to distinguish from each other, and equally from a numerous small unidentifiable flagellates, and were therefore excluded from the counts. We relied on HPLC analysis to estimate the quantity and composition of small sized phytoplankton. Dinoflagellates  $>20\text{ }\mu\text{m}$  that could not be classified were counted as “undefined dinoflagellates”.

### 2.4. Statistical analyses

Changes in the mean species richness of microphytoplankton at each station under upwelling and downwelling conditions were investigated via a Two-Way Crossed Analysis of Variance (ANOVA) using Minitab V16. We defined two factors within the ANOVA: “Oceanographic condition” (OC; composed of the two levels downwelling and upwelling) and “Station ID” (SI; composed of the three levels CH1, CH3 and CH5), which were tested for their combined and separate effects on the microphytoplankton species richness. Residual plots were visually checked and no violations were obvious. As aimed at detecting differences in the tropical species input into the study area depending on the oceanographic context, the temperate silicoflagellate species found were excluded from this ANOVA (see results, Table 2).

**Table 2**  
Concentrations of diatom, dinoflagellate and silicoflagellate (cells L<sup>-1</sup>) per sampled depths (m) at the three sampling stations CH1, CH3 and CH5 during downwelling and upwelling events.

Station	Downwelling (28 May 2011)							Upwelling (07 June 2011)						
	CH1		CH3		CH5			CH1		CH3		CH5		
	0	21	0	20	40	69	0	0	26	0	30	50	0	62
<b>Centric diatoms (cells L<sup>-1</sup>)</b>														
<i>Anaulus minutus</i>	0	0	0	0	0	0	0	0	41	0	0	0	0	0
<i>Asteromphalus</i> spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	10
<i>Bacteriastrium elongatum</i>	0	0	0	10	0	0	41	0	0	7	0	0	0	5
<i>Bacteriastrium furcatum/delicatulum</i>	0	0	0	0	41	0	0	0	0	0	0	0	0	0
<i>Bacteriastrium hyalinum</i>	0	0	31	0	31	0	0	20	0	0	0	0	0	0
<i>Bacteriastrium</i> spp.	20	0	31	0	0	0	0	20	0	62	0	0	29	0
<i>Chaetoceros atlanticus</i>	20	20	51	15	31	20	10	20	0	103	41	41	4	27
<i>Chaetoceros coronatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chaetoceros curvisetus</i>	0	0	0	0	0	0	0	0	0	41	0	0	0	0
<i>Chaetoceros lorenzianus</i>	0	0	0	0	0	0	0	0	0	41	0	0	25	0
<i>Chaetoceros peruvianus</i>	82	0	10	0	10	0	0	0	0	0	0	0	0	5
<i>Chaetoceros</i> spp. <i>Hyalochaete</i>	0	31	51	31	123	10	143	41	0	290	184	368	90	92
<i>Chaetoceros</i> spp. <i>Phaeoceros</i>	61	10	82	10	41	20	51	20	0	83	20	29	29	14
<i>Cerataulina pelagica</i>	41	0	0	5	20	0	0	0	0	0	0	20	4	14
<i>Climacodinium frauenfeldianum</i>	41	61	31	36	0	82	102	143	27	166	102	286	25	46
<i>Coscinodiscus</i> spp.	0	20	20	0	31	0	10	0	7	0	0	0	4	10
<i>Cyclotella</i> spp.	0	0	0	5	0	0	0	20	0	0	0	0	0	7
<i>Dactylosolen fragilissimus</i>	41	71	10	20	31	51	71	20	0	331	286	204	41	15
<i>Guinardia flaccida</i>	20	0	0	0	0	0	0	20	0	0	327	41	0	7
<i>Guinardia striata</i>	245	265	265	184	31	133	214	143	48	331	592	1184	74	117
<i>Helicotheca tamesis</i>	20	31	0	0	0	0	0	143	95	41	286	102	4	15
<i>Hemidius hauckii</i>	0	0	0	5	0	0	0	0	0	21	41	20	8	5
<i>Hemidius membranaceus</i>	0	0	0	0	20	0	0	0	0	0	0	41	12	20
<i>cf. Lauderia annulata</i>	41	31	0	0	0	0	0	20	7	21	225	490	8	27
<i>Leptocylindrus danicus</i>	0	0	0	0	0	0	163	20	0	310	531	817	41	10
<i>Leptocylindrus mediterraneus</i>	143	0	31	0	0	0	0	0	0	21	0	0	0	10
<i>Paralia sulcata</i>	123	51	0	0	0	0	0	245	136	124	0	0	0	0
<i>Planktoniella sol</i>	0	0	0	5	0	0	0	0	0	0	41	0	0	5
<i>Proboscia alata</i>	0	0	31	20	10	0	41	0	0	41	0	0	0	14
<i>Rhizosolenia fallax</i> -type	123	174	41	51	61	31	51	61	20	62	163	306	49	20
<i>Rhizosolenia setigera</i> -type	61	20	10	5	10	0	0	0	20	0	41	123	0	20
<i>Thalassiosira</i> spp.	368	235	123	66	204	51	20	470	320	352	1715	1429	131	102
<i>Triceratium dubium</i>	0	0	0	5	0	0	0	0	7	0	0	0	0	0
Undefined centric	41	31	123	77	71	41	306	82	14	269	551	368	4	454
<b>Pennate diatoms</b>														
<i>Amphora</i> spp.	0	0	0	10	0	0	0	61	14	0	0	20	0	7
<i>Asterionellopsis glacialis</i>	0	0	0	0	0	0	0	0	0	0	0	82	0	0
<i>Ceratoneis closterium/Nitzschia longissima</i>	102	766	347	362	153	306	225	1389	197	1014	551	1144	69	157
<i>Climacophenia moniligera</i>	82	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis</i> spp.	551	204	0	15	51	0	0	204	82	21	41	0	16	82
<i>Meuniera membranacea</i>	20	0	0	0	10	0	31	0	0	290	0	0	4	5
<i>Navicula</i> spp.	490	163	61	31	41	51	10	41	20	41	41	20	29	75
<i>Nitzschia/Lioloma/Thalassiothrix</i> spp.	204	31	235	107	133	102	61	306	95	372	327	653	65	56
<i>Pleurosigma</i> spp.	61	41	41	20	143	61	0	5	41	27	41	20	4	34
<i>Pseudo-nitzschia</i> spp.	347	327	745	148	541	153	20	163	163	869	368	1368	90	197
<i>cf. Pseudo-nitzschia subcurvata</i>	429	112	82	199	61	408	163	1389	61	1200	1674	898	41	116
<i>Rhabdonema adriaticum</i>	20	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Thalassionema nitzschoides/frauenfeldii</i>	0	0	0	0	0	0	8	0	41	124	20	82	4	54
Undefined pennate < 40 µm	3614	1072	306	112	317	255	276	3288	1191	952	470	1123	486	694
Undefined pennate > 40 µm	388	194	225	77	143	61	163	408	61	269	265	225	57	109

<b>Dinoflagellates (cells L<sup>-1</sup>)</b>	225	71	61	148	102	71	153	53	58	449	82	331	184	306	86	136	20
<i>Alexandrium</i> /Gonyaulax/ <i>Heterocapsa</i> / <i>Scrippsiella</i> spp.	20	0	10	0	0	0	10	0	0	0	0	0	0	0	0	0	0
<i>Ceratium fusus</i>	0	0	10	5	20	0	0	4	11	20	7	21	20	41	4	0	0
<i>Ceratium lineatum</i>	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0
<i>Ceratium massiliense</i>	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0
<i>Ceratium pentagonum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceratium ranipes</i>	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	5
<i>Ceratium tripos</i>	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0
<i>Ceratium</i> spp.	0	0	10	15	10	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceratocorys horrida</i>	0	0	0	5	0	0	0	0	0	0	7	0	0	0	0	0	0
<i>Dinophysis</i> spp.	0	0	0	0	0	0	0	0	0	0	0	21	0	0	4	0	5
<i>Gymnodinium</i> spp.	163	51	41	31	10	31	20	25	21	82	20	124	102	61	4	27	0
<i>Gyrodinium</i> spp.	265	123	112	46	71	133	82	131	69	143	27	145	265	327	57	299	71
<i>Karlodinium</i> spp.	0	20	10	0	10	0	10	0	5	0	0	0	0	0	0	41	0
<i>Ornithocercus thumii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
<i>Oxytoxum constrictum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oxytoxum laticeps</i>	0	10	41	36	31	51	20	12	11	82	0	0	0	41	12	34	5
<i>Oxytoxum milneri</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0
cf. <i>Oxytoxum variabile</i>	61	92	20	56	71	51	82	49	85	41	27	103	123	61	41	61	0
<i>Oxytoxum scolopax</i>	0	10	0	5	0	0	10	4	11	0	0	21	0	20	4	7	0
<i>Podolimpas bipes</i>	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Podolimpas palmipes</i>	0	0	0	0	0	0	0	0	0	0	0	0	20	0	4	0	0
<i>Pronoctiluca</i> spp.	0	0	0	0	0	20	0	8	0	61	0	62	41	41	16	0	0
<i>Prorocentrum cordatum</i>	102	41	102	26	153	61	10	25	79	20	34	21	41	163	0	14	0
<i>Prorocentrum dentatum</i>	0	20	0	5	10	0	0	4	11	0	0	124	82	0	0	0	0
<i>Prorocentrum lima</i>	41	0	31	0	0	0	10	0	0	20	0	0	0	20	0	0	0
<i>Prorocentrum rostratum</i>	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prorocentrum</i> spp.	143	51	10	112	61	51	20	61	32	286	75	228	143	368	110	88	0
<i>Protoperidinium elegans</i>	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Protoperidinium bipes</i>	0	0	0	0	0	0	0	0	5	0	0	21	82	61	4	0	0
<i>Protoperidinium</i> spp.	0	61	20	15	20	10	41	25	21	163	20	83	143	163	8	68	20
<i>Pyrocystis lunula</i>	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0
<i>Schuetziella mitra</i>	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
<b>Silicoflagellates (cells L<sup>-1</sup>)</b>	102	123	92	66	102	143	133	90	26	163	14	124	163	184	53	82	56
<i>Dictyocha fibula</i>	82	20	31	31	31	31	71	37	101	265	102	310	163	265	102	41	26
<i>Dictyocha speculum</i>	0	0	10	0	0	0	10	0	37	123	0	21	0	20	0	0	0
<i>Dictyocha</i> spp.	0	10	10	5	0	10	31	4	48	0	14	124	41	41	33	14	15
<b>Total abundance (cells L<sup>-1</sup>)</b>	9005	4676	3604	2251	3073	2501	2890	1572	2074	10761	2941	9827	10578	13723	2022	3403	2185

Grey shading indicates the most abundant species for each sampling station (by combining the five most abundant species per depth at each station).

Similarities in microphytoplankton species composition between SI and OC were tested via Crossed Analysis of Similarities (ANOSIM) (Clarke, 1993). To visualise multivariate interactions and to ordinate sampling locations based on their similarity in microphytoplankton composition we applied non-metric multidimensional scaling (nMDS) using PRIMER Version 6.1.12 (Clarke and Gorley, 2006). Distance-based redundancy analysis following Legendre and Anderson (1999) was used to test interactions between environmental and/or physico-chemical variables and microphytoplankton species distribution using the DistLM (Distance-based linear Model) procedure of the PRIMER add on PERMANOVA+ Version 1.0.2. Variables chosen were depth, temperature, salinity, nitrate and silicate concentrations at the respective microphytoplankton sampling locations.

Within the ANOVA and the ANOSIM we chose to average the species richness per station, i.e. all samples collected at the same station were treated as replicates (depth had no significant impact on the microphytoplankton community distribution; see results Section 3.7). ANOSIM, nMDS and DistLM were conducted based on  $\log_{10}(X+1)$  transformed microphytoplankton abundance data and Bray Curtis similarity. In the DistLM we used forward selection on Akaike's Information Criterion (AIC) to select the minimally adequate model.

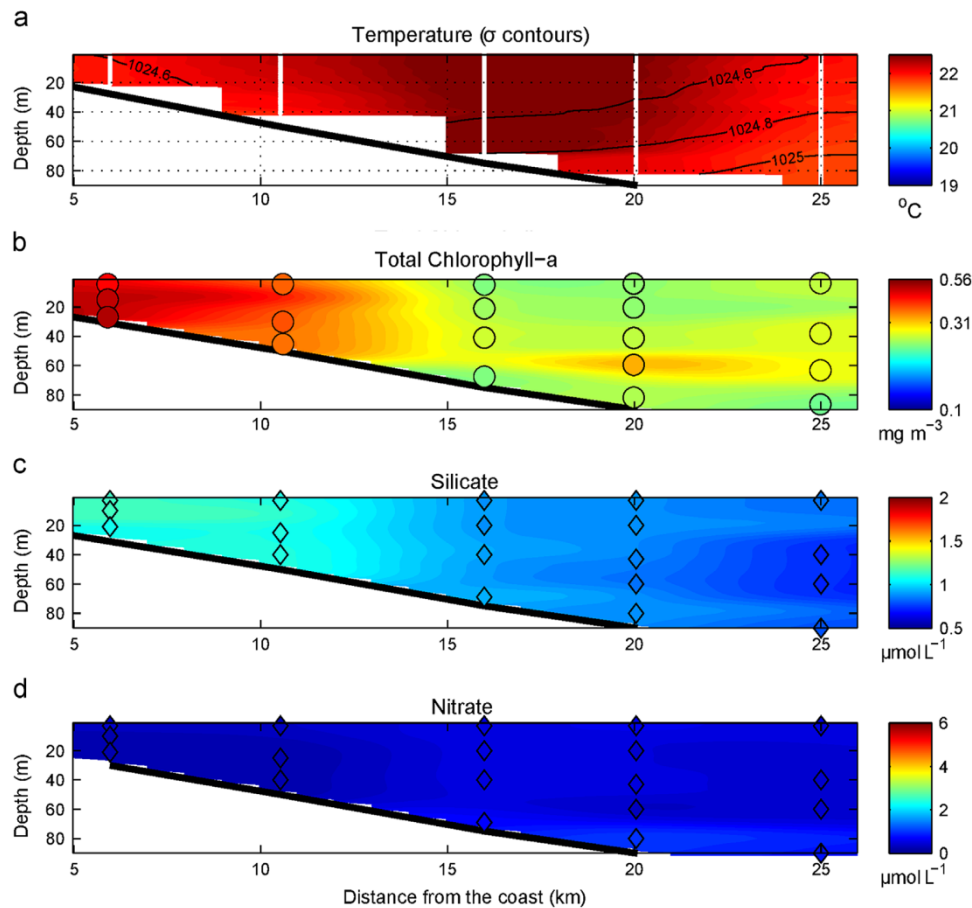
### 3. Results

#### 3.1. Oceanographic conditions and nutrient concentrations

Moored current and temperature measurements (CH070) revealed a sequence of changes in the oceanographic conditions at the study site. Our samplings took place on 28 May and 07 June 2011, thus within a  $\sim 1$  week interval.

Three days prior to our first sampling, a strong downwelling favourable wind was blowing (southerly wind, 25–26 May,  $|v| > 0.1 \text{ N m}^{-2}$ , Fig. 2a). The immediate ocean response (observed at the mid-shelf mooring CH070) was a northward coastal flow (up to  $0.6 \text{ ms}^{-1}$ , May 26–27, Fig. 2b) and a gradual homogenisation of the water column ( $T \sim 22.5^\circ\text{C}$ , 27–29 May, Fig. 2c). The southerly wind-induced vertical mixing was noticeable over the entire continental shelf: during our sampling (28 May), the temperature range was  $< 1^\circ\text{C}$  over a 90 m gradient (Fig. 3a). The downward sloping isopycnals (towards the coast) confirm a downwelling response to the along-shore wind stress. While the EAC waters were homogenised over the mid-shelf, we noted a local density maximum at the inner shelf (within a 12 km distance from the coast).

Shelf waters could be divided into two water masses. The inner shelf was characterised by a local maximum of silicate ( $\sim 1 \mu\text{mol L}^{-1}$ )



**Fig. 3.** Interpolated cross-shore sections (distance versus depth) along the CH-line (30.25°S) on 28 May 2011. (a) Temperature ( $^\circ\text{C}$ ) (with density  $\sigma$  in  $\text{kg m}^{-3}$  contoured) as measured by the CTD; white lines represent the positions of the cast. (b) Total chlorophyll *a* (Tchl *a*) concentrations (in  $\text{mg m}^{-3}$ ). (c) Dissolved silicate and (d) nitrate ( $\mu\text{mol L}^{-1}$ ). Black circles/diamonds represent the sampling depths for Tchl *a*/nutrients, coloured according to their measured values.



(Fig. 3c) and depleted nitrate within the water column ( $<0.3 \mu\text{mol L}^{-1}$ ) (Fig. 3d). The mid- and outer shelf was homogeneous with both low silicate ( $0.7\text{--}0.9 \mu\text{mol L}^{-1}$ ) (Fig. 3c) and low nitrate ( $<0.6 \mu\text{mol L}^{-1}$ ) (Fig. 3d) concentrations.

The period immediately prior to, and during, the second sampling date (07 June) was characterised by weak winds and a southward along-shelf current flow (Fig. 2). The southward flow reached velocities between  $0.2$  and  $0.5 \text{ ms}^{-1}$  (Fig. 2b) and was associated with SSTs of  $\sim 22^\circ\text{C}$  at the mooring CH070 (Fig. 2c). Satellite derived geostrophic velocities and SSTs (Fig. 1; 06–09 June, 2011) showed this along-shelf current with an associated EAC encroachment onto the shelf. The EAC was characterised by warm sea surface temperatures (approx.  $23\text{--}24^\circ\text{C}$ ), while SSTs on the shelf were colder by  $2\text{--}3^\circ\text{C}$  (Fig. 1). On 07 June, during the second sampling period, the temperature decreased in the bottom layer ( $\sim 19.5^\circ\text{C}$ , CH070, Fig. 2c), leading to a vertical temperature gradient of  $\sim 4^\circ\text{C}$  in  $90 \text{ m}$  of water (Fig. 4a). A slight upward slope (towards the coast) of the isopycnals was observed at the seafloor with a vertical uplift of  $20 \text{ m}$  over a distance of  $10 \text{ km}$  between  $16$  and  $26 \text{ km}$  offshore (Fig. 4a). The dense/cold bottom waters had high nutrient concentrations ( $\sim 6 \mu\text{mol L}^{-1}$  of nitrate and  $\sim 1.75 \mu\text{mol L}^{-1}$  of silicate) (Fig. 4c and d), while the inner shelf

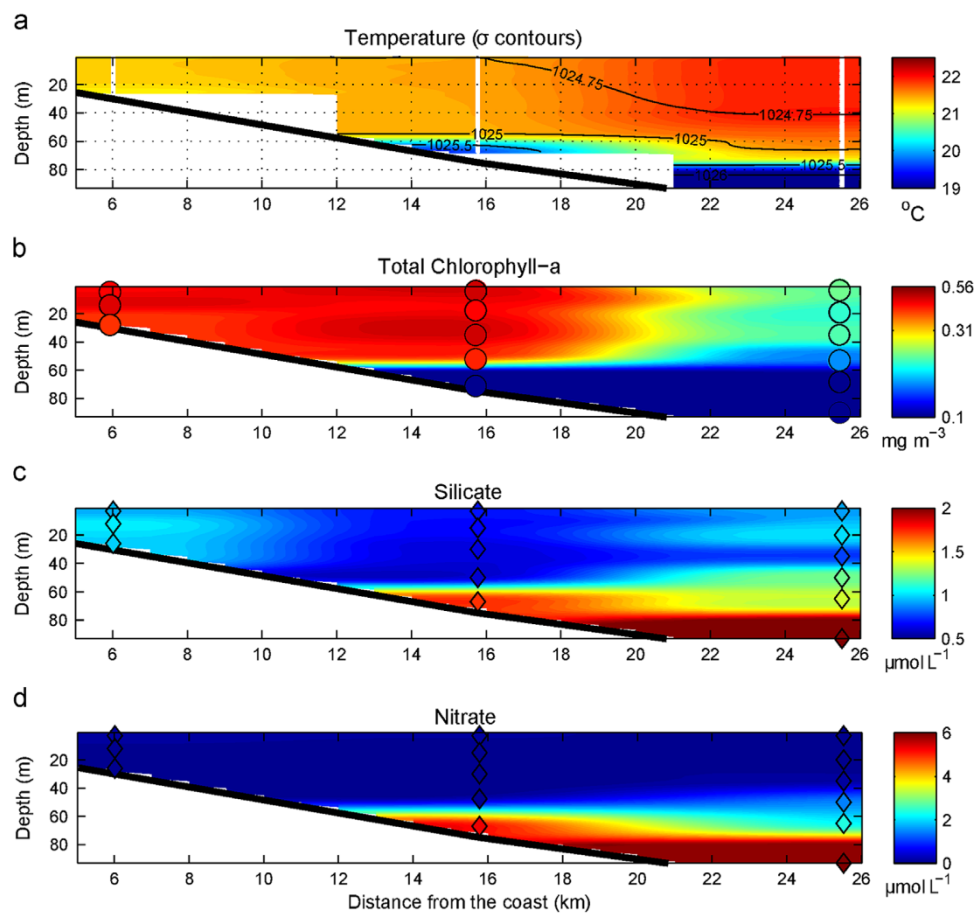
was characterised by a local silicate maximum ( $\sim 1 \mu\text{mol L}^{-1}$ ) (Fig. 4c).

Prior to our sampling (21–25 May, 2011) another (stronger) upwelling event was identified from the current and temperature observations. Again, encroachment of a strong and warm EAC ( $0.8 \text{ ms}^{-1}$ ,  $22\text{--}23^\circ\text{C}$ ), lead to the uplift of cold water of  $18\text{--}19^\circ\text{C}$  (Fig. 2).

### 3.2. Total Chlorophyll *a* (Tchl *a*) concentrations

During the downwelling event (28 May), the inner shelf was characterised by high Tchl *a* concentrations ( $0.3\text{--}0.6 \text{ mg m}^{-3}$ , Fig. 3b). On the mid- and outer shelf (homogenous water mass) Tchl *a* concentrations were, by comparison to the inner shelf, patchy ( $0.1\text{--}0.3 \text{ mg m}^{-3}$ ).

During the upwelling event (07 June), most of the shelf (inner and mid-shelf) was characterised by relatively high Tchl *a* concentrations ( $0.3\text{--}0.5 \text{ mg m}^{-3}$ ) (Fig. 4b). In the core of the EAC (offshore), the Tchl *a* concentrations were an order of magnitude lower ( $\sim 0.03 \text{ mg m}^{-3}$ ). Offshore below the EAC (at  $90 \text{ m}$  depth), Tchl *a* concentrations were very low ( $0.02 \text{ mg m}^{-3}$ ) (Fig. 4b).



**Fig. 4.** Interpolated cross-shore sections (distance versus depth) along the CH-line ( $30.25^\circ\text{S}$ ) on 07 June 2011. (a) Temperature ( $^\circ\text{C}$ ) (with density  $\sigma \text{ kg m}^{-3}$  contoured) as measured by the CTD; white lines represent the positions of the cast, (b) Total chlorophyll *a* (Tchl *a*) concentrations ( $\text{mg m}^{-3}$ ). (c) Dissolved silicate and (d) nitrate ( $\mu\text{mol L}^{-1}$ ). Black circles/diamonds represent the sampling depths for Tchl *a*/nutrients, coloured according to their measured values.

### 3.3. Distribution of micro-, nano-, and picoplankton derived from HPLC

TChl *a* determined by fluorometry was slightly underestimated in comparison to HPLC derived values but both measurements were highly positively correlated ( $R^2=0.80$ , linear regression analysis). HPLC-determined TChl *a* was used to calculate the relative contribution of the different size classes to the whole phytoplankton community. As TChl *a* values did not vary considerably through the water column, depth integrated averages were plotted for each size class per station (Fig. 5).

We found that the phytoplankton community was mainly composed of nanoplankton at all stations (generally 40–50% of TChl *a*), except for CH1 during the downwelling event where less nanoplankton was found (Fig. 5a). Picoplankton represented 22–38% of the phytoplankton during both oceanographic conditions (Fig. 5). The greatest variance in abundance derived from HPLC was found in the microphytoplankton size class, which made up 16–34% of TChl *a* depending on the station (Fig. 5).

We consistently found high DV Chl *a* and DV Chl *b* (representative of prochlorophytes) at all locations during both, upwelling and downwelling (data not shown). The photoprotective pigment Zea was also consistently found, indicating the presence of cyanobacteria, chrysophytes and raphidophytes. Zea-containing chlorophytes and prasinophytes may also have been present as suggested by low concentrations of Neo/Viola/Lut (chlorophytes) and Pras (prasinophytes) at nearly all sampling locations (data not shown) (for pigment abbreviations and characteristics of phytoplankton taxa see Table 1 and Jeffrey et al. (2005)).

In the nanoplankton size range, relatively high concentrations of Hex-fuco simultaneously with But-fuco suggest the presence of prymnesiophytes. Hex-fuco may originate from rarely occurring, Hex-fuco containing dinoflagellates, such as few *Karlodinium* spp. (undefined species of this genus were found sporadically at most sampling stations, Table 2), But-fuco and low concentrations of Allo (not shown) might suggest the presence of chrysophytes and cryptomonads, respectively. However, both groups were not included in our microscopy analyses, therefore, their presence remains speculative.

The main fraction of the microphytoplankton was linked to the presence of diatoms, which were characterised by relatively high concentrations of the marker pigment Fuco (data not shown). The dominance of diatoms over other microphytoplankton was supported by our microscopy data (see, Section 3.4; Table 2).

### 3.4. Microphytoplankton abundance

Microphytoplankton counts revealed 46 diatom, 32 dinoflagellate and three silicoflagellate taxa (excluding the three groups of undefined pennate and centric diatoms and dinoflagellates). Total abundances of all taxa counted ranged from  $1.57 \times 10^3$  cells  $L^{-1}$  (CH5 60 m) to

$9.01 \times 10^3$  cells  $L^{-1}$  (CH1 0 m) during the downwelling event and from  $2.02 \times 10^3$  cells  $L^{-1}$  (CH5 0 m) to  $13.72 \times 10^3$  cells  $L^{-1}$  (CH3 50 m) during the upwelling event (Table 2).

During both oceanographic events, high microphytoplankton abundances were found at CH1, with maximum abundances at the surface while microphytoplankton abundances at CH5 were much lower (Table 2). Generally, diatoms contributed the major proportion of microphytoplankton in this study (Table 2).

Microphytoplankton abundance patterns at CH3 differed greatly during upwelling and downwelling events. During downwelling, the microphytoplankton abundance at CH3 was similar to values found at CH5, whereas during upwelling microphytoplankton abundances were much higher at CH3 than those encountered at CH5 (Table 2). Total microphytoplankton abundance at CH3 increased with depth until the maximum value observed during this study was reached close to the seafloor (Table 2). A full species list documenting phytoplankton abundances at each station is recorded in Table 2.

### 3.5. Microphytoplankton species richness

Average species richness at the stations CH1, CH3 and CH5 changed from  $33 \pm 1$ ,  $33 \pm 3$  and  $31 \pm 4$  during downwelling to  $32 \pm 4$ ,  $40 \pm 2$ ,  $38 \pm 4$  during upwelling, respectively). A statistically significant difference in species richness was found between upwelling and downwelling events (Table 3) but not between stations. The increased species richness during the upwelling event, particularly at CH3 and CH5, was partly due to the appearance of some temperate diatoms (although these occurred in relatively low cell numbers) such as *Asterionellopsis glacialis*, *Asteromphalus* spp., *Chaetoceros curviretus* and *C. lorenzianus* (Table 2). Moreover, tropical dinoflagellate species such as *Ceratium massiliense*, *C. ranipes*, *Ornithocercus thumii*, *Oxytoxum milneri*, *Podolompa palmipes* and *Pyrocystis lunula*, which were absent during downwelling, were found during the upwelling event.

### 3.6. Microphytoplankton species composition

Non-metric MDS (nMDS) of microphytoplankton abundance data per sampling location revealed a reliable ordination as indicated by a 2D stress of 0.17 (Fig. 6) and demonstrated consistency with the microphytoplankton abundance patterns described in Section 3.4.

The phytoplankton composition characteristic for a sampling location was highly dependent on a complex correlation of OC and SI as determined by ANOSIM: Two-way Crossed ANOSIM revealed statistically significant differences in the variability of microphytoplankton composition between all stations and between both oceanographic regimes (Table 3). Inter-correlation of OC and SI can also be inferred from the scattered distribution of sampling locations via nMDS (Fig. 6).

nMDS showed that phytoplankton composition at all stations during the downwelling event was relatively dissimilar. Ordination of station CH1, CH3 and CH5, in a vertical direction on the nMDS plot (Fig. 6) suggests a cross-shelf transition in microphytoplankton composition from an offshore to an inshore community (or vice-versa). The inshore station's community was characterised by the increased presence of three diatom genera: *Diploneis*, *Navicula* and *Pleurosigma* (Table 2). We also found maximum abundances determined during this oceanographic regime and elevated abundances of the dinoflagellate taxa *Alexandrium*/*Gonyaulax*/*Heterocapsa*/*Scrippsiella* spp. at CH1 (Table 2). Taxa typically found at the mid-shelf station were *Pseudo-nitzschia* spp., cf. *Pseudo-nitzschia subcurvata* and *Nitzschia*/*Lioloma*/*Thalassiothrix* spp. (Table 2). In contrast, the offshore station was characterised by elevated abundances of *Chaetoceros* spp. (*Hyalochaetes*), *Dactylosolen fragilissimus*, *Leptocylinthus*

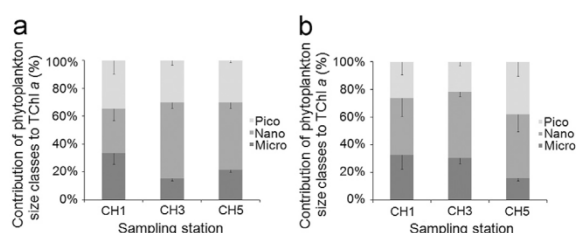
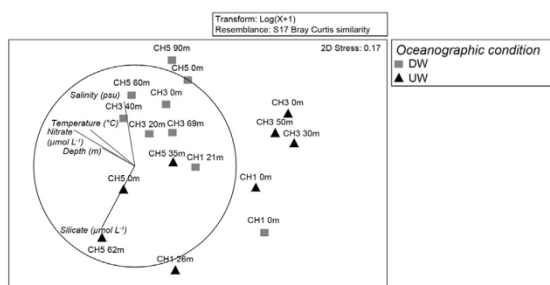


Fig. 5. Phytoplankton size class fractionation. Percentages of pico-, nano- and microphytoplankton associated with total chlorophyll *a* (TChl *a*) derived from HPLC analysis during (a) the downwelling, 28 May and (b) the upwelling, 07 June 2011. Error bars represent standard deviations from the mean.

**Table 3**  
Summary of ANOVA, ANOSIM and DistLM results.

ANOVA	Crossed (OC and SI), differences between OC	Crossed (OC and SI), differences between SI	Separate (OC)	ANOSIM	Crossed (OC and SI), differences between SI	Crossed (OC and SI), differences between OC
<i>p</i>	0.041	0.455	0.455	<i>p</i> , <i>R</i> (Global)	0.001, 0.604	0.003, 0.82
<i>F</i>	5.17	0.84	4.96	<i>p</i> , <i>R</i> (CH1 and CH3)	0.007, 0.609	
<i>DF</i>	1	2	1	<i>p</i> , <i>R</i> (CH1 and CH5)	0.01, 0.708	
				<i>p</i> , <i>R</i> (CH3 and CH5)	0.56	
DistLM	Depth (m)		Temperature (°C)	Silicate ( $\mu\text{mol L}^{-1}$ )	Nitrate ( $\mu\text{mol L}^{-1}$ )	Salinity (psu)
<b>Marginal tests</b>	<i>p</i>	0.326	0.006	0.024	0.097	0.32
<b>(Res. DF=15)</b>	Pseudo- <i>F</i>	1.0886	2.6145	1.993	1.5272	1.1873
	Proportional	0.0677	0.1484	0.1173	0.0924	0.0733
	<i>p</i>	–	0.003	0.002	0.032	0.053
	Pseudo- <i>F</i>	–	2.6145	2.2245	1.8096	1.8662
<b>Sequential tests</b>	Cumulative	–	0.1484	0.26519	0.35497	0.44178
	Proportional	–	0.1484	0.11676	0.089786	0.086812
	Res. DF	–	15	14	13	12
<b>Best solution</b>	AIC=104.1, $R^2=0.44178$ , RSS=4308.4, No. of variables=4 (temperature, silicate, nitrate, and salinity)					

OC = oceanographic condition, SI = station ID, proportional/cumulative = proportional/cumulative variance explained, AIC = Akaike's information criterion, RSS = residual sum of squares. *p*-Value at a significance level of 0.05, test statistics: *F* (ANOVA), *R* (ANOSIM) and Pseudo-*F* (DistLM), and *DF*=degrees of freedom.



**Fig. 6.** Phytoplankton species composition. nMDS plot showing ordinated sampling locations (sampled during upwelling and downwelling) based on microphytoplankton abundance data. Vectors visualise the fitted environmental/physico-chemical variables (depth, temperature, salinity, nitrate and silicate) as suggested by our DistLM model. Vectors are based on Spearman correlation, their length/direction indicate strength of effect/correlation of the variable on the ordination plot. The circle is a unit circle.

*danicus* and undefined centric diatoms (Table 2). It should be noted that although inshore, mid-shelf and offshore communities could be categorised, some taxa occurred in high abundances at all stations during the downwelling event, e.g. *Guinardia striata*, *Ceratoneis closterium*/*Nitzschia longissima* and undefined pennate diatoms < 40  $\mu\text{m}$  (Table 2).

Ordination of the microphytoplankton abundance data collected during the upwelling event revealed a similar species composition at station CH3 throughout all depths (Fig. 6). This station differed in species composition from all other sampling locations during both oceanographic events. Under upwelling, ordination of the microphytoplankton abundance data did not show a cross-shelf gradient in community composition as seen during downwelling. Microphytoplankton species driving the similarity in community composition between all depths at CH3 were generally diatoms identified as an “offshore-community” during downwelling; viz. *D. fragilissimus*, *Chaetoceros* spp. (Hyalochaetes), *L. danicus*. High abundances of the taxa described as a “mid-shelf community” during downwelling in addition to the presence of the diatoms *Chaetoceros atlanticus*, *G. striata* and *Thalassiosira* spp. at CH3 characterised the upwelling event. This community generally showed increasing abundances with depth

(Table 2). At the inshore station the dissimilarity in species composition between depths (0 m and 26 m) (Fig. 6) could be described by a few species found either only at the surface (e.g. *Chaetoceros* spp. (Hyalochaetes and Phaeoceros)) where total abundance was generally elevated, or only at 26 m (e.g. *Coscinodiscus* spp., *Thalassionema nitzschioides*/[*frauenfeldii*] (Table 2). Elevated abundances of the grouped dinoflagellate taxa *Alexandrium*/*Gonyaulax*/*Heterocapsa*/*Scrippsiella* spp., *Prorocentrum* spp. and the silicoflagellate *Dictyocha fibula* were noted during upwelling at the inshore and the mid-shelf station (Table 2).

### 3.7. Influence of environmental variables on phytoplankton distribution

Marginal tests in the DistLM revealed temperature and silicate concentrations were significant variables in explaining the variation in microphytoplankton composition at all three sampling stations during downwelling and upwelling (Table 3). Depth, salinity and nitrate were defined as statistically insignificant in explaining the variability in microphytoplankton composition (Table 3). Temperature alone was responsible for explaining 15% of the variation in microphytoplankton composition. The sequential (cumulative) inclusion of silicate, nitrate and salinity increased the explained variation in microphytoplankton composition to 27%, 36% and 44%, respectively (Table 3). It should be noted that upon the addition of salinity into the sequential test, the analysis became insignificant (Table 3). Therefore, salinity seemed to play a minor role in the global model explaining the variation in microphytoplankton distribution compared to the variables temperature, silicate and nitrate (in descending order).

The variables temperature, nitrate and depth were positively correlated as indicated by the clustering of vectors in the ordination plot (Fig. 6). The strength of these three variables increased towards offshore (indicated by the left-oriented vectors simultaneously with the allocation of all offshore sampling locations in the ordination plot). Temperature, nitrate and depth therefore had an overall impact on the microphytoplankton community along a horizontal rather than a vertical gradient during both oceanographic events. Increased silicate concentrations were correlated with offshore microphytoplankton composition during the upwelling (Fig. 6). The local silicate maximum inshore during



downwelling (see Section 3.1, Fig. 3) explains the direction of the respective vector to the lower part of the MDS plot, where all inshore sampling locations were ordinated (Fig. 6). The positioning of the salinity vector slightly towards the right relative to the temperature, depth and nitrate vectors, indicates a strong impact of salinity at station CH3 during upwelling (Fig. 6), which is consistent with increasing density at depth in the water column (Fig. 4a). Nevertheless, the impact of salinity should be interpreted with caution (see first paragraph of Section 3.7).

#### 4. Discussion

Our observations demonstrated marked and rapid changes in phytoplankton composition that occur within a few days under contrasting oceanographic conditions of wind- and current-forcing in the Coffs Harbour region ( $\sim 30^\circ\text{S}$ ) (Fig. 7), and confirm the local importance of short-term fluctuations in nutrient supply and vertical mixing in driving cross-shelf community structure.

##### 4.1. Oceanographic conditions

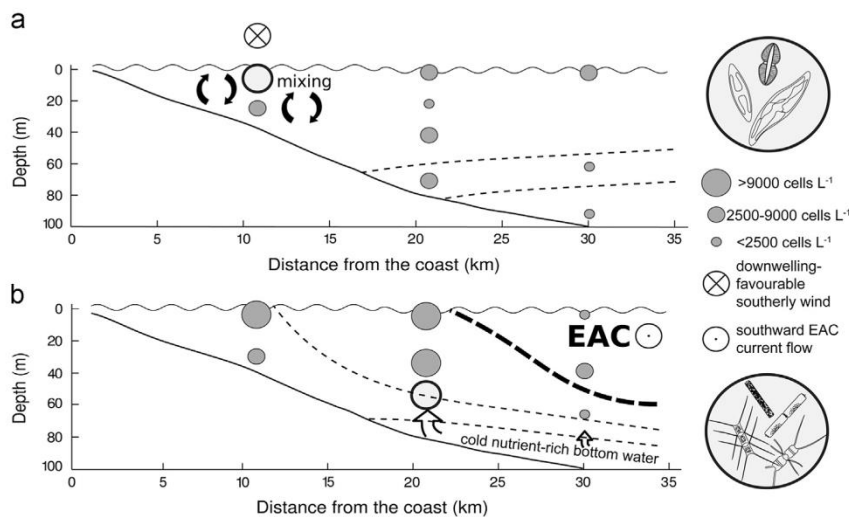
Large and high frequency fluctuations in coastal water temperature off the Coffs Harbour region, Eastern Australia, are usually either wind-driven, or caused by flow patterns of the EAC (Rossi et al., in preparation). Our study was conducted in early winter when the EAC is generally weaker than in summer (Ridgway and Godfrey, 1997). We observed significant spatial patchiness in temperature across the shelf, as well as rapid temporal changes in temperature. Such variability has previously been shown significantly to alter the abundance of larval fish assemblages in the area (Fowler et al., 2006). In this study, we have shown that the local phytoplankton communities also respond rapidly to changing oceanographic regimes, which concurs with changes observed in temperature and nutrient availability.

Our analysis suggested that phytoplankton responded to changes in hydrological properties driven by the wind (downwelling case) and

by the EAC encroachment onto the shelf (upwelling case). Other processes might also affect the water masses over the continental shelf. In fact, satellite images of sea-surface temperature showed that small scale instabilities (fronts, sub-mesoscale eddies) were simultaneously occurring on the shoreward flank of the EAC. Because of their relatively small scale and distance offshore, we did not see an impact on the inner shelf circulation. However, this observation highlights the potential periodic influence of such sub-mesoscale and mesoscale features in modifying the circulation and stratification of the shelf waters. Such features could add significantly to the complexity of oceanographic forcing impacting phytoplankton populations under the right conditions.

##### 4.2. Historical context

TChl *a* concentrations and frequently found phytoplankton taxa were consistent with previous investigations from the Port Hacking long-term station,  $\sim 530$  km south of the SIMP, during winter (Jeffrey and Carpenter, 1974; Hallegraeff and Reid, 1986; Ajani et al., 2001). The total number of microphytoplankton taxa found during this study ( $\sim 80$ ) is lower than values reported by Ajani et al. (2001) probably due to our comparatively short sampling period (2 days versus weekly sampling across one year by Ajani et al. (2001)). Our results confirm the dominance of nanoplankton during winter along the Australian east coast (40–50%), in line with findings by Hallegraeff (1981) from Port Hacking. Small-sized phytoplankton can efficiently acquire nutrients due to their high surface:volume ratio (Eppley et al., 1969) and thus occur constantly throughout the year at the Port Hacking station (50–80% of TChl *a*), except in spring/summer (10–20% TChl *a*) when elevated nutrient uplift favors diatom growth (Hallegraeff, 1981). Here we found that, although on a very short temporal and small regional scale, a current-driven upwelling event and local nutrient enrichment lead to a decrease/increase in nanoplankton/diatom abundance over the mid-shelf relative to the downwelling event.



**Fig. 7.** Phytoplankton response to oceanographic conditions. Schematic cross-shore sections showing total phytoplankton abundance (circles; including diatoms, dinoflagellates and silicoflagellates) at each sample location during (a) downwelling (28 May 2011) and (b) upwelling (07 June 2011). Dashed lines represent schematic isopycnals reproduced from Figs. 3 and 4, with the bold dashed line (in b) being the schematic EAC delimitation. Direction and size of arrows indicate effects and movement of water masses influencing phytoplankton abundance and distribution. The two dark-ringed circles indicate locations of maximum phytoplankton abundance found under each oceanographic condition. Respective enlarged circles indicate the difference in species composition resulting from (a) downwelling: *Diploneis*, *Navicula* and *Pleurosigma* spp. and (b) upwelling: *Chaetoceros* spp., *Hyalochaetes*, *C. atlanticus*, *Dactylosolen fragilissimus* and *Leptocylindrus danicus* (see text).

#### 4.3. Microphytoplankton abundance and composition during downwelling

During the downwelling event, we found a clear transition from an inshore to an offshore phytoplankton community while TChl *a* concentrations and microphytoplankton abundances were maximised inshore.

The elevated abundances of species representative of the genera *Diploneis*, *Navicula* and *Pleurosigma* at the inshore station, especially at the surface, are consistent with vertical re-suspension of benthic diatoms during the downwelling event. All three diatom genera are regarded as benthic indicator species (Crosby and Wood, 1959; Hallegraeff et al., 2010) and their elevated abundances in the surface waters (and low concentrations during upwelling conditions) is of note. *Diploneis*, *Pleurosigma* and *Navicula* do not dominate the diatom community during the winter months at the Port Hacking station (Jeffrey and Carpenter, 1974; Hallegraeff and Reid, 1986; Ajani et al., 2001) and latitudinal differences are unlikely to play a role as these species generally occur in most Australian waters (Hallegraeff et al., 2010).

Changes in the cross-shelf microphytoplankton community varied clearly with nutrient (nitrate and silicate) concentrations. During downwelling, a local silicate maximum concurrent with a minimum in nitrate concentration was determined inshore and may explain the high abundances of the (silica-requiring) diatoms *Diploneis*, *Navicula* and *Pleurosigma*. However, as silica and nitrate concentrations were generally low (the majority of diatoms do not grow below a silica concentration of 2  $\mu\text{mol L}^{-1}$ ; Smetacek, 1999), we assume that most of the available nutrients had been already taken up by the microphytoplankton prior to sampling.

Terrestrial nutrient inputs originating from local river systems might have played a role during this time, especially for silicate, classically delivered via weathering from land (Limmer et al., 2012). However, while April rainfall was more than twice the annual average, May rainfall was substantially lower than average (<http://www.bom.gov.au/climate/data/>, Station MO 59040, Coffs Harbour, NSW, 30.31°S, 153.12°E), complicating a clear assessment of the potential for runoff to influence coastal nutrient supply.

Alternatively, the preceding strong upwelling event (21–25 May 2011, Section 3.1) might have enhanced nutrient availability and phytoplankton growth in the area. Highly silicic acid-requiring phytoplankton (such as diatoms) had most likely grown prior to our sampling until they reached limitation. At this point other taxa, which do not require large amounts of silica for growth, became more dominant. This would explain the presence of a high percentage of nano- and picoplankton (~30% each of TChl *a*) and increased abundances of the dinoflagellates *Alexandrium*/*Gonyaulax*/*Heterocapsa*/*Scrippsiella* spp. A similar situation occurs off the west coast of Australia, where the strength of the Leeuwin Current, known as a significant source of silica in autumn, can determine the probability of diatom growth regionally (Lourey et al., 2006), with nanoplankton favoured when this supply is not present.

#### 4.4. Microphytoplankton abundance and composition during upwelling

During the upwelling event the response of microphytoplankton to elevated nutrient input was identified on the mid-shelf. This response was the greatest recorded microphytoplankton abundance found during this study (as reflected in TChl *a* concentrations, microscopy, increased species richness and a change in species composition). As our sampling was timed in the middle of the upwelling event microphytoplankton might have not yet fully responded to the enhanced nutrient availability. This would explain why TChl *a* and microphytoplankton abundances were only slightly

higher at the mid-shelf during the upwelling than at the inshore station during the downwelling event.

The uplifted cold slope water was enriched in both silica (2–2.5  $\mu\text{mol L}^{-1}$ ) and nitrate (8–9  $\mu\text{mol L}^{-1}$ ), which, in all likelihood, led to the enhanced growth of microphytoplankton. Statistically, the impact of depth-related environmental variables on the microphytoplankton composition was overridden by small-scale, cross-shelf differences in our model. This might be because most sampling locations were in the upper water column (0–40 m) (maintaining consistency with the shallow depth inshore) and did not include deep measurements. However, the statistical result supports the importance of short-term nutrient pulses (> 5  $\mu\text{g L}^{-1}$  of nitrate) in enhancing the growth of diatoms, which can then divide rapidly under nutrient-repletion (Hallegraeff, 1981; Hallegraeff and Reid, 1986).

Diatom species found at CH3 have been commonly identified at the offshore (located above the 100 m isobath) Port Hacking station but were never reported as important contributors to the winter phytoplankton community at Port Hacking (e.g. *Guinardia striata*, *Pseudo-nitzschia* spp.; Jeffrey and Carpenter, 1974; Hallegraeff and Jeffrey, 1984; Hallegraeff and Reid, 1986; Ajani et al., 2001). In addition to these common species, diatoms from the “offshore community” during the preceding downwelling (*D. fragilissimus*, *L. danicus*, *Chaetoceros* spp.; see Section 3.6) were also abundant at the mid-shelf station during upwelling. This coastward movement of the “offshore community” along with the appearance of tropical dinoflagellates at CH3 and the increased percentage of tropical picoplankton (e.g. *Prochlorococcus*) at CH5 would appear to be indicative of the strong EAC influence on phytoplankton communities during upwelling.

Most of the tropical dinoflagellates we found during upwelling conditions (see Section 3.5), were previously recorded by Hallegraeff and Jeffrey (1984) from Northern Australia but are not listed as part of the temperate phytoplankton community of Australian coastal waters (Hallegraeff et al., 2010). The occurrence of these dinoflagellates in the SIMP region during the investigated upwelling event implies the southward transport of these species by the EAC. Our classification of a “tropical” species is based on recent regional and international literature (Hallegraeff and Jeffrey, 1984; Hallegraeff and Reid, 1986; Tomas, 1997; Ajani et al., 2001; Hallegraeff et al., 2010).

An increased abundance of dinoflagellates (*Alexandrium*/*Gonyaulax*/*Heterocapsa*/*Scrippsiella* spp., *Prorocentrum* spp.) and the silicoflagellate, *Dictyocha fibula*, was also detected during the upwelling event. The presence of these taxa during winter is consistent with previous investigations off Port Hacking, where these taxa usually range between 10–10<sup>3</sup> cells L<sup>-1</sup> (Jeffrey and Carpenter, 1974; Hallegraeff and Reid, 1986). We assume that the increased abundances of the dinoflagellates and *D. fibula* observed during upwelling, particularly on the mid-shelf, were associated with enhanced nutrient availability. Increased silica concentrations are likely to have favoured the growth of *D. fibula*.

#### 5. Conclusions

This study is the first detailed taxonomic investigation of the winter microphytoplankton community in the SIMP (~30°S, east Australian tropical–temperate transition zone, upstream of the EAC separation point). We provide key groundwork towards the explanation of cross-shelf phytoplankton responses to distinctive oceanographic regimes in this region, where physical conditions change rapidly (on daily to weekly scales). During the observed downwelling period we found a transition in phytoplankton composition from an inshore to an offshore community. In contrast, during upwelling conditions we found phytoplankton abundances to peak on the mid-shelf. Elevated species richness



offshore, mainly due to the appearance of tropical dinoflagellates, was indicative of a strong EAC influence during upwelling conditions. During both oceanographic events we found diatoms to be the phytoplankton class that was most sensitive to changes in temperature and nutrient, especially silicic acid, concentrations across the continental shelf.

Longitudinal (cross-shelf) phytoplankton distribution patterns (as described here) might be affected by the strengthening EAC beyond the expected poleward range expansions of warm-water phytoplankton species, as a result of climate change in the oceanic environment. Specifically, our investigation allows us to hypothesize that a potential increase in the frequency of upwelling events along the east Australian coast (caused by EAC strengthening) may lead to a persistent shift of “offshore” phytoplankton communities towards the coast. Baseline investigations aimed at natural variability of phytoplankton temporally (seasonally and annually) and spatially (tropical–temperate) are required as a reference for future research monitoring long-term changes in oceanography and associated phytoplankton dynamics along the east Australian coast and WBC delimited coastlines.

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## Chapter 5

Environmental variables determine cross-shelf phytoplankton community structures:  
Two case studies from the Kimberley and Coffs Harbour regions (Australia)

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**Key words:** Continental shelf, Microscopy, CHEMTAX, Phytoplankton size-classes, Diatoms, *Synechococcus*.

**Abbreviations:** ITF = Indonesian Throughflow; DistLM = distance-based Linear Model; CCA = Canonical Correspondence Analysis.

**Abstract**

Global phytoplankton composition and distribution are expected to change due to climate change-induced modifications in the oceanographic environment. However, little is known about environmental driving forces of compositional and distributional patterns in natural phytoplankton communities. We investigate the preferences of phytoplankton (pico-, nano-, and microphytoplankton, determined by microscopy and CHEMTAX) for a variety of environmental variables along cross-shelf gradients. Two case studies were conducted in two highly distinct oceanographic regions of Australia: the tropical-temperate Coffs Harbour region (~30°S, 153°E), where the shelf is narrow (~30 km), and the tropical Kimberley region (~15°S, 122°E), where the shelf is wide (~200 km). We distinguished two water masses in both study regions: nutrient-rich, well-mixed inshore waters and oligotrophic, stratified offshore waters. Cyanobacteria, cryptophytes, dinoflagellates, haptophytes and prasinophytes each showed taxon-specific preferences for similar environmental variables in both regions. Diatoms preferred nutrient-rich inshore waters in the Kimberley, whereas they were widely spread across the narrow continental shelf at Coffs Harbour. Off Coffs Harbour, a senescent bloom of the diatom *Leptocylindrus danicus* caused shelf-scale surface nutrient depletion and re-distribution of dinoflagellates and pelagophytes to inshore waters, where local nutrient maxima prevailed. Thus, while microphytoplankton (pico-, nanophytoplankton) increased (decreased) clearly with distance from the coast over the wide shelf in the Kimberley region, such an increase/decrease in cross-shelf abundances of individual phytoplankton size-classes was not determined across the narrow Coffs Harbour shelf. Our study provides important information on phytoplankton habitat preferences in shelf-systems and will benefit future studies investigating phytoplankton dynamics in a changing oceanographic environment.

**1 Introduction**

Global phytoplankton dynamics are expected to be modified as a result of climate-change-induced changes in the oceanographic environment (Hays et al., 2005; Hallegraeff, 2010). Such oceanographic changes include sea level rise, sea surface temperature warming, ocean acidification, changes in ocean current circulation, level of vertical mixing/stratification and nutrient availability in the euphotic zone (Falkowski

and Oliver, 2007; Hallegraeff, 2010; IPCC, 2013). Sea surface temperature warming is expected to induce a reduction of vertical mixing, ultimately decreasing nutrient availability in the euphotic zone, especially in open oceans (Behrenfeld et al., 2006; Falkowski and Oliver, 2007). Small-sized phytoplankton with high surface:volume (S:V) ratios and diazotrophs are thought to be favoured under such oligotrophic and stratified conditions (at the expense of diatoms), which may reduce export production (Bopp, 2005; Falkowski and Oliver, 2007). Such worldwide changes seem to be already underway and include a global decline in productivity and phytoplankton biomass (Behrenfeld et al., 2006; Boyce et al., 2010) and an increase of small-sized (pico) phytoplankton in a warmer ocean (Morán et al., 2010). Within this context, the study of the natural variability of all phytoplankton size-classes, including pico-, nano- and microphytoplankton (0.2 – 2, 2 – 20, 20 – 200  $\mu\text{m}$ , respectively) in their oceanographic environment is crucial.

Coastal and shelf regions provide suitable locations for studies focussed on resolving interactions between gradients of environmental variables and phytoplankton dynamics. Depending on the width of the continental shelf, gradients in vertical mixing, nutrient availability and light regime can be found within a few kilometres (or a few hundred kilometres) across the shelf. Inshore waters are generally nutrient-rich and well-mixed while offshore regions are rather oligotrophic and stratified with a deep euphotic zone (Smayda and Reynolds, 2001). Frequently, near-shore waters are additionally impacted by anthropogenic nutrient input, which has been demonstrated to significantly shape coastal phytoplankton community structures (Fehling et al., 2012; Goodman et al., 2012; Greenwood and Craig, 2014). High turbulence, usually found in highly mixed inshore waters, has been shown to be a prerequisite for large and heavy cells, such as silicified diatoms, to stay suspended and to acquire nutrients (Kiørboe, 1993). On the contrary, small cells with high S:V ratios have been reported to prefer oligotrophic offshore regions, where their small size prevents them from sinking and nutrients can be taken up efficiently (Kiørboe, 1993).

Along the Australian coast, research aimed at understanding cross-shelf variability in phytoplankton abundance, composition and distribution along environmental variable gradients, such as mixing/stratification and nutrient-accessibility, is still in its infancy. It has been shown that microphytoplankton (in particular diatom) abundance decreases with increased distance from the coast, while nano- and picophytoplankton (particularly the picoplanktonic cyanobacteria *Synechococcus* and *Prochlorococcus*) are more

prolific offshore (Thompson et al., 2011a). Such size-dependent distribution patterns have been reported on a large spatial scale from the east and west coasts of Australia between 27.5°S and 34.5°S (Thompson et al., 2011a) and also in regional studies from the Coffs Harbour (30°S, 153°E, Eastern Australia; Armbrrecht et al., 2014) and Kimberley coasts (~16°S, 126°E, North-Western Australia; Thompson and Bonham, 2011). Yet, detailed information on how such cross-shelf distribution patterns can be explained based on gradients in specific environmental variables, associated with coastal and offshore water masses, is still missing along the Australian coastline.

Within this study we aim to determine the preferences of pico- to microphytoplankton for specific environmental variables (including temperature, stratification, sample depth, salinity and nutrient availability) that occur along cross-shelf gradients. To additionally test the importance of the extent of the continental shelf in influencing environmental variables and phytoplankton variability, we compare two distinct study sites along the east and west Australian coast. The tropical-temperate Coffs Harbour region is located upstream of the point where the East Australian Current (EAC) separates eastward from the coast (~32°S). Frequent EAC-, wind-, and topographically-driven upwelling has been reported in the region, with topographically-driven upwelling induced by a very narrow (~30 km wide) continental shelf (Roughan and Middleton, 2002). Upwellings have been shown to promote nutrient input and phytoplankton blooms in the coastal euphotic zone along the east Australian coast (Ajani et al., 2001; Pritchard et al., 2003; Armbrrecht et al., 2014), especially during spring and summer when winds and EAC strength are at their annual maximum (Schaeffer et al., 2013; Rossi et al., 2014). The tropical Kimberley region is characterised by a broad continental shelf of about ~200 km width that interacts with the Indonesian Throughflow (ITF) to generate massive tides (~10 m) promoting extensive vertical mixing (Mustoe and Edmunds, 2008; Thompson and Bonham, 2011). Despite the low seasonality in this tropical region (Tranter and Leech, 1987), phytoplankton biomass has been reported to increase as a result of the strengthening ITF during winter (Thompson and Bonham, 2011; Thompson et al., 2011b).

In this investigation oceanographic, phytoplankton count and pigment data collected during the formation of the winter phytoplankton bloom off the Kimberley coast in April 2010 (Thompson and Bonham, 2011) is re-analysed alongside oceanographic, phytoplankton count and pigment data collected during a spring bloom period in the Coffs Harbour region in September 2012. Interactions between environmental

variables and the microscopy- and pigment-derived phytoplankton community are investigated via multivariate analyses (where pigment data is optimised in the software CHEMTAX; Mackey et al., 1996). We hypothesise that the combination of different environmental variables across the shelf determines the cross-shelf phytoplankton abundance, composition and distribution. We expect to find a decrease (increase) in microphytoplankton (pico-, nanophytoplankton) with distance from the coast, which may be more pronounced in the Kimberley region where the continental shelf is much wider than at Coffs Harbour. More specifically, we expect diatoms to be highly abundant in nutrient-rich and well-mixed inshore waters while all other taxa are predicted to prefer different combinations of temperature, stratification, depth and nutrients. In the subsequent methods and results sections we will present the case studies from the Coffs Harbour and Kimberley regions separately. Similarities and/or differences between phytoplankton responses to environmental gradients determined in the two case studies are discussed within a regional and global context at the end of the paper.

## 2 Methods

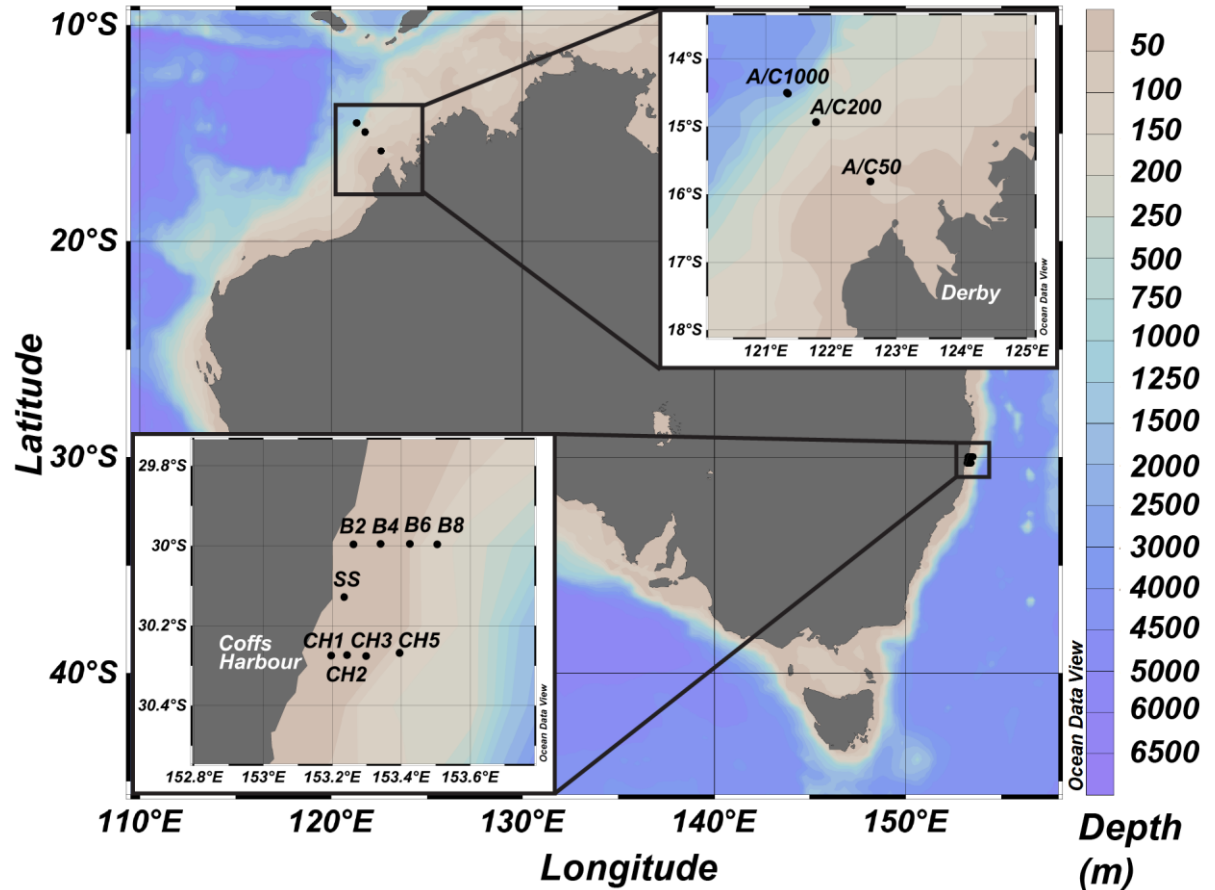
### 2.1 Coffs Harbour, Eastern Australia, ~30°S

#### 2.1.1 Hydrographic sampling

Water sampling for phytoplankton and pigments was undertaken along two cross-shelf transects (henceforth B- (north) and CH- (south) Line, respectively) and one coastal intermediate station (SS) on 11 and 12 September 2012 (Fig. 1, Table 1). The terminology used for all sampling stations is consistent with previous phytoplankton research in the Coffs Harbour region (Armbrecht et al., 2014). Along the B- and CH-Line, four sampling stations were located at the 25, 50, 70 and 100 m isobaths (B2 and CH1, B4 and CH2, B6 and CH3, B8 and CH5, respectively; Fig. 1).

Sampling was undertaken on the *RV Bombora* (NSW Office of Environment and Heritage, OEH, vessel) equipped with a SBE 911*plus* conductivity-temperature-depth (CTD) profiler (Sea-Bird Electronics, USA) and an ECO FLNTU fluorescence sensor (Wetlabs, USA). Surface samples were collected with a 10 L plastic bucket. Samples from the depth of the deep chlorophyll *a* maximum (DCM) were retrieved with 5 L Niskin bottles (General Oceanics, USA) fitted on the CTD rosette (Table 1). Data post-

processing of fluorescence profiles was conducted using the Seabird SBE Data Processing software (Sea-Bird Electronics, USA) following IMOS CTD processing protocols (<http://imos.org.au/anmndocuments.html>). Based on CTD data, stratification was determined as the difference between surface (5 m depth) and bottom density.



**Figure 1. Map of sampling locations.** The upper and lower expansions show the sampling transects in the Kimberley and Coffs Harbour regions, respectively. The colour scale of water depths was adapted from default settings in Ocean Data View (Schlitzer, R., Ocean Data View, <http://odv.awi.de>, 2012).

**Table 1. Sampling locations and dates.** Numbers in brackets indicate the depths at which a second water sample for HPLC analysis was taken at the end of the occupation of each respective station. In Tables 3, 4, 7, 8: '0 m' corresponds to 'surface' and sampling depths below the surface correspond to the Chl *a* maximum (DCM) depth.

Site	Station	Depth (m)	Date (UTC)
Coffs Harbour	B2	0, 8	11.09.2012
	B4	0, 20	11.09.2012
	B6	0, 12	11.09.2012
	B8	0, 12	11.09.2012
	SS	0, 8	11.09.2012
	CH1	0, 12	12.09.2012
	CH2	0, 8	12.09.2012
	CH3	0, 20	12.09.2012
	CH5	0, 24	11.09.2012
Kimberley	A50	0, 25 (0, 10)	17.04.2010
	A200	0, 75	16.04.2010
	A1000	0, 75 (0, 75)	15.04.2010
	C50	0, 25 (0, 20)	25.04.2010
	C200	0, 25 (0, 35)	23.04.2010
	C1000	0, 75	22.04.2010

### 2.1.2 Nutrients

Nutrient samples were analysed at the OEH, Sydney, by flow injection analysis using a LACHAT® Quik-Chem instrument. Standard methods following the American Public Health Association (APHA) were used for the detection of dissolved inorganic nitrogen (DIN; nitrate + nitrite, Method 4500 – NO<sub>3</sub>, detection limit ~0.02 µM; ammonium, Method 4500 – NH<sub>3</sub> H, detection limit ~0.06 µM), phosphate (Method 4500 – P G, detection limit ~0.01 µM) and silicate (Method 4500 – Si F, modified using stannous chloride as the reducing agent, detection limit ~0.08 µM) (APHA-AWWA-WEF1999).

### 2.1.3 Phytoplankton

Two litres of seawater were preserved by adding 6 mL Lugol's acid solution in plastic containers immediately after collection. After sedimentation in the laboratory (for 48 hrs), phytoplankton identification and enumeration under an inverted microscope (Leica DMI 3000B) followed Utermöhl (1958). The counting procedure is described in Armbrrecht et al. (2014), with the exception that all dinoflagellates belonging to the genera *Alexandrium*/*Gonyaulax*/*Heterocapsa* spp. were grouped to a complex.



#### **2.1.4 Pigments**

One replicate sample for High-Performance Liquid Chromatography (HPLC) analyses was prepared by directly filtering 2 L of water collected at all sampling locations onto 25 mm GF/F filter papers (Whatman, UK). Subsequently, filters were frozen in liquid nitrogen until further analysis (at the laboratories of the Commonwealth Scientific and Industrial Research Organisation, CSIRO, Hobart). HPLC analysis followed Clementson (2010), where pigments were extracted in acetone (for 15 - 18 hrs), and then separated and detected at 436 nm using a Waters Alliance® HPLC system (a binary gradient system with a C<sub>8</sub> column at 55°C). Pigments were identified by retention time and absorption spectrum from a photo-diode array detector and quantified against commercial and international standards (Sigma Aldrich, USA or DHI, Denmark).

### **2.2 Kimberley region, North-Western Australia (~14 - 16°S)**

#### **2.2.1 Hydrographic sampling**

Sampling for phytoplankton and pigments was undertaken twice along one cross-shelf transect at approximately 15°S between 15 and 26 April 2010 (hereafter referred to as transects A (spring tide) and C (neap tide), respectively; Fig. 1). Three sampling stations were located along both transects at the 50, 200 and 1000 m isobaths, being positioned approximately 80, 210 and 280 km offshore, respectively.

The *RV Southern Surveyor* was equipped with a SBE911 CTD (Sea-Bird Electronics, USA) fitted with an Aquatracka™ fluorometer (Chelsea Technologies Ltd., UK), which enabled the determination of the DCM in real-time. Water samples were collected at the surface and DCM at least once during the occupation of each station (Table 1) using 5 L Niskin bottles (General Oceanics, USA) and kept dark and cool until preserved or filtered (described below). CTD data processing followed the procCTD procedures manual (Beattie 2010) applying quality adjustments described in (Pender, 2000; Underwood, 2010a, b, c, d, e). Based on CTD data, stratification was determined as the difference between surface (5 m depth) and bottom density, except at stations A/C1000 where bottom depth density was replaced with density values at 200 m. At station A200, the deepest measurement at 150 m was used instead of the bottom density. The latter was not expected to change the stratification proxy, as the pycnocline was always shallower than 150 m.

### **2.2.2 Nutrients**

Nutrient samples for DIN, phosphate and silicate were collected at the same sampling locations and analysed at CSIRO, Hobart. Protocols followed Quik-Chem<sup>TM</sup> methods on a flow injection LACHAT® instrument as per the following protocols for nitrate + nitrite (Method 31-107-04-1-A; detection limit ~0.03  $\mu\text{M}$ ; adapted from Wood et al. (1967), silicon (Method 31-114-27-1-D; detection limit 0.05  $\mu\text{M}$ ; adapted from Armstrong (1951) and phosphate (Method 31-115-01-1-G; detection limit 0.02  $\mu\text{M}$ ; adapted from Murphy and Riley (1962). Samples were analysed for ammonium using the technique of K  rouel and Aminot (1997) adapted for flow injection (detection limit ~0.05  $\mu\text{M}$ ).

### **2.2.3 Phytoplankton**

One litre water samples were preserved using acid Lugol's solution for phytoplankton identification and enumeration by microscopy (Parsons et al., 1984). After sedimentation (for 24 hrs), phytoplankton composition was analysed in 1 mL Sedgwick Rafter counting chambers under an inverted microscope (Olympus IX 71). For a detailed description of sedimentation and counting procedures see Thompson and Bonham (2011).

One sample for the taxonomic determination of coccolithophores (Prymnesiophyceae) was collected at station A50. A volume of 250 mL seawater was filtered onto cellulose membrane filters (Millipore HAWP), rinsed twice with 1 mL distilled water, filtered to dryness and air-dried. Species were identified using scanning electron microscopy and polarised light microscopy (Olympus). Absolute abundances (coccoliths  $\text{mL}^{-1}$ , coccospheres  $\text{mL}^{-1}$ ) were calculated from 4 replicate counts of 25 field-of-views (FOV) under polarised light microscopy (at 1000x magnification; calibrated FOV area = 9702  $\mu\text{m}^2$ ).

### **2.2.4 Pigments**

Samples for pigment analyses were obtained by filtering 1 - 5 L seawater through a stacked filtration apparatus with a nylon mesh with 5  $\mu\text{m}$  square holes followed by GF/F filter papers (Whatman Ltd, UK) from both the surface and DCM. Immediately after collection samples were stored in liquid nitrogen and analysed separately by HPLC

following (Clementson, 2010) as described in section 2.1.4. For the purpose of this study pigment concentrations of both size-fractions were totalled.

### **2.3 CHEMTAX**

Regional literature (Jeffrey et al., 1975; Hallegraeff, 1981; Hallegraeff and Jeffrey, 1981; Hallegraeff and Reid, 1986) in combination with HPLC pigment and microscopy data determined algal classes to be included in the CHEMTAX analysis. Ten and twelve algal classes were selected for the Coffs Harbour and Kimberley datasets, respectively (Table 2).

Initial pigment ratio and ratio limit matrices were created following S. Wright (unpublished) and pigment optimisation followed Latasa (2007) and Wright et al. (2009). The complete procedure is described in Armbrrecht et al. (a, under review) and terminology follows Mackey et al. (1996). At first, optimisation was applied to the two complete HPLC datasets ( $n_{\text{Coffs Harbour}} = 18$ ,  $n_{\text{Kimberley}} = 20$ ). Running CHEMTAX consecutively resulted in an increasing number of unreasonable ratios after each run when compared to minimum and maximum ratios given in Higgins et al. (2011). Therefore, we used the output of the first run as initial input ratios for the following runs on binned data. Data was binned according to sampling transect (A and C for the Kimberley dataset; B-Line including SS and CH-Line for the Coffs Harbour dataset) and depth (surface and DCM; Lohrenz et al., 2003). Up to nine consecutive runs were conducted on the binned data until the number of unreasonable ratios increased. Absolute concentrations of Chl a ( $\mu\text{g L}^{-1}$ ) assigned to each algal class per sample from the best solutions of the run that delivered the most reasonable output were used in the subsequent statistical analyses (section 2.5).

**Table 2. Algal classes included in CHEMTAX analyses of the Coffs Harbour and Kimberley datasets.** Example species and characteristic pigments for each algal class are also listed, for a detailed definition of algal classes see Higgins et al. (2011).

Algal class	Example species	Characteristic pigments	Coffs Harbour	Kimberley
Cryptophytes	<i>Chroomonas salina</i>	Alloxanthin	x	x
Chrysophytes	"equatorial species" following Mackey et al., 1996	19'Butanoyloxyfucoxanthin, Fucoxanthin		x
Cyanobacteria-1	<i>Trichodesmium</i> sp.	Zeaxanthin		x
Cyanobacteria-2	<i>Synechococcus</i> sp.	Zeaxanthin	x	x
Cyanobacteria-4	<i>Prochlorococcus marinus</i>	Chl <i>b</i> , Zeaxanthin		x
Diatoms-1	<i>Chaetoceros didymus</i>	Chl <i>c</i> <sub>1</sub> , Chl <i>c</i> <sub>2</sub> , Fucoxanthin	x	x
Diatoms-2	<i>Pseudo-nitzschia</i> sp.	Chl <i>c</i> <sub>3</sub> , Chl <i>c</i> <sub>2</sub> , Fucoxanthin	x	x
Diatoms-4	<i>Ceratoneis closterium/Nitzschia longissima</i> , following Armbrrecht et al., a, under review	Chl <i>c</i> <sub>2</sub> , Fucoxanthin (lacking Chl <i>c</i> <sub>3</sub> , Chl <i>c</i> <sub>1</sub> )	x	x
Dinoflagellates-1	<i>Amphidinium carterae</i>	Peridinin	x	x
Haptophytes-6	<i>Emiliania huxleyi</i> , <i>Gephyrocapsa oceanica</i>	19'Hexanoyloxyfucoxanthin	x	x
Haptophytes-8	<i>Phaeocystis pouchetii</i>	19'Hexanoyloxyfucoxanthin	x	
Pelagophytes	<i>Pelagococcus subviridis</i>	19'Butanoyloxyfucoxanthin	x	x
Prasinophytes-3	<i>Micromonas pusilla</i> , <i>Pycnococcus provasolii</i>	Prasinoxanthin	x	x

## 2.4 Cross-shelf rates of change in pigment-based phytoplankton size-classes

The fractions of pico-, nano- and microphytoplankton to the total Chl *a* biomass ( $f_{\text{pico}}$ ,  $f_{\text{nano}}$ ,  $f_{\text{micro}}$ ), as well as the total Chl *a* biomass associated with each size-class ( $\text{TChl } a_{\text{pico}}$ ,  $\text{TChl } a_{\text{nano}}$ ,  $\text{TChl } a_{\text{micro}}$ ) were calculated following (Vidussi et al., 2001; Uitz et al., 2006; Ras et al., 2008). There are some uncertainties associated with this method as the same pigment can occur in several phytoplankton taxa and/or not all species are limited to one size-class (Uitz et al., 2006). However,  $f$  and  $\text{TChl } a$  assigned to each phytoplankton size-class per sample were tested for a correlation with distance from the coast. The slope of subsequent regression analyses (conducted in the software Minitab 16, 2010) provided a quantifiable measure to estimate the rates of change in the abundance of each phytoplankton size-class with distance from the coast.

## 2.5 Multivariate analyses

In order to identify relationships between environmental variables and phytoplankton species distribution determined by microscopy and CHEMTAX for each region (i.e. four separate datasets) we applied two consecutive statistical approaches. 1. Distance-based redundancy analysis following Legendre and Anderson (1999) was applied to

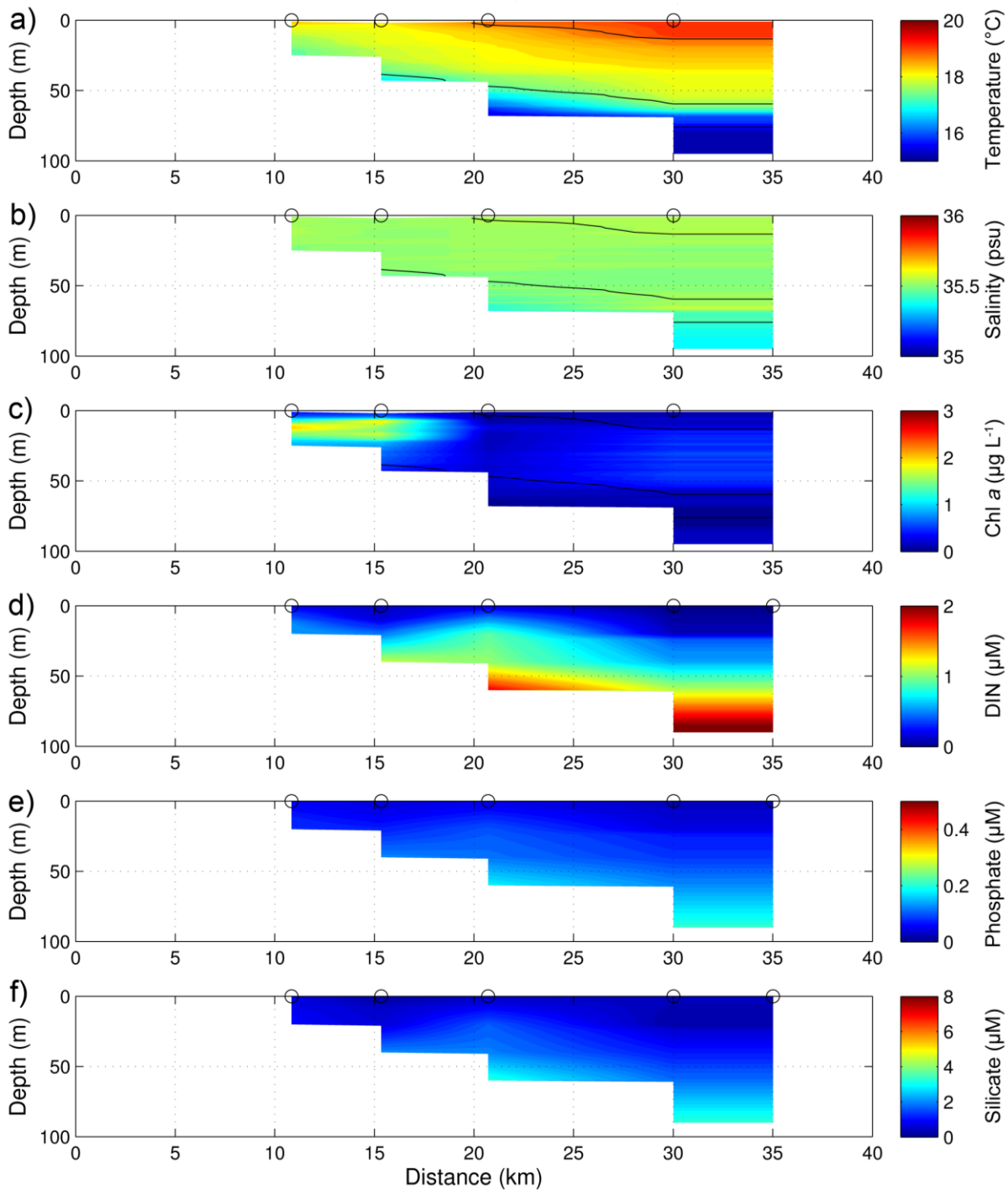
each dataset via the Distance-based Linear Modelling (DistLM) procedure in the software primer PRIMER Version 6.1.12 using the add on PERMANOVA Version 1.0.2 (Clarke and Gorley, 2006). Prior to the analysis, abundance data was  $\log(X+1)$  transformed. We used Bray Curtis similarity, forward selection and AIC selection criteria in the DistLM (Armbrecht et al., 2014). Environmental variables included in the analysis for both regions were stratification ( $\text{kg m}^{-3}$ ), sample depth (m), distance from the coast (km), temperature ( $^{\circ}\text{C}$ ), salinity (psu), total nitrogen, phosphate and silicate (all nutrients in  $\mu\text{M}$ ). In order to investigate the response of individual phytoplankton taxa to specific environmental variables, we applied Canonical Correspondence Analysis (CCA) via the software PAST (Hammer et al., 2001). We included all environmental variables named above independent of their importance in explaining the variability in phytoplankton community distribution as defined by the prior DistLM. This procedure was chosen in order to achieve the highest resolution of interactions between phytoplankton taxa and environmental variables.

### 3 Results

#### 3.1 Coffs Harbour

##### 3.1.1 Oceanographic conditions

While an intense wind-driven upwelling (04 – 07 September 2012, wind-stress up to  $0.15 \text{ N m}^{-2}$ ) preceded our sampling, winds were moderately upwelling-favourable on 12 September 2012 (wind-stress measured at Coffs Harbour airport station by the Bureau of Meteorology; Mantovanelli et al., under review). Southward depth-averaged EAC velocities were  $>0.3 \text{ m s}^{-1}$  (measured by moored Acoustic Doppler Current Profilers off Coffs Harbour, data not shown). The combined action of wind and EAC was responsible for EAC encroachment onto the shelf, which was evidenced in warm offshore waters, the uplift of the isotherms, isopycnals and nutrients toward the coast associated with increased Chl *a* concentration inshore (up to  $2 \mu\text{g L}^{-1}$ ; Fig. 2).



**Figure 2. Water column properties in the Coffs Harbour region.** Interpolated cross-shelf sections (distance versus depth) along CH-Line (~30°S) on 12 September 2012. Circles at the surface represent the positions of the casts. a) Temperature (°C); b) salinity (psu); c) chlorophyll a (Chl a;  $\mu\text{g L}^{-1}$ ); d) dissolved inorganic nitrogen (DIN;  $\mu\text{M}$ ); e) dissolved phosphate ( $\mu\text{M}$ ); f) dissolved silicate ( $\mu\text{M}$ ). a) – c) Density ( $\text{kg m}^{-3}$ ) contoured as measured by the CTD.

### 3.1.2 Total phytoplankton abundance determined by microscopy

Total phytoplankton abundance was at bloom level and ranged between  $2.8 \times 10^4$  cells  $\text{L}^{-1}$  (CH3, DCM) and  $8 \times 10^5$  cells  $\text{L}^{-1}$  (CH2, DCM; Table 3). Diatoms and dinoflagellates

contributed ~96% and ~4% to the total phytoplankton community, respectively (Table 3). Generally, diatom abundance was higher inshore than offshore with maximum abundance determined at station CH2 (surface and DCM; Table 3). *Leptocylindrus danicus* was the dominant diatom species at all stations (phytoplankton abundance data publicly available at: <http://catalogue.aodn.org.au/geonetwork/srv/en/metadata.show?uuid=f7502841-a2c7-4437-b557-20ef89e754e0>). A detailed list of phytoplankton taxa determined and their abbreviations used in the CCA is presented in Supplementary Material Table 1. To facilitate comparisons between microscopy and CHEMTAX abundance estimates, all phytoplankton taxa were classified into pigment-types following Armbrrecht et al., (a, under review; Supplementary Material Table 1).

**Table 3. Microscopically determined phytoplankton abundance in the Coffs Harbour region.** Phytoplankton abundance is given in cells L<sup>-1</sup> with individual taxa being classified into pigment-types (see Supplementary Material Table 1). Average abundances for each phytoplankton pigment-type across all samples and standard deviations are given as a percentage. DCM = Chl a maximum depth.

Station	Depth	Diatoms-1	Diatoms-2	Diatoms-4	Dino- flagellates -1	Dino- flagellates -2	Total abundance
B2	Surface	7,487	206,586	4,424	19,059	340	237,897
B2	DCM	6,807	481,240	2,042	3,403	681	494,173
B4	Surface	0	67,923	145	5,214	434	73,716
B4	DCM	123	37,982	245	858	82	39,289
B6	Surface	0	300,860	681	11,572	4,084	317,196
B6	DCM	1,225	277,308	204	3,267	1,225	283,230
B8	Surface	54,795	152,472	13,614	6,807	1,702	229,389
B8	DCM	98,018	202,161	5,445	3,403	0	309,028
SS	Surface	10,210	492,811	4,084	12,933	0	520,038
SS	DCM	6,126	384,583	1,361	2,042	681	394,793
CH1	Surface	9,529	407,046	2,042	42,202	2,042	462,861
CH1	DCM	17,102	613,637	2,736	684	1,368	635,528
CH2	Surface	8,849	699,057	2,042	21,101	0	731,049
CH2	DCM	8,849	784,822	681	6,126	2,042	802,520
CH3	Surface	12,661	55,850	3,982	12,967	1,532	86,991
CH3	DCM	694	25,444	449	1,470	327	28,384
CH5	Surface	4,492	68,340	1,497	3,676	1,089	79,095
CH5	DCM	26,683	70,382	9,393	681	1,225	108,364
Average		0.07	0.87	0.02	0.04	0.01	1.00
Standard deviation		0.10	0.13	0.02	0.04	0.01	0.00

### 3.1.3 *Phytoplankton composition estimate of CHEMTAX*

The initial ratio and ratio limit matrix, as well as the optimised output ratios for the complete Coffs Harbour sample set and for the binned data (including the run number delivering these ratios) are given in Supplementary Material Table 2.

TChl *a* concentrations ranged between 0.45  $\mu\text{g L}^{-1}$  (CH3, DCM) and 4.19  $\mu\text{g L}^{-1}$  (CH1, DCM) with generally higher concentrations inshore than offshore (Table 4). Absolute pigment composition of algal taxa to TChl *a* ( $\mu\text{g L}^{-1}$ ) for each sample is given in Table 4. On average (across all samples) CHEMTAX assigned 69% of TChl *a* to diatoms (29% diatoms-1, 16% diatoms-2, 24% diatoms-3; Table 4). Haptophytes-6 were assigned 14% of TChl *a* while all other taxa (cryptophytes, cyanobacteria-2, dinoflagellates-1, haptophytes-8, pelagophytes and prasinophytes) were each assigned 6% of TChl *a* or less (Table 4).

**Table 4. Phytoplankton abundance determined by CHEMTAX in the Coffs Harbour region.** Abundance is estimated in  $\mu\text{g Chl } a \text{ L}^{-1}$ . Average abundances for each phytoplankton pigment-type across all samples and standard deviations are given as percentage. DCM = Chl *a* maximum depth.

Sample ID	Depth	Crypto- phytes	Cyano- bacteria -2	Diatom s -1	Diatom s -2	Diatom s -4	Dino- flagellates -1	Hapto- phytes -6	Hapto- phytes -8	Pelago- phytes	Prasino- phytes -3	Total Chl <i>a</i>
B2	Surface	0.029	0.022	0.582	0.035	0.319	0.059	0.305	0.169	0.001	0.176	1.698
B2	DCM	0.000	0.000	1.408	0.245	0.979	0.000	0.459	0.214	0.007	0.176	3.488
B4	Surface	0.000	0.023	0.212	0.090	0.075	0.013	0.101	0.056	0.057	0.043	0.669
B4	DCM	0.012	0.000	0.188	0.042	0.134	0.000	0.108	0.148	0.046	0.091	0.770
B6	Surface	0.000	0.000	0.461	0.163	0.198	0.014	0.314	0.031	0.007	0.028	1.216
B6	DCM	0.078	0.000	0.720	0.000	0.556	0.000	0.223	0.067	0.000	0.038	1.682
B8	Surface	0.012	0.047	0.000	1.291	0.497	0.000	0.130	0.061	0.041	0.031	2.111
B8	DCM	0.000	0.000	0.000	1.049	1.612	0.000	0.730	0.004	0.021	0.036	3.451
SS	Surface	0.019	0.000	1.099	0.860	0.076	0.032	0.211	0.178	0.019	0.166	2.659
SS	DCM	0.140	0.000	1.459	0.173	0.651	0.000	0.574	0.486	0.010	0.305	3.797
CH1	Surface	0.000	0.000	0.591	0.072	0.005	0.055	0.332	0.028	0.003	0.022	1.108
CH1	DCM	0.000	0.000	0.754	0.443	2.361	0.000	0.437	0.014	0.006	0.174	4.188
CH2	Surface	0.000	0.000	0.703	0.246	0.906	0.034	0.304	0.043	0.006	0.021	2.264
CH2	DCM	0.034	0.000	0.923	0.791	0.519	0.000	0.232	0.031	0.009	0.184	2.724
CH3	Surface	0.000	0.023	0.109	0.051	0.083	0.044	0.054	0.084	0.013	0.007	0.469
CH3	DCM	0.072	0.014	0.036	0.044	0.047	0.000	0.021	0.027	0.030	0.157	0.448
CH5	Surface	0.000	0.025	0.227	0.225	0.068	0.005	0.007	0.076	0.016	0.007	0.657
CH5	DCM	0.032	0.000	0.391	0.005	0.929	0.000	0.268	0.006	0.008	0.029	1.667
Average		0.02	0.01	0.29	0.16	0.24	0.01	0.14	0.06	0.02	0.06	1.00
Standard deviation		0.04	0.02	0.15	0.16	0.17	0.02	0.07	0.06	0.03	0.08	0.00



### 3.1.4 Cross-shelf rates of change in abundance of phytoplankton size-classes

TChl *a* of each phytoplankton size-class decreased with distance from the coast, this decrease was significant in the nano- and picophytoplankton size-ranges only (at rates of  $8.00 \times 10^3$  and  $1.02 \times 10^4$  pg L<sup>-1</sup> km<sup>-1</sup>, respectively;  $p_{\text{nano}} = 0.01$ ,  $p_{\text{pico}} < 0.001$ ; Table 5).

Proportionally, only picoplankton decreased significantly ( $p = 0.041$ ) with distance from the coast at a rate of 0.004% km<sup>-1</sup> (Table 5).

**Table 5. Cross-shelf rates of change in the abundance of phytoplankton size-classes.** Rates of change are given for TChl *a* assigned to each phytoplankton size-class and in the proportional fraction per size-class (%). TChl *a* and the size fraction determined in each sample was tested for a correlation with distance from the coast ( $r$  = Pearson correlation coefficient). The slope of subsequent regression analyses was used to estimate the rate of change (in pg L<sup>-1</sup> km<sup>-1</sup> for TChl *a* and % km<sup>-1</sup> for fractions) in the abundance of each size-class with distance from the coast.

		Coffs Harbour			Kimberley		
	Size class	Correlation coefficient $r$	p-value	Rate of change	Correlation coefficient $r$	p-value	Rate of change
TChl <i>a</i> (pg Chl <i>a</i> L <sup>-1</sup> km <sup>-1</sup> )	Micro	-0.181	0.471	-2.34E+04	-0.676	0.001	-1.36E+03
	Nano	-0.589	0.010	-8.00E+03	-0.015	0.950	-1.30E+01
	Pico	-0.761	<0.001	-1.02E+04	-0.365	0.114	-2.38E+02
Fraction (% km <sup>-1</sup> )	Micro	0.363	0.139	0.005	-0.856	<0.001	-0.002
	Nano	-0.229	0.360	-0.002	0.774	<0.001	0.001
	Pico	-0.486	0.041	-0.004	0.626	0.003	0.001

### 3.1.5 Importance of environmental variables for species distribution

In the Coffs Harbour region, DistLM revealed stratification, phosphate, DIN and distance from the coast as environmental variables being important for the phytoplankton species distribution as determined by microscopy (in descending order, in total 47% variability explained, Table 6).

Silicate, temperature, distance from coast, phosphate, DIN, stratification and salinity were defined as having an influence (in descending order) on the phytoplankton taxa distribution determined by CHEMTAX with an explained variability of 65% (Table 6).

**Table 6. Summary of DistLM results.** Proportional/Cumulative = Proportional/Cumulative variance explained, AIC = Akaike's Information Criterion, RSS = Residual Sum of Squares. P-value at a significance level of 0.05, Test Statistic = Pseudo-F, DF = Degrees of Freedom.

			Distance from coast (km)	Temperature (°C)	Sample depth (m)	Salinity (psu)	Phosphate (µM)	DIN (µM)	Silicate (µM)	Stratification
DistLM: Microscopy (Coffs Harbour)	Marginal tests	p	0.032	0.167	0.197	0.974	0.14	0.333	0.421	0.02
		Pseudo-F	2.2911	1.4324	1.4039	0.31096	1.6209	1.1124	1.0212	2.7129
		Proportional	0.12526	0.082171	0.080666	0.019065	0.091989	0.065004	0.059996	0.14498
	Sequential tests	p	0.033				0.107	0.009		0.017
		Pseudo-F	2.1564				1.7751	3.4837		2.7129
		Cumulative	0.47489				0.23545	0.38779		0.14498
		Proportional	0.087104				0.090475	0.15234		0.14498
	Res. DF		13				15	14		16
		Best solution: AIC = 119.66, $R^2 = 0.47489$ , RSS = 7964.2, No. of Variables = 4 (Stratification, Phosphate, DIN, Distance from coast)								
DistLM: CHEMTAX (Coffs Harbour)	Marginal tests	p	0.102	0.111	0.304	0.371	0.072	0.153	0.042	0.063
		Pseudo-F	1.8911	1.8244	1.3075	1.0732	2.2037	1.6485	2.779	3.3215
		Proportional	0.1057	0.10236	0.075547	0.062857	0.12106	0.093407	0.14799	0.12671
	Sequential tests	p	0.111	0.028		0.229	0.187	0.039	0.034	0.209
		Pseudo-F	1.8456	3.0353		1.4419	1.5628	2.748	2.779	1.529
		Cumulative	0.37391	0.29138		0.65105	0.4411	0.54524	0.14799	0.60074
		Proportional	0.082536	0.14339		0.050313	0.067188	0.10414	0.14799	0.05497
	Res. DF		14	15		10	13	12	16	11
		Best solution: AIC = 126.88, $R^2 = 0.65105$ , RSS = 8519.5, No. of Variables = 7 (Silicate, Temperature, Distance from coast, Phosphate, DIN, Stratification, Salinity)								
DistLM: Microscopy (Kimberley)	Marginal tests	p	0.222	0.209	0.235	0.814	0.013	0.146	0.209	0.18
		Pseudo-F	1.3505	1.3156	1.3211	0.55536	3.0382	1.6533	1.3396	1.4667
		Proportional	0.11898	0.11626	0.1167	0.052614	0.23303	0.14188	0.11813	0.12791
	Sequential tests	p					0.01			
		Pseudo-F					3.0382			
		Cumulative					0.23303			
		Proportional					0.23303			
	Res. DF						10			
		Best solution: AIC = 73.25, $R^2 = 0.23303$ , RSS = 3849.8, No. of Variables = 1 (Phosphate)								
DistLM: CHEMTAX (Kimberley)	Marginal tests	p	0.069	0.07	0.067	0.341	0.001	0.003	0.004	0.308
		Pseudo-F	2.1888	2.2584	2.2876	1.1478	7.9009	5.1163	4.2337	1.1192
		Proportional	0.17958	0.18423	0.18617	0.10296	0.44137	0.33846	0.29744	0.10066
	Sequential tests	p	0.066	0.003	0.147	0.554	0.001	0.172	0.498	0.631
		Pseudo-F	1.8611	3.0132	1.7451	0.7959	7.9009	1.5157	0.92631	0.61654
		Cumulative	0.66047	0.58149	0.78379	0.84786	0.44137	0.72091	0.81758	0.87379
		Proportional	0.078987	0.14012	0.062883	0.030273	0.44137	0.060432	0.033795	0.025937
	Res. DF		8	9	6	4	10	7	5	3
		Best solution: AIC = 84.115, $R^2 = 0.87379$ , RSS = 2964.6, No. of Variables = 8 (Phosphate, Temperature, Distance from coast, DIN, Sample depth, Silicate, Salinity, Stratification)								

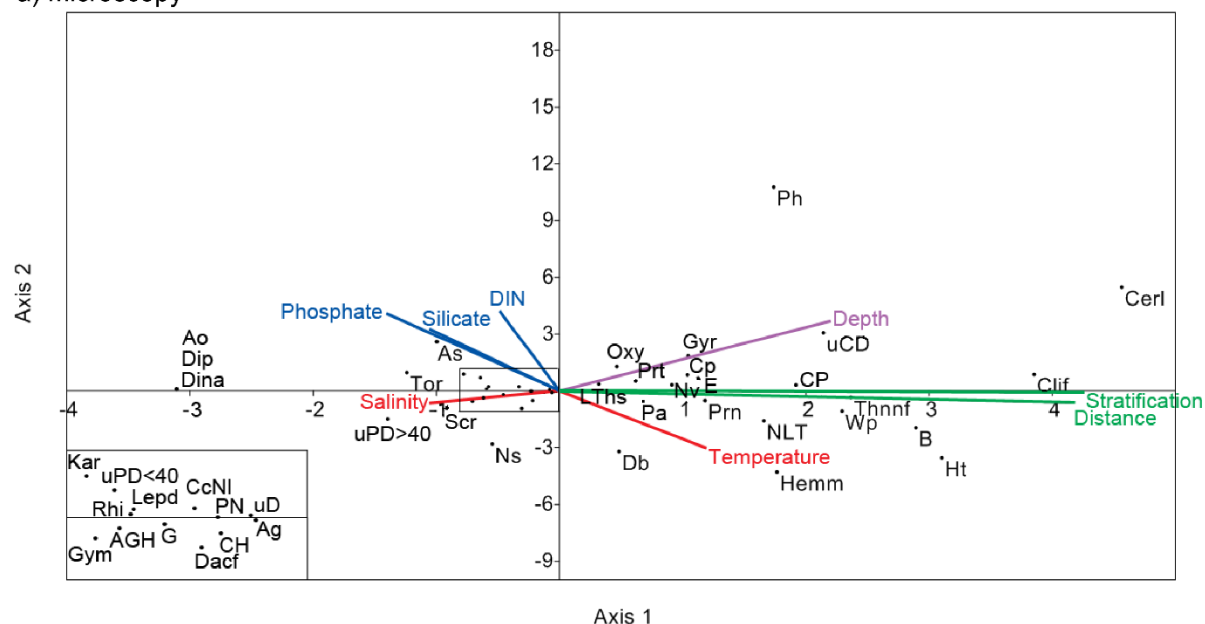
### 3.1.6 Response of individual taxa to environmental variables

CCA revealed that nutrients (phosphate, DIN and silicate) were strongly and positively correlated (as indicated by their vectors, which are arranged in acute angles and point in the same direction in Fig. 3a, b). Distance from the coast and stratification were strongly positively correlated with each other and to a lesser degree with elevated temperature (Fig. 3a, b). Sample depth was weakly correlated with increased distance from the coast/stratification and increased nutrient concentrations in the microscopy and CHEMTAX datasets, respectively (Fig. 3a, b). Salinity values were nearly identical throughout all samples, thus an obvious correlation with other variables could not be determined in the microscopy dataset (Fig. 3a) and exclusion from the CHEMTAX

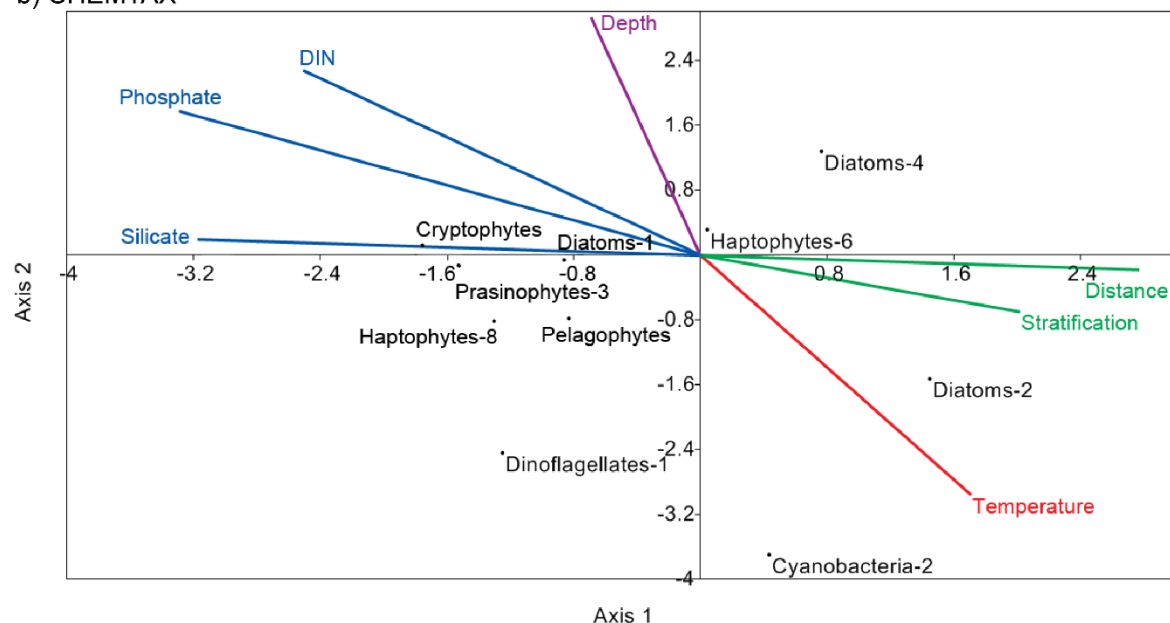
analysis was necessary. We were able to define two water masses in the Coffs Harbour region: nutrient-rich waters weakly associated with sample depth and warm stratified offshore waters.

Most microscopically determined diatom taxa, including the blooming diatom *Leptocylindrus danicus*, and dinoflagellates preferred nutrient-rich inshore waters (Fig. 3a). However, we also found many diatom and dinoflagellate taxa to only be weakly associated with nutrient concentrations, but responding to increased distance from the coast, temperature and depth gradients (Fig. 3a). CCA results based on CHEMTAX abundance estimates were consistent with the relatively wide spread of diatoms across the shelf. Diatoms-1 were associated with nutrient-rich inshore waters while diatoms-2 preferred warm temperature and offshore waters. Diatoms-4 were strongly associated with offshore waters and increasing sample depth rather than with warm temperature (Fig. 3b). CCA on the CHEMTAX dataset suggested that dinoflagellates generally preferred warm nutrient-rich waters (Fig. 3b; despite some dinoflagellate species being spread out across the shelf, Fig. 3a). Pelagophytes and haptophytes-8 were associated with high temperature and nutrient concentrations while cryptophytes and prasinophytes were mainly associated with elevated nutrient concentrations (Fig. 3b). Haptophytes-6 preferred deep waters and cyanobacteria-2 warm waters (Fig. 3b).

## a) Microscopy



## b) CHEMTAX



**Figure 3. Phytoplankton response to environmental variables in the Coffs Harbour region.** Triplots produced via Canonical Correspondence Analyses in PAST based on phytoplankton abundance determined by a) microscopy and b) CHEMTAX in the Coffs Harbour region. Phytoplankton taxa are ordinated in a two-dimensional space along two arbitrary axes, vectors visualise the fitted environmental/physico-chemical variables (distance from the coast, stratification, sample depth, temperature, salinity, DIN, phosphate, silicate). The length/direction of the vectors indicates the strength of effect/correlation of the variable on the ordination plot. Note that vectors indicating environmental variables are exaggerated by a factor of five for better visualisation in both (a) and (b). Insert shows the enlarged ordination of closely clustered species in the centre of the plot. For species abbreviations see Supplementary Material Table 1.

## 3.2 Kimberley

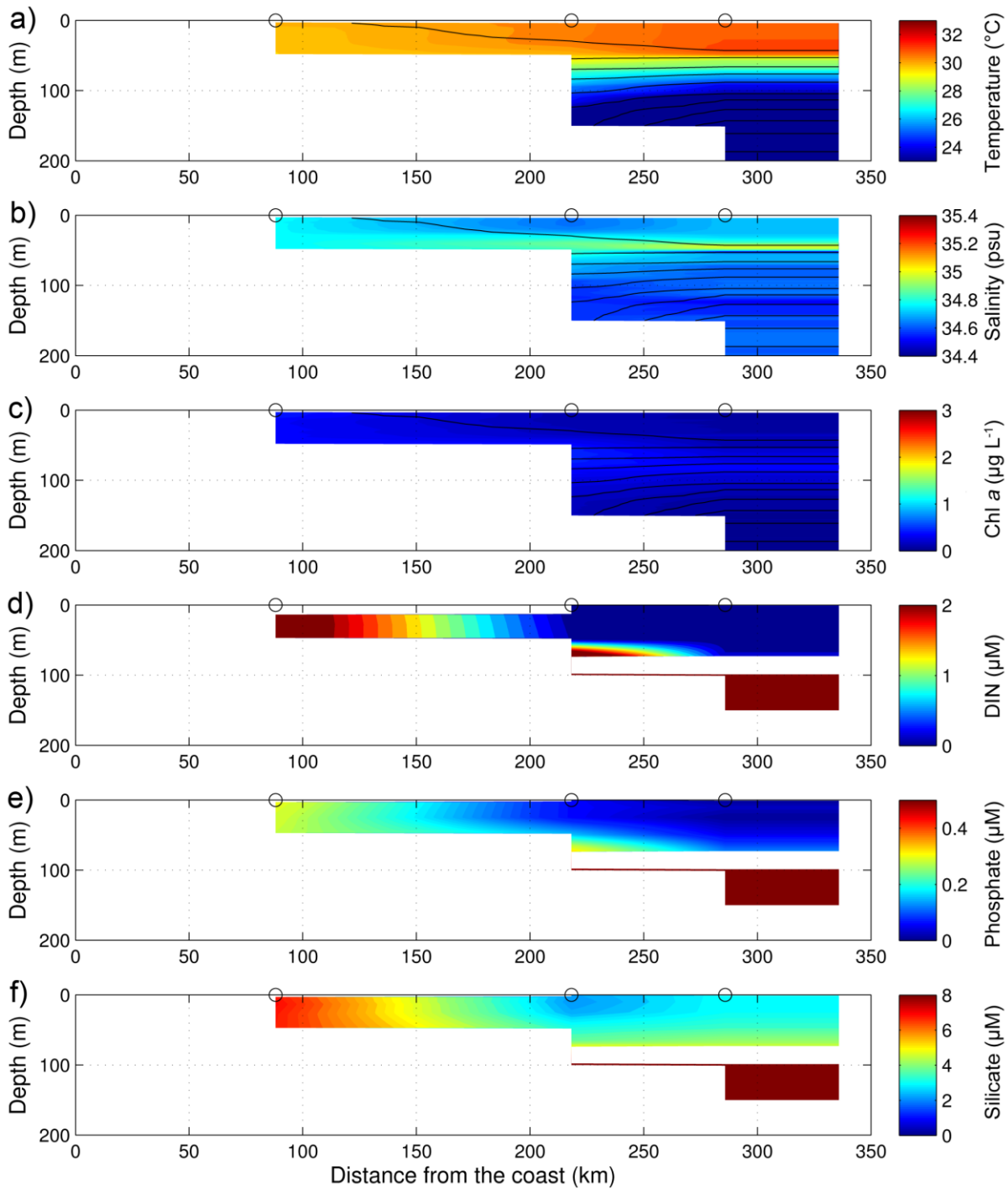
### 3.2.1 Oceanographic conditions

During April 2010 the ITF was weak and not intruding onto the shelf, as indicated by geostrophic velocities derived from altimetry products (data not shown). Thus, tidal activity was driving the inshore dynamics. Although sampling along transects A and C was undertaken during the spring (9.4 m range) and neap tides (6.5 m range) hydrographic conditions were similar. A homogenised water column on the shelf resulted in vertical mixing of nutrients (DIN, phosphate and silicate) and elevated Chl *a* concentrations inshore (although Chl *a* concentrations were low relative to Coffs Harbour; Fig. 4). Offshore, warm and fresh water was characteristic of tropical water, overlying nutrient-rich colder water and the DCM (Fig. 4).

### 3.2.2 Total phytoplankton abundance determined by microscopy

Total abundance ranged between  $2.03 \times 10^5$  (C1000, surface) and  $7.90 \times 10^5$  cells L<sup>-1</sup> (A200, DCM; Table 7). Generally, total abundance was higher inshore than offshore (Table 7). Averaging across all samples and excluding the small undefined flagellates, cryptophytes, diatoms, dinoflagellates and prymnesiophytes showed the highest abundance (each ~23% of the phytoplankton community; Table 7). Prasinophytes were less abundant (~7%), while chrysophytes and *Trichodesmium* sp. showed the lowest abundances (~0.7% and ~0.4%, respectively; Table 7). For a detailed species list of phytoplankton taxa determined in the Kimberley region, their abbreviation as used in the CCA and their classification into pigment-types see Supplementary Material Table 1. For exact abundances of all phytoplankton taxa at each sampling location see Thompson and Bonham (2011).

Complementary coccolithophore counts from station A50 revealed *Gephyrocapsa oceanica* as the dominant coccolithophore species ( $6.85 \times 10^3$  cells L<sup>-1</sup> and  $2.15 \times 10^5$  -  $1.10 \times 10^6$  loose liths L<sup>-1</sup>; Table 7). Other coccolithophore species were present but much less abundant (data not shown).



**Figure 4. Water column properties in the Kimberley region.** Interpolated cross-shelf sections (distance versus depth) along transect A ( $\sim 14.5^{\circ}\text{S}$  -  $16^{\circ}\text{S}$ ) on 15 April 2010 (start casts only). Circles at the surface represent the positions of the casts. a) Temperature ( $^{\circ}\text{C}$ ); b) salinity (psu); c) chlorophyll *a* (Chl *a*;  $\mu\text{g L}^{-1}$ ); d) dissolved inorganic nitrogen (DIN;  $\mu\text{M}$ ); e) dissolved phosphate ( $\mu\text{M}$ ); f) dissolved silicate ( $\mu\text{M}$ ). a) – c) Density ( $\text{kg m}^{-3}$ ) contoured as measured by the CTD. d) – f) Interpolation based on nutrient concentrations measured between 0 and 75 m.

**Table 7. Microscopically determined phytoplankton abundance in the Kimberley region.** Phytoplankton abundance is given in cells L<sup>-1</sup> with individual taxa being classified into pigment-types (see Supplementary Material Table 1). Average abundances for each phytoplankton pigment-type across all samples and standard deviations are given as percentage. DCM = Chl *a* maximum depth. Live cells and loose liths of the coccolithophore *Gephyrocapsa oceanica* were counted at A50 only.

Station	Depth	Chryso- phytes	Crypto- phytes	Tricho- desmium sp.	Diatoms- 1	Diatoms- 2	Diatoms- 4	Dino- flagellates- 1	Dino- flagellates- 2	Prasino- phytes	Prymnesio- phytes	Flagellates <10 µm, <i>Mesodinium rubrum</i>	Total abundance	<i>Gephyro- capsa oceanica</i> at A50
A50	Surface	90	4,481	269	2,483	12,189	3,943	11,382	45	2,241	13,443	658,726	709,292	6850 Cells L <sup>-1</sup>
A50	DCM	49	7,850	294	2,453	3,680	2,159	4,171	49	1,963	11,776	261,028	295,472	
A200	Surface	52	3,107	0	52	570	466	3,275	78	207	3,107	298,252	309,165	
A200	DCM	283	26,415	943	7,943	20,868	943	22,830	283	7,547	7,547	694,340	789,943	
A1000	Surface	310	6,196	0	774	9,500	1,084	6,402	52	2,065	12,391	183,804	222,579	215,000 - 1,100,000 loose liths L <sup>-1</sup>
A1000	DCM	2,299	9,195	0	1,686	1,609	1,379	4,726	77	0	11,494	222,989	253,155	
C50	Surface	46	3,689	0	20,522	6,132	1,660	7,655	184	3,689	12,913	391,068	447,559	
C50	DCM	340	32,264	0	4,585	2,420	1,061	19,104	212	3,396	10,189	580,755	654,325	
C200	Surface	45	7,129	401	757	3,252	3,431	4,322	624	8,911	7,129	406,337	442,337	loose liths L <sup>-1</sup>
C200	DCM	45	23,524	45	181	2,940	2,217	12,712	136	1,810	10,857	401,714	456,181	
C1000	Surface	40	4,800	0	280	540	320	8,120	20	1,600	1,720	185,600	203,040	
C1000	DCM	54	3,257	271	2,931	2,660	1,221	8,250	163	1,628	6,513	332,184	359,133	
Average		0.001	0.025	0.0004	0.008	0.012	0.004	0.022	0.0004	0.024	0.007	0.897	1.00	-
Standard deviation		0.003	0.016	0.0005	0.012	0.012	0.002	0.009	0.0003	0.015	0.005	0.036	0.00	-
Average		0.01	0.24	0.004	0.07	0.11	0.04	0.23	0.004	0.23	0.07	excluded	1.00	-
Standard deviation		0.02	0.12	0.005	0.10	0.08	0.03	0.10	0.005	0.09	0.06	excluded	0.00	-

### 3.2.3 Phytoplankton composition estimate of CHEMTAX

The initial ratio and ratio limit matrix, as well as the optimised output ratios for the complete Kimberley sample set and for the binned data (including the run number delivering these ratios) are given in Supplementary Material Table 3.

TChl *a* ranged from 0.05 µg L<sup>-1</sup> (C1000, surface) to 0.67 ± 0.12 µg L<sup>-1</sup> (A50, DCM) (Table 8). Generally, Chl *a* concentrations were higher inshore than offshore. On average (across all samples), CHEMTAX determined diatoms as the most abundant phytoplankton group (26% of TChl *a*, with diatoms-1 contributing 16%, diatoms-2 4%, and diatoms-4 6%; Table 8). Cyanobacteria-2, haptophytes-6 and prasinophytes-3 followed closely (each contributing ~20% to TChl *a*) while pelagophytes, cryptophytes, cyanobacteria-1, -4, chrysophytes and dinoflagellates-1 were each assigned less than 5% of Chl *a* (Table 8).

**Table 8. Phytoplankton abundance determined by CHEMTAX in the Kimberley region.** Abundance is estimated in  $\mu\text{g Chl } a \text{ L}^{-1}$ , Average abundances for each phytoplankton pigment-type across all samples and standard deviations are given as percentage. DCM = Chl *a* maximum depth. At depths where two samples were taken (see Table 1), the abundance estimates were averaged (standard deviations are shown).

Sample ID	Depth	Chryso-phytes	Crypto-phytes	Cyano-bacteria-1	Cyano-bacteria-2	Cyano-bacteria-4	Diatoms-1	Diatoms-2
A50	Surface	0 $\pm$ 0	0.034 $\pm$ 0.042	0 $\pm$ 0	0.045 $\pm$ 0.005	0 $\pm$ 0	0.396 $\pm$ 0.157	0.004 $\pm$ 0.005
A50	DCM	0 $\pm$ 0	0.033 $\pm$ 0.02	0 $\pm$ 0	0.043 $\pm$ 0.011	0 $\pm$ 0	0.008 $\pm$ 0.011	0.108 $\pm$ 0.07
A200	Surface	0.016	0.000	0.000	0.047	0.000	0.000	0.000
A200	DCM	0.000	0.023	0.000	0.000	0.000	0.020	0.081
A1000	Surface	0.013 $\pm$ 0.007	0.002 $\pm$ 0.003	0.005 $\pm$ 0.007	0.03 $\pm$ 0.005	0 $\pm$ 0	0.005 $\pm$ 0.007	0.002 $\pm$ 0.003
A1000	DCM	0.007 $\pm$ 0.00006	0 $\pm$ 0	0 $\pm$ 0	0.02 $\pm$ 0.011	0.022 $\pm$ 0.007	0.044 $\pm$ 0.009	0 $\pm$ 0
C50	Surface	0.008 $\pm$ 0.01	0.003 $\pm$ 0.004	0 $\pm$ 0	0.026 $\pm$ 0.037	0 $\pm$ 0	0.044 $\pm$ 0.062	0.004 $\pm$ 0.006
C50	DCM	0 $\pm$ 0	0.02 $\pm$ 0.028	0.001 $\pm$ 0.002	0.038 $\pm$ 0.004	0 $\pm$ 0	0.058 $\pm$ 0.082	0 $\pm$ 0
C200	Surface	0 $\pm$ 0	0.001 $\pm$ 0.001	0 $\pm$ 0	0.058 $\pm$ 0.02	0 $\pm$ 0	0.018 $\pm$ 0.025	0.001 $\pm$ 0.001
C200	DCM	0 $\pm$ 0	0.017 $\pm$ 0.005	0.053 $\pm$ 0.006	0.003 $\pm$ 0.005	0 $\pm$ 0	0.0004 $\pm$ 0.0004	0.009 $\pm$ 0.012
C1000	Surface	0.001	0.000	0.001	0.038	0.000	0.000	0.000
C1000	DCM	0.000	0.001	0.000	0.040	0.097	0.000	0.000
Average		0.033	0.023	0.018	0.207	0.026	0.159	0.036
Standard deviation		0.060	0.027	0.042	0.229	0.072	0.222	0.066
Sample ID	Depth	Diatoms-4	Dino-flagellates-1	Hapto-phytes-6	Pelago-phytes	Prasino-phytes-3	Total Chl <i>a</i>	
A50	Surface	0 $\pm$ 0	0 $\pm$ 0	0.031 $\pm$ 0.021	0.007 $\pm$ 0.01	0.152 $\pm$ 0.051	0.667 $\pm$ 0.118	
A50	DCM	0.263 $\pm$ 0.165	0 $\pm$ 0	0.018 $\pm$ 0.013	0.009 $\pm$ 0.012	0.12 $\pm$ 0.011	0.602 $\pm$ 0.242	
A200	Surface	0.000	0.000	0.023	0.000	0.000	0.086	
A200	DCM	0.000	0.000	0.061	0.065	0.149	0.401	
A1000	Surface	0 $\pm$ 0	0.001 $\pm$ 0.002	0.024 $\pm$ 0.008	0 $\pm$ 0	0 $\pm$ 0	0.083 $\pm$ 0.028	
A1000	DCM	0 $\pm$ 0	0 $\pm$ 0	0.029 $\pm$ 0.0004	0.032 $\pm$ 0.007	0.031 $\pm$ 0.0008	0.186 $\pm$ 0.004	
C50	Surface	0 $\pm$ 0	0.006 $\pm$ 0.008	0.051 $\pm$ 0.005	0.0002 $\pm$ 0.0003	0.038 $\pm$ 0.005	0.179 $\pm$ 0.04	
C50	DCM	0.118 $\pm$ 0.167	0.012 $\pm$ 0.01	0.109 $\pm$ 0.134	0.004 $\pm$ 0.003	0.109 $\pm$ 0.121	0.469 $\pm$ 0.387	
C200	Surface	0 $\pm$ 0	0.002 $\pm$ 0.003	0.039 $\pm$ 0.055	0.006 $\pm$ 0.0005	0.028 $\pm$ 0.024	0.153 $\pm$ 0.034	
C200	DCM	0.032 $\pm$ 0.046	0.005 $\pm$ 0.006	0.151 $\pm$ 0.06	0.035 $\pm$ 0.006	0.165 $\pm$ 0.054	0.469 $\pm$ 0.133	
C1000	Surface	0.000	0.000	0.003	0.003	0.000	0.046	
C1000	DCM	0.000	0.000	0.108	0.050	0.045	0.342	
Average		0.063	0.010	0.193	0.052	0.180	1.000	
Standard deviation		0.144	0.016	0.131	0.064	0.123	0.000	

### 3.2.4 Cross-shelf rates of change in abundance of phytoplankton size-classes

TChl *a* of each phytoplankton size-class decreased with distance from the coast (Table 5). In the microphytoplankton size-class this decrease was significant ( $p = 0.001$ ) at a rate of  $1.36 \times 10^3 \text{ pg L}^{-1} \text{ km}^{-1}$  (Table 5).

Proportionally, the microphytoplankton fraction decreased significantly ( $p < 0.001$ ) in inshore offshore direction at a rate of  $-0.002\% \text{ km}^{-1}$ , while the pico- and nanophytoplankton fractions increased significantly ( $p_{\text{pico}} = 0.003$ ,  $p_{\text{nano}} < 0.001$ ) at a rate of  $0.001\% \text{ km}^{-1}$  each (Table 5).



### **3.2.5 Importance of environmental variables for species distribution**

In the Kimberley region, the DistLM revealed phosphate as the only environmental variable with a significant ( $p = 0.01$ ) influence on phytoplankton species distribution determined by microscopy. This variable was attributed to explain 23% of the variability in the phytoplankton composition (Table 6).

All eight environmental variables explained 87% of the variability in the phytoplankton composition as determined by CHEMTAX in the Kimberley region (Table 6). In descending order these variables were phosphate, temperature, distance from coast, DIN, sample depth, silicate, salinity and stratification.

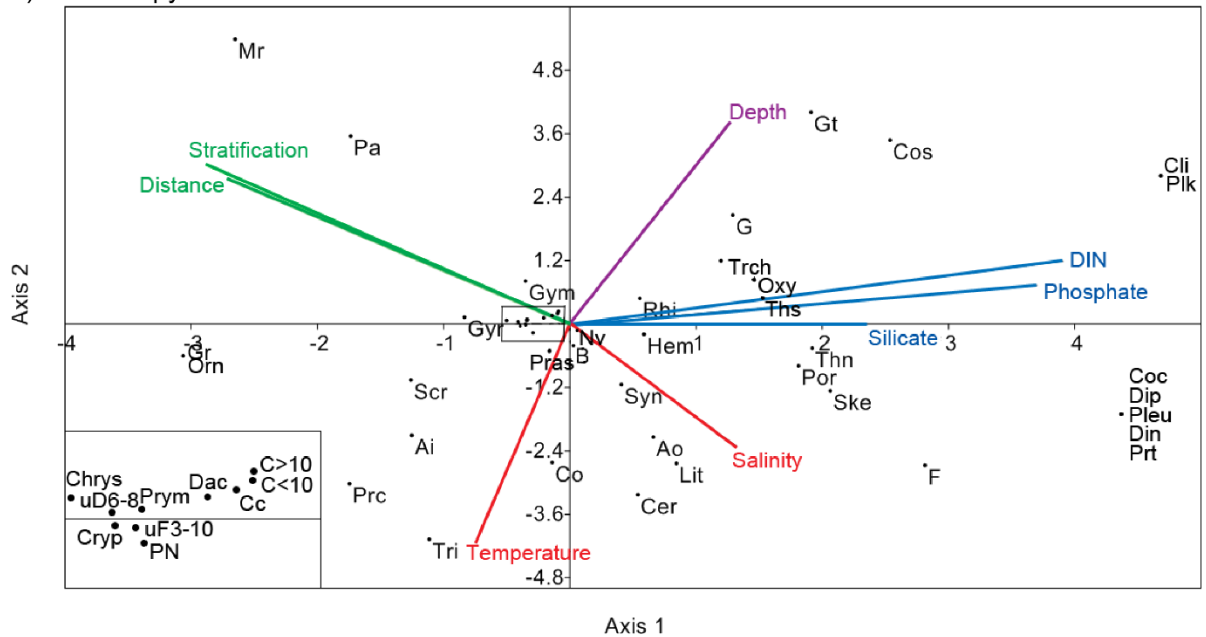
### **3.2.6 Response of individual taxa to environmental variables**

CCA on the phytoplankton abundance data, determined by both microscopy and CHEMTAX, showed that nutrients (phosphate, DIN and silicate) were highly positively correlated (Fig. 5a, b). Increased concentrations of all three nutrients were associated with inshore waters (Fig. 5a, b). Distance from the coast and stratification were also positively correlated (Fig. 5a, b). The latter two variables were anti-correlated with salinity (Fig. 5a; due to nearly identical salinity values this variable had to be excluded from the CCA analysis on the CHEMTAX dataset). Temperature was weakly correlated with salinity and nutrients based on the microscopy and CHEMTAX datasets, respectively (Fig. 5a, b). Sample depth was weakly correlated with nutrients and distance from the coast/stratification based on the microscopy and CHEMTAX dataset, respectively (Fig. 5a, b). We defined two distinct water masses in the Kimberley region: nutrient-rich, well-mixed inshore waters and relatively stratified nutrient-poor offshore waters (as in the Coffs Harbour region, see section 3.1.6).

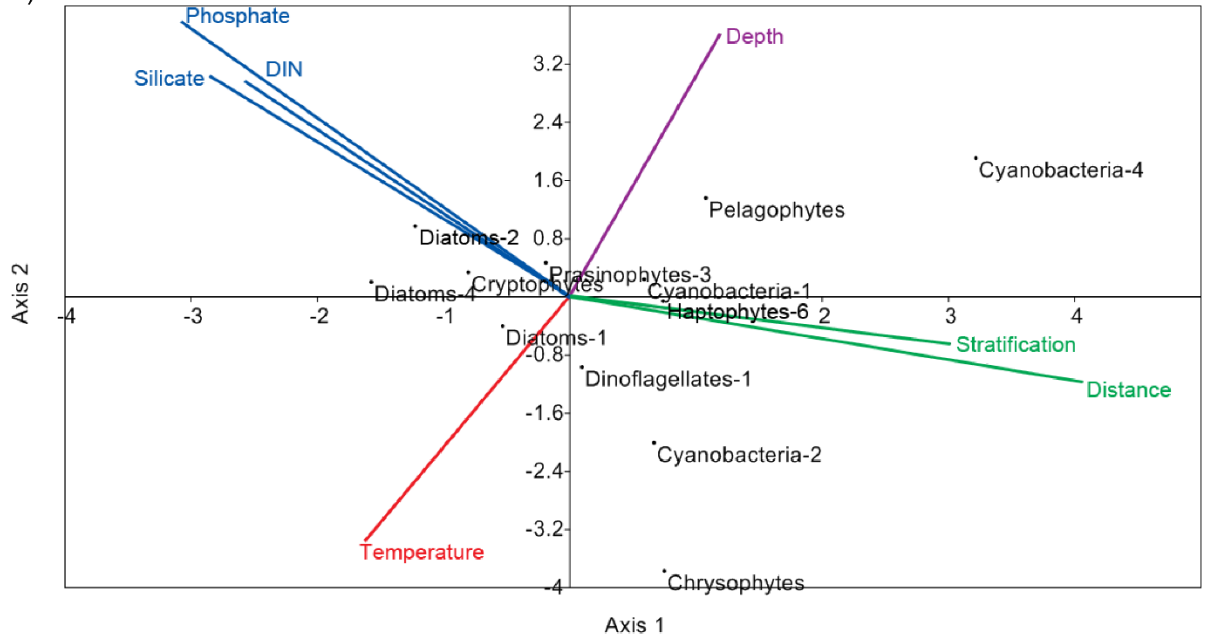
The phytoplankton community response to environmental variables was consistent within the microscopy and CHEMTAX datasets. Diatoms (individual species and diatoms-1, -2, -4) were ordinated close to the nutrient vectors (Fig. 5a, b, for abbreviations of taxa see Supplementary Material Table 1). Cryptophytes, and to a lesser degree prasinophytes, showed a similar preference for nutrient-rich waters (Fig. 5b). Dinoflagellates preferred warm waters (Fig. 5a, b). Cyanobacteria-2 and chrysophytes preferred increased temperature and distance from the coast (Fig. 5b). Haptophytes-6, cyanobacteria-1, -2, -4, chrysophytes and pelagophytes were associated with increased distance from the coast and elevated stratification (Fig. 5b).

Cyanobacteria-4 and pelagophytes were additionally associated with increased sample depth (Fig. 5b). Cyanobacteria-2 and chrysophytes also preferred warm waters. While cyanobacteria-1 responded to enhanced distance from the coast, stratification and sample depth based on the CHEMTAX dataset (Fig. 5b), its type-species, *Trichodesmium* sp., showed a clear preference for warm water (Fig. 5a).

## a) Microscopy



## b) CHEMTAX



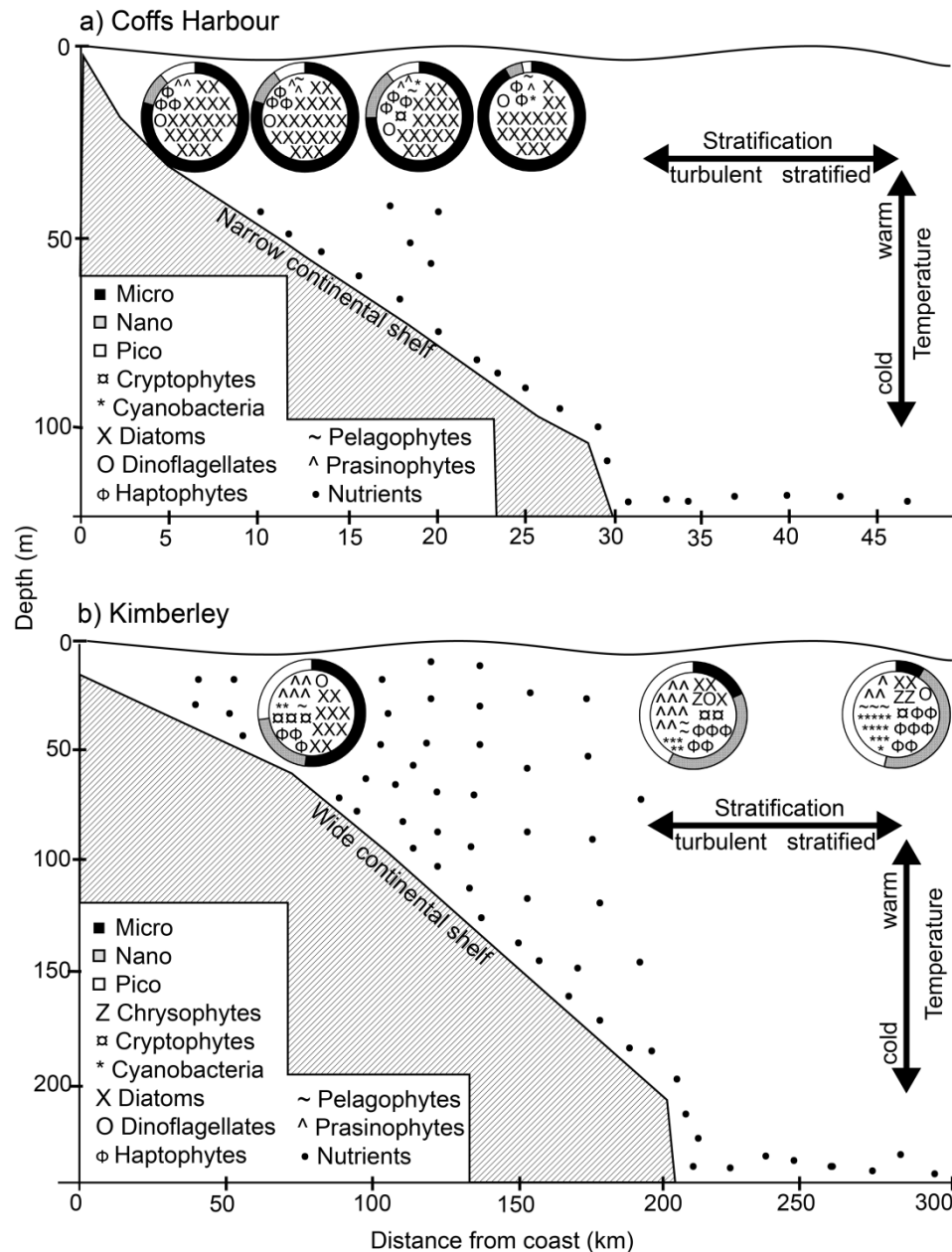
**Figure 5. Phytoplankton response to environmental variables in the Kimberley region.** Triplots produced via Canonical Correspondence Analyses in PAST based on phytoplankton abundance determined by a) microscopy and b) CHEMTAX in the Kimberley region. Phytoplankton taxa are ordinated in a two-dimensional space along two arbitrary axes, vectors visualise the fitted environmental/physico-chemical variables (distance from the coast, stratification, sample depth, temperature, salinity, DIN, phosphate, silicate). The length/direction of the vectors indicate the strength of effect/correlation of the variable on the ordination plot. Note that vectors indicating environmental variables are exaggerated by a factor of five for better visualisation in both (a) and (b). Insert shows the enlarged ordination of closely clustered species in the centre of the plot. For species abbreviations see Supplementary Material Table 1.

## 4 Discussion

### 4.1 *Phytoplankton distribution in response to environmental variables in the Coffs Harbour region*

At the time of sampling the EAC was encroaching onto the continental shelf. This is a frequent condition upstream of the EAC separation zone, occurring more than 30% of the time (Schaeffer et al., 2013). We assume that the wind-driven upwelling event preceding our sampling induced a nutrient pulse resulting in a short-lived (5 - 8 days) spring diatom bloom (Fig. 6). The blooming diatom, *Leptocylinthus danicus*, was abundant at all stations across the shelf indicating a bloom surface area of at least 1100 km<sup>2</sup> (Fig. 6). The frequently deformed shape of living *L. danicus* cells, the presence of numerous empty *L. danicus* frustules, and the detection of the Chl *a* degradation products chlorophyllide *a*, phaeophytin *a* and phaeophorbide *a* in nearly all pigment samples (data not shown) suggested a senescent phase of the bloom. Senescence explains the high microscopy abundance estimate of diatoms-2 (amongst which *L. danicus* was classified) versus the low Chl *a*-based CHEMTAX estimate (Armbrecht et al., a, under review).

We speculate that the diatom bloom caused the pronounced cross-shelf nutrient depletion, especially between the surface and about 20 m from the bottom, affecting the distribution of other phytoplankton taxa (Fig. 6, Table 9). Irrespective of their size, nearly all other taxa (i.e. dinoflagellates, cryptophytes, pelagophytes, haptophytes-8, prasinophytes) primarily responded to local nutrient maxima (Fig. 6, Table 9). This preference was unexpected for pelagophytes and dinoflagellates, and contradictory to the insensitivity of these two taxa to nutrient concentrations found in the Kimberley region (Fig. 6, Table 9). We suggest that dinoflagellates and pelagophytes re-distributed to zones where local nutrient maxima occurred (inshore) during the post-bloom oligotrophic conditions at Coffs Harbour. Whether such a distribution pattern was caused by advective mechanisms or by active movement remains speculative, however, given the nature of both taxa being flagellated, active movement seems more likely. The only phytoplankton taxon not responding to elevated nutrient levels were cyanobacteria-2 (*Synechococcus*), which confirms the cosmopolitan abundance of this cyanobacterium (Table 9; Partensky et al., 1999).



**Figure 6. Phytoplankton community structure driven by cross-shelf gradients of environmental variables in the Coffs Harbour and Kimberley regions.** Both study sites (note difference in the shelf widths) were characterised by relatively cold nutrient-rich inshore waters and warm, oligotrophic offshore waters (indicated by black arrows). The contribution of depth-averaged proportions of the micro-, nano- and picophytoplankton (Micro, Nano, Pico; derived from pigment-based size-class fractionation) at each sampling station are shown as doughnut charts. Inside the charts, the depth-averaged relative abundance of individual phytoplankton taxa (derived from microscopy and CHEMTAX) are schematically indicated by different symbols. a) Diatoms (in particular *Leptocylindrus danicus*) dominated the narrow continental shelf off Coffs Harbour, resulting in shelf-scale surface nutrient depletion and low abundance of other phytoplankton taxa. b) Microphytoplankton (in particular diatoms) decreased with distance from the coast over the wide shelf in the Kimberley region, while pico- and nanophytoplankton (in particular *Synechococcus* sp. and *Gephyrocapsa oceanica*) increased.

**Table 9. Summary of preferences of individual phytoplankton taxa for specific environmental variables.** Preferences determined in the Coffs Harbour and Kimberley regions, and determined in previous investigations, are listed per phytoplankton taxon. Where only one characteristic is listed, this characteristic applies to the respective taxon in both the Coffs Harbour and the Kimberley regions. S:V = surface to volume ratio.

Taxon	Abundance Coffs Harbour / Kimberley	Habitat preference Coffs Harbour / Kimberley	Preferences for environmental variables previously reported and potentially explaining preferences detected in this study	Literature
Diatoms-1	High	Nutrient-rich inshore waters (both regions)	Turbulent waters prerequisite to stay in suspension for large, heavy, silicified frustules. Passive movement by buoyancy control, chain/mucilage formation to enhance buoyancy, adaptation to fluctuating light in highly mixed regions.	Margalef, 1978; Richardson et al., 1983; Kjørbe, 1993; Wyatt, 2014
Diatoms-2	High	Distance from coast, warm surface waters / nutrient-rich inshore waters	The diatom <i>Leptocylindrus danicus</i> was spread out across the shelf during its bloom at Coffs Harbour. Kimberley: same as diatoms-1 but stronger preference for cold waters.	Margalef, 1978; Richardson et al., 1983; Kjørbe, 1993; Wyatt, 2014
Diatoms-4	Medium	Distance from coast, cold deep waters / nutrient-rich inshore waters	Includes benthic diatom species (e.g. <i>Navicula</i> spp.) found in higher abundances and more widely distributed across the shelf in the Coffs Harbour than the Kimberley region where only present inshore. Generally, same as diatoms-1.	Thompson and Bonham, 2011; Armbrrecht et al., 2014
Dinoflagellates-1	Medium	Increased temperature and nutrients / increased temperature and distance from coast	Small/unarmored, high S:V ratios (e.g. <i>Gymnodinium</i> ): well-mixed, nutrient-rich inshore waters to remain suspended and efficient nutrient uptake. Highly ornamented/laterally flattened (e.g. <i>Ornithocercus</i> ): stratified offshore waters, morphology/fast swimming facilitates nutrient uptake/vertical migration.	Margalef, 1978; Kjørbe, 1993; Smayda and Reynolds, 2001; Smayda, 2010; Wyatt, 2014
Chrysophytes	Low	Increased temperature and distance from coast (Kimberley coast only)	Dictyochales: below 15°C; naked Dictyochales and Parmales: cosmopolitan. Minor role in marine waters (confirmed in this study). Mixotrophy, ingestion/use of bacteria as symbionts, vertical migration to acquire phosphate.	Van Valkenburg and Norris, 1970; Holen and Boraas, 1995; Jeffrey and Vesik, 2005; Kristiansen, 2009
Cryptophytes	Medium	Nutrient-rich, deep inshore waters (both regions)	Mixotrophy, vertical migration, sulphated deep waters, osmotolerance, some euryhaline forms. Pico- to microphyto-plankton. Our study: Microscopy suggested larger forms and independence of frequent symbiotic relationships. Low CHEMTAX abundance estimate potentially due to size-variable Chl a content.	Klaveness, 1989; Jeffrey and Vesik, 2005; Kaňa et al., 2013; Armbrrecht et al., a, under review
Cyanobacteria-1 ( <i>Trichodesmium</i> spp.)	Low	Nutrient-rich, deep offshore water (Kimberley only)	Diazotroph, common surface blooms, photoprotection by zeaxanthin, vertical migration by buoyancy control (varying carbohydrate:protein composition, ascending several m h <sup>-1</sup> ), acquisition of phosphate in deep waters.	Walsby, 1978; Carpenter and Capone, 1992; Villareal and Carpenter, 2003; Jeffrey et al., 2005
Cyanobacteria-2 ( <i>Synechococcus</i> spp.)	Low / High	Increased temperature and distance from coast (both regions)	Cosmopolitan, globally less abundant than <i>Prochlorococcus</i> but highly abundant in Kimberley region, warm tropical waters, efficient nutrient uptake due to high S:V ratios, photoprotection by zeaxanthin.	Kjørbe, 1993; Partensky et al., 1999
Cyanobacteria-4 ( <i>Prochlorococcus</i> spp.)	Low	Increased temperature and distance from coast (Kimberley only)	Cosmopolitan, globally more abundant than <i>Synechococcus</i> , warm tropical waters, efficient nutrient uptake due to high S:V ratios, photoprotection by zeaxanthin.	Kjørbe, 1993; Partensky et al., 1999
Haptophytes-6	High	Weak preference for deep waters and increased distance from the coast (both regions)	Regions of intermediate nutrient-fluxes and increased day lengths. <i>Gephyrocapsa oceanica</i> identified as major haptophyte-6 species in the Kimberley region in this study.	Balch, 2004; Falkowski and Oliver, 2007
Haptophytes-8	Low	Nutrient-rich, warm inshore waters (Coffs Harbour only)	Example species <i>Phaeocystis pouchetii</i> known from 34°S, Eastern Australia, and reported as ubiquitous.	Hallegraeff and Reid, 1986
Pelagophytes	Low	Warm nutrient-rich inshore waters / deep offshore waters	Efficient nutrient uptake due to high S:V ratios, lack of photoprotective pigment zeaxanthin, thus positioning in deeper waters beneficial (as in Kimberley region, this study).	Jeffrey et al., 2005
Prasinophytes-3	Medium	Nutrient-rich inshore water (both regions)	Preference for nutrient-rich deep waters shown previously. High CHEMTAX abundance estimate potentially due to incorrect assignment of shared Chl b, and/or missed/absent during microscopy.	Thomsen and Buck, 1998; Zingone et al., 1999; Not et al., 2012; Armbrrecht et al., a, under review

#### ***4.2 Phytoplankton distribution in response to environmental variables in the Kimberley region***

The sampling period (April 2010) was characterised by quasi-no rainfall and weak winds, preceding the winter maximum of ITF transport (Holliday et al., 2011; Thompson et al., 2011b). At the time of sampling (autumn), the phytoplankton were in the middle of building up biomass (>50% increase) as part of the annual winter phytoplankton bloom (Thompson and Bonham, 2011). Our data suggests nutrient transport onto the shelf by the intrusion of cold bottom water, as shown previously from the north-west Australian shelf just south of the Kimberley region during this time of the year (Tranter and Leech, 1987). Coastal nutrient concentrations might have been enhanced due to runoff from the closely located large King Sound embayment, although rainfall was not pronounced during 2010 (Holliday et al., 2011).

The sharp decrease in nutrient concentrations between the 50 m and 200 m stations, and a gradual increase in stratification, impacted on the phytoplankton community distribution. While microphytoplankton were associated with turbulent nutrient-rich inshore waters and decreased rapidly in abundance towards offshore, pico- and nanophytoplankton preferred warm, nutrient-poor waters and increased towards offshore (proportionally to TChl *a*; Fig. 6). This finding is consistent with recent investigations from the west Australian coast reporting similar cross-shelf changes in phytoplankton composition over a distance of about five longitudinal degrees (Thompson et al., 2011a). Individual phytoplankton taxa responded differently to specific combinations of environmental variables along the shelf and a concise summary and of the environmental preferences of all phytoplankton taxa including further background information is given in Table 9.

#### ***4.3 Comparison of the cross-shelf phytoplankton variability in the Coffs and Kimberley regions***

##### ***4.3.1 Similarities in the phytoplankton response to environmental variables***

We identified similar water masses in the oceanographically distinct Kimberley and Coffs Harbour regions. Both shelf-regions were characterised by relatively cold nutrient-rich inshore waters and relatively stratified warm, oligotrophic offshore waters (with nutrient concentrations being generally very low at Coffs Harbour, section 4.1). Surface temperatures increased ~1°C with distance from the coast and ~5°C with

depth in both study regions, with surface temperatures being  $\sim 10^{\circ}\text{C}$  higher in the Kimberley than in the Coffs Harbour region. Despite the differences in temperature, continental shelf width and nutrient supply mechanism (upwelling versus tidal mixing) in the two study regions, we determined similar habitat preferences of several phytoplankton at both study sites. For example, cryptophytes and prasinophytes both preferred nutrient-rich inshore waters, cyanobacteria-2 stratified offshore waters, and haptophytes-6 nutrient-poor waters with an additional weak preference for deep and offshore waters (Fig. 6, Table 9). Diatoms were highly abundant at both study sites, and associated with well-mixed inshore waters, particularly in the Kimberley region. Dinoflagellates and cyanobacteria-2 (*Synechococcus* sp.) were much more abundant in the Kimberley region, which we attribute to the high temperature at this location. Dinoflagellates and *Synechococcus* sp. have been found to prefer warm and tropical waters (Armbrecht et al., b, under review; Partensky et al., 1999, respectively). The above outlined taxon-specific distributional patterns associated with distinct oceanographic variables are consistent with previous research and have been detailed in Table 9. In summary, the similarities in phytoplankton preferences for different cross-shelf gradients of environmental variables in both study regions showed the sensitivity of the phytoplankton community to changes in such variables.

#### **4.3.2 Importance of the width of the continental shelf**

In the Kimberley region, our sampling transect extended about 280 km off the coast, covering two shelf stations and one offshore station (Fig. 6). In the Coffs Harbour region, transects extended about 28 km off the coast with all sampling stations located on the continental shelf (Fig. 6). Therefore, large-scale patterns in phytoplankton abundance, composition and distribution are highlighted in our Kimberley case study, while shelf-scale patterns are better resolved in our Coffs Harbour case study. Thus it is plausible that diatoms were dominant at all sampling stations off Coffs Harbour (over a distance of  $\sim 28$  km) and at the inshore station in the Kimberley (located  $\sim 80$  km from the coast). The increasing (decreasing) rates of change in microphytoplankton (pico-, nanophytoplankton) abundance observed in the Coffs Harbour region reflect small-scale community changes that might be similar shoreward of the inshore station in the Kimberley region. Conversely, a clear decrease (increase) in microphytoplankton (pico-, nanophytoplankton) as found across the wide Kimberley shelf, might also occur over comparable distances in the Coffs Harbour region. Further studies aimed at



resolving shelf-scale phytoplankton community structures in the Kimberley region (e.g. at closely positioned sampling stations) and offshore community structures in the Coffs Harbour region (e.g. sampling in open waters) might enable direct distance-based comparisons.

#### **4.3.3 *Phytoplankton bloom development***

Our sampling in the Coffs Harbour and Kimberley regions took place during annual bloom periods. Seasonality is inconspicuous in the Kimberley region and basically divided into monsoon and non-monsoonal periods (Tranter and Leech, 1987). However, an annual winter phytoplankton bloom is formed as a consequence of a strengthening ITF and was sampled during its log-phase in this study (Thompson and Bonham, 2011; Thompson et al., 2011b). Seasonality is pronounced in the tropical-temperate Coffs Harbour region whereby upwelling during spring and summer leads to the formation of diatom blooms (this study; Armbrrecht et al., b, under review). Diatoms have been shown to overgrow small-sized phytoplankton taxa during such short-lived bloom periods, increasing from an annual background contribution of ~20% to the total Chl *a* biomass to 80 - 90%, at the expense of small-sized phytoplankton (at ~34°S, Eastern Australia; Hallegraeff, 1981). Our Coffs Harbour case study revealed that post-bloom oligotrophic conditions created by such diatom blooms force successive phytoplankton taxa to redistribute across the shelf. Potentially, the developing bloom at the Kimberley coast experienced a similar condition, however, this is speculative and more research aimed at investigating terminal bloom processes will be necessary to confirm this.

#### **4.3.4 *Phytoplankton community estimates by microscopy and CHEMTAX***

In both case studies different environmental variables drove the cross-shelf phytoplankton variability. This difference relied on whether our multivariate analyses were based on microscopy or CHEMTAX community assessments. Different sources of errors (due to classification, analysis, sampling and omissions) involved in both microscopy and CHEMTAX might have influenced our analyses and phytoplankton abundance estimates. However, analysing the error sources of microscopy and CHEMTAX exceeds the purpose of this study and is the topic of another paper (Armbrrecht et al., a, under review). In this study, the different outcomes indicate the

varying importance of particular variables for different phytoplankton taxa that were included in the microscopy and CHEMTAX analyses. These preferences are summarised in Table 9.

Additionally, a higher percentage of variability in the phytoplankton community was explained when our analyses were based on CHEMTAX (up to 87%). Such a high percentage in the CHEMTAX-based analysis suggests that the software achieves a comprehensive picture of the phytoplankton community including small taxa, which can be fitted along environmental gradients. However, microscopy analyses were crucial in our study with regard to assessing the terminal state of the diatom bloom off Coffs Harbour. Even though a large percentage of the variability in the phytoplankton community composition was explained in our datasets, a fraction of the variability still remained unexplained. Environmental variables that were not addressed in our study were, for example, irradiance, turbidity, additional nutrients (e.g. iron), and predation, and should be included in future studies.

### **4.4 Global importance of our findings**

We provided important information on how the oceanographic environment drives cross-shelf phytoplankton community structures including pico-, nano- and microphytoplankton. Such information has been missing along the Australian coast. Previous investigations have shown that individual phytoplankton taxa inhabit specific marine niches characterised by gradients in turbulence, nutrient accessibility and irradiance in a seasonal context (Balch, 2004; Margalef, 1978; Wyatt, 2014). Our study shows that such habitat preferences are also expressed on small scales, i.e. across the shelf, where gradients of environmental variables occur within relatively short distances from the coast. A better understanding of the preferences of individual phytoplankton taxa for specific environmental variables in natural systems will help us predict and monitor community changes expected due to climate change-induced modifications in the oceanographic environment. For example, the preference of cyanobacteria-2 (*Synechococcus* sp.) for warm, oligotrophic, stratified offshore waters in both our case studies suggests that this taxon might be favoured under the continuous increase in sea surface temperature and stratification (Morán et al., 2010). Along the east Australian coast, an increase in the abundance of *Synechococcus* at the expense of diatoms (Thompson et al., 2009) might decrease export production.

However, this is speculative and long-term research will be necessary to make any statements regarding climate change-induced changes in phytoplankton communities and distribution along the Australian coastline or elsewhere.

## 5 Conclusions

Despite differences in the oceanographic conditions, topography and seasonality in the Coffs Harbour and Kimberley regions we found that most phytoplankton taxa responded to the same combination of environmental variables indicating taxon-specific adaptations to specific marine niches across the shelf. Changes in the community structure from a microphytoplankton-dominated inshore community to a picophytoplankton-dominated offshore community were more pronounced across the wide Kimberley shelf (~200 km) than across the narrow Coffs Harbour shelf (~30 km). Off Coffs Harbour, post-bloom nutrient depletion caused by a shelf-scale diatom bloom in its terminal stage led to the re-distribution of dinoflagellates and pelagophytes to zones of local nutrient maxima (inshore). In both case studies, the complementary application of microscopy and CHEMTAX was highly suitable to investigate natural phytoplankton communities including all size-classes. Our study fills a void regarding the habitat preferences of individual phytoplankton taxa in shelf-systems along the Australian coast and will benefit future studies investigating phytoplankton dynamics under long-term changing oceanographic conditions.

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**Supplementary Material Table 1. List of microscopically determined phytoplankton taxa in the Coffs Harbour and Kimberley regions.** Also given are abbreviations as used in Figures 3 and 5 and the assigned pigment-types for each species following Armbrrecht et al., a (under review), and references therein. For exact cell numbers for each species in each sample in the Coffs Harbour and Kimberley regions please see <http://catalogue.aodn.org.au/geonetwork/srv/en/metadata.show?uuid=f7502841-a2c7-4437-b557-20ef89e754e0> and Thompson and Bonham (2011), respectively.

	Phytoplankton taxa determined in the Coffs Harbour region	Pigment type	Abbreviation	Phytoplankton taxa determined in the Kimberley region	Pigment type	Abbreviation
Diatoms	<i>Amphora</i> spp.	1	Ao	<i>Amphora</i> sp.	1	Ao
	<i>Asterionellopsis glacialis</i>	1	Ag	<i>Bacteriastrum</i> spp.	1	B
	<i>Asteromphalus</i> spp.	1	As	<i>Chaetoceros</i> spp. <10µm	1	C<10
	<i>Bacteriastrum</i> spp.	1	B	<i>Chaetoceros</i> spp. >10µm	1	C>10
	<i>Ceratoneis closterium</i> / <i>Nitzschia longissima</i>	4	CcNI	<i>Climacodium</i> sp.	1	Cli
	<i>Cerataulina pelagica</i>	1	Cp	<i>Cocconeis</i> spp.	2	Coc
	<i>Chaetoceros</i> spp. Hyalochaete	1	CH	<i>Coscinodiscus</i> spp.	1	Cos
	<i>Chaetoceros</i> spp. Phaeoceros	1	CP	<i>Dactyliosolen</i> spp.	2	Dac
	<i>Climacodium frauenfeldianum</i>	1	Clif	<i>Diploneis</i> sp.	4	Dip
	<i>Dactyliosolen fragilissimus</i>	2	Dacf	<i>Fragilariopsis</i> spp.	2	F
	<i>Diploneis</i> spp.	4	Dip	<i>Gossleria tropica</i>	2	Gt
	<i>Ditylum brightwellii</i>	1	Db	<i>Grammatophora</i> sp.	1	Gr
	<i>Eucampia</i> spp.	1	E	<i>Guinardia</i> spp.	2	G
	<i>Guinardia</i> spp.	2	G	<i>Hemiaulus</i> spp.	1	Hem
	<i>Helicotheca tamesis</i>	1	Ht	<i>Leptocylindrus</i> spp.	2	Lep
	<i>Hemiaulus membranaceus</i>	1	Hemm	<i>Lithodesmium</i> sp.	1	Lit
	<i>Lauderia annulata</i> / <i>Thalassiosira</i> spp.	1	LThs	<i>Navicula</i> spp.	4	Nv
	<i>Leptocylindrus danicus</i>	2	Lepd	<i>Nitzschia closterium</i> (now <i>Ceratoneis closterium</i> )	4	Cc
	<i>Navicula</i> spp.	4	Nv	<i>Planktoniella sol</i>	1	Plk
	<i>Nitzschia/Lioloma/Thalassiothrix</i> spp.	4	NLT	<i>Pleurosigma</i> spp.	1	Pleu
	<i>Proboscia alata</i>	2	Pa	<i>Porosira</i> sp.	1	Por
	<i>Pseudo-nitzschia</i> spp.	2	PN	<i>Proboscia alata</i>	2	Pa
	<i>Rhizosolenia</i> spp.	2	Rhi	<i>Pseudo-nitzschia</i> spp.	2	PN
	<i>Thalassionema nitzschioides/frauenfeldii</i>	2	Thnnf	<i>Rhizosolenia</i> spp.	2	Rhi
	Undefined centric diatom	1	uCD	<i>Skeletonema</i> spp.	1	Ske
	Undefined pennate diatom <40µm	2	uPD<40	<i>Synedra</i> sp.	2	Syn
	Undefined pennate diatom >40µm	2	uPD>40	<i>Thalassionema</i> spp.	2	Thn
				<i>Thalassiosira</i> spp.	1	Ths
				<i>Trigonium</i> spp.	1	Tri
Dinoflagellates	cf. <i>Alexandrium/Gonyaulax</i> / <i>Heterocapsa</i> spp.	1	AGH	Dinoflagellates 6 - 8 µm	1	uD6-8
	<i>Ceratium lineatum</i>	1	Cerl	<i>Amphidinium</i> spp.	1	Ai
	<i>Dinophysis acuminata</i>	1	Dina	<i>Ceratium</i> spp.	1	Cer
	<i>Gymnodinium</i> spp.	1	Gym	<i>Cochlodinium</i> sp.	1	Co
	<i>Gyrodinium</i> spp.	2	Gyr	<i>Dinophysis</i> spp.	1	Din
	<i>Karlodinium</i> spp.	2	Kar	<i>Gymnodinium</i> spp.	1	Gym
	<i>Noctiluca scintillans</i>	1	Ns	<i>Gyrodinium</i> spp.	2	Gyr
	<i>Oxytoxum</i> spp.	1	Oxy	<i>Ornithocercus</i> sp.	1	Orn
	<i>Phalacroma</i> spp.	1	Ph	<i>Oxytoxum</i> sp.	1	Oxy
	<i>Pronoctiluca</i> spp.	1	Prn	<i>Prorocentrum</i> spp.	1	Prc
	<i>Prorocentrum</i> spp.	1	Prc	<i>Protoperidinium</i> spp.	2	Prt
	<i>Protoperidinium</i> spp.	2	Prt	<i>Scrippsiella</i> sp.	1	Scr
	<i>Scrippsiella trochoidea</i>	1	Scr			
	<i>Torodinium</i> spp.	1	Tor			
	<i>Warnowia polyphemus</i>	1	Wp			
	Undefined dinoflagellate	1	uD			
Others				Cyanobacteria ( <i>Trichodesmium</i> )		Trch
				Chrysophytes		Chrys
				Cryptophytes		Cryp
				Prasinophytes		Pras
				Prymnesiophytes		Prym
				Flagellates 3 - 10 µm		uF3-10
				<i>Mesodinium rubrum</i>		Mr

**Supplementary Material Table 2. Initial and optimised biomarker pigment:Chl *a* ratios for the Coffs Harbour region.** The initial ratio matrix (a) and the ratio limit matrix (b) were created following S. Wright (unpublished; see text). For diatoms-4, we additionally considered the Fuco:Chl *a* ratio of *Ceratoneis closterium* (previously *Nitzschia closterium*) given in Stauber and Jeffrey (1988). Ratios for chrysophytes were adapted from Mackey et al. (1996). c) Optimised ratios for complete Coffs Harbour HPLC sample set (n = 18) after one CHEMTAX run. d - g) Optimised ratios for each subset (Transect: B-line and SS or CH-Line, sampling depth: surface or deep Chl *a* maximum, DCM) after one to eight CHEMTAX run following Latasa, (2007) using ratio matrix (c) as starting ratio. Ratios below/exceeding the reasonable literature range are indicated by italic/bold font, respectively (this excludes chrysophytes as no ranges were given in Higgins et al., 2011). For pigment abbreviations see Jeffrey et al. (2005a).

Class / Pigment	Chl <i>c</i> <sub>3</sub>	Chl <i>c</i> <sub>2</sub>	Chl <i>c</i> <sub>1</sub>	Peridinin	19'-Butanoyloxy-fuco-xanthin	Fuco-xanthin	Neo-xanthin	Prasino-xanthin	19'-Hexanoyloxy-fuco-xanthin	Allo-xanthin	Zea-xanthin	Chl <i>b</i>	Chl <i>a</i>
a) Initial input ratio matrix for the Coffs Harbour region following S. Wright (unpublished)													
Cryptophytes	0.000	0.169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.359	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.362	0.000	1
Diatoms-1	0.000	0.061	0.017	0.000	0.000	0.571	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.065	0.221	0.000	0.000	0.000	1.025	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4	0.000	0.067	0.000	0.000	0.000	0.597	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.226	0.000	0.541	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	0.159	0.222	0.000	0.000	0.006	0.066	0.000	0.000	0.236	0.000	0.000	0.000	1
Haptophytes-8	0.082	0.146	0.000	0.000	0.108	0.124	0.000	0.000	0.597	0.000	0.000	0.000	1
Pelagophytes	0.220	0.000	0.000	0.000	0.547	0.660	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.051	0.145	0.000	0.000	0.084	0.368	1
b) Ratio limits matrix Coffs Harbour following S. Wright (unpublished)													
Cryptophytes	500	338	500	500	500	500	500	500	500	220	500	500	100
Cyanobacteria-2	500	500	500	500	500	500	500	500	500	500	476	500	100
Diatoms-1	500	508	338	500	500	299	500	500	500	500	500	500	100
Diatoms-2	409	170	500	500	500	124	500	500	500	500	500	500	100
Diatoms-4	500	559	500	500	500	519	500	500	500	500	500	500	100
Dinoflagellates-1	500	251	500	190	500	500	500	500	500	500	500	500	100
Haptophytes-6	144	153	500	500	126	1097	500	500	638	500	500	500	100
Haptophytes-8	390	198	500	500	252	1130	500	500	239	500	500	500	100
Pelagophytes	142	500	500	500	228	208	500	500	500	500	500	500	100
Prasinophytes-3	500	500	500	500	500	500	281	606	500	500	420	281	100
c) Optimised ratio matrix after one CHEMTAX run on complete Coffs Harbour HPLC data set (n = 18)													
Cryptophytes	0.000	0.172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.350	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.372	0.000	1
Diatoms-1	0.000	0.151	<b>0.098</b>	0.000	0.000	0.373	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.080	0.216	0.000	0.000	0.000	0.731	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4	0.000	0.078	0.000	0.000	0.000	0.520	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.152	0.000	0.701	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	<b>0.429</b>	<b>0.612</b>	0.000	0.000	<b>0.017</b>	0.152	0.000	0.000	0.063	0.000	0.000	0.000	1
Haptophytes-8	0.094	0.115	0.000	0.000	0.107	0.098	0.000	0.000	0.765	0.000	0.000	0.000	1
Pelagophytes	0.181	0.000	0.000	0.000	0.480	0.884	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.167	0.000	0.000	0.021	0.800	1
d) Optimised ratio matrix based on surface samples of B-Line and SS (n = 5) after two CHEMTAX runs													
Cryptophytes	0.000	0.215	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.462	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.241	0.000	1
Diatoms-1	0.000	0.166	<b>0.135</b>	0.000	0.000	0.214	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.077	0.253	0.000	0.000	0.000	<i>0.457</i>	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4		0.172	0.000	0.000	0.000	0.772	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.193	0.000	0.756	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	<b>0.334</b>	0.251	0.000	0.000	<b>0.009</b>	0.214	0.000	0.000	0.039	0.000	0.000	0.000	1
Haptophytes-8	0.044	0.161	0.000	0.000	0.064	0.083	0.000	0.000	0.760	0.000	0.000	0.000	1
Pelagophytes	0.183	0.000	0.000	0.000	0.265	0.323	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.096	0.258	0.000	0.000	<i>0.009</i>	<b>1.296</b>	1
e) Optimised ratio matrix based on DCM samples of B-Line and SS (n = 5) after one CHEMTAX run													
Cryptophytes	0.000	0.138	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.320	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.328	0.000	1
Diatoms-1	0.000	0.162	<b>0.112</b>	0.000	0.000	0.468	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.097	0.168	0.000	0.000	0.000	0.930	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4		0.098	0.000	0.000	0.000	0.364	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.198	0.000	0.857	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	<b>0.468</b>	<b>0.723</b>	0.000	0.000	<b>0.014</b>	0.165	0.000	0.000	0.067	0.000	0.000	0.000	1
Haptophytes-8	0.052	0.063	0.000	0.000	0.044	0.071	0.000	0.000	0.351	0.000	0.000	0.000	1
Pelagophytes	0.218	0.000	0.000	0.000	0.418	0.909	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.184	0.000	0.000	<i>0.005</i>	0.746	1

## Supplementary Material Table 2. Continued.

Class / Pigment	Chl $c_3$	Chl $c_2$	Chl $c_1$	Peridinin	19'- Butanoyloxy- fucoxanthin	Fuco- xanthin	Neo- xanthin	Prasino- xanthin	19'- Hexanoyloxy- fucoxanthin	Allo- xanthin	Zea- xanthin	Chl $b$	Chl $a$
f) Optimised ratio matrix based on surface samples of CH-Line (n = 4) after eight CHEMTAX runs													
Cryptophytes	0.000	0.211	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.587	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.390	0.000	1
Diatoms-1	0.000	0.178	<b>0.201</b>	0.000	0.000	0.272	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.211	0.213	0.000	0.000	0.000	0.719	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4		0.068	0.000	0.000	0.000	0.330	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.138	0.000	0.905	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	<b>0.268</b>	<b>0.606</b>	0.000	0.000	<b>0.012</b>	0.173	0.000	0.000	0.042	0.000	0.000	0.000	1
Haptophytes-8	0.104	0.223	0.000	0.000	0.039	0.092	0.000	0.000	0.429	0.000	0.000	0.000	1
Pelagophytes	0.158	0.000	0.000	0.000	0.587	1.123	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.051	0.032	0.000	0.000	6.9E-06	<b>3.309</b>	1
g) Optimised ratio matrix based on DCM samples of CH-Line (n = 4) after seven CHEMTAX runs													
Cryptophytes	0.000	0.187	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.367	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.260	0.000	1
Diatoms-1	0.000	<b>0.424</b>	<b>0.253</b>	0.000	0.000	0.447	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.149	0.699	0.000	0.000	0.000	0.459	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4	0.000	<b>0.041</b>	0.000	0.000	0.000	0.452	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.136	0.000	0.612	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	<b>0.663</b>	0.192	0.000	0.000	<b>0.017</b>	0.160	0.000	0.000	0.045	0.000	0.000	0.000	1
Haptophytes-8	0.055	0.102	0.000	0.000	0.137	0.085	0.000	0.000	1.307	0.000	0.000	0.000	1
Pelagophytes	0.293	0.000	0.000	0.000	0.541	0.624	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.093	0.000	0.000	1.6E-06	0.314	1



**Supplementary Material Table 3. Initial and optimised biomarker pigment:Chl a ratios for the Kimberley region.** The initial ratio matrix (a) and the ratio limit matrix (b) were created following S. Wright (unpublished; see text). For diatoms-4, we additionally considered the Fuco:Chl *a* ratio of *Ceratoneis closterium* (previously *Nitzschia closterium*) given in Stauber and Jeffrey (1988). Ratios for chrysophytes were adapted from Mackey et al. (1996). c) Optimised ratios for complete Kimberley HPLC sample set (n = 20) after one CHEMTAX run. d - g) Optimised ratios for each subset (Transect: A or C, sampling depth: surface or deep Chl *a* maximum, DCM) after one CHEMTAX run using ratio matrix (c) as starting ratio. Ratios below/exceeding the reasonable literature range are indicated by italic/bold font, respectively (this excludes chrysophytes as no ranges were given in Higgins et al., 2011). For pigment abbreviations see Jeffrey et al. (2005a).

Class / Pigment	Chl <i>c</i> <sub>3</sub>	Chl <i>c</i> <sub>2</sub>	Chl <i>c</i> <sub>1</sub>	Peridinin	19'- Butanoyloxy- fucoxanthin	Fuco- xanthin	Neo- xanthin	Prasino- xanthin	19'- Hexanoyloxy- fucoxanthin	Allo- xanthin	Zea- xanthin	Divinyl Chl <i>b</i>	Chl <i>b</i>	Chl <i>a</i>
a) Initial input ratio matrix for the Kimberley region following S. Wright (unpublished)														
Chrysophytes	0.000	0.000	0.000	0.000	0.366	0.976	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Cryptophytes	0.000	0.169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.359	0.000	0.000	0.000	1
Cyanobacteria-1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.059	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.362	0.000	0.000	1
Cyanobacteria-4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.224	0.495	0.000	1
Diatoms-1	0.000	0.061	0.017	0.000	0.000	0.571	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.065	0.221	0.000	0.000	0.000	1.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4	0.000	0.067	0.000	0.000	0.000	0.597	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.226	0.000	0.541	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	0.159	0.222	0.000	0.000	0.006	0.066	0.000	0.000	0.236	0.000	0.000	0.000	0.000	1
Pelagophytes	0.220	0.000	0.000	0.000	0.547	0.660	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.051	0.145	0.000	0.000	0.084	0.000	0.368	1
b) Ratio limits matrix Kimberley following S. Wright (unpublished)														
Chrysophytes	500	500	500	500	500	500	500	500	500	500	500	500	500	100
Cryptophytes	500	338	500	500	500	500	500	500	500	220	500	500	500	100
Cyanobacteria-1	500	500	500	500	500	500	500	500	500	500	1469	500	500	100
Cyanobacteria-2	500	500	500	500	500	500	500	500	500	500	476	500	500	100
Cyanobacteria-4	500	500	500	500	500	500	500	500	500	500	447	707	500	100
Diatoms-1	500	508	338	500	500	299	500	500	500	500	500	500	500	100
Diatoms-2	409	170	500	500	500	124	500	500	500	500	500	500	500	100
Diatoms-4	500	559	500	500	500	519	500	500	500	500	500	500	500	100
Dinoflagellates-1	500	251	500	190	500	500	500	500	500	500	500	500	500	100
Haptophytes-6	144	153	500	500	126	1097	500	500	638	500	500	500	500	100
Pelagophytes	142	500	500	500	228	208	500	500	500	500	500	500	500	100
Prasinophytes-3	500	500	500	500	500	500	281	606	500	500	420	500	281	100
c) Optimised ratio matrix after one CHEMTAX run on complete Kimberley HPLC data set (n = 20)														
Chrysophytes	0.000	0.000	0.000	0.000	0.305	1.215	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Cryptophytes	0.000	0.218	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.322	0.000	0.000	0.000	1
Cyanobacteria-1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.079	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.574	0.000	0.000	1
Cyanobacteria-4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.180	0.447	0.000	1
Diatoms-1	0.000	0.064	0.038	0.000	0.000	0.379	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.074	0.195	0.000	0.000	0.000	1.224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4	0.000	0.052	0.000	0.000	0.000	0.547	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.241	0.000	0.487	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	0.110	0.182	0.000	0.000	0.007	0.076	0.000	0.000	0.267	0.000	0.000	0.000	0.000	1
Pelagophytes	0.250	0.000	0.000	0.000	0.623	0.503	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.138	0.000	0.000	0.106	0.000	0.468	1
d) Optimised ratio matrix based on surface samples of Transect A (n = 5) after one CHEMTAX run														
Chrysophytes	0.000	0.000	0.000	0.000	0.418	0.477	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Cryptophytes	0.000	0.283	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.276	0.000	0.000	0.000	1
Cyanobacteria-1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.089	0.000	0.000	1
Cyanobacteria-4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.134	0.554	0.000	1
Diatoms-1	0.000	0.012	<b>0.058</b>	0.000	0.000	0.496	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.048	0.210	0.000	0.000	0.000	0.857	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4	0.000	0.049	0.000	0.000	0.000	0.472	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.288	0.000	0.372	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	0.070	0.077	0.000	0.000	0.007	0.050	0.000	0.000	0.925	0.000	0.000	0.000	0.000	1
Pelagophytes	0.205	0.000	0.000	0.000	0.583	0.433	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.041	0.147	0.000	0.000	0.100	0.000	0.345	1

## Supplementary Material Table 3. Continued.

Class / Pigment	Chl <i>c</i> <sub>3</sub>	Chl <i>c</i> <sub>2</sub>	Chl <i>c</i> <sub>1</sub>	Peridinin	19'- Butanoyloxy- fucoxanthin	Fuco- xanthin	Neo- xanthin	Prasino- xanthin	19'- Hexanoyloxy- fucoxanthin	Allo- xanthin	Zea- xanthin	Divinyl Chl <i>b</i>	Chl <i>b</i>	Chl <i>a</i>
e) Optimised ratio matrix based on DCM samples of Transect A (n = 5) after one CHEMTAX run														
Chrysophytes	0.000	0.000	0.000	0.000	0.294	0.348	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Cryptophytes	0.000	0.219	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.275	0.000	0.000	0.000	1
Cyanobacteria-1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.866	0.000	0.000	1
Cyanobacteria-4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.124	0.708	0.000	1
Diatoms-1	0.000	0.025	<b>0.233</b>	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.033	0.238	0.000	0.000	0.000	0.939	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4	0.000	0.043	0.000	0.000	0.000	0.369	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.336	0.000	0.409	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	0.065	0.126	0.000	0.000	0.006	0.070	0.000	0.000	<b>1.769</b>	0.000	0.000	0.000	0.000	1
Pelagophytes	<b>0.530</b>	0.000	0.000	0.000	0.906	0.202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.117	0.000	0.000	0.084	0.000	0.628	1
f) Optimised ratio matrix based on surface samples of Transect C (n = 5) after one CHEMTAX run														
Chrysophytes	0.000	0.000	0.000	0.000	0.212	1.630	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Cryptophytes	0.000	0.287	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.349	0.000	0.000	0.000	1
Cyanobacteria-1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.074	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.823	0.000	0.000	1
Cyanobacteria-4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.189	0.308	0.000	1
Diatoms-1	0.000	0.019	<b>0.183</b>	0.000	0.000	0.305	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.051	0.158	0.000	0.000	0.000	<b>1.426</b>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4	0.000	0.059	0.000	0.000	0.000	0.719	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.318	0.000	0.649	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	0.158	0.155	0.000	0.000	<b>0.010</b>	0.108	0.000	0.000	0.610	0.000	0.000	0.000	0.000	1
Pelagophytes	0.191	0.000	0.000	0.000	1.046	0.360	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.114	0.000	0.000	0.136	0.000	0.570	1
g) Optimised ratio matrix based on DCM samples of Transect C (n = 5) after one CHEMTAX run														
Chrysophytes	0.000	0.000	0.000	0.000	0.325	1.106	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Cryptophytes	0.000	0.181	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.331	0.000	0.000	0.000	1
Cyanobacteria-1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.059	0.000	0.000	1
Cyanobacteria-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.615	0.000	0.000	1
Cyanobacteria-4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.180	0.403	0.000	1
Diatoms-1	0.000	0.014	<b>0.229</b>	0.000	0.000	0.476	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-2	0.059	0.176	0.000	0.000	0.000	<b>1.459</b>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Diatoms-4	0.000	0.040	0.000	0.000	0.000	0.586	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Dinoflagellates-1	0.000	0.255	0.000	0.627	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Haptophytes-6	<b>0.302</b>	0.215	0.000	0.000	<b>0.016</b>	0.125	0.000	0.000	0.828	0.000	0.000	0.000	0.000	1
Pelagophytes	0.288	0.000	0.000	0.000	1.184	0.196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
Prasinophytes-3	0.000	0.000	0.000	0.000	0.000	0.000	0.061	0.124	0.000	0.000	0.104	0.000	0.647	1

## Chapter 6

Interactions between seasonality and oceanic forcing drive the phytoplankton variability in the tropical-temperate transition zone (~30°S) of Eastern Australia

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**Key Words:** Time-series survey; Coffs Harbour; Water-types; East Australian Current; Diatoms; *Trichodesmium erythraeum*.

**Abbreviations:** MLD = Mixed layer depth; MDS = non-metric Multidimensional Scaling; DistLM = Distance based Linear Model.

## **Abstract**

The East Australian Current (EAC) has been shown to be warming rapidly, which is expected to cause latitudinal shifts in phytoplankton abundance, distribution and composition along the east Australian coast. Yet a lack of phytoplankton information exists northward of 34°S. Here, we provide the first detailed taxonomic time-series survey (monthly sampling for about one annual cycle, 2011 - 2012) in the east Australian tropical-temperate transition zone (~30°S, upstream of the EAC separation point at ~32°S). All phytoplankton (categorised depending on their association with specific water-types) showed a seasonal signal with abundance maxima (minima) during summer (winter). This seasonal signal was most pronounced in the seasonal/bloom category and least expressed by deep-water taxa, which preferred cold, saline and dense bottom water independent of the season. Different extents of EAC encroachment onto the continental shelf drove the cross-shelf phytoplankton composition and distribution, such that a weak EAC was associated with the phytoplankton community being organised along 'depth' and 'distance from the coast' gradients and a strong EAC favoured the occurrence of warm-water taxa offshore. We conclude that the phytoplankton community in the tropical-temperate transition zone of Eastern Australia is driven by an interaction of intrinsic seasonal cycles and primarily EAC-driven oceanic forcing. Our findings benefit studies located in Western Boundary Current systems worldwide, in which warming and strengthening of these currents are predicted to severely impact phytoplankton dynamics.

## **1 Introduction**

Global Western Boundary Currents (WBCs) are being modified as a result of the present anthropogenically-induced climate change (Wu et al., 2012; IPCC, 2013). Specific changes include the surface warming of these currents at a two to three times faster rate than the global mean ocean surface warming, as well as an intensification and/or poleward shifts of the extent of the WBCs (Wu et al., 2012). Such modifications in ocean circulation, and associated alterations of physical and chemical properties of the ocean itself, are expected to affect phytoplankton distribution and community structure worldwide (Hallegraeff, 2010). Predicted climate-change induced long-term changes include a shift towards an earlier onset of annual phytoplankton blooms (peak production periods), changes in the marine food web structure, disruption of established seasonal succession patterns, poleward range expansions of tropical

species, enhanced stratification impeding on nutrient accessibility and an increase in the frequency of harmful algal blooms (Hays et al., 2005; Hallegraeff, 2010; Winder et al., 2012). Recent investigations suggest that such alterations in the marine ecosystem have already started as evidenced in a global decline of global net primary productivity (between 1996 and 2006; Behrenfeld et al., 2006) and phytoplankton biomass (over the last century; Boyce et al., 2010).

The East Australian Current (EAC) is the South Pacific WBC flanking the east Australian coast, transporting warm-water masses from tropical to temperate latitudes (Godfrey et al., 1980). During the past 60 years, the southward penetration of the EAC has extended, as evidenced by a warming trend of  $2.28^{\circ}\text{C century}^{-1}$  and an increase in salinity of  $0.34 \text{ psu century}^{-1}$  recorded at  $\sim 43^{\circ}\text{S}$  (Ridgway, 2007). These modifications in the EAC characteristics have been attributed to changes in basin-scale wind forcing influencing the Subtropical Gyre (Hill et al., 2008). Generally, the EAC flows southward with a speed of up to  $2 \text{ m s}^{-1}$  from its formation point at about  $10 - 15^{\circ}\text{S}$  until it bifurcates at  $\sim 32^{\circ}\text{S}$  into an eastward and southward component (Tasman Front and Southern Extension, respectively; Godfrey et al., 1980; Ridgway and Dunn, 2003). In addition to the poleward residual flow, cyclonic and anti-cyclonic eddies are regularly shed at the separation zone (Suthers et al., 2011; Ridgway and Hill, 2012). The strength of the EAC varies seasonally, reaching maximum velocities during the austral summer and being weakest in winter (Ridgway and Godfrey, 1997), although this seasonality is weak in shelf velocities (Wood, 2014). However, it seems that a clear relationship between EAC dynamics and large scale variations in surface chlorophyll *a* (Chl *a*) concentrations (a proxy for phytoplankton biomass) exists (Everett et al., 2014). For example, it has been shown that south of the separation zone ( $\sim 34^{\circ}\text{S}$ ), the combination of wind and current driven upwelling, both of which are at their maximum in summer, promotes nutrient input and phytoplankton growth and therefore controls the onset of the annual spring bloom (Hallegraeff and Jeffrey, 1993; Ajani et al., 2001; Pritchard et al., 2003).

Changes in the oceanographic environment seem to already impact on the phytoplankton community structure along the south-east Australian coast. For example, the warming and strengthening EAC has been associated with the recent poleward range expansions of several phytoplankton species, such as the red-tide dinoflagellate *Noctiluca scintillans* (Hallegraeff et al., 2008; McLeod et al., 2012) and several species of the dinoflagellate genus *Ceratium* (Buchanan et al., 2014). Thompson et al. (2009) reported increasing phosphate and nitrate versus decreasing

silicate concentrations off South-Eastern Australia (34°S) within the last 60 years. Considering the altered nutrient availability and the warming and strengthening of the EAC, diatoms were expected to decline and dinoflagellates to increase (Thompson et al., 2009). Yet a recent study of ten years of phytoplankton data found the opposite pattern (declining dinoflagellates and increasing diatoms; Ajani et al., 2014). However, the unexpected shift towards diatoms in the latter study seemed to be caused by a short-term (decadal) decline in water-column temperature, highlighting the sensitivity of phytoplankton to periodical fluctuations in water properties (particularly related to El Niño Southern Oscillations; Holbrook et al., 2011). When considered within long-term trends in EAC warming and overall silicate decline, the temporarily phytoplankton variability shown by Ajani et al. (2014) remains consistent with the predicted long-term decrease (increase) in diatom (dinoflagellate) abundance (Thompson et al., 2009). With diatoms playing a key role in primary production and the carbon cycle (Smetacek, 1999; Uitz et al. 2010) and dinoflagellates being the most frequent cause for harmful algal blooms (Hallegraeff, 2010), changes in their relative abundance patterns severely affect the local biogeochemical processes and economical values along the east Australian coast.

Phytoplankton research along the east Australian coast has mainly been conducted at the long-term sampling station Port Hacking (34°S) and a clear absence of modern phytoplankton investigations exists convening the region northward of the EAC separation zone. Upstream of the EAC separation point, in the Coffs Harbour region at about 30°S, upwelling has been shown to occur ~30% of the time (Schaeffer et al., 2013). Intense EAC driven upwelling (through encroachments of the current onto the shelf) occurs sporadically throughout the year with 1 - 8 current driven upwelling days estimated per month and the maximum number of such upwelling days generally occurring during the austral spring/summer (Rossi et al., 2014). Armbrrecht et al. (2014) provided the first study of insights into the cross-shelf responses of phytoplankton to different oceanographic conditions north of the EAC separation zone (~30°S). These authors showed that during an EAC-driven upwelling event, the major phytoplankton response was found on the mid-shelf and is constituted of primarily oceanic diatoms species. Simultaneously, an increase in species richness, mainly due to the appearance of tropical dinoflagellates, was found offshore, which is consistent with long-term predictions of EAC-driven dinoflagellate distribution (Thompson et al., 2009; Ajani et al., 2014).

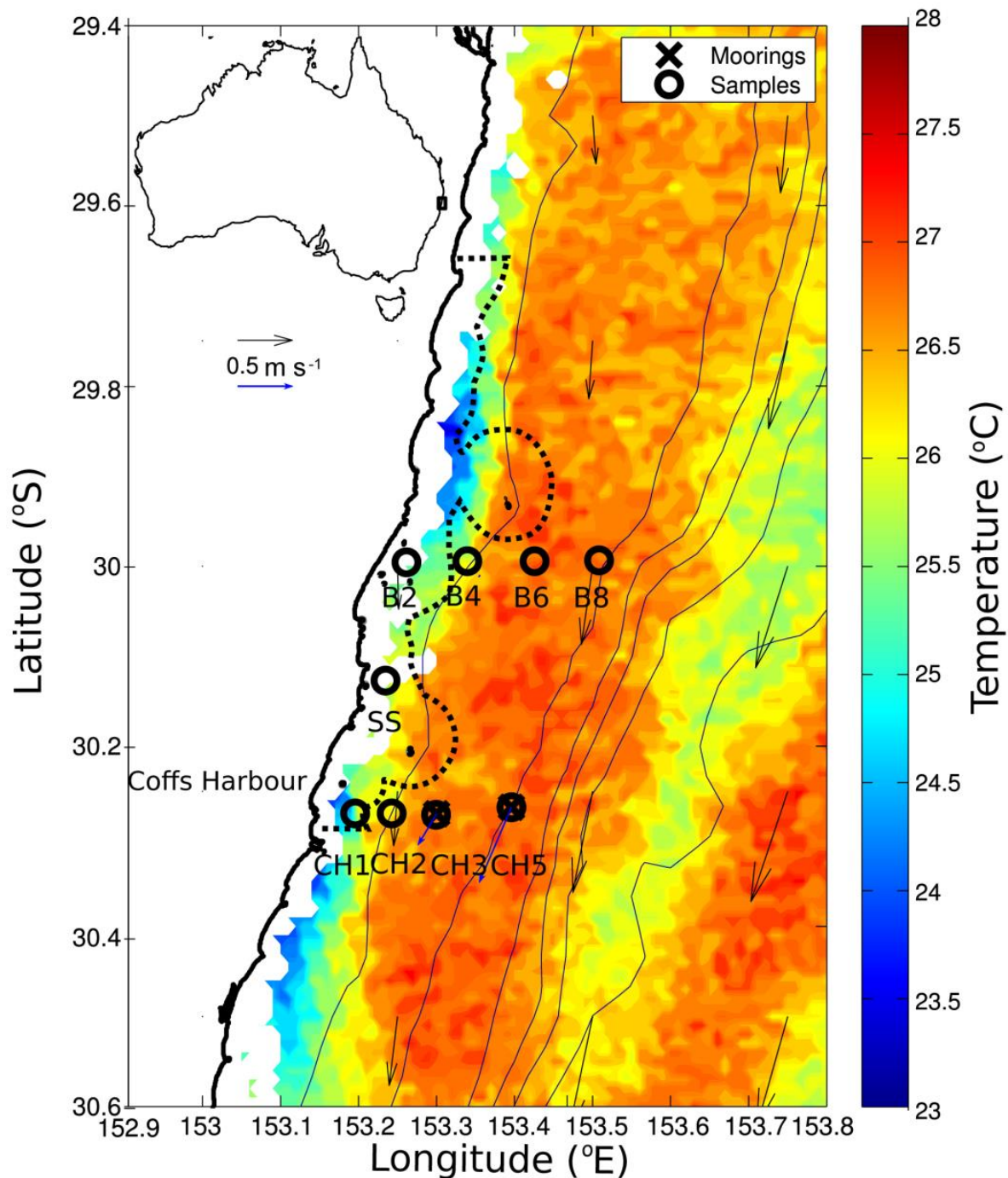
In this study we provide the first detailed taxonomic phytoplankton time-series survey in the east Australian tropical-temperate transition zone ( $\sim 30^\circ\text{S}$ ), covering about one annual cycle between 2011 and 2012. As the study location, Coffs Harbour, is under the influence of the main flow of the EAC, a wide range of tropical and temperate phytoplankton species is encompassed (Armbrecht et al., 2014). The specific aims of our study are to: (i) determine seasonal patterns and the natural variation and in the phytoplankton abundance, composition and distribution in the course of nearly one year; (ii) determine the impact of the EAC encroachment onto the shelf on the cross-shelf phytoplankton composition, in particular investigate the link between tropical input of phytoplankton species into the study area and EAC influence. A statistical approach is applied to pursue these two aims and the importance of the results is discussed within a regional and global context.

## 2 Materials and methods

### 2.1 Sampling design and instrumentation

Monthly sampling was undertaken at the 25, 50, 70 and 100 m isobaths along two cross-shelf transects and one intermediate station off the east coast of Australia between May 2011 and February 2012 with one extra sampling in September 2012 (Fig. 1, Table 1). The cross-shelf transects, henceforth identified as the CH-line (south) and the B-line (north), were about 30 km apart and extended  $\sim 26$  km and  $\sim 28$  km offshore, respectively (Fig. 1). An intermediate station (SS) is located about 3 km off the coast near Southwest Solitary (Groper) Island. All three inshore stations are located within a marine park (Solitary Islands Marine Park, Fig. 1).

As part of the NSW Integrated Marine Observing System (IMOS), stations CH3 and CH5 were also the site of long-term *in-situ* oceanographic moorings (CH070 and CH100, respectively, Roughan et al., 2010, 2013; Schaeffer et al., 2013). These moorings recorded temperature in 8 m distance intervals from the bottom to about 15 m below the sea surface. Current measurements from the bottom-mounted Acoustic Doppler Current Profiler (ADCP) were provided at 4 m bins in the vertical. Daily depth-averaged along-shelf velocity was used as a proxy for the EAC encroachment onto the shelf during each sampling.



**Figure 1. Map of sampling locations (B-Line, SS, CH-Line) off Coffs Harbour (~30°S).** Insert showing sea surface temperature (GHRSSST from NOAA AVHRR products) and geostrophic current velocity vectors from altimetry (NOAA provided by the Commonwealth Scientific and Industrial Research Organisation; black arrows) and from the IMOS moorings (blue arrows) on 25 January 2012. Black circles indicate sampling locations; crossed circles indicate IMOS moorings CH70 and CH100. The thick black line indicates the contour of the coastline while thin black lines indicate the 50, 100, 250, 500, 1000, 2000 m isobaths. The dashed black line indicates the offshore delimitation of Solitary Islands Marine Park.



## **2.2 Hydrographic sampling**

Sampling was either undertaken on the *RV Bombora* (NSW Office of Environment and Heritage, OEH, vessel) or on the *RV Circe* (provided by the National Marine Science Centre, NMSC, Coffs Harbour) (Table 1).

On the *RV Bombora* an automatic SBE32 carousel water sampler (Sea-Bird Electronics, Inc., USA) with 12 x 5 L Niskin-bottles (General Oceanics, USA) was deployed via an A-Frame. The CTD rosette was fitted with a SBE 911*plus* Conductivity-Temperature-Depth (CTD) profiler (Seabird Electronics, Inc., USA) and an ECO FLNTU fluorescence sensor (Wetlabs, Inc., USA). At each station, the CTD was allowed a surface soak of 3 minutes and then lowered to within 5 m of the seabed. The depth of the Chl *a* maximum was determined in real-time during the downward cast and sampled during the upward cast. In addition, water samples were taken in 20 m depth intervals, except at the shallow inshore stations where 10 m intervals were sampled, and one interval of 30 m between 60 and 90 m depth at CH5. Surface samples were collected in a 10 L plastic bucket. Cast start and finish times and positions were recorded, however as CTD casts lasted for a maximum of 20 minutes and boat drift with the mean current was negligible, we assumed that the CTD and rosette sampled the same water and refer to the start times and coordinates only.

On the *RV Circe*, Niskin bottles (5 L capacity, General Oceanics, USA) were attached to a rope manually at fixed intervals as described above. The rope and the bottles were lowered into the water with an electrical winch and after reaching the respective sampling depths the bottles were automatically shut by releasing messengers. Surface water was collected in a 10 L bucket as on the *Bombora*. One of three different CTD profilers were used depending on availability (Table 1), mounted at the end of the rope and before lowering a 3 minutes surface soak was allowed. Depending on the volume of water required at each station 1 - 2 casts were conducted, lasting for a maximum of ~45 minutes.

Data post-processing was conducted using the Seabird SBEData Processing software (Sea-Bird Electronics, Inc., USA) following IMOS CTD processing protocols (<http://imos.org.au/anmndocuments.html>).

**Table 1. Sampling dates and locations.** Also listed are the *Research Vessel (RV)* and the Conductivity-Temperature-Depth (CTD) profiler used. Asterisks (\*) indicate the Chl *a* maximum depths measured in real-time on *RV Bombora*. Accent (^) indicates samples that were excluded from multivariate analyses as no CTD data was recorded.

Date	Station ID	Sample depths (m)	Research Vessel, CTD	Date	Station ID	Sample depths (m)	Research Vessel, CTD
27 May 2011	CH3	0, 20, 40, 60	<i>RV Bombora</i> , <i>SBE911plus</i>	07 November 2011	CH1	0, 10, 20	
	CH1	0, 10, 21			CH2	0, 10, 20, 40	
28 May 2011	CH2	0, 25, 40			CH3	0, 10, 20, 40, 60	
	CH3	0, 20, 40, 69			CH5	0, 20, 40, 60, 90	
	CH5	0, 40, 60, 90			B2	0, 10, 20	
	B6	0, 25	<i>RV Bombora</i> , <i>SBE911plus</i>	08 November 2011	B4	0, 20, 40	
06 June 2011	B8	0, 20, 45*			B6	0, 20, 40, 60	
	SS^	0^			B8	0, 20, 40, 60, 90^	
	CH1	0, 12, 26			SS	0, 20	
07 June 2011	CH3	0, 15, 30, 50			B2	0, 10, 20	<i>RV Circe</i> , <i>SBE19+</i> <i>SEACAT</i> Profiler
	CH5	0, 20, 35, 62	<i>RV Circe</i> , <i>SBE25</i> Sealogger	06 December 2011	B4	0, 20, 40	
	CH1	0, 10, 20			B6	0, 20, 40, 60	
13 July 2011	CH2	0, 10, 25, 40			SS	0, 20	
	CH3	0, 10, 20, 40, 60		07 December 2011	CH1	0, 10, 20	
	CH5	0, 20, 40, 60, 90	<i>RV Circe</i> , <i>SBE25</i> Sealogger		CH2	0, 10, 20, 40	
	B2^	0^, 10^, 20^			CH3	0, 10, 20, 40, 60	
14 July 2011	B4	0, 40,			CH5	0, 20, 40, 60, 90	
	B6	0, 20, 40, 65			CH1	0, 10, 20	
	B8	0, 20, 40, 60, 80		24 January 2012	CH2	0, 10, 20, 40	
	SS	0^	<i>RV Bombora</i> , <i>SBE911plus</i>		CH3	0, 10, 20, 40, 60	
	CH1	0, 20			CH5	0, 20, 40, 60, 90	
09 August 2011	CH2	0, 20, 40,			B2	0, 10, 20	
	CH3	0, 20, 40, 60		27 February 2012	B4	0, 20, 40	
	CH5^	0^, 10^, 20^, 40^, 90^			B6	0, 20, 40, 60	<i>RV Bombora</i> , <i>SBE911plus</i>
	B2	0, 10, 20	<i>RV Bombora</i> , <i>SBE911plus</i>		B8	0, 20, 40, 60, 80	
06 September 2011	B4	0, 40			SS	0, 20	
	B6	0, 20, 40, 60		28 February 2012	CH1	0, 10, 20	
	B8	0, 20, 40			CH2	0, 10, 20, 40	
	SS	0, 20	<i>RV Bombora</i> , <i>SBE911plus</i>		CH3	0, 10, 20, 40, 60	
	CH1	0, 10, 20			CH5	0, 20, 40, 60, 90	
07 September 2011	CH2	0, 10, 20, 40			B2	0, 8*, 20	<i>RV Bombora</i> , <i>SBE911plus</i>
	CH3	0, 10, 20, 40, 60		11 September 2012	B4	0*, 20, 40	
	CH5	0, 20, 40, 60, 90			B6	0, 12*, 20, 40, 60	
	B2	0, 10, 20	<i>RV Circe</i> , <i>SBE19+</i> <i>SEACAT</i> Profiler		B8	0, 12*, 20, 40, 60, 80	
10 October 2011	B4	0, 20, 40			SS	0, 8*, 20	
	B6	0, 20, 40, 60		12 September 2012	CH1	0, 12*, 20	
	B8	0, 20, 40, 60, 80			CH2	0, 8*, 20, 40	
	SS	0, 20			CH3	0, 20, 40, 60	
	CH1	0, 10, 20			CH5	0, 20, 24*, 40, 60, 90	
12 October 2011	CH2	0, 10, 20, 40					
	CH3	0, 10, 20, 40, 60					
	CH5	0, 20, 40, 60, 90					

### 2.3 Phytoplankton (>10 µm) identification and enumeration

Subsamples of 2 L were fixed in plastic containers with 6 mL of Lugol's acid solution for later concentration by sedimentation (48 hrs), identification and enumeration under an inverted microscope. Phytoplankton enumeration was conducted following the Utermöhl method (Utermöhl, 1958). A minimum of 400 cells were counted per sample at 200x magnification and identification and enumeration was made at the lowest taxonomic level possible by an expert analyst. Identification was based on taxonomic guides by Dakin and Colefax (1940); Crosby and Wood (1958, 1959); Wood et al.

(1959); Wood (1954, 1961a, 1961b); Tomas (1997); Hallegraeff et al. (2010) and further studies by Hallegraeff and Reid (1986); Ajani et al. (2001); Gómez et al. (2008) and Stidolph et al. (2012).

As the purpose of this study was to determine functional phytoplankton groups that are characteristic for distinct water-types (for a definition of the term 'water-type' see section 2.4), species belonging to the same genus were grouped. In some cases morphologically similar taxa were grouped into a complex, such as the diatoms *Ceratoneis closterium/Nitzschia longissima*, *Lauderia annulata/Thalassiosira* spp., *Nitzschia/Lioloma/Synedra/Thalassiothrix* spp. and the dinoflagellates *Alexandrium/Gonyaulax/Heterocapsa* spp. If the genus could not be determined (e.g. due to overlying particles in the sample or degradation of distinct morphological structures) diatoms were classified as "undefined centric" or "undefined pennate" and dinoflagellates as "undefined dinoflagellates". A Calcofluor White Stain (Sigma Aldrich, USA) solution was added to each 3 mL sample 30 minutes prior to counting, at a final concentration of 20  $\mu\text{g mL}^{-1}$ , to facilitate identification of thecate dinoflagellates (Fritz and Triemer, 1985). Distinct *Gymnodinium* spp. and *Gyrodinium* spp. >20  $\mu\text{m}$  were included in the counts. Smaller individuals were difficult to distinguish from each other and from a large number of small unidentifiable flagellates, and were therefore excluded from the counts.

To quantify the abundance of the filamentous cyanobacterium *Trichodesmium erythraeum*, the average cell length of ten random filaments in a randomly picked sample was determined prior to counting (average = 7.5  $\mu\text{m}$ ; standard deviation = 1.9  $\mu\text{m}$ ). Subsequently (during counting), each filament was measured using an eyepiece micrometer and cell numbers of *T. erythraeum* were calculated by dividing the filament length by the average cell length.

Zooplankton (copepods and their larvae) including microzooplankton (ciliates, including tintinnids) were counted following the Utermöhl method in order to estimate the potential impact of grazing on the phytoplankton community. However, as the primary focus of this study is to provide a phytoplankton time-series survey, the total abundance of the zoo and micro-zooplankton is only used as a proxy of their potential predatory impact.

## 2.4 *Multivariate analyses*

In order to determine seasonal patterns in phytoplankton composition we conducted non-metric Multidimensional Scaling (MDS) on the  $\log(X+1)$  transformed phytoplankton abundance data (based on Bray-Curtis similarity and Kruskal-fit scheme 1) using PRIMER Version 6.1.12 (Clarke and Gorley, 2006).

To categorise phytoplankton taxa depending on their association with distinct water-types (where a water-type shall be defined as being characterised by one, or a combination of, abiotic environmental condition(s)/variable(s) within this study) we applied distance-based redundancy analysis following (Legendre and Anderson, 1999) via the Distance-based Linear Modelling (DistLM) procedure in PRIMER. Forward selection of the Akaike's Information Criterion were used to select the minimally adequate model (Armbrecht et al., 2014). Environmental variables included in the DistLM were month, temperature, salinity, density, distance from the coast, depth, mixed layer depth (MLD) and southward velocity. Months were integrated in the analyses by assigning each calendar month its respective number, e.g. January = 1, February = 2, etc. Temperature ( $^{\circ}\text{C}$ ), salinity (psu) and density ( $\text{kg m}^{-3}$ ) were recorded during CTD casts. Mixed layer depth (MLD, in m) was calculated for each station as the depth at which temperature < surface temperature – 0.5  $^{\circ}\text{C}$  (Levitus, 1982). In the case that a station was too shallow and no MLD could be determined, the deepest sampled depth at that station was used instead. EAC velocities ( $\text{m s}^{-1}$ ) on the shelf were derived from the moored ADCP measurements at the sampling depths of CH070 and CH100 on the respective sampling day. Measurements from CH070 and CH100 were extrapolated, assigning the velocities measured at CH070 to all inshore and mid-shelf stations (CH1 - CH3, SS, B2 - B6) and velocities measured at CH100 to the offshore stations (CH5 and B8). In the event that measurements were missing at a certain depth, the measurement at the closest sampling depth of the same station was used. As velocity measurements were missing between July and August 2011 at CH3, and May and August 2011 at CH5 due to instrument failure, the average velocity measured over all other sampling days at the respective station was used during these two months. Eleven samples had to be excluded from the DistLM due to missing environmental information (Table 1).

In order to determine seasonal phytoplankton abundance and composition patterns, and to define specific ranges of physical parameters associated with each category, we summarised the monthly- and depth-averaged abundance of each category and counted predators in temperature-salinity (T-S) plots. Monthly- and depth-averaged

abundance of the most abundant phytoplankton taxa (contributing on average >1% to the total phytoplankton community across all samples) were also summarised in T-S plots. In addition to establishing a visual overview of seasonal contributions by individual taxa to the phytoplankton community of Coffs Harbour, the plotting of individual taxa in T-S plots was also completed to verify the selection of individual taxa into water-type categories.

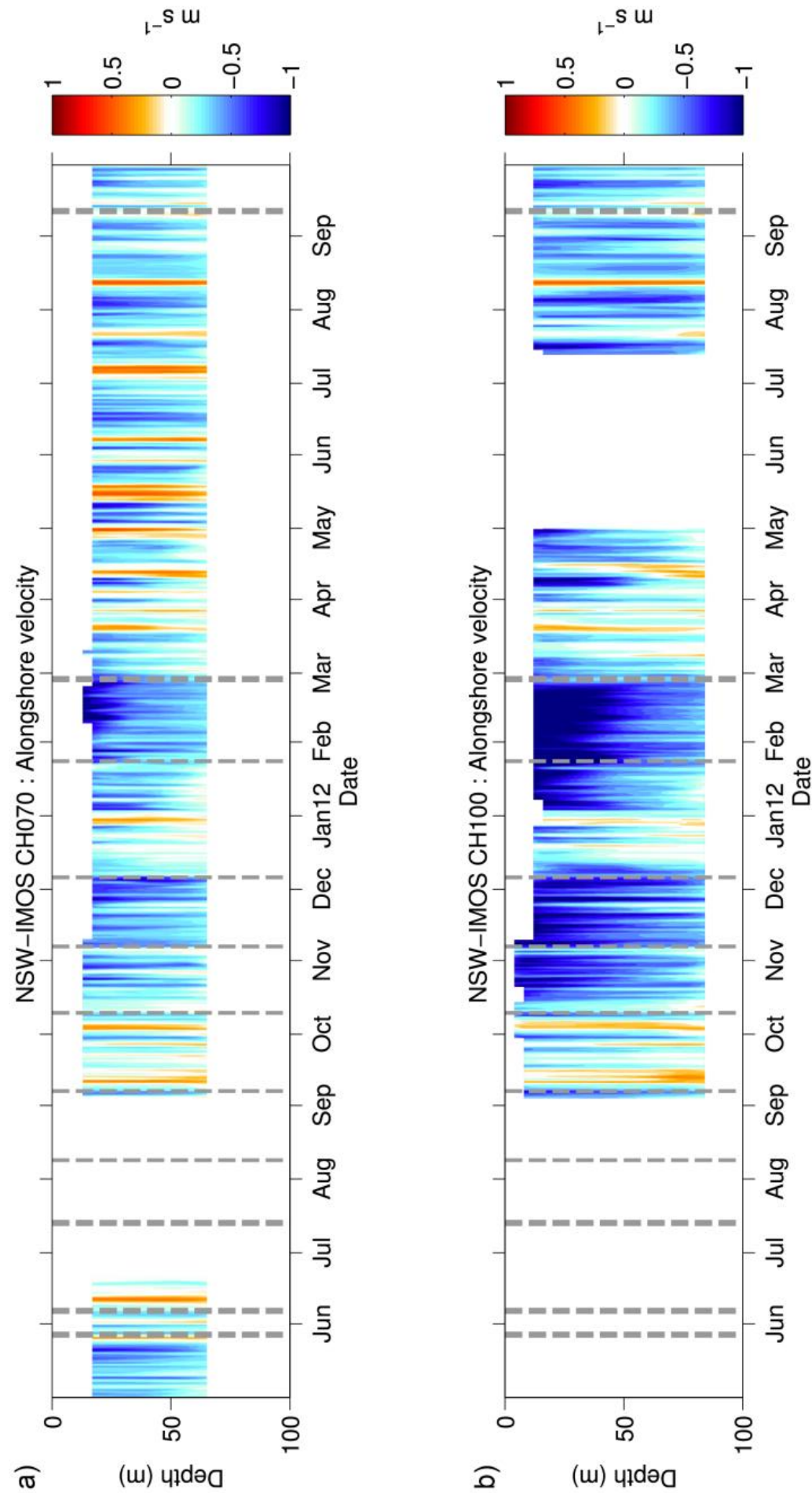
We investigated the influence of the EAC encroachment on the cross-shelf phytoplankton community structure by choosing the two months characterising the weakest and the strongest events of EAC influence (as determined by ADCP velocity data) and conducted an additional MDS and DistLM for each of these months (using PRIMER Version 6.1.12).

### 3 Results

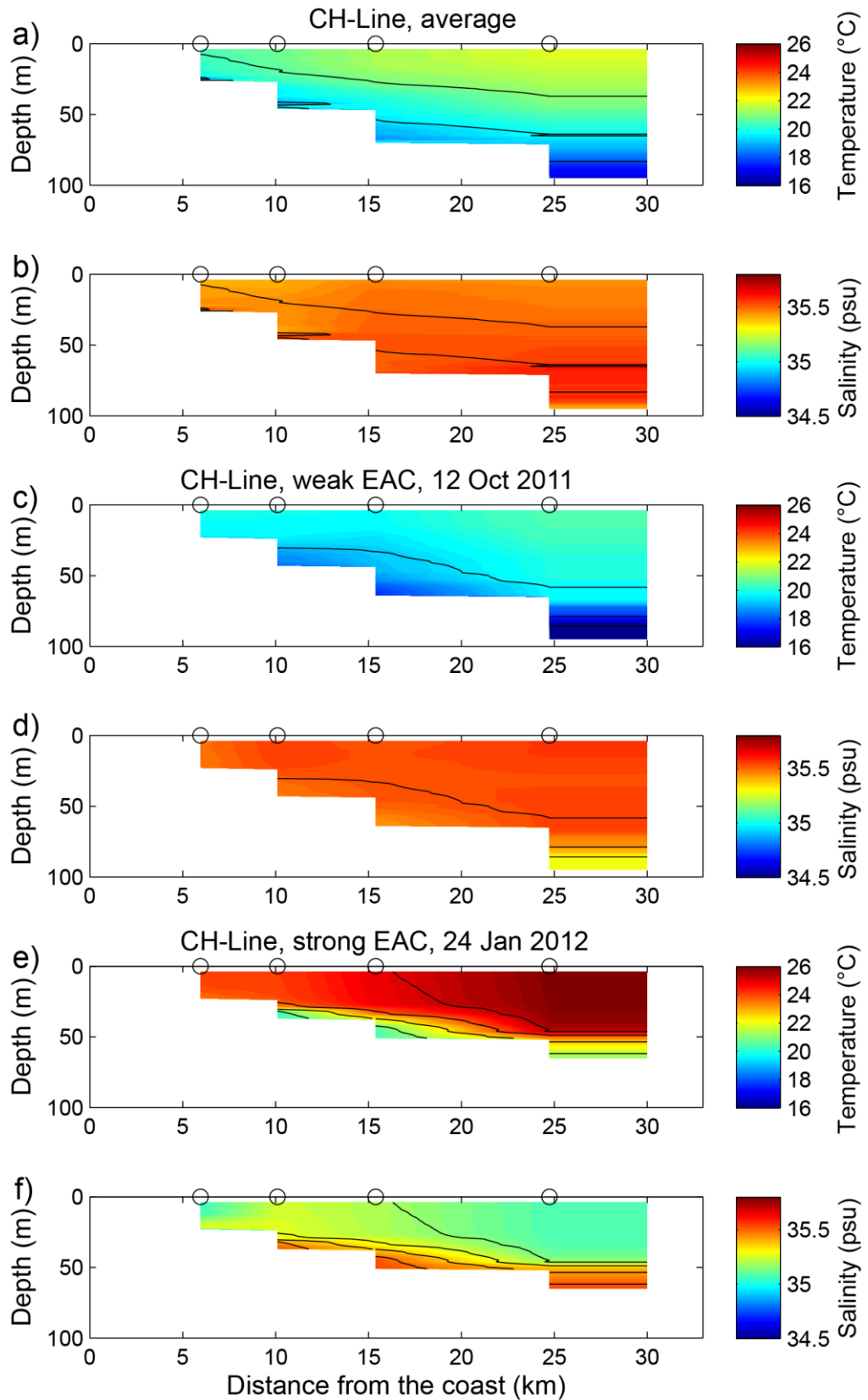
#### 3.1 Oceanography

The depth-averaged poleward along-shelf velocity over the 20 sampling days was  $0.24 \text{ m s}^{-1}$  and  $0.37 \text{ m s}^{-1}$  at CH070 and CH100, respectively (Fig. 2). The average temperature in the Coffs Harbour region across all sampling days, stations and depths was  $20.6^{\circ}\text{C} \pm 2^{\circ}\text{C}$  (Fig. 3a).

Weak current velocities occurred in October 2011, when the circulation on the shelf was less than half the mean, with poleward depth-averaged velocities of  $0.06 \text{ m s}^{-1}$  at CH070 and  $0.17 \text{ m s}^{-1}$  at CH100 (Fig. 2), and weak cross-shelf gradients of temperature, salinity and density (Fig. 3c, d). This scenario is chosen to characterise the weak EAC influence on the phytoplankton community (section 3.4). The strongest current velocities of all samplings occurred in January 2012, with depth-averaged velocities of  $0.34 \text{ m s}^{-1}$  at CH070 and  $0.77 \text{ m s}^{-1}$  at CH100 (Fig. 2). The intrusion of the EAC onto shelf is characterised by a warm, oligotrophic surface layer, resulting in an uplift of the isopycnals (Fig. 3e, f). This scenario represents the strong EAC influence on phytoplankton community composition (section 3.4).



**Figure 2. Current observations.** Along-shelf current velocity ( $\text{m s}^{-1}$ ) measured at the a) CH070 and b) CH100 moorings between 01 May 2011 - 30 September 2012. The grey dashed lines indicate the sampling dates. Positive (red) values indicate northward, negative (blue) values indicate southward velocities.



**Figure 3. Water properties.** Interpolated cross-shore sections (distance versus depth) of the CH-Line showing temperature (°C) and salinity (psu) (both including density contours,  $\text{kg m}^{-3}$ ) recorded during CTD casts. a) and b) averages across all samplings; c) and d) during weak EAC influence (12 October 2011); e) and f) during strong EAC influence (24 January 2012). Open circles at the surface indicate the locations of the CTD casts.

### 3.2 *Phytoplankton abundance and composition*

A total of 74 phytoplankton genera were determined throughout the complete study period, with 19 taxa (12 diatom taxa, six dinoflagellate taxa and *T. erythraeum*) occurring (across all samples) on average >1% (Table 2). Most abundant were *Leptocylindrus* spp. (18%, mainly *L. danicus* and very rarely *L. mediterraneus*), *Pseudo-nitzschia* spp. (14%), *T. erythraeum*, *Chaetoceros* spp. (Hyalochaete) and undefined pennate diatoms (each about 6%, Table 2). The 19 abundant taxa, except undefined pennate diatoms and undefined dinoflagellates, were selected as representatives of the water-type categories (section 3.4). A complete list of all phytoplankton genera, their abbreviations and average percent abundance across all samples including standard deviation is given in Table 2.

The exact abundances per sample of each individual taxon (of a total of 137 taxa) within the 74 genera and of zooplankton are publicly available at: <http://catalogue.aodn.org.au/geonetwork/srv/en/metadata.show?uuid=f7502841-a2c7-4437-b557-20ef89e754e0>. Total abundances of the sum of diatoms, dinoflagellates and silicoflagellates ranged between  $2.00 \times 10^2$  cells L<sup>-1</sup> (CH5, 90 m, July 2011) and  $1.94 \times 10^6$  cells L<sup>-1</sup> (CH1, 20 m, December 2011). *Trichodesmium erythraeum* occurred in abundances up to  $4.33 \times 10^5$  cells L<sup>-1</sup> (SS, 0 m, October 2011). Thus, low phytoplankton abundances were found in late autumn and winter and high abundances in spring and summer. Phytoplankton abundances generally decreased with increasing distance from the coast and sample depth. Diatoms dominated the phytoplankton community throughout all samplings. Zooplankton abundance reached  $1.75 \times 10^4$  specimens L<sup>-1</sup> (CH1, 0 m, October 2011) but remained below  $1 \times 10^3$  specimens L<sup>-1</sup> in most instances (with frequently being 0). High zooplankton abundances were found in surface samples during spring and summer (<http://catalogue.aodn.org.au/geonetwork/srv/en/metadata.show?uuid=f7502841-a2c7-4437-b557-20ef89e754e0>).



**Table 2. List of taxa included in each water-type category.** Note that although some taxa may be considered as belonging to more than one category (e.g. warm-water and offshore), they were grouped under one category only based on their ordination (see Fig. 4b). Also given are the abbreviations used in Figs. 4b – 7 and the proportional abundances (averaged across all samples) including standard deviations of each taxon. Abundances of taxa that occurred on average >1% are indicated in italic font.

Taxon	Abbreviation	Category	Average abundance (%)	Standard deviation (%)
Diatoms				
<i>Amphora</i> spp.	<i>Amp</i>	Deep	0.05	0.16
<i>Anaulus minutus</i>	<i>Am</i>	Deep	0.005	0.045
<i>Asterionellopsis glacialis</i>	<i>Ag</i>	Cosmopolitan	3.13	7.84
<i>Asteromphalus</i> spp.	<i>Ast</i>	Deep	0.03	0.10
<i>Bacteriastrum</i> spp.	<i>Bac</i>	Cosmopolitan	0.72	1.50
<i>Ceratoneis closterium</i> / <i>Nitzschia longissima</i>	<i>Cc/Nl</i>	Cosmopolitan	4.62	4.91
<i>Cerataulina pelagica</i>	<i>Cp</i>	Cosmopolitan	0.24	0.85
<i>Chaetoceros</i> spp. (Hyalochaete)	<i>ChH</i>	Cosmopolitan	6.49	9.79
<i>Chaetoceros</i> spp. (Phaeoceros)	<i>ChP</i>	Deep	0.60	1.01
<i>Climacodium frauenfeldianum</i>	<i>Cf</i>	Offshore	0.52	0.92
<i>Climacosphenia moniligera</i>	<i>Cm</i>	Deep	0.005	0.065
<i>Corethron</i> spp.	<i>Cor</i>	Cosmopolitan	0.07	0.19
<i>Coscinodiscus</i> spp.	<i>Cos</i>	Deep	0.14	0.41
<i>Cyclotella</i> spp.	<i>Cyc</i>	Offshore	0.02	0.09
<i>Dactyliosolen fragilissimus</i>	<i>Df</i>	Cosmopolitan	2.36	2.72
<i>Detonula pumila</i>	<i>Dpu</i>	Deep	0.02	0.30
<i>Diploneis</i> spp.	<i>Dip</i>	Deep	0.94	2.00
<i>Ditylum brightwellii</i>	<i>Db</i>	Seasonal/bloom	0.01	0.07
<i>Entomoneis</i> spp.	<i>Ent</i>	Deep	0.06	0.24
<i>Eucampia</i> spp.	<i>Euc</i>	Cosmopolitan	0.75	1.81
<i>Grammatophora</i> spp.	<i>Gra</i>	Offshore	0.02	0.10
<i>Guinardia</i> spp.	<i>Gui</i>	Cosmopolitan	3.95	3.81
<i>Gyrosigma/Pleurosigma</i> spp.	<i>G/Pleu</i>	Deep	0.34	0.83
<i>Helicotheca tamesis</i>	<i>Ht</i>	Deep	0.24	0.50
<i>Hemiaulus</i> spp.	<i>Hem</i>	Seasonal/bloom	0.15	0.34
<i>Lauderia annulata</i> / <i>Thalassiosira</i> spp.	<i>La/Ths</i>	Cosmopolitan	3.73	5.18
<i>Leptocylindrus</i> spp.	<i>Lep</i>	Seasonal/bloom	18.29	25.95
<i>Mastogloia rostrata</i>	<i>Mr</i>	Warm	0.06	0.70
<i>Meuniera membranacea</i>	<i>Mm</i>	Deep	0.41	0.69
<i>Navicula</i> spp.	<i>Nav</i>	Deep	1.86	5.10
<i>Neodenticula seminae</i>	<i>Ns</i>	Deep	0.004	0.030
<i>Nitzschia/Lioloma</i> / <i>Synedra/Thalassiothrix</i> spp.	<i>Nit/Lio/Syn/Thx</i>	Cosmopolitan	1.26	1.89
<i>Odontella</i> spp.	<i>Odo</i>	Deep	0.008	0.086
<i>Paralia sulcata</i>	<i>Psul</i>	Deep	0.26	1.11
<i>Planktoniella sol</i>	<i>Psol</i>	Deep	0.02	0.07
<i>Proboscia alata</i>	<i>Pal</i>	Seasonal/bloom	0.39	0.52
<i>Pseudo-nitzschia</i> spp.	<i>PN</i>	Seasonal/bloom	14.20	20.05
<i>Rhabdonema adriaticum</i>	<i>Ra</i>	Deep	0.0008	0.0132
<i>Rhizosolenia</i> spp.	<i>Rhi</i>	Cosmopolitan	0.85	1.10
<i>Skeletonema</i> sp.	<i>Ske</i>	Seasonal/bloom	0.04	0.26
<i>Surirella fastuosa</i>	<i>Sf</i>	Seasonal/bloom	0.0005	0.0086
<i>Thalassionema</i> spp.	<i>Thn</i>	Cosmopolitan	1.20	2.53
<i>Trachyneis aspera</i>	<i>Ta</i>	Deep	0.13	0.39
<i>Triceratium</i> spp.	<i>Tri</i>	Offshore	0.10	0.32
<i>Trigonium alternans</i>	<i>Talt</i>	Deep	0.01	0.10
Undefined centric	<i>Udc</i>	Deep	0.77	1.87
Undefined pennate	<i>Udp</i>	Cosmopolitan	6.07	7.96

**Table 2.** Continued.

Taxon	Abbreviation	Category	Average abundance (%)	Standard deviation (%)
Dinoflagellates				
cf. <i>Alexandrium</i> / <i>Gonyaulax</i> / <i>Heterocapsa</i> spp.	<i>Al/Gon/Het</i>	Warm	0.62	0.99
<i>Amphisolenia bidentata</i>	<i>Ab</i>	Cosmopolitan	0.0004	0.0069
<i>Ceratium</i> spp.	<i>Cer</i>	Warm	0.20	0.39
<i>Ceratocorys horrida</i>	<i>Ch</i>	Offshore	0.002	0.023
<i>Dinophysis</i> spp.	<i>Din</i>	Offshore	0.29	2.37
<i>Dissodinium pseudolunula</i>	<i>Dp</i>	Offshore	0.006	0.050
<i>Gymnodinium</i> spp.	<i>Gym</i>	Warm	0.83	1.28
<i>Gyrodinium</i> spp.	<i>Gyr</i>	Seasonal/bloom	1.24	1.70
<i>Karlodinium</i> spp.	<i>Kar</i>	Warm	2.14	5.94
<i>Noctiluca scintillans</i>	<i>Nsc</i>	Warm	0.02	0.09
<i>Ornithocercus</i> spp.	<i>Orn</i>	Offshore	0.006	0.042
<i>Oxytoxum</i> spp.	<i>Oxy</i>	Warm	1.75	2.67
<i>Phalacroma</i> spp.	<i>Pha</i>	Offshore	0.02	0.13
<i>Podolampas</i> spp.	<i>Pod</i>	Offshore	0.02	0.07
<i>Pronoctiluca</i> spp.	<i>Pm</i>	Offshore	0.21	0.36
<i>Prorocentrum</i> spp.	<i>Prc</i>	Warm	3.94	6.30
<i>Protoperdinium</i> spp.	<i>Prt</i>	Seasonal/bloom	0.83	0.88
<i>Schuettilia mitra</i>	<i>Sm</i>	Offshore	0.0009	0.0148
<i>Scrippsiella trochoidea</i>	<i>Scr</i>	Warm	3.06	6.30
cf. <i>Torodinium robustum</i>	<i>Tor</i>	Warm	0.97	1.54
<i>Warnowia</i> spp.	<i>War</i>	Offshore	0.07	0.22
Undefined dinoflagellate	<i>Udd</i>	Seasonal/bloom	1.06	1.45
Silicoflagellates				
<i>Dictyocha</i> spp.	<i>Dic</i>	Offshore	0.65	1.65
Cyanobacteria				
<i>Trichodesmium erythraeum</i>	<i>Te</i>	Warm	6.93	17.16

### 3.3 Characterisation of the phytoplankton community through environmental variables

Multivariate analyses on the complete phytoplankton abundance dataset enabled to identify a seasonal pattern in phytoplankton composition in the Coffs Harbour region (Fig. 4a). Species composition differed slightly in each month as reflected by the relatively close clustering of the same symbols in Fig. 4a. A gradual transition in species composition is indicated from May (austral autumn) through to August 2011 (winter; as indicated by black and purple to blue filled symbols, which are arranged from right to left in Fig. 4a), September to November 2011 (spring; ordination of light to dark green symbols from the right to the left in Fig. 4a) and December to February (summer; ordination of light to dark orange/red open symbols from the left to the right in Fig. 4a). The phytoplankton population found in September 2012 (grey open triangles) seemed to differ from the population in September 2011 (light green crosses, Fig. 4a).

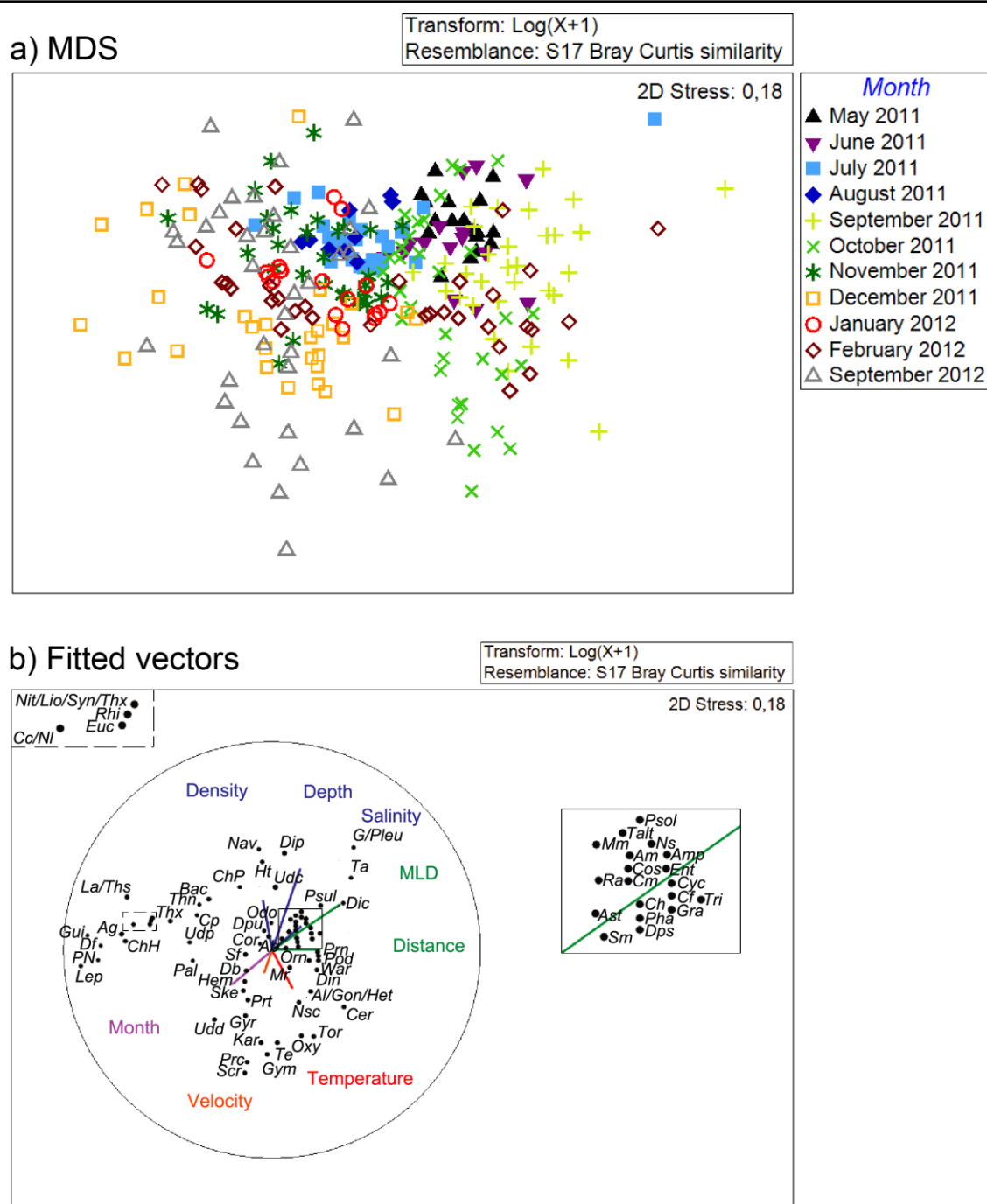
DistLM determined seven of the eight environmental variables measured as being important drivers of the phytoplankton community structure. In descending order these variables were: MLD, temperature, distance from the coast, depth, month, velocity and

density; in total explaining 18.7% of the variability in phytoplankton composition (Table 3). Fitted vectors represented the strength (length of a vector) and correlation (Spearman correlation, where a strong (weak) correlation is indicated by acute (obtuse) angles between vectors, Fig. 4b). Thus, velocity and month, and velocity and temperature, were closely correlated, as were density, depth, MLD and salinity (Fig. 4b). Distance from the coast was correlated with temperature, increased depth, MLD and salinity (Fig. 4b). The correlation of velocity with temperature as well as month indicated that seasonal and EAC-driven patterns in phytoplankton composition are partially related.

Superimposed ordination of individual phytoplankton taxa suggested groups of phytoplankton taxa that specifically responded to different environmental variables (i.e. ordination along vectors representing those variables, Fig. 4b). Consequently, we determined five phytoplankton categories associated with different water-types:

1. Seasonal/bloom taxa: Taxa associated with the variable month and, to a lesser degree, southward velocity.
2. Cosmopolitan taxa: Taxa not particularly responding to any environmental variable, i.e. tolerant towards a relatively wide range of environmental conditions.
3. Deep-water taxa: Taxa associated with increased sample depth, salinity and density
4. Warm-water taxa: Taxa strongly associated with increased temperature and, to a lesser degree, southward velocity.
5. Offshore taxa: Taxa responding to increased distance from the coast and increased MLD.

While diatoms were the major phytoplankton class in categories one to three, dinoflagellates dominated categories four and five (Table 2). Silicoflagellates only occurred in category five and *Trichodesmium erythraeum* was assigned to the warm-water group (Table 2). All taxa and their assigned category are listed in (Table 2). It should be noted that although some taxa may be considered as belonging to more than one category (e.g. warm-water and offshore), they were grouped under one category only based on their ordination (Fig. 4b).



**Figure 4. Phytoplankton characterisation through environmental variables.** a) MDS plot showing ordinated sampling locations (all samples, see Table 1) based on phytoplankton abundance data. b) Based on (a), the vectors visualise the fitted environmental variables (month, distance from the coast, velocity, temperature, salinity, density, depth, mixed layer depth (MLD)) as suggested by our DistLM model. Vectors are based on Spearman correlation, their length/direction indicate strength of effect/correlation of the variable on the ordination plot. Also plotted are the abbreviated species names (see Table 2), but for visual facilitation the individual vectors are replaced by a black dot at the ending position of the respective species vector. The circle is a unit circle and valid for both, environmental variables and ordinated species correlations. Expanded boxes show closely clustered phytoplankton taxa in higher resolution.

**Table 3. Summary of DistLM results.** AIC = Akaike's information criterion,  $R^2$  = coefficient of determination, RSS = residual sum of squares, p-value at a significance level of 0.05, Pseudo-F = test statistic, DF = degrees of freedom.

DistLM			MLD (m)	Temperature (°C)	Distance from the coast (km)	Density (kg m <sup>-3</sup> )	Depth (m)	Month	Salinity (psu)	Velocity (m s <sup>-1</sup> )
All months	Marginal tests	p	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002
		Pseudo-F	21.060	11.545	13.053	10.117	9.259	7.113	6.248	6.155
		Proportional	0.069	0.039	0.044	0.034	0.032	0.024	0.022	0.021
	Sequential tests	p	0.001	0.001	0.001	0.012	0.001	0.001	-	0.009
		Pseudo-F	21.060	11.979	8.163	3.022	6.876	5.381	-	3.252
		Cumulative	0.069	0.107	0.132	0.187	0.153	0.169	-	0.178
		Proportional	0.069	0.038	0.025	0.009	0.021	0.016	-	0.001
		Res. DF	284	283	282	278	281	280	-	279
		Best solution: AIC = 1914.4, $R^2$ = 0.18709, RSS = 218330, No. of variables = 7 (MLD, temperature, distance from coast, depth, month, velocity, density)								
Weak EAC; 10/12 October 2011	Marginal tests	p	0.001	0.003	0.001	0.001	0.001	-	0.388	0.109
		Pseudo-F	7.911	3.362	10.822	5.556	12.047	-	1.000	1.844
		Proportional	0.198	0.095	0.253	0.148	0.274	-	0.031	0.054
	Sequential tests	p	-	-	0.001	-	0.001	-	-	0.009
		Pseudo-F	-	-	7.067	-	12.047	-	-	2.200
		Cumulative	-	-	0.408	-	0.274	-	-	0.449
		Proportional	-	-	0.135	-	0.274	-	-	0.040
		Res. DF	-	-	31	-	32	-	-	30
		Best solution: AIC = 207.92, $R^2$ = 0.44879, RSS = 12167, No. of variables = 3 (depth, distance from coast,								
Strong EAC; 24 January 2012	Marginal tests	p	0.112	0.030	0.037	0.043	0.262	-	0.142	0.008
		Pseudo-F	1.674	2.501	2.211	2.193	1.238	-	1.561	3.211
		Proportional	0.100	0.143	0.128	0.128	0.076	-	0.094	0.176
	Sequential tests	p	0.09	0.02	0.0010	-	-	-	-	0.004
		Pseudo-F	1.7451	2.7268	3.5424	-	-	-	-	3.211
		Cumulative	0.52702	0.31061	0.4582	-	-	-	-	0.176
		Proportional	0.06878	0.13427	0.1476	-	-	-	-	0.176
		Res. DF	12	14	13	-	-	-	-	15
		Best solution: AIC = 97.68, $R^2$ = 0.52702, RSS = 2953.9, No. of variables = 4 (velocity, temperature, distance from coast, MLD)								

### 3.4 Seasonal variation of the phytoplankton community in relation to the physical environment

Monthly averaged abundance of all phytoplankton taxa assigned to each of the five water-type categories (and their most abundant representative taxa), total phytoplankton and zooplankton were summarised in T-S plots (Figs. 5, 6). The exact values of averaged abundances including standard deviations are given in Supplementary Material Table 1.

T-S plots visualised the seasonal change in total phytoplankton abundance and the seasonal contribution of each category (and representative species) with respect to the physical oceanographic environment (Figs. 5, 6). Generally, we found the abundance of total phytoplankton and within each phytoplankton category to be higher in summer (characterised by warm, low-salinity/density water) than late autumn and winter (characterised by cold, saline/dense water) (Fig. 5a-c, e, f). This seasonal signal was most pronounced in the seasonal/bloom category (Fig. 5b) and least expressed in the deep-water category (Fig. 5d). The seasonal/bloom, cosmopolitan, deep and warm water category were sporadically abundant in individual months during spring

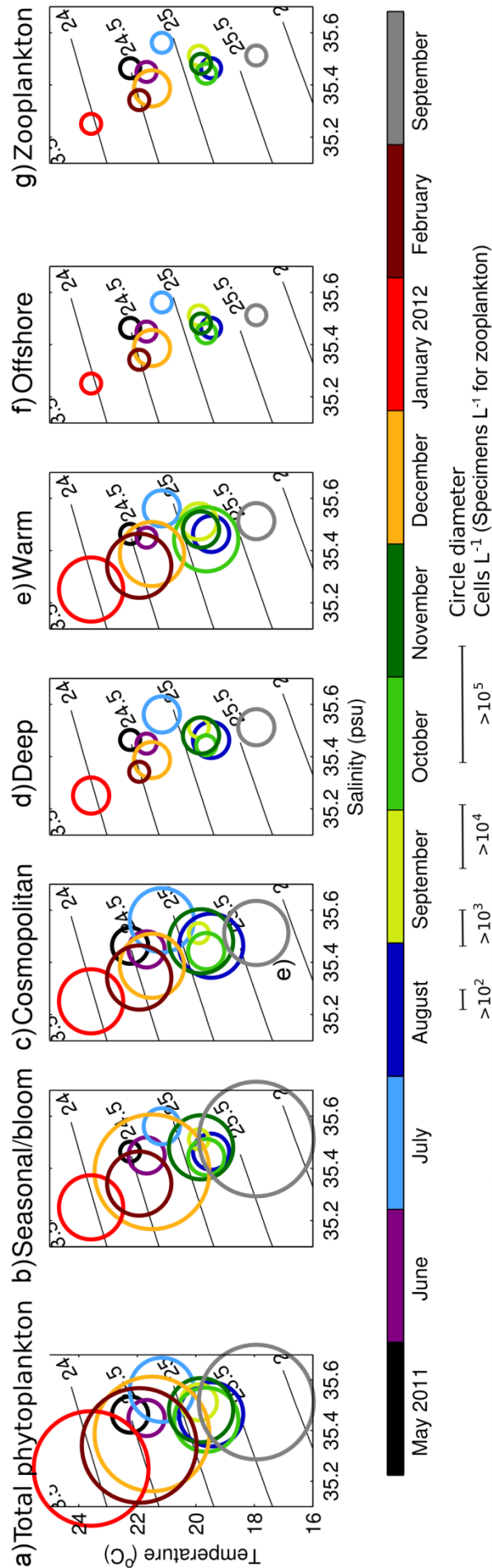
(September – November 2011 and September 2012), which was characterised by relatively cold, saline/dense water, especially during September 2012 (Fig. 5b, c, d, e). Highest abundances were found within the seasonal/bloom category during December 2011 ( $4.71 \times 10^5$  cells L<sup>-1</sup>) and September 2012 ( $2.19 \times 10^5$  cells L<sup>-1</sup>) (Fig. 5b) and could be assigned to blooms of the diatoms *Leptocylindrus danicus* (September 2012) and *Pseudo-nitzschia* spp. (December 2011) (Fig. 6a, b). Despite the identical appearance of individual *Pseudo-nitzschia* cells counted in the September 2012 samples we cannot exclude that more than one species of this diatom genus was present (due to the relatively low resolution of light microscopy) and therefore refer to *Pseudo-nitzschia* spp. in the following. Average temperature, salinity and density during the *Pseudo-nitzschia* spp. bloom were 21°C, 35.5 psu and 24.74 kg m<sup>-3</sup>, respectively whilst during the *L. danicus* bloom 18°C, 35.5 psu and 25.75 kg m<sup>-3</sup>, respectively (Figs. 5b, 6a, b). The depth-averaged southward velocity was 0.24 m s<sup>-1</sup> and 0.42 m s<sup>-1</sup> at CH070 and CH100, respectively, during the blooms in December 2011 (averaged between the two sampling days) and 0.04 m s<sup>-1</sup> and 0.02 m s<sup>-1</sup>, at CH070 and CH100, respectively, during the September 2012 bloom (Fig. 2). Dinoflagellates of the genus *Gyrodinium* were also representatives of the seasonal/bloom group (Fig. 6c). Although occurring in much lower abundances than *L. danicus* and *Pseudo-nitzschia* spp., a peak of *Gyrodinium* spp. was determined during December 2011 (the month of the *Pseudo-nitzschia* spp. bloom, Fig. 6b, c). Relatively high abundance of the cosmopolitan category was found throughout all months (compared to warm-, deep-water and offshore taxa) and over a wide range of temperature, salinity and density (~17 - 24°C, 35.2 - 35.5 psu and 24 - 25.75 kg m<sup>-3</sup>, respectively, Fig. 5c). The seasonal abundance of this category followed the general pattern, with slightly higher abundances during summer than in late autumn and winter (Fig. 5c). Most (i.e. eight) of the 19 taxa that contributed >1% to the total phytoplankton community were assigned to the cosmopolitan category, with *Asterionellopsis glacialis* and *Chaetoceros* spp. (Hyalochaetes) being the most abundant ( $1.77 \times 10^4$  and  $2.05 \times 10^4$  cells L<sup>-1</sup>, respectively, Fig. 6d-k). *Chaetoceros* spp. deviated from the seasonal pattern, being relatively abundant in June and August (Fig. 6f). The deep-water category was present in low abundance and did not follow the general seasonal signal as abundance within this group was elevated during winter and spring (especially August 2012, Fig. 5d). The most abundant representative of the deep-water category was the diatom genus *Navicula*, which peaked during August 2011, and, to a lesser degree, during September 2012 (Fig. 6m). We added the diatom genus

*Diploneis* as a second representative of the deep-water category (*Diploneis* spp. contributed on average 0.9% to the total phytoplankton community, Table 2). However, *Diploneis* was most abundant during December 2011 and showed its second highest abundance during August 2011 (Fig. 6l).

Warm-water taxa were most prominent during summer (December 2011 - February 2012, Fig. 5e). Within this category, *Trichodesmium erythraeum* was the most abundant phytoplankton taxon (Fig. 6o). Despite exhibiting high abundances during summer (up to  $2.42 \times 10^4$  cells L<sup>-1</sup>), *T. erythraeum* also showed relatively high abundances during October 2011 ( $1.85 \times 10^4$  cells L<sup>-1</sup>, Fig. 6o). Warm-water dinoflagellates of the genera *Oxytoxum*, *Karlodinium* and *Prorocentrum* and the species *Scrippsiella trochoidea* showed highest abundances during summer (Fig. 6n, p, q, r). All genera revealed high abundance during December, however, elevated abundance of *Karlodinium* ( $6.95 \times 10^3$  cells L<sup>-1</sup>), *Prorocentrum* ( $2.43 \times 10^3$  cells L<sup>-1</sup>) and *Scrippsiella trochoidea* ( $2.78 \times 10^3$  cells L<sup>-1</sup>) were also determined during November 2011, October 2011 and September 2012, respectively (Fig. 6p, q, r, respectively).

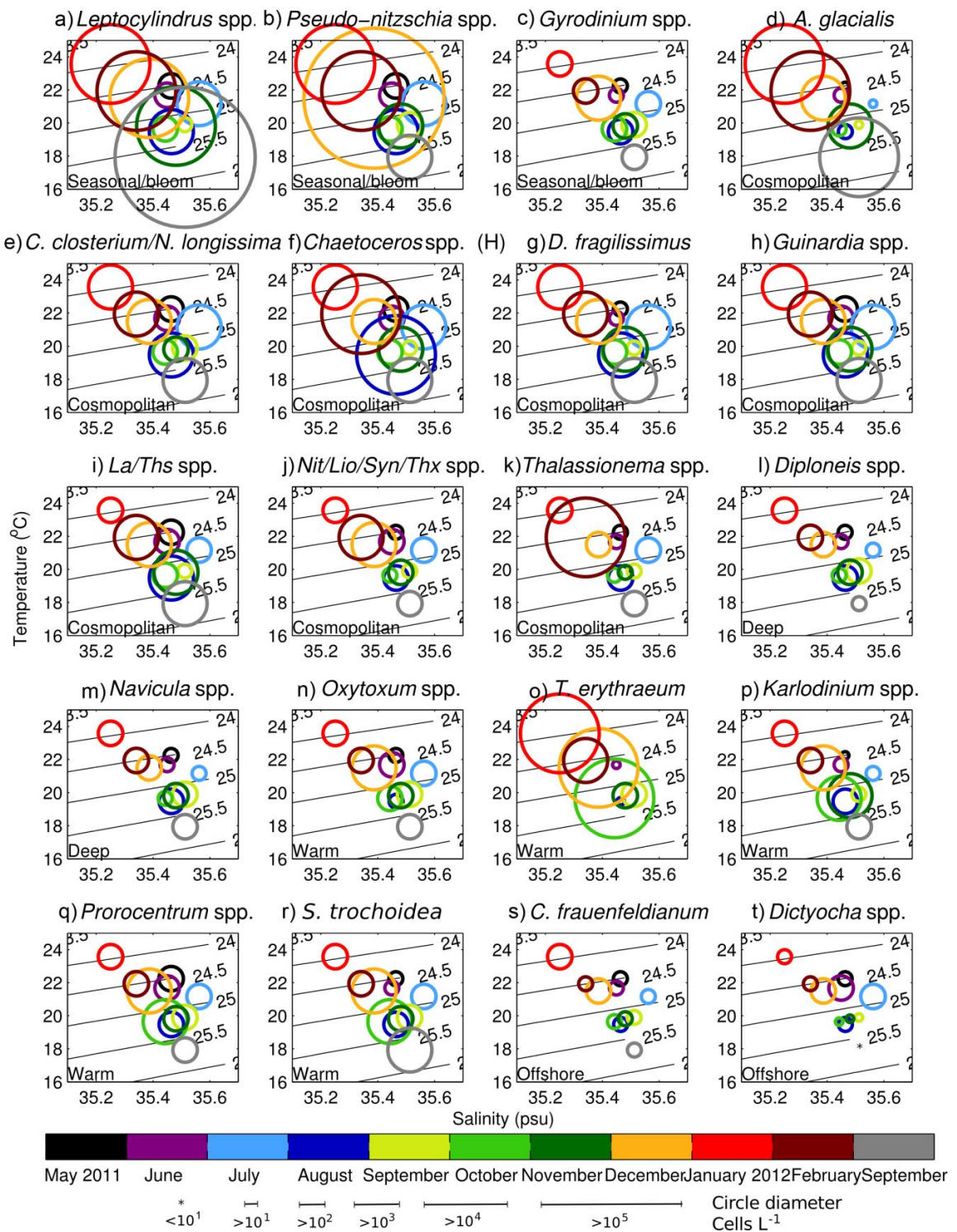
Lowest abundances were found within the offshore category (compared to all other categories), reaching small maxima during summer (Fig. 5f). As no taxon assigned to this group contributed >1% to the total phytoplankton community, we selected the two most abundant offshore taxa, namely the silicoflagellate genus *Dictyocha* and the diatom *Climacodium frauenfeldianum* as representative taxa for this group (contributing on average 0.65% and 0.52% to the total phytoplankton community, Table 2). *C. frauenfeldianum* had highest abundances during January 2012 ( $4.64 \times 10^2$  and  $4.23 \times 10^2$  cells L<sup>-1</sup>) and *Dictyocha* spp. during December 2011, respectively, Fig. 6s, t).

Zooplankton occurred in cell numbers between 88 and  $2.67 \times 10^3$  specimens L<sup>-1</sup> (Fig. 5g). Maximum abundances within this group occurred during December 2011 and September 2012 (Fig. 5g), i.e. coinciding with bloom periods of the diatoms *Pseudo-nitzschia* and *Leptocylindrus* spp. (Fig. 6a, b).



**Figure 5. Seasonal abundance of phytoplankton categories.** Temperature-salinity plots showing average abundance of total phytoplankton (a), phytoplankton taxa belonging to the five water-type categories (b - f) and zooplankton (g) per month (averaged across all samples collected during each month; for complete species list and exact monthly averaged abundances including standard deviations see Table 2 and Supplementary Material Table 1, respectively). Phytoplankton abundances are given in cells L<sup>-1</sup> and zooplankton abundances in specimens L<sup>-1</sup>.





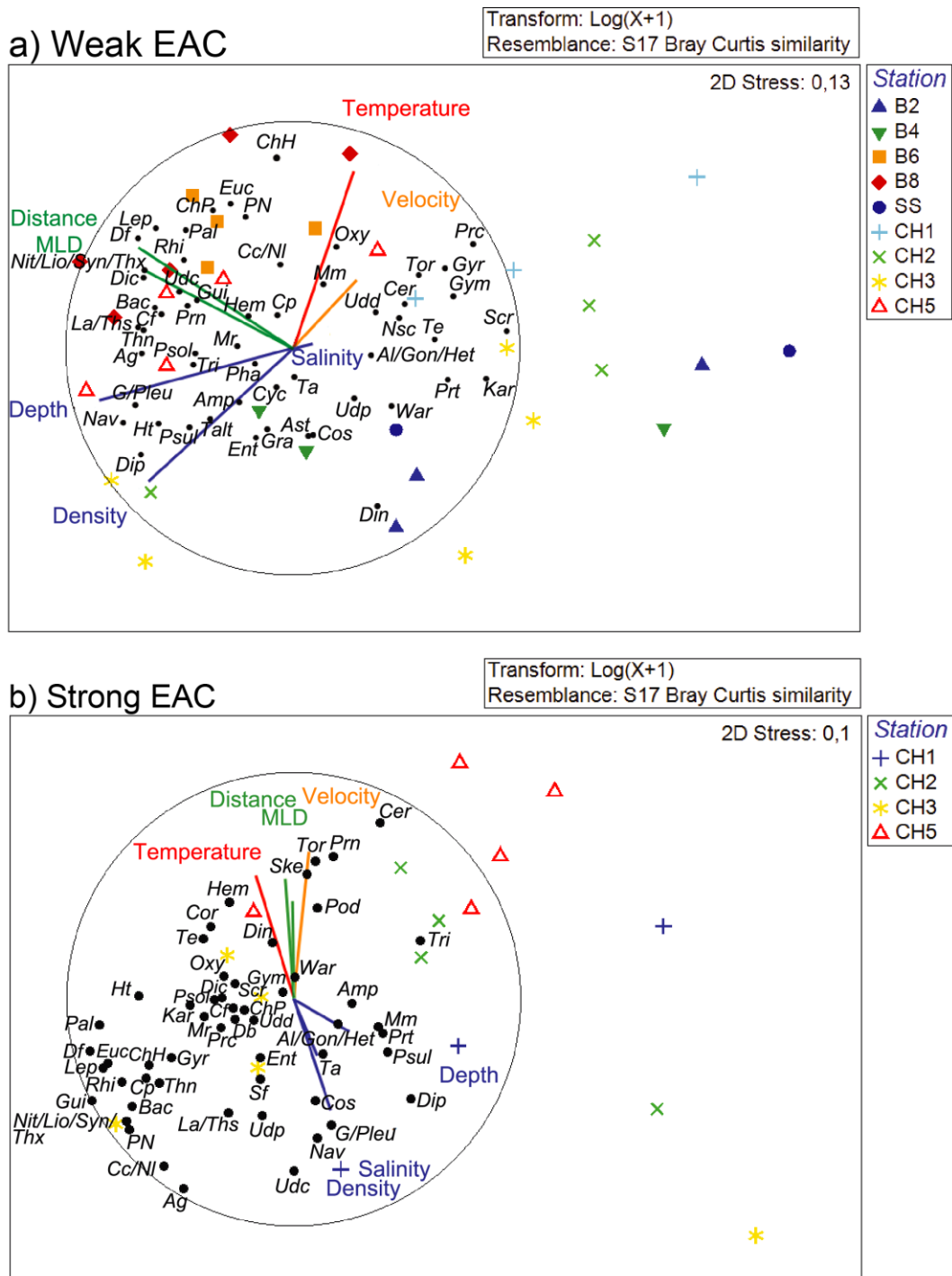
**Figure 6. Seasonal abundance of most abundant taxa.** Temperature-salinity plots showing taxa contributing on average (across all samples) >1% to the total phytoplankton community (for exact monthly averaged abundances including standard deviations see Supplementary Material Table 1). Exceptions are *Diploneis* spp. (l), *Climacodium frauenfeldianum* (s) and *Dictyocha* spp. (t), which contributed 0.9, 0.51 and 0.65% to the total phytoplankton community, respectively, and were included to represent the deep-water (*Diploneis* spp.) and offshore category (*C. frauenfeldianum* and *Dictyocha* spp.). The water-type to which each taxon belongs is indicated in the lower corner of each panel. For complete list of species belonging to the five water-type categories and taxon name abbreviations see Table 2.

### 3.5 Influence of EAC encroachments on phytoplankton composition

During October 2011, the weak EAC scenario, DistLM revealed depth, distance from the coast and southward velocity as being the most important variables in explaining the variability in phytoplankton composition (in descending order) (Table 3). In total, these variables explained 45% of the variability in the phytoplankton composition (Table 3). Depth was strongly correlated with density, distance from the coast with MLD and temperature with southward velocity (Fig. 7a). Unexpectedly, salinity was not correlated with depth/density, which we account to slightly lower salinity values measured at the bottom depths of the offshore stations (data not shown). However, the vector pair distance from the coast/MLD was ordinated in between the vector pair temperature/velocity and the vector pair depth/density, indicating a weak correlation with both these vector pairs (Fig. 7a). This ordination pattern suggested a cross-shelf gradient of increasing temperature/velocity and depth/density with distance from the coast. Simultaneously, we determined a cross-shelf transition in phytoplankton composition, indicated by the ordination of inshore to offshore stations following the direction of the distance from coast vector (blue to red symbols from the right to the left, Fig. 7a). The majority of diatoms were found to be associated with increased distances from the coast, including taxa from the cosmopolitan, offshore and seasonal/bloom category (Fig. 7a). Dinoflagellates, including warm-water and seasonal/bloom taxa, seemed to mainly respond to warm temperature (Fig. 7a). Taxa associated with depth included taxa that had been predominantly assigned to the deep-water category (e.g. *Navicula* spp., *Diploneis* spp. Fig. 7a). The cyanobacterium *Trichodesmium erythraeum* was only correlated with temperature (although relatively weakly) and was highly abundant in surface waters of inshore stations (Fig. 7a, <http://catalogue.aodn.org.au/geonetwork/srv/en/metadata.show?uuid=f7502841-a2c7-4437-b557-20ef89e754e0>).

During January 2012, when the EAC influence was strong, DistLM determined southward velocity as being most important in explaining the variability of the phytoplankton composition, followed by distance from the coast and MLD (in descending order; Table 3). In total, these three variables explained 53% of the variability in the phytoplankton composition (Table 3). Velocity, MLD, temperature and distance from the coast were strongly positively correlated, as were depth, salinity and density (Fig. 7b). The first combination of vectors confirms the strong influence of the warm EAC and its impact on the phytoplankton composition offshore, the latter being indicated by the ordination of the offshore stations close to the velocity vector (Fig. 7b).

The second combination of vectors confirms the intrusion of cold bottom water onto the shelf, which was observed to impact on the inshore phytoplankton community (Fig. 7b). The phytoplankton community composition on the mid-shelf seemed to be relatively mixed (as indicated by the wide-spread distribution of mid-shelf station symbols) and influenced by both the warm and strong EAC and the intrusion of cold, saline and dense bottom water (Fig. 7b). On average (across all samples) total phytoplankton abundance was eight times higher in the strong EAC scenario than in the weak EAC scenario (Fig. 5a, Supplementary Material Table 1). Warm-water and offshore taxa, including several dinoflagellates and *Trichodesmium erythraeum*, were clearly associated with elevated temperature and velocities in this strong EAC scenario. Deep-water taxa, such as *Navicula* spp. and *Diploneis* spp. were associated with the cold, saline and dense bottom water encroaching onto the shelf (Fig. 7b).



**Figure 7. Phytoplankton response to weak and strong EAC influence.** MDS plot showing ordinated sampling locations in the months characterised by a) weak EAC influence (October 2011) and b) strong EAC influence (January 2012) based on phytoplankton abundance data. Vectors visualise the fitted environmental variables (distance from the coast, temperature, salinity, density, depth, mixed layer depth (MLD)) as suggested by our DistLM model. Vectors are based on Spearman correlation, their length/direction indicate strength of effect/correlation of the variable on the ordination plot. Also plotted are the abbreviated species names (see Table 2), but for visual facilitation the individual vectors are replaced by a black dot at the ending position of the respective species vector. The circle is a unit circle and valid for both, environmental variables and ordinated species correlations.

## 4 Discussion

Our study provides the first detailed taxonomic phytoplankton time-series survey in the tropical-temperate transition zone ( $\sim 30^{\circ}\text{S}$ ) of Eastern Australia. Within this investigation, covering a nearly complete annual cycle, we were able to determine 74 phytoplankton genera that occurred in the Coffs Harbour region between 2011 and 2012. In addition, we were able to link seasonal phytoplankton abundance and composition patterns (water-type categories and most abundant taxa) as well as cross-shelf distribution patterns to the strength of the EAC.

### 4.1 Seasonal patterns in the phytoplankton composition off Coffs Harbour

The average velocity encountered on the continental shelf at  $\sim 30^{\circ}\text{S}$  between May 2011 and September 2012 is in agreement with the two-year averaged values presented in Schaeffer et al. (2013). It is known that the EAC exhibits a seasonal cycle strengthening during the austral summer (Godfrey et al., 1980; Ridgway and Godfrey, 1997), although this seasonal cycle is not as clear in the circulation on the continental shelf (Schaeffer et al., 2013; Wood, 2014). Yet, we found the seasonal phytoplankton abundance and composition to strongly depend on the strength of the current along the Coffs Harbour coast. This dependence was particularly supported by the strong correlation between environmental variables serving as proxies for temporal evolution in phytoplankton composition (month) and EAC influence (southward velocity and temperature). All phytoplankton categories defined in this study, particularly the seasonal/bloom category, exhibited their highest abundances during summer. The exception was deep-water taxa, which seemed to generally prefer relatively cold, saline and dense water independently of the season. Maximum phytoplankton abundance was reached during December 2011, when southward velocities were high ( $\sim 0.24 \text{ m s}^{-1}$ ). During this month we observed a bloom of the diatom *Pseudo-nitzschia* spp., a ubiquitous taxon previously reported as one of the most abundant genera in the temperate region of Eastern Australia (Hallegraeff and Reid; 1986; Ajani et al., 2013). Although we are unable to make any statements regarding the longevity of this *Pseudo-nitzschia* spp. bloom, the morphology and appearance of the diatom during our counts suggested that it was in its stationary growth phase. A second prominent diatom bloom, this time attributed to *Leptocylindrus danicus*, was noted in September 2012, unexpectedly when southward velocities were relatively weak. The *L. danicus* bloom was most likely induced by a wind-driven upwelling event occurring four to five

days prior to our sampling and already in its senescent phase (discussed in detail in Armbrrecht et al., under review). Assuming that the diatom blooms were already approaching or in their end-phases, the increased abundance of predatory zooplankton in December 2011 and September 2012 is also consistent with our bloom observations and suggests that grazing had an impact on bloom termination in both months. The latter assumption is plausible, considering that successive patterns in phytoplankton can cover short periods (a few weeks) until a physical disturbance “resets” the oceanographic and nutrient resource conditions (Hallegraeff and Reid, 1986; Wyatt, 2014).

### **4.2 Cross-shelf phytoplankton distribution during weak and strong EAC influence**

The importance of the EAC in affecting the cross-shelf oceanographic evolution and phytoplankton community structure was obvious from our contrasting approach investigating physical and biological associations during times of minimum and maximum EAC influence. When the influence of the EAC was weak (October 2011), phytoplankton community structure was organised along ‘depth’ and ‘distance from the coast’ gradients. The upper water column (0 - 20 m depth) was characterised by homogenous temperature, salinity and density across the whole shelf (20 - 20.5°C, ~35.5 psu and 24.6 - 25 kg m<sup>-3</sup>), thus creating a similar habitat (with regard to temperature and vertical mixing) for all taxa. The homogenous condition of the upper water column might explain the overlapping cross-shelf distribution of, in particular, cosmopolitan, seasonal/bloom and offshore taxa (i.e. mainly diatoms) and also the relatively weak correlation of very few warm-water (e.g. *Trichodesmium erythraeum*) taxa with temperature (as there was no major difference in temperature).

When a strong EAC prevailed during January 2012, southward velocity was the most important variable driving the cross-shelf phytoplankton composition and distribution alongside temperature, distance from the coast and MLD. In this scenario, a horizontal increase in temperature of about 2°C was observed in the surface waters across the shelf (with a larger temperature gradient, 3 - 5°C, at depth). Warm-water and offshore taxa were highly associated with both warm temperature and increased distances from the coast, verifying our selection of taxa into the warm-water and offshore categories. Phytoplankton abundances were highly elevated during this month, especially within the warm-water category at the expense of seasonal/bloom taxa (relative to December

2011 and February 2012). These results are consistent with previous results from the Coffs Harbour region, where the appearance of tropical dinoflagellates at the offshore station CH5 had been associated with pronounced EAC intensification (Armbrecht et al., 2014).

The cyanobacterium *Trichodesmium erythraeum* was the main contributor to the high phytoplankton abundance determined within the warm-water category during January 2012. *T. erythraeum* is a warm-water species commonly found in oligotrophic tropical regions (Capone et al., 1997); hence its appearance in the Coffs Harbour region during January 2012 may be indicative of its increased transport with the EAC. Nevertheless, we found a high density of *T. erythraeum* under both strong and weak EAC conditions. We hypothesise that *T. erythraeum* may have established itself permanently in the east Australian tropical-temperate transition zone and speculate that the species is undergoing a seasonal cycle with pronounced growth during spring and summer. This hypothesis is supported by our observation of a *T. erythraeum* bloom exactly one year later, around the 30<sup>th</sup> October 2012, in which cell concentrations of  $1.87 \times 10^6$  cells L<sup>-1</sup> were reached at the surface at CH3 (data not shown). Alternatively, the elevated abundance of *T. erythraeum* during our sampling in October 2011 might have been caused by EAC intrusion prior to our sampling (as exemplified by the *Leptocylindrus danicus* bloom in September 2012).

#### **4.3 Coffs Harbour phytoplankton variability in the regional context**

Seasonal changes in phytoplankton abundance and composition have been described previously from the long-term sampling station Port Hacking (~34°S), and have generally been associated with seasonal variability of wind and EAC strengths (Hallegraeff and Jeffrey, 1993; Ajani et al., 2001; Pritchard et al. 2003). It has been shown recently that, in the Coffs Harbour region, EAC-driven upwelling occurs sporadically and most frequently during the austral spring and summer (October to March; Rossi et al., 2014). Our study is the first to show that seasonal phytoplankton abundance patterns also exist in the Coffs Harbour region, which is located upstream of the EAC separation zone and highly exposed to the influence of the EAC. Our results are consistent with previous investigations from the more southerly Port Hacking station showing the importance of the EAC in driving phytoplankton dynamics. For example, phytoplankton composition is very similar between our study and previous investigations from the Port Hacking station. Furthermore, taxa that were classified as

warm-water or seasonal/bloom taxa through our analyses have been summarised in comparable groups in the Port Hacking studies (e.g. *Leptocylindrus danicus* as a bloom-species and *Pseudo-nitzschia* sp. (in particular *P. fraudulenta*) as consistently present; Hallegraeff and Reid, 1986). *Trichodesmium erythraeum* has been described as warm-water species and its frequent abundance and extended bloom formation in temperate east Australian waters has been reported before (Ajani et al., 2011). It is believed that *T. erythraeum* is transported to temperate regions of Eastern Australia with the EAC, EAC-derived warm core eddies and occurs in higher abundances during El-Niño events (Hallegraeff and Reid, 1986; Ajani et al., 2001). Considering that we found *T. erythraeum* being highly abundant regardless of EAC strength and during a La Niña period lasting from 2010 - 2012 (<http://www.bom.gov.au/watl/enso/>) supports our hypothesis that the cyanobacterium has established itself permanently in the east Australian tropical-temperate transition zone and is undergoing an intrinsic seasonal cycle (see section 4.3).

#### **4.4 Relevance of the Coffs Harbour phytoplankton survey in the global context**

It has been proposed that the phytoplankton communities along the Australian east coast will proportionally increase in dinoflagellates and decrease in diatoms due to climate-change derived modifications in physical and chemical parameters (e.g. strengthening EAC, warmer temperatures, higher nitrate and phosphate versus lower silicate concentrations; Thompson et al., 2009; Ajani et al., 2014). Our short-term study supports this theory as we found warm-water and offshore species to be dominated by dinoflagellates, including tropical taxa that were most likely transported to the Coffs Harbour region with the EAC (with the term tropical being based on distributional ranges given in Hallegraeff and Reid, 1986; Tomas, 1997; Ajani et al., 2001 and Hallegraeff et al., 2010). However, the observed long-term strengthening of the EAC might increase the occurrence of upwelling events along the east Australian coast, in which case we would expect diatoms to be favoured by their competitiveness during sporadic nutrient pulses. Our study demonstrated that diatoms dominate the microphytoplankton community in the tropical-temperate transition zone. The predicted decline of this crucial group of the phytoplankton community due to reduced silicate availability might have severe consequences for succession patterns and biogeochemical processes, especially in an area that is as exposed to the EAC influence as the Coffs Harbour region.



The cyanobacterial genus *Trichodesmium* is ubiquitous in tropical to subtropical oligotrophic oceans and WBC systems, where it can thrive due to its capability to fix nitrogen and with negligible grazing impact (Capone et al., 1997). *Trichodesmium* has gas vacuoles that enable buoyancy control, thus it can migrate vertically between the surface to fix N<sub>2</sub> (a light-bound process) and deep waters to acquire phosphate (Villareal and Carpenter, 2003). These characteristics and its fast growth rate make *Trichodesmium* a competitive species that frequently forms extensive blooms in warm surface waters, promoted through wind dispersal (Capone et al., 1997). Such widespread blooms, which can be seen on satellite images (Subramaniam and Carpenter, 1994; Subramaniam et al., 1999), create shading in the upper water layer and thus decrease the light availability for purely photosynthetic organisms (Oliver and Ganf, 1988; Capone et al., 1998). Seasonal succession patterns of photoautotrophic phytoplankton that are adapted to high light conditions (such as many diatoms) can therefore be impacted (Devassy et al., 1978; Capone et al., 1997).

With the predicted strengthening and warming of the EAC, the species *Trichodesmium erythraeum* (along with tropical dinoflagellates) might become more prominent in the east Australian tropical-temperate transition zone with consequences such as disruptions of established successional patterns. Our study provides important insights into interactions of oceanic forcing and the cross-shelf phytoplankton distribution. However, long-term investigations assessing the distribution and abundance of *Trichodesmium* should be the focus of future research. Increased nitrogen fixation rates at elevated CO<sub>2</sub> levels have been reported as well as the expansion of worldwide cyanobacterial blooms (Hutchins et al., 2007; Paerl and Paul, 2012). Therefore, the need to monitor in particular *Trichodesmium* in its role as a major contributor in the N-cycle, is not a task restricted to the east Australian coast but one of global importance under a changing climate.

## 5 Conclusion

Our study provides the first detailed taxonomic time-series survey in the east Australian tropical-temperate transition zone (~30°S) upstream of the EAC separation zone. Within this study we determined 74 phytoplankton genera, including mainly diatom taxa, followed by dinoflagellates, silicoflagellates and the cyanobacterium *Trichodesmium erythraeum*. Five phytoplankton categories associated with different water-types were determined and confirmed via temperature-salinity plots. Four of the

categories (seasonal/bloom, cosmopolitan, warm-water and offshore taxa), and representative taxa therein, revealed a seasonal signal (with increased/decreased abundance during summer/winter) while one category (deep-water taxa) occurred relatively independent of the season in cold, saline dense bottom water. The most extensive blooms were caused by diatoms (*Pseudo-nitzschia* spp., December 2011 and *Leptocylindrus danicus*, September 2012) and most likely initiated by upwelling resulting from increased southward velocities (strong EAC). The extent of EAC encroachment onto the shelf influences the cross-shelf and vertical phytoplankton abundance, composition and distribution. The high abundance of the diazotroph, warm-water cyanobacterium *Trichodesmium erythraeum*, seemingly undergoing a seasonal cycle off Coffs Harbour, might influence the local nitrogen cycle considerably. We conclude that the phytoplankton community in the east Australian tropical-temperate transition zone is driven by seasonal, as well as, EAC signals and the interaction between both. Our baseline investigation is an important reference for future phytoplankton research aimed at determining latitudinal range expansions along the east Australian coast and improves our understanding of interactions between oceanic forcing and phytoplankton dynamics in a WBC system, potentially being applicable to such systems worldwide.

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**Supplementary Material Table 1. Monthly averaged phytoplankton abundance.**

Monthly averaged abundance (across all samples of most abundant phytoplankton taxa (see Table 2 and Figure 6), water-type categories, total phytoplankton and zooplankton including standard deviation (in cells L<sup>-1</sup> for phytoplankton and specimen L<sup>-1</sup> for zooplankton).

Phyto- and zooplankton abundance (Cells L <sup>-1</sup> and specimen L <sup>-1</sup> , respectively)	Month of sampling/ Taxon	May 2011	June 2011	July 2011	August 2011	September 2011	October 2011	November 2011	December 2011	January 2012	February 2012	September 2012
Monthly averaged abundance of dominant phytoplankton taxa	<i>Asterionellopsis glacialis</i>	3	11	4	61	4	19	3319	2354	14066	17713	11164
	<i>Ceratoneis closterium</i> / <i>Nitzschia longissima</i>	249	453	1231	2174	390	490	840	2256	4458	2937	4283
	<i>Chaetoceros</i> spp. ( <i>Hyalochaete</i> )	124	115	5552	16505	56	288	1317	5494	4630	20499	3958
	<i>Climacodium frauenfeldianum</i>	48	97	66	14	23	23	77	224	423	25	96
	<i>Dactyliosolen fragilissimus</i>	71	79	1080	1518	17	111	1428	4639	7846	2434	1939
	<i>Diploneis</i> spp.	61	46	35	191	141	84	108	436	171	160	98
	<i>Guinardia</i> spp.	190	272	2054	3343	62	302	1940	4664	3893	2561	3163
	<i>Lauderia annulata</i> / <i>Thalassiosira</i> spp.	110	456	698	1888	33	294	6567	3657	492	2550	5048
	<i>Leptocylindrus</i> spp.	112	172	2525	2001	38	296	27120	14220	22061	59241	211599
	<i>Navicula</i> spp.	91	40	80	875	428	89	220	350	139	207	690
	<i>Nitzschia/Lioloma</i> / <i>Synedra/Thalassiothrix</i> spp.	68	146	453	122	23	52	198	3171	872	1150	270
	<i>Pseudo-nitzschia</i> spp.	294	765	4925	7130	145	535	1931	451340	14953	22521	5865
	<i>Thalassionema</i> spp.	31	86	438	278	15	36	70	876	329	11594	296
	Undefined pennate diatom	533	1040	363	711	324	805	967	2493	1741	1454	1471
	<i>Gyrodinium</i> spp.	36	76	127	267	100	130	270	1307	329	175	409
	<i>Karlodinium</i> spp.	5	35	30	257	71	1039	6951	1174	226	175	302
	<i>Oxytoxum</i> spp.	78	125	166	70	107	162	150	3501	414	199	130
	<i>Prorocentrum</i> spp.	104	241	451	911	390	2431	570	1683	998	826	771
	<i>Scrippsiella trochoidea</i>	31	96	141	313	235	1726	676	3510	509	350	2776
	Undefined dinoflagellate	61	70	94	168	64	91	184	1553	280	395	474
	<i>Dictyocha</i> spp.	66	256	135	36	10	9	1	464	24	11	0
	<i>Trichodesmium erythraeum</i>	0	1	0	5	193	18486	890	24079	22690	9628	0
Standard deviation from monthly averaged abundance of dominant phytoplankton taxa	<i>Asterionellopsis glacialis</i>	8	11	4	61	4	19	3319	2354	14066	17713	11164
	<i>Ceratoneis closterium</i> / <i>Nitzschia longissima</i>	215	453	1231	2174	390	490	840	2256	4458	2937	4283
	<i>Chaetoceros</i> spp. ( <i>Hyalochaete</i> )	116	115	5552	16505	56	288	1317	5494	4630	20499	3958
	<i>Climacodium frauenfeldianum</i>	40	97	66	14	23	23	77	224	423	25	96
	<i>Dactyliosolen fragilissimus</i>	53	79	1080	1518	17	111	1428	4639	7846	2434	1939
	<i>Diploneis</i> spp.	132	46	35	191	141	84	108	436	171	160	98
	<i>Guinardia</i> spp.	100	272	2054	3343	62	302	1940	4664	3893	2561	3163
	<i>Lauderia annulata</i> / <i>Thalassiosira</i> spp.	111	456	698	1888	33	294	6567	3657	492	2550	5048
	<i>Leptocylindrus</i> spp.	119	172	2525	2001	38	296	27120	14220	22061	59241	211599
	<i>Navicula</i> spp.	132	40	80	875	428	89	220	350	139	207	690
	<i>Nitzschia/Lioloma</i> / <i>Synedra/Thalassiothrix</i> spp.	33	146	453	122	23	52	198	3171	872	1150	270
	<i>Pseudo-nitzschia</i> spp.	219	765	4925	7130	145	535	1931	451340	14953	22521	5865
	<i>Thalassionema</i> spp.	32	86	438	278	15	36	70	876	329	11594	296
	Undefined pennate diatom	898	1040	363	711	324	805	967	2493	1741	1454	1471
	<i>Gyrodinium</i> spp.	28	76	127	267	100	130	270	1307	329	175	409
	<i>Karlodinium</i> spp.	8	35	30	257	71	1039	6951	1174	226	175	302
	<i>Oxytoxum</i> spp.	46	125	166	70	107	162	150	3501	414	199	130
	<i>Prorocentrum</i> spp.	70	241	451	911	390	2431	570	1683	998	826	771
	<i>Scrippsiella trochoidea</i>	34	96	141	313	235	1726	676	3510	509	350	2776
	Undefined dinoflagellate	41	70	94	168	64	91	184	1553	280	395	474
	<i>Dictyocha</i> spp.	46	256	135	36	10	9	1	464	24	11	0
	<i>Trichodesmium erythraeum</i>	0	1	0	5	193	18486	890	24079	22690	9628	0
Monthly averaged phytoplankton abundance within each water-type category	Warm water	376	684	1041	1841	1218	24172	9566	37451	25620	11626	4608
	Offshore	127	385	231	128	101	171	178	1160	483	140	272
	Deep water	443	466	1161	2283	727	412	1192	2032	1013	870	1401
	Cosmopolitan	1498	2770	13433	29157	941	2510	22090	30730	40207	67593	33287
	Seasonal/bloom	562	1190	8045	9772	398	1326	30036	470597	38391	82913	218807
Standard deviation from monthly averaged phytoplankton abundance within each water-type category	Total	3006	5495	23913	43182	3386	28592	63062	541971	105713	163142	258375
	Warm water	226	404	569	1347	1016	79093	16201	75593	19253	20293	8251
	Offshore	82	292	140	95	90	195	217	1852	543	167	807
	Deep water	422	354	887	979	1002	293	979	2247	1366	1518	1846
	Cosmopolitan	1198	1986	13215	23341	774	1496	25469	27892	43274	87675	46615
Monthly averaged total phytoplankton abundance	Seasonal/bloom	275	1148	9566	6767	312	913	34449	664478	42384	149471	216487
	Total	2074	3733	23430	30832	2028	79616	68798	675709	90508	232685	218928
	Diatoms, dinoflagellates, silicoflagellates, <i>Trichodesmium erythraeum</i>	3006	5495	23913	43182	3386	28592	63062	541971	105713	163142	258375
	Standard deviation from monthly averaged total phytoplankton abundance	2074	3733	23430	30832	2028	79616	68798	675709	90508	232685	218928
	<i>Trichodesmium erythraeum</i>	3006	5495	23913	43182	3386	28592	63062	541971	105713	163142	258375
Monthly averaged zooplankton abundance	Copepods, larvae and ciliates	104	176	88	962	326	807	673	1559	776	362	2667
	Standard deviation from monthly averaged zooplankton abundance	72	154	96	660	220	3010	800	2486	655	566	3078

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## **7 General Discussion**

In order to comprehensively summarise the major findings of each chapter and explore them within in a broader regional and global context, this discussion is divided into three sections relating to the three principal aims of this thesis. Abundance and distribution of the phytoplankton determined by microscopy and pigment analysis are discussed in section 7.1 and 7.2, respectively. Insights into the response of phytoplankton including micro-, nano- and picophytoplankton to oceanographic conditions are discussed in 7.3. Conclusions drawn from this thesis are included at the end of this chapter (section 7.4).

### ***7.1 The principal aim of this thesis was to provide the first taxonomic phytoplankton survey in the east Australian tropical-temperate transition zone as a reference for future research.***

#### ***7.1.1 Phytoplankton data collection overview***

Monthly samplings were conducted in the Coffs Harbour region over eleven field campaigns (May 2011 to February 2012 and September 2012). Throughout the eleven field collections 297 samples were obtained for microscopic analysis. A total of 137 microphytoplankton taxa within 74 genera were determined and photographed including diatoms, dinoflagellates, silicoflagellates and the cyanobacterium *Trichodesmium erythraeum* (Appendix 2). Microphytoplankton abundance data collected throughout this time-series survey has been made publicly available via the Australian Ocean Data Network (AODN; <http://portal.aodn.org.au/aodn/>, Appendix 3). Public access will benefit future comparison and continued documentation of Australian phytoplankton community composition and biogeographic distribution, and ultimately facilitate prospective long-term investigations that will require large spatial and temporal phytoplankton data coverage.

#### ***7.1.2 Proportion of tropical and temperate microphytoplankton species***

The initial hypothesis was that the phytoplankton community composition off Coffs Harbour was characterised by a high proportion of tropical relative to temperate species. This hypothesis was proposed because the study region is exposed to the

warm-water influence of the EAC (section 2.1.1). The determination of a tropical:temperate phytoplankton species ratio confirmed this hypothesis. The ratios were resolved from analysing the biogeography of microphytoplankton taxa (i.e. diatoms, dinoflagellates, silicoflagellates and *Trichodesmium erythraeum*) using established regional and taxonomic literature (Dakin & Colefax 1933, 1940, Wood 1954, Crosby & Wood 1958, 59, Wood et al. 1959, Wood 1961a, b, 1963, Grant & Kerr 1970, Hallegraeff & Reid 1986, Tomas 1997, Ajani et al., 2001, Hallegraeff et al. 2010). As predicted, a latitudinal difference north and south of the EAC separation zone found the tropical:temperate ratio stronger at Coffs Harbour (0.54), than at Port Hacking (~34°S, Eastern Australia; ratio of 0.28 based on data in Ajani et al. 2001).

The preliminary tropical:temperate phytoplankton species ratios reported here will be prepared for publication with additional collaborators post-thesis submission. Documenting the proportions of tropical and temperate phytoplankton species along a latitudinal gradient is of particular importance to on-going marine park zoning and characterisation efforts. Such studies, where the aim is to define bioregions or long-term trends in poleward species migrations, often overlook or have little understanding of the phytoplankton community. This thesis represents the first phytoplankton survey in the SIMP, filling the void of knowledge that existed at the primary producer level in this marine park.

### **7.1.3 New phytoplankton species records for Australia**

Three diatom species were identified for the first time along the Australian coast as a result of this thesis' investigations. These species are *Bacteriastrum elongatum*, *Neodenticula seminae* and *Triceratium obtusum*. The first two diatoms are representatives reported from tropical regions external to Australia. Tomas (1997) has reported that the diatom *N. seminae* occurs in the North and tropical Pacific and described *B. elongatum* as warm-water species. The third species, *Triceratium obtusum*, is considered a temperate species, having previously been reported from New Zealand by Harper et al. (2012). None of the three diatom species has ever been listed in existing studies used to determine tropical:temperate phytoplankton taxa classification, nor have they appeared in studies along the west Australian coast (Griffin et al. 1997, Twomey et al. 2006, Thompson & Bonham 2011). Also, these three species were not included in any of the currently available AusCPR records from

around Australia (<http://imos.aodn.org.au/imos123/home>) or listed within the Codes of Australian Aquatic Biota (<http://www.marine.csiro.au/caab/>). Whether the presence of *N. seminae* and *B. elongatum* in the Coffs Harbour region is the result of the increased southward transport of the EAC remains speculative. The two species might have simply been unnoticed in previous phytoplankton investigations along the east Australian coast. However, the presence of all three newly recorded diatoms within this thesis will be an important reference for regional future studies aimed at identifying phytoplankton species range expansions due to climate change.

#### **7.1.4 Phytoplankton distribution and abundance within a regional context**

Several diatom and dinoflagellate species determined within this thesis, which commonly predominate in warm and tropical regions, have been found to occur sporadically along the south-east Australian coast. For example, the warm-water diatoms *Bacteriastrum hyalinum* and *Chaetoceros* cf. *compressus* were previously reported from ~34°S (Dakin & Colefax 1940). While *B. hyalinum* was reported as rare in Dakin & Colefax (1940), *C. compressus* was found to be part of a larger *Chaetoceros* species complex that notably increased in abundance during austral spring and late summer. The warm-water and tropical dinoflagellates *Ceratium arietinum*, *C. extensum*, *C. massiliense*, and *C. symmetricum*, *Ceratocorys horrida*, *Oxytoxum compressum*, *O. constrictum* and *O. tessellatum* were identified in samples taken at Coffs Harbour. These dinoflagellate species have never been included in the extensive species lists from repeated studies undertaken to the south at 34°S; the location of Australia's longest time-series station Port Hacking (Dakin & Colefax 1933, 1940, Hallegraeff & Reid 1986, Ajani et al. 2001). All aforementioned dinoflagellates have been associated with warm and tropical waters off Northern and Eastern Australia (Wood 1954). However, the latter author also reported the occasional transport of the four *Ceratium* species with warm water along the east Australian coast as far south as Tasmania. In fact, *C. horrida* was reported to occur at ~43°S (Maria Island, Tasmania) during late winter (Wood 1954), in agreement to a recent study, which reported the appearance of several *Ceratium* species (including *C. symmetricum*) at ~43.5°S during spring (Buchanan et al. 2014). Today, we know that Wood's warm-water transport mechanism is the southward flowing EAC (Ridgway & Godfrey 1997). The current has been attributed with significantly influencing the seasonal phytoplankton abundance

and composition patterns far to the south of its source (Ajani et al. 2001, Pritchard et al. 2003, Buchanan et al. 2014).

The Coffs Harbour time-series survey revealed diatoms as the predominant phytoplankton taxon at all sampling times, with highest abundances determined during two bloom periods encountered in December 2011 and September 2012 (Chapter 5 and 6). The diatoms *Leptocylindrus danicus* and *Pseudo-nitzschia* spp. represented the most abundant taxa during these blooms, followed by *Chaetoceros* spp. and *Ceratoneis closterium/Nitzschia longissima* (Chapter 6). The same diatom species have also been found to represent the most abundant taxa in other coastal studies around Australia, including Port Hacking, South-Eastern Australia (~34°S; Ajani et al. 2001), the Gascoyne region, Western Australia (~22 - 27°S; Hanson et al. 2007), the Kimberley region, Western Australia (~15°S; Chapter 5; Thompson & Bonham 2011) and the Huon Estuary, Tasmania (~43°S; *Chaetoceros* and *Pseudo-nitzschia* only; Thompson et al. 2008). In Australian CPR samples these four diatom taxa are recorded at the genus-level and have been encountered frequently in high numbers (<http://imos.aodn.org.au/imos123/home>). The consistency in the frequency and abundance of the same diatom genera in different regions along the Australian coast confirms the global cosmopolitan nature of these taxa (Tomas 1997).

### **7.1.5 Phytoplankton abundance and distribution within a global context**

Diatoms are clearly one of the most abundant phytoplankton taxa in coastal and shelf regions of other WBC systems (Lohrenz et al. 2003, Carreto et al. 2003, 2008, Barlow et al. 2008, 2010, 2013, Moreno et al. 2012). Microscopic investigations in shelf regions along the Mozambique coast, influenced by the Agulhas Current system, identified *Chaetoceros* spp., *Pseudo-nitzschia* spp. and *Ceratoneis closterium* as the most abundant diatom taxa in this coastal system (Sá et al. 2013). The diatoms *Pseudo-nitzschia* spp., *Rhizosolenia setigera*, *Thalassiosira* spp. and *Thalassionema nitzschioides* were determined as the most dominant taxa in a coastal region under the influence of the Brazil Current along the east coast of South America (Carreto et al. 2003, 2008). These latter four diatom taxa were also found to be amongst the top 20 most abundant microphytoplankton taxa in the Coffs Harbour region (Chapter 6).

In agreement with this thesis' findings, dinoflagellates and silicoflagellates have been reported in relatively low abundances in other WBC systems (Lohrenz et al. 2003,



Carreto et al. 2003, 2008, Barlow et al. 2008, 2010, 2013, Moreno et al. 2012). The dinoflagellate and silicoflagellate species composition observed at Coffs Harbour (Chapter 4 - 6) exhibits close similarities to other WBC systems (Lohrenz et al. 2003, Carreto et al. 2003, 2008). For example, Carreto et al. (2003, 2008) reported the frequent abundance of *Prorocentrum cordatum*, *Dinophysis acuminata*, *D. caudata*, *Ceratium furca* and *C. tripos*, *Scrippsiella trochoidea* and *Noctiluca scintillans* from the east coast of South America. Lohrenz et al. (2003) also identified the genera *Ceratium*, *Protoperidinium*, *Scrippsiella* and *Alexandrium* off North Carolina, USA. More recently, Kim et al. (2013) have reported the novel presence of 24 tropical dinoflagellates around Jeju Island (Korea) due to the increased influence of the Kuroshio Current. Many of the 24 new species listed in Kim et al. (2013) were also found at Coffs Harbour (Chapter 4 – 6). There is little mention of silicoflagellates in studies from other WBC systems, however, the species *Dictyocha fibula* and *D. speculum* have been identified by Carreto et al. (2003, 2008). In one of their studies they reported a bloom of *D. fibula* reaching cell densities of 14,700 cells L<sup>-1</sup> (Carreto et al. 2008). Although no bloom by this species was identified within this survey, *D. fibula* was the most abundant silicoflagellate at Coffs Harbour (Chapter 4 and 6).

Global WBCs have been described as natural laboratories in which the early effects of ocean warming on marine organisms can be studied and where adaptation lessons can be learned and shared (Frusher et al. 2014, Pecl et al. 2014). While this thesis is limited to a one-year survey, it lays the groundwork towards a better knowledge of phytoplankton community composition in a changing WBC system. The similarities that can now be drawn from the studies of microphytoplankton composition between the global subtropical WBC systems, including the east Australian coast, suggest close parallels in the functioning of these systems. Diatoms are known to dominate phytoplankton communities in upwelling regions worldwide and to make a considerable contribution to global primary production (Uitz et al. 2010, Chavez et al. 2011). As a result of the strengthening of the WBCs, community regime shifts at the primary producer level are expected (Thompson et al. 2009). Recent studies from different WBC systems have reported an increased poleward migration of warm-water and tropical dinoflagellates (Kim et al. 2013, Buchanan et al. 2014). At higher trophic levels, regime shifts of a number of species including rock lobsters and several fish species have been reported to occur as a result of the warming and strengthening of WBCs (the EAC and Kuroshio Current) in Tasmanian and Korean waters (Hamon et al. 2014,

Jung et al. 2014). Hamon et al. (2014) further reported that the future of the Tasmanian rock lobster fishery, currently worth AUD\$70 Million, is highly uncertain due to climate change and insecurities linked to market conditions. Within this context, the investigation of the role of phytoplankton as the food source for higher trophic levels is crucial to marine and commercial fisheries management.

### **7.1.6 Recommendations for future research**

This thesis provides a starting point for future phytoplankton research in the tropical-temperate Coffs Harbour region. While over 100 microphytoplankton taxa could be distinguished, the low resolution of light microscopy did not allow the discrimination of phytoplankton taxa that are substantially morphologically similar. Some taxa were only identified to genus level (most species within the genera *Pseudo-nitzschia*, *Chaetoceros* and *Protoperdinium*) and 16 taxa were consistently grouped into complexes (*Bacteriastrum furcatum/delicatulum*, *Lauderia annulata/Thalassiosira* spp., *Nitzschia/Lioloma/Thalassiothrix* spp., *Thalassionema nitzschioides/frauenfeldii*, *Gyrosigma/Pleurosigma* spp., and *Alexandrium/Gonyaulax/Heterocapsa* spp.). Future research can re-use the 297 Lugol's preserved phytoplankton samples archived within this thesis for the high-resolution examination of such genera and complexes. Specifically, *Prorocentrum* spp., *Pseudo-nitzschia* spp. and *Alexandrium* spp. would be suitable for extended species determination as several species within these genera have been shown to produce toxins (Glibert et al. 2012, Ajani et al. 2013, Farrell et al. 2013, respectively). Toxin production in species of these phytoplankton genera is well known to result in shellfish poisoning with significant impacts on the coastal ecosystem, aquaculture, economy and human health (Ajani 2013, Farrell et al. 2013). Alternatively, several dinoflagellates species have been shown to be good indicators for changing environmental conditions, including eutrophication (e.g. *Prorocentrum cordatum*; Glibert et al. 2012), sea surface temperature warming and tropicalisation (e.g. several species within the genera *Ceratium*, *Ceratocorys*, *Ornithocercus*, *Oxytoxum*, *Ornithocercus*; Kim et al. 2013, Buchanan et al. 2014). Species-specific research will thus significantly benefit managing authorities, such as the Solitary Island Marine Parks Authority, who need to provide sound evidence behind the implementation of ecosystem protection and zoning measures (NSW MPA 2009a, b).

Long-term investigations of phytoplankton upstream of the EAC separation zone should be anticipated as an extension of this Coffs Harbour survey. For example, modern phytoplankton abundance, composition and distribution patterns determined within this thesis can be placed into the historical context based on sediment data. The composition of fossilised taxa and geochemical tracers may reveal insights into past climatic and oceanographic conditions (Giuliani et al. 2006). Such information will be invaluable for an improved understanding and evaluation of the physical, chemical and biological changes currently happening along the east Australian coast. Molecular analysis of phytoplankton is another avenue that can be explored to further improve the knowledge of phytoplankton species abundance, diversity and range expansions (Zwirgmaier et al. 2008, Ryneerson & Palenik 2011, Read et al. 2013). Long-term phytoplankton investigations in combination with the advanced oceanographic infrastructure established by IMOS will provide an excellent platform with which to simultaneously monitor climate-change induced changes in the EAC alongside any phytoplankton response. In order to achieve a complete picture of the marine ecosystem function in a biological hotspot such as Coffs Harbour, whole food-chain studies from physical oceanography to fish populations should be an additional focus of future research.

The preliminary assessment of the contribution of tropical and temperate species determined within this thesis enabled a rapid comparison to the temperate phytoplankton community of South-Eastern Australia (34°S, Port Hacking). Determining the tropical:temperate phytoplankton species ratios based on existing studies or future studies from other WBC regions may equally provide a rapid comparison of the phytoplankton community between different WBC systems. If regularly determined over the long-term, such ratios will comprise a suitable measure of tropicalisation at the primary producer level. Ultimately, these ratios will contribute a useful tool to the standardised reporting of global phytoplankton communities, which is urgently needed in the context of climate change (Pecl et al. 2014). Measures of tropicalisation in phytoplankton communities will not only be of use in WBC systems, but will provide a simple assessment of the phytoplankton community in warming oceans worldwide.

**7.2 The second aim of this thesis was focussed on including and quantifying pico- and nanophytoplankton in the time-series survey by applying pigment analyses through CHEMTAX. Similarities, differences and the compatibility of both microscopy and CHEMTAX abundance estimates were statistically determined.**

**7.2.1 Overview of the phytoplankton community derived from CHEMTAX analysis**

Chapter 3 and 5 document the success of applying pigment and CHEMTAX analyses to the time-series survey, whereby a total of nine phytoplankton classes were found at Coffs Harbour. In Chapter 3 these were designated by the taxonomic categories of diatoms, cyanobacteria (*Trichodesmium erythraeum*, *Synechococcus* sp. and *Prochlorococcus marinus* pigment-types), haptophytes (*Phaeocystis pouchetii* and *Emiliana huxleyi*/Gephyrocapsa oceanica pigment-types), prasinophytes, chrysophytes, euglenophytes, cryptophytes and dinoflagellates (in descending order of relative abundances averaged across all pigment samples). Pelagophytes had been included in the comparative analysis of the phytoplankton communities off the Kimberley and Coffs Harbour coasts, yet contributed only a minute fraction to the phytoplankton community off Coffs Harbour (Chapter 5).

A pre-selection of taxa based on our Coffs Harbour pigment data and regional literature had to be made due to the current limitations of CHEMTAX (Jeffrey et al. 1975, Hallegraeff 1981, Hallegraeff & Jeffrey 1981, Hallegraeff & Reid 1986). This pre-selection of taxa, included in the CHEMTAX analyses, would suggest that the CHEMTAX-based assessment of the phytoplankton community was unlikely to be comprehensive. Taxa that were excluded from the analysis but share pigments with taxa that were included, may have been present but were not recorded. For example, chlorophytes were not included in the analysis and have a similar pigment composition to prasinophytes, a taxon that was included and encountered in the Coffs Harbour time-series (Chapter 3 and 5) and Kimberley case study (Chapter 5). Equally, different subgroups of haptophytes and prasinophytes, which exhibit intra-class variations in pigment composition, cannot be considered as absent from this study as there were no analytical means by which these taxa could be separately identified using CHEMTAX (Jeffrey & Vesk 2005, Laza-Martinez et al. 2007).

### **7.2.2 Pigment-based phytoplankton composition in the regional and global context**

The integration of this time-series survey into the small pool of Australian coastal phytoplankton pigment studies enhances the limited knowledge of pico- to microphytoplankton distribution and abundance along the east Australian coast. Previous pigment studies from South-Eastern Australia have primarily focussed on the nanophytoplankton size-class, with little information available regarding picophytoplankton (Jeffrey & Carpenter 1974, Jeffrey & Hallegraeff 1980, 1987, Hallegraeff 1981, Hallegraeff & Jeffrey 1981). Chapter 3 and 5 reported on the occurrence of the picoplanktonic *Prochlorococcus marinus* at ~30°S, Eastern Australia. *Prochlorococcus* has been found in the tropical Kimberley region (~15°S, Western Australia; Chapter 5 and Thompson & Bonham 2011) and reported to decrease in abundance poleward (Thompson et al. 2011). The higher abundance of *Prochlorococcus* in the Kimberley region compared to the Coffs Harbour region (Chapter 5) is thus consistent with the biogeographic distribution of *Prochlorococcus* shown in Thompson et al. (2011). In contrast, the cyanobacteria *Synechococcus* sp. and *Trichodesmium erythraeum* have been reported to occur to the south of Coffs Harbour (34°S, Hallegraeff & Reid 1986, Ajani et al. 2001), thus their distribution appears more widespread along the east Australian coastline.

Size-class fractionation following of all pigment data collected within this thesis revealed that micro-, nano and picophytoplankton contributed on average  $42 \pm 29\%$ ,  $30 \pm 16\%$  and  $28 \pm 16\%$ , respectively, to the total phytoplankton community (across all samples; data not shown). The larger variation in microphytoplankton abundance compared to nano- and picophytoplankton abundances is consistent with previous pigment-based studies from 34°S, Port Hacking, Eastern Australia (Hallegraeff 1981) as well as global studies applying the size-fractionation approach (Uitz et al. 2010).

A diverse assemblage of the nine phytoplankton classes identified by CHEMTAX within this thesis (section 7.2.1) has also been found to comprise the phytoplankton community in shelf and coastal regions in other WBC systems (Lohrenz et al. 2003, Carreto et al. 2003, 2008, Barlow et al. 2008, 2010, Moreno et al. 2012). All these studies have applied microscopy and pigment analyses to characterise the phytoplankton communities. The increased awareness that small-sized phytoplankton contribute a considerable fraction to the total phytoplankton community in different WBC systems reinforces the complementary use of microscopy and CHEMTAX to

comprehensively characterise natural phytoplankton communities. However, a direct comparison of the abundance estimates of the two quantification techniques based on different measurement (Chl *a* and cell numbers) remains difficult (Lohrenz et al. 2003) and thus their statistical comparison was a further focus of this thesis as summarised in 7.2.3, below.

### **7.2.3 Outcomes of the statistical comparison between microscopy and CHEMTAX**

Current literature suggests good correlations have been found between phytoplankton abundance estimates made by light microscopy and CHEMTAX (summarised in Higgins et al. 2011). Yet, frequently, researchers acknowledge CHEMTAX abundance estimates include taxa that share specific pigments (e.g. fucoxanthin in diatoms and dinoflagellates) that result in errors in taxa categorisation (e.g. Rodriguez et al. 2002, Lohrenz et al. 2003). Quantitatively, this thesis has shown that comparing phytoplankton abundance estimates made by light microscopy and CHEMTAX remains complicated. Chapter 3 focussed on revealing how both techniques have strengths and weaknesses, which, if recognised, can determine the appropriate use of either technique, and subsequent interpretation of their results.

The strength of microscopy was clearly the high resolution of the microphytoplankton community as evidenced by the identification of 137 taxa within this thesis (thesis aim 1). Based on this detailed microscopy data, individual taxa could be statistically linked to different water-types (Chapter 6), an achievement never previously completed to this extent in studies from Eastern Australia. Microscopy was also invaluable for detecting the diatom blooms during December 2011 (*Pseudo-nitzschia* spp.) and September 2012 (*Leptocylindrus danicus*), which were in their stationary and senescent phases, respectively (Chapter 5 and 6). Consequently, senescence was identified as an important factor that can confuse phytoplankton abundance estimates made by microscopy and CHEMTAX (Chapter 3). Increased chlorophyllide *a* concentrations were linked to senescent material derived from the terminal staged *L. danicus* bloom (Chapter 3 and 5). However, chlorophyll *a* degradation to chlorophyllide *a* through the activation of chlorophyllase *a* during sample processing prior to HPLC analysis has been shown to be particularly pronounced in diatoms (Jeffrey & Hallegraeff 1987). The latter might partly explain why microscopy abundance

estimates of diatoms are frequently higher than CHEMTAX abundance estimates of diatoms (Schlüter & Møhlenberg 2003, Havskum et al. 2004, Vidussi et al. 2004).

The strength of the CHEMTAX-based analysis was the comprehensive quantitative assessment of the phytoplankton community from pico- to microphytoplankton (Chapter 5). When the complete phytoplankton community was tested for their interaction with the environment, a maximum of 87% of the variability in the phytoplankton composition off the Kimberley coast was explained by CHEMTAX (Chapter 5). A maximum of ~45% of this environmental variability in the phytoplankton composition could be explained based on microscopic analyses (Chapter 4 - 6). One limitation of CHEMTAX is the resolution of phytoplankton abundance at a class level (except for *Prochlorococcus marinus*, which is the only species containing divinyl Chl *a* and *b*; Jeffrey et al. 2005). Also, the attribution of shared pigments (e.g. fucoxanthin in diatoms, dinoflagellates, chrysophytes, raphidophytes and haptophytes) is a difficult task for CHEMTAX. Most studies recommend the complementary use of CHEMTAX and microscopy to characterise natural phytoplankton communities (Carreto et al. 2003).

In the event that a methodological choice may depend on limited financial resources, equipment or time, this thesis provides recommendations that may help in selecting the most suitable method for the purpose of directed studies. In Chapter 3, regression and Bland-Altman analysis showed that the two quantification techniques agreed for the most part at the phytoplankton class level. When taxonomic discrimination was extended beyond the diatom class level to different diatom pigment-types the agreement was poor. The classification of microscopically determined diatoms into pigment-types based on the literature was a significant issue. The resulting classification errors suggested that intra-specific pigment variation in diatoms is likely to be higher than previously assumed, so that the use of CHEMTAX beyond the class level is currently not feasible (Chapter 3).

#### **7.2.4 Recommendations for future research**

The high resolution of microphytoplankton taxa achieved during microscopy analyses shows that this technique is particularly suited to investigate phytoplankton communities that are dominated by large cells (Chapter 3 - 6). Thus, studies aimed at investigating diatom-dominated regions, such as upwelling and coastal WBC systems

will benefit from microscopy analysis. Conversely, CHEMTAX is a powerful tool with which to characterise phytoplankton communities dominated by small-sized taxa that are invisible under the microscope. CHEMTAX is thus highly suited to investigate open ocean waters or WBCs (as shown by Carreto et al. 2008).

In order to improve the conformity of phytoplankton abundance estimates made by microscopy and CHEMTAX, and taxonomic discrimination beyond the class level in CHEMTAX, two relevant forward steps can be suggested.

- i) Elimination of classification errors. Investigations of monocultured and multi-strained phytoplankton pigment compositions will lead to the clarification of intra-specific pigment variation. To date, such elaborate studies have been rarely attempted (e.g. Laza-Martinez et al. 2007). Environmental variables, such as nutrient concentrations, temperature and light conditions, have been shown to influence pigment ratios. For example, prymnesiophytes, pelagophytes, cryptophytes and cyanobacteria increase their ratio of diagnostic pigments (19'hexanoyloxyfucoxanthin/19'butanoyloxyfucoxanthin, 19'butanoyloxyfucoxanthin, alloxanthin and zeaxanthin, respectively) under high irradiances (Schlüter et al. 2000). Thus, in order to detect intra-specific variation in pigment composition, experimental studies will have to be performed under stable culture conditions. Once ranges of intra-specific pigment variations are determined, they can be further examined under different environmental conditions (e.g. light, temperature, nutrients). This procedure will ultimately help to select the most suitable starting ratios to load into CHEMTAX, which will improve the correct assignment of pigments to a respective algal class. Upon the elimination of classification errors, Bland and Altman analysis is likely to become more meaningful in phytoplankton research by delivering specific ranges of over-/underestimates between pigment and microscopy estimates allowing a more precise assessment of true phytoplankton abundance in field samples.
- ii) Quantification of senescent material during microscopy and CHEMTAX analyses. Senescent and dead cells may be easier to distinguish during microscopy than CHEMTAX analyses. For example, epifluorescence microscopy is an easy approach to distinguish live phytoplankton by the red autofluorescent signal of Chl *a* (for photoautotrophic organisms) from dead



cells. Additionally, specific dyes such as SYTOX® green enable simple identification of dead cells (Armbrecht et al. 2014). In the past 20 years, epifluorescence has been increasingly applied to identify and enumerate pico- and nanophytoplankton (Booth 1987, 1993, Hall 1991). Thus, the application of epifluorescence microscopy will enable a comparison of abundance estimates made by microscopy and CHEMTAX for more taxa than were comparable in this thesis (Chapter 3). In CHEMTAX, the attribution of senescent and dead material is beyond the program's present capacity, and would involve a considerable re-development and extension of the software by experts.

**7.3 The third and final aim of this thesis was to determine the responses of individual phytoplankton taxa to different oceanographic conditions on a local (upwelling/downwelling), regional (Eastern/Western Australia) and temporal scale (near annual cycle).**

**7.3.1 Phytoplankton response to upwelling and downwelling**

Coffs Harbour is characterised by highly variable oceanographic conditions due to a complex coastal topography, including a narrowing continental shelf, and considerable influence of the EAC main flow. Upwellings in this region have been estimated to occur ~30 % of the time with about eight EAC-driven upwellings per month (Schaeffer et al. 2013, Rossi et al. 2014). Detailed investigation of a short upwelling and downwelling event closely following each other during austral winter 2011 was undertaken within this thesis (Chapter 4). This investigation provided evidence of rapidly changing oceanographic conditions (within ten days) and quantified the impact on the phytoplankton abundance, composition and cross-shelf distribution (Chapter 4).

During the downwelling event we found an increased abundance of benthic diatoms in the water column of the inshore station, where high vertical mixing and a local silicate maximum prevailed (Chapter 4). Specific taxa that showed enhanced abundance in these well-mixed inshore waters were *Navicula*, *Diploneis* and *Pleurosigma* spp. (Chapter 4). These three diatom genera are commonly regarded as benthic (Chapter 4) and were classified as deep-water taxa in Chapter 6. Their preference for increased depths (Chapter 6) confirmed our theory that strong vertical mixing led to the elevated abundance of these taxa in the water column during the downwelling event (Chapter

4). Additionally, lower species richness occurred during the downwelling event relative to the upwelling event. This finding is consistent with a study from North Carolina (exposed to the influence of the Gulf Stream), which showed a decrease in species diversity during downwelling conditions (Lohrenz et al. 2003).

During the current-driven upwelling event, the peak phytoplankton abundance was localised at ~50 m depth on the mid-shelf (~75 m isobath; Chapter 4). EAC encroachment led to increased nutrient concentrations (silicate and nitrate) and low temperatures from intruding bottom water (below 50 m depth). Species that showed high abundances at the mid-shelf station were *Chaetoceros* spp. (Hyalochaete), *Dactyliosolen fragilissimus* and *Leptocylindrus danicus* (Chapter 4). These taxa had been found at the offshore station during the preceding downwelling event, which suggested their coastward movement during the upwelling event (Chapter 4). The same three diatom taxa were part of the Coffs Harbour phytoplankton community during January 2012, when southward velocities were at their maximum and the EAC was encroaching onto the shelf (Chapter 6). A closer investigation of the abundance and distribution of the three diatom taxa at this time revealed that, again, their highest abundances were found at the mid-shelf (~16 km away from the coast at the 75 m isobath; Fig. 1). As with during the winter upwelling (Chapter 4), total phytoplankton abundances peaked at ~40 m depth at this mid-shelf-station (Appendix 3). This localised response of phytoplankton to EAC encroachment on the mid-shelf is a new finding along the east Australian coast. Yoder et al. (1985) have reported a major phytoplankton (>10 µm size fraction) response on the mid-shelf between the 20 – 40 m isobaths along the coast of the South-Eastern United States (exposed to the Gulf Stream). The recurrent phytoplankton response on the mid-shelf at ~40 m depth as determined within this thesis and in another WBC system confirmed it to be a phenomenon of greater spatial importance. Such identified occurrences of concentrated phytoplankton abundances on the mid-shelf in WBCs might influence the distribution of higher trophic organisms during upwelling-induced peak production periods.

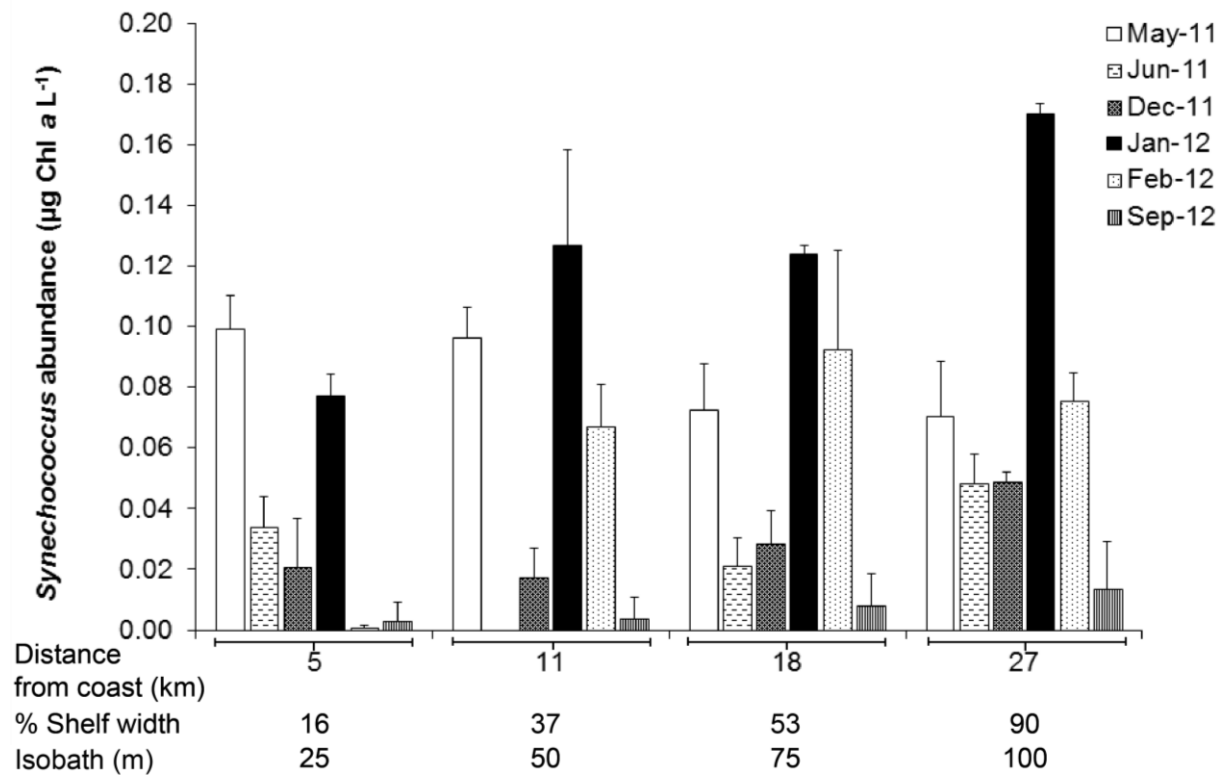
Upwelling has been reported to control the formation of phytoplankton blooms south of the separation zone (mainly described from Port Hacking; Ajani et al. 2001, Lee et al. 2001, Pritchard et al. 2003). The two diatom blooms determined in this Coffs Harbour study were almost certainly induced by current- (*Pseudo-nitzschia* spp.) and wind-driven (*Leptocylindrus danicus*) upwelling events, respectively (Chapter 5 and 6). Thus, it appears that the upwelling-mechanism itself was not critical to the bloom formation, however, the major factor appears to be the resulting nutrient-supply to the euphotic zone (i.e. the upwelling 'symptom'). Most notably, silicate supply from the upwelling of nutrients resulted in the increased abundance of diatoms inshore on the

mid-shelf during the winter upwelling event (Chapter 4). Silicate was found to be one of the most important variables influencing the cross-shelf phytoplankton community in both the Kimberley and Coffs Harbour regions (Chapter 5) and similarly vital in promoting diatom abundance inshore during the downwelling event (Chapter 4). We can expect that the Coffs Harbour phytoplankton community, in particular diatoms, will be seriously impacted by the predicted long-term silicate decline caused by the strengthening of the EAC (Thompson et al. 2009).

Evidence of tropical dinoflagellate dispersion by the southward flowing EAC was originally signalled by Wood (1954). Thus it is not surprising that today, with the recognised EAC intensification, more recent studies have also reported on the southward range expansion of warm-water dinoflagellates (Hallegraeff et al. 2008, McLeod et al. 2012, Buchanan et al. 2014). The short time-series survey represented by this thesis showed conclusively, for the first time, that transport of tropical species into the study area was enhanced during times when the EAC was strongest (Chapter 4 and 6). Species richness analyses based on microscopy data revealed the increased abundance of tropical dinoflagellates at the mid-shelf and offshore stations during the EAC encroachment event in winter 2011 (which resulted in upwelling; Chapter 4). In addition, warm-water taxa, mainly composed of dinoflagellates and the cyanobacterium *Trichodesmium erythraeum*, increased in abundance at the expense of seasonal/bloom taxa when the EAC was strong (Chapter 6).

Pigment data collected over six months (Chapter 3) revealed that small-sized phytoplankton, especially *Synechococcus*, also increased in abundance when the EAC was strong. Figure 2 illustrates the averaged abundance per month and per station for *Synechococcus* determined by CHEMTAX (based on Chapter 3, Supplementary Material Table 5). During January 2012, when southward velocities were at their maximum, *Synechococcus* showed its highest abundances of the study period (Fig. 2). During this month, *Synechococcus* abundance was highest ( $\sim 0.17 \mu\text{g Chl } a \text{ L}^{-1}$ ) at the offshore station (100 m isobath; Fig. 2). To a lesser degree, increased abundances of *Synechococcus* were also found at the offshore station during the upwelling event in June 2011, and a relatively strong EAC period in December 2011 (data shown in Chapter 3, Supplementary Material Table 5). *Synechococcus* was found in association with warm, oligotrophic offshore waters in both the Kimberley and Coffs Harbour regions and was highly abundant in the tropical Kimberley region (Chapter 5). The cyanobacterium's known preferences for warm tropical waters support this thesis'

observation that *Synechococcus* is transported to tropical-temperate Coffs Harbour regions with the EAC, and that this transport is pronounced when the current is strong.



**Figure 2. Monthly averaged abundance and distribution of *Synechococcus* in the Coffs Harbour region.** Monthly averaged *Synechococcus* abundances and standard deviations ( $\mu\text{g Chl a L}^{-1}$ ) determined at stations positioned in ~5, ~11, ~18 and ~27 km from the coast (with distances from coast averaged for B2/CH1/SS, B4/CH2, B6/CH3 and B8/CH5, respectively) at ~30°S, Coffs Harbour, Eastern Australia. Also given are the % shelf width (total shelf width ~30 km) and the isobath at which each station is located. During January 2012 (black bars) the EAC was strong, which resulted in maximum abundances of *Synechococcus* at the offshore station (100 m isobath, near the shelf break).

### 7.3.2 Cross-shelf phytoplankton distribution in Eastern and Western Australia

Phytoplankton have previously been shown to prefer certain marine niches characterised by gradients in environmental variables, in particular nutrient accessibility, turbulence and irradiance in a seasonal context (Margalef 1978, Smayda & Reynolds 2001, Wyatt 2014). The conceptual models developed by Margalef (1978) and Smayda & Reynolds (2001) have focussed on diatom and dinoflagellate distribution, due to the importance of these taxa during annual spring bloom and

harmful algal bloom formation (Wyatt 2014). Coccolithophores, which are also a highly productive taxon in temperate waters, were later integrated in the conceptual model of Balch (2004). By integrating pico- to microphytoplankton, this thesis demonstrates that habitat preferences are expressed across the continental shelf, following gradients of environmental variables in close proximity to the coast (Chapter 5).

The Coffs Harbour and Kimberley regions are oceanographically and topographically distinct (Chapter 5). Nevertheless, similarities were determined in the habitat preferences of individual phytoplankton taxa for specific combinations of environmental variables occurring along cross-shelf gradients. Cryptophytes and prasinophytes were particularly responsive to nutrient-rich inshore waters in both study regions (Chapter 5). Haptophytes preferred intermediate nutrient concentrations and deep waters while dinoflagellates and *Synechococcus* preferred warm, oligotrophic and stratified offshore waters (Chapter 5). Diatoms were highly abundant at the inner shelf station in the Kimberley region (~80 km distance from the coast) while a pronounced bloom of the diatom *Leptocylindrus danicus* dominated the whole shelf (~28 km) at Coffs Harbour (Chapter 5). Post-bloom nutrient depletion led to the re-distribution of pelagophytes and dinoflagellates to zones of local nutrient-maxima (Chapter 5). In summary, it appears that the phytoplankton community in the Coffs Harbour and Kimberley regions cannot be exclusively explained by any single one most important variable but by the interaction of many environmental factors occurring at certain times. These results support the idea that coastal phytoplankton communities are highly stochastic as shown previously in studies of dinoflagellate distribution by Smayda & Reynolds (2001).

Investigations targeting phytoplankton responses to environmental variables across the shelf are now increasingly common in other WBC regions. Several of these studies have found diatoms, cryptophytes and prasinophytes to specifically respond to increased nutrient concentrations leading to their elevated abundances near the coast and in estuaries (Lohrenz et al. 2003, Carreto et al. 2003, 2008, Barlow et al. 2008). Haptophytes (in particular *Emiliania huxleyi*) have been reported to occur at increased abundances in relatively deep waters, consistent with our findings from the Kimberley region, where the coccolithophore *Gephyrocapsa oceanica* was associated with elevated depths (Chapter 5). Carreto et al. (2003, 2008) applied microscopy and CHEMTAX analyses to characterise the phytoplankton community along the east coast of South America (near the Río de la Plata Estuary) influenced by the Brazil Current.

They found *Synechococcus* and *Prochlorococcus* to contribute up to 45% and 41% to the Brazil Current phytoplankton community (Carreto et al. 2008). Their study observation is consistent with our finding of *Synechococcus* and *Prochlorococcus* preferring warm offshore waters (Chapter 5).

### **7.3.3 Phytoplankton dynamics within a near annual cycle**

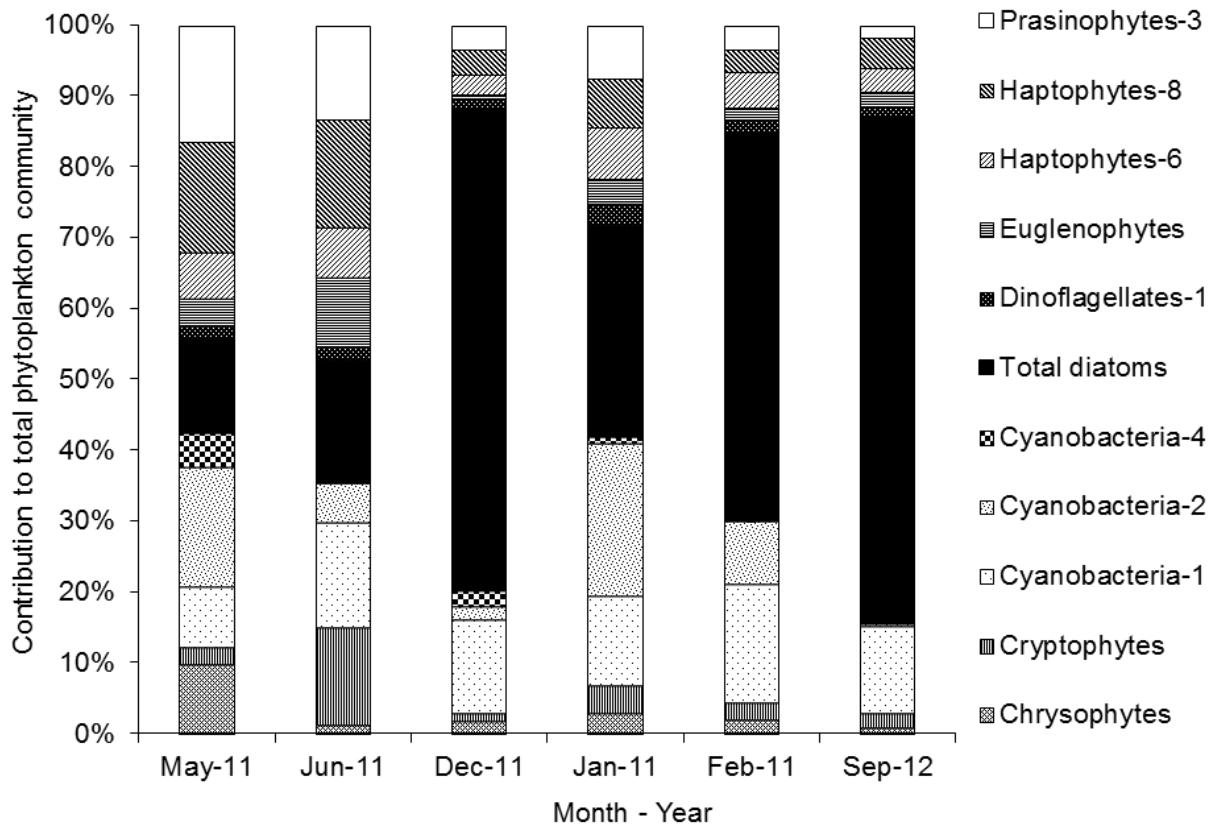
Chapter 6 of this thesis provides the first statistical study linking the phytoplankton community at Coffs Harbour, Eastern Australia, to the complex interactions between seasonality and oceanic forcing. Previous studies from 34°S (Port Hacking, Eastern Australia) reported seasonal variability in phytoplankton abundance and composition to be driven by wind- and EAC-induced upwelling (Hallegraeff & Jeffrey 1993). Their results are different from other temperate regions where seasonal cycles are considered the direct result of seasonal climatic changes (Hallegraeff & Jeffrey 1993). The Coffs Harbour survey, however, revealed the variables ‘month’ and ‘southward velocity/temperature’ to be significant drivers of the phytoplankton community structure throughout the annual cycle (Chapter 6). These two variables were used as proxies of the temporal evolution in phytoplankton composition and the degree of EAC influence (Chapter 6). This approach confirmed the existence of an independent seasonal cycle that interacted with oceanic forcing in driving the temporal phytoplankton variability at Coffs Harbour through 2011 – 2012 (Chapter 6).

The 74 microscopically determined phytoplankton genera were classified into different water-types, i.e. seasonal/bloom, cosmopolitan, deep, warm-water and offshore taxa (Chapter 6). As expected, the highest variation in abundance was exhibited by the seasonal/bloom category (Chapter 6). Two diatom taxa that were considered representatives of the latter category bloomed during December 2011 (*Pseudonitzschia* spp.) and September 2011 (*Leptocylindrus danicus*, Chapter 6). CHEMTAX analyses confirmed the high abundance of diatoms during these two months (Chapter 3). Figure 3 shows the monthly averaged abundance of eleven phytoplankton taxa derived from CHEMTAX analysis of pigment data over six months at Coffs Harbour (based on Chapter 3, Supplementary Material Table 5). Diatoms were totalled due to the difficulty of classifying them into pigment-types (Fig. 3). Based on the CHEMTAX data, we now estimate that diatoms contributed ~70% to the total phytoplankton community during bloom periods in December 2011 and September 2012 (Fig. 3). This

percentage is slightly lower than those previously reported from 34°S (Port Hacking), Eastern Australia, where diatom contributions were ~80 – 90 % to the total phytoplankton community during bloom periods (Hallegraeff 1981). It should be noted that the latter study had not accounted for cyanobacteria, which were found to contribute 12 – 15% to the Coffs Harbour phytoplankton community during bloom periods (Fig. 3). *Trichodesmium* and *Synechococcus* have been identified at Port Hacking in the past (Hallegraeff & Reid 1986). It is likely that the consideration of these two cyanobacteria in phytoplankton community estimates at Port Hacking would approximate the proportions of diatoms at Port Hacking and Coffs Harbour.

The winter phytoplankton community at Coffs Harbour was characterised by a mix of the eleven individual phytoplankton taxa (considering total diatoms only) included in the CHEMTAX analysis (Chapter 3). Each taxon contributed ~15% to the total phytoplankton community during May and June 2011, with the exception of dinoflagellates and cyanobacteria-4 (*Prochlorococcus*), whose contribution was <10% (Fig. 3). Such broadly equivalent taxon contributions to the total phytoplankton community might be explained by the rapidly changing oceanographic conditions prevailing during winter at Coffs Harbour (Chapter 4). It is widely accepted that rapid environmental fluctuations prevent highly competitive species from outgrowing others; a theory well known as the 'Intermediate Disturbance Hypothesis' (Hutchinson 1961, Wilson 1994). As a consequence, a diverse mix of species can co-exist between episodes of physical disturbances (Hutchinson 1961, Wilson 1994). Lohrenz et al. (2003) have referred to this hypothesis as being an underlying force behind the rapid wind-induced changes in local oceanography that influenced phytoplankton size and community structure in North Carolina shelf waters. The rapid alternations that occurred between upwelling and downwelling events during austral winter at Coffs Harbour may thus have contributed to the relatively even abundances of co-existing taxa during May and June 2011 (Fig. 3).





**Figure 3. Monthly averaged phytoplankton composition at Coffs Harbour derived from CHEMTAX.** Monthly averaged proportions (% based on  $\mu\text{g Chl } a \text{ L}^{-1}$ ) of eleven phytoplankton taxa (pigment-types) derived from CHEMTAX analysis of pigment profiles. A mixed population of equally abundant phytoplankton taxa characterised the phytoplankton community during winter (May and June 2011). Diatoms dominated the phytoplankton community during summer, especially during December 2011 and September 2012, when blooms of the diatoms *Pseudo-nitzschia* spp. and *Leptocylindrus danicus*, respectively, were detected. During January 2012 the EAC was strong and enhanced abundances of the cyanobacteria *Trichodesmium erythraeum* (cyanobacteria-1) and *Synechococcus* (cyanobacteria-2) were determined.

The annual cycle survey revealed that *Trichodesmium erythraeum* is permanently established in the Coffs Harbour region and undergoes a seasonal cycle (Chapter 6). Commonly, *T. erythraeum* has been regarded as an indicator species of increased EAC influence and has been reported as appearing in high abundances at Port Hacking during El Niño years (Ajani et al. 2011). In the Coffs Harbour region, *T. erythraeum* appeared during times when the EAC was weak in October 2011, as well as when the EAC was strong in January 2012 (acknowledging a much higher abundance of the cyanobacterium during strong EAC conditions; Chapter 6). The cyanobacterium also bloomed about one year later, during October 2012, confirming

an annual cycle pattern. The complete survey period represented by this thesis fell within a La Niña phase lasting from 2010 to 2012 (<http://www.bom.gov.au/climate/enso/Inlist/>). Pigment data analysed by CHEMTAX (Chapter 3) confirmed the permanent presence of *T. erythraeum* in revealing that this cyanobacterium contributed on average between 9% and 17% to the total phytoplankton community per month (shown as cyanobacteria-1 in Figure 3).

### **7.3.4 Similarities in shelf-scale phytoplankton dynamics between Coffs Harbour and other WBC regions**

Several phytoplankton taxa that were associated with specific water types in this thesis have been shown to prefer similar environmental conditions in the coastal waters of other WBC systems. For example, diatoms have been shown to be indicative of cold, nutrient-rich water in other subtropical WBC systems (Lohrenz et al. 2003, Carreto et al. 2003, 2008, Barlow et al. 2008, 2010, 2013, Moreno et al. 2012, Sá et al. 2013). Along the south-east African coast, which is strongly influenced by the Agulhas Current and frequent upwelling, diatoms have been found to be highly adaptable to sudden nutrient-pulses and changing light conditions due to a flexible light-harvesting physiology (Barlow et al. 2010, 2013). In contrast, dinoflagellates, other flagellates and prokaryotes including *Synechococcus* and *Prochlorococcus* have been associated with warm offshore regions, with picoplankton preferring highly irradiated surface waters and nanoplankton occupying deeper waters (Carreto et al. 2008, Sá et al. 2013, Barlow et al. 2013). Upwelling has been found to be the major driving force of productivity in all four subtropical WBC systems (Gong et al. 1997, Lohrenz et al. 2003, Carreto et al. 2008, Jackson et al. 2012). Such numerous parallels in interactions between oceanographic conditions and phytoplankton responses between the Coffs Harbour region and the other four major subtropical WBC systems confirm that this thesis provides valuable data on shelf-scale phytoplankton dynamics that are applicable to studying changes in global WBC systems.

### **7.3.5 Importance of oceanographically-driven phytoplankton dynamics off Coffs Harbour within the context of a long-term-strengthening EAC**

Phytoplankton communities in the Coffs Harbour region were investigated over an almost complete annual cycle incorporating the major spring bloom period. As this is

considered a short period of time on 'climatic' timescales, no conclusions can be drawn regarding climate change-induced long-term changes in phytoplankton dynamics. Yet the results allow for an understanding of the interactions between phytoplankton communities and shelf-scale oceanographic processes. Such knowledge is fundamental in predicting potential effects of long-term oceanographic changes on phytoplankton community composition and response to longer-term oceanic change. In this context, the expectation of a decreasing diatom to dinoflagellate ratio along the south-east Australian coast, caused by long-term changes in oceanographic processes (Thompson et al. 2009), raises two points addressed by the results of this thesis.

The first point focuses on the predicted increase in upwelling frequency along the east Australian coast as a result of EAC strengthening (Chapter 4). This thesis has shown that diatoms, in particular, increased their abundance in response to cold nutrient-rich slope water intrusions (Chapter 4 - 6). Therefore, one might expect a rise of this phytoplankton class along the east Australian coast should the frequency in upwellings increase. In contrast, Thompson et al. (2009) reported a long-term silicate decline attributed to the strengthening of the EAC, which was expected to reduce the abundance of silicic-acid requiring diatoms along the east Australian coast. Resolving this conflicting diatom abundance response to increased upwelling events requires the replenishment of silicate (or indeed other trace nutrients) in the upwelled water. It is equally possible to envision that silicate-limited diatoms may adapt to low silicate concentrations by producing thinner or smaller frustules. Variations in cellular Si content towards increased silicification have been shown to occur when diatoms are exposed to iron-deficiency (Marchetti & Cassar 2009). Alternatively, species shifts might occur towards lightly silicified diatoms or diatoms with low half-saturation constants ( $K_s$ ) for silicate uptake. Thompson et al. (2008) already reported a shift from a *Pseudo-nitzschia* spp. to a *Skeletonema* spp. (low  $K_s$ ; Paasche 1973) dominated phytoplankton community in coastal waters at ~43°S, Tasmania. The lightly silicified species *Thalassiosira* cf. *partheneia* (Brzezinski 1985) has been shown to be the most abundant diatom at 34°S, Port Hacking, Eastern Australia over the past decade (Ajani et al. 2014). However, whether the abundance of this lightly silicified diatom at Port Hacking is an early warning signal of the long-term silicate decline can only be determined through decadal length studies.

Secondly, dinoflagellates were expected to increase proportionally in abundance along the east Australian coast as a consequence of the decrease in diatom abundances caused by the anticipated silicate-decline (Thompson et al. 2009). In addition, the strengthening of the EAC had been predicted to facilitate the poleward migration of tropical species (Hallegraeff 2010). This thesis has demonstrated that the transport of tropical dinoflagellates (e.g. *Ceratium massiliense*, *C. ranipes*, *Ornithocercus thumii*, *Oxytoxum milneri*, *Podolompas palmipes*) to the Coffs Harbour region was increased during times when the EAC was strong (Chapter 4 and 6). Therefore, the findings within this thesis are consistent with the predictions by Thompson et al. (2009) and Hallegraeff (2010), whereby a shift towards a higher proportion of dinoflagellates with a strengthening EAC seems plausible. A distinct spatial preference of dinoflagellates was determined for warm, offshore waters associated with EAC influence (Chapter 6). It is proposed here that the hypothesis of Hallegraeff (2010) can be refined, and that elevated abundances of dinoflagellates will be most noticeable in offshore regions where the main flow of the warm EAC prevails. Continued study within the Coffs Harbour region is best suited to test this hypothesis as it remains a region where the EAC is in close proximity to the coast (~30 km) and is upstream of the EAC separation point.

### **7.3.6 Recommendations for future research**

Upwelling-promoted nutrient supply, in particular of silicate, was identified as the main driver of diatom productivity in the Coffs Harbour region (Chapter 4 - 6, section 7.3.1). The conflict of increased diatom abundance due to the predicted amplification in upwelling frequencies versus decreased diatom abundances caused by the long-term silicate-decline as a result of the EAC strengthening raised many questions regarding the future fate of diatom abundance along the east Australian coast (section 7.3.1). In order to clarify the future performance of diatoms under such dramatically changing conditions, laboratory- and field-based research will be required. Laboratory-based experiments with locally isolated diatom species may be specifically suited to investigate physiological responses of members of the regional diatom community to changes in silicic acid availability (e.g. size reduction, degree of silicification and/or community shifts). Preferably, such experiments should be carried out within mesocosms, which are highly suited to simulate natural conditions (e.g. Lassen et al. 2010). Field-based studies should focus on determining impacts of the silicate-decline

on phytoplankton communities at different latitudes along the east Australian coast. While silicate concentrations have decreased at a rate of  $\sim 2 \mu\text{M century}^{-1}$  over the past 60 years at  $34^\circ\text{S}$ , Port Hacking, Eastern Australia, a decrease of  $\sim 5.8 \mu\text{M century}^{-1}$  has been determined at  $\sim 43^\circ\text{S}$ , Maria Island, Tasmania (Thompson et al. 2009). The latter decrease was reported alongside a 50% reduction in the phytoplankton spring bloom biomass from 1997 to 2009 (Thompson et al. 2009). The latitudinal difference in the rates of silicate-decline may partly be due to variations in silicate uptake by phytoplankton during the EAC's southward progression from the tropics, or because silicate along the east Australian coast is largely replenished via runoff from several coastal embayments (e.g. Yamba at  $\sim 29^\circ\text{S}$ , Port Macquarie at  $\sim 31^\circ\text{S}$ , Port Stephens at  $\sim 32^\circ\text{S}$ , Broken Bay at  $\sim 33^\circ\text{S}$ ). Annual rainfall and runoff in south-east Australian catchments is predicted to increase due to climate change (<http://www.climatechange.gov.au/climate-change/climate-science/climate-change-impacts/new-south-wales>) promoting an increased silicate supply from the land in the future. More research in this context will provide crucial information on whether regions at high southern latitudes, such as the Tasmanian east coast, will be the hotspots of long-term diatom productivity changes.

Silicoflagellates, which also require silicic acid to grow, are often forgotten due to their very low abundance. Within this thesis, the generally minor importance of this phytoplankton group was confirmed. Yet we were able to classify *Dictyocha* spp. as offshore taxon in Chapter 6, which suggests that *Dictyocha* spp. might become more pronounced in the Coffs Harbour region as a direct result of the increased influence of the EAC. On the contrary, long-term silicate decline and sea surface temperature warming might reduce the already low abundances of silicoflagellates further, potentially driving its disappearance from the Coffs Harbour region. *Dictyocha speculum*, which was identified to occur in lower abundances than its close relative *D. fibula* within this thesis, has been shown elsewhere to cope poorly with enhanced temperatures (Lewandowska et al. 2014). Future research should focus on the largely unknown role of silicoflagellates in coastal ecosystems, and the effects a potential disappearance of this taxon might have on the functioning of the marine food web in the Coffs Harbour region.

Future research in the Coffs Harbour region should be directed toward exploring the phytoplankton community during highly productive upwelling periods more closely. Specifically, sampling during phytoplankton bloom periods should be carried out at

daily intervals, to investigate species succession patterns, and be coupled with investigations of carbon, nitrogen and silicon export. Such coupled investigations will reveal important insights into taxon-specific export production and biogeochemical cycles that are altogether missing from the east Australian coast. Sampling throughout phytoplankton spring bloom periods should furthermore focus on determining the longevity of the pronounced mid-shelf response of phytoplankton determined within this thesis. The extensive diatom bloom across the whole Coffs Harbour shelf, investigated in Chapter 5, suggests that a mid-shelf response to upwelling, as found during June 2011 and January 2012 (Chapter 4 and section 7.3.1), characterises an early stage in bloom progression. According to the Intermediate Disturbance Hypothesis (IDH), mono-specific bloom formation is the result of a relatively long stagnant period between physical fluctuations (Wilson 1994, Huisman et al. 1999). Future investigations of the applicability of the IDH within the context of Coffs Harbours rapidly changing oceanographic conditions and responsive phytoplankton community dynamics, is warranted. However, the recurrent mid-shelf response represents a food-patch to higher trophic organisms, thus investigating distributional patterns of zooplankton and fish may be especially worthwhile during early upwelling periods. Within this thesis, we found zooplankton abundances to be elevated at the mid-shelf during the strong EAC period in January 2012 (Appendix 3), coinciding with the major phytoplankton response in this location (section 7.3.1). Knowledge about the cross-shelf distributional patterns of phyto- and zooplankton may benefit fisheries (to the south of the SIMP) and marine park authorities for zone planning (as the SIMP).

Having identified the preferences of individual phytoplankton taxa for different environmental variables in two shelf regions of Australia (Chapter 5), we can now use such data to improve ecosystem models. A recent study by Everett et al. (2014) has identified surface Chl *a* hotspots along the east Australian coast by using satellite-derived Chl *a* data in combination with a hydrodynamic model. Such hydrodynamic models could be extended by integrating information on associations between specific environmental variables (e.g. temperature, nutrient concentrations, salinity, density) and individual phytoplankton taxa. Using such an approach, the abundance of specific phytoplankton taxa could be modeled according to the prevailing oceanographic regime. The integration of phytoplankton preference data may yield central insights into the seasonal progression of individual phytoplankton taxa and their contribution to identified Chl *a* hotspots. Specific ranges of environmental variables that favour the

growth of individual phytoplankton taxa remain to be identified but once established will enable the large-scale modelling of taxon-specific productivity along the east Australian coast.

Ultimately, there is an urgent need for continuous physico-chemical and biological sampling over long time-periods. Definite long-term changes in the oceanographic environment and phytoplankton abundance, composition and distribution along the east Australian coast can only be addressed through many consecutive years of research. In the Coffs Harbour region, IMOS has initiated a continuous monitoring of the EAC. This thesis provides a crucial baseline study of pico- to microphytoplankton dynamics during one annual cycle within this oceanographic monitoring framework. Building on this starting point, long-term research within the context of EAC strengthening should be focussed on phytoplankton composition changes and southward range expansions. Microscopy analyses will be of crucial importance to monitor changes on the microphytoplankton species level (diatoms, dinoflagellates, silicoflagellates) expected due to long-term changes in physico-chemical parameters along the east Australian coast. In addition, CHEMTAX and molecular analyses will be of vital importance to monitor the composition, abundance and distribution of small-sized phytoplankton taxa. For example, the picoplanktonic cyanobacterium *Synechococcus* was suggested to be indicative of increased EAC influence within this thesis (section 7.3.1). Very recently, Pittera et al. (2014) have shown that different clades of *Synechococcus* are adapted to specific temperature ranges typically occurring at their latitude of origin. Thus, molecular analyses will be required to resolve where *Synechococcus*, identified within this thesis, was truly sourced. Additionally, such distributional information will permit the inclusion of small-sized phytoplankton taxa in a tropical:temperate species ratio assessment (section 7.1.2). The application of this ratio alone will benefit a comprehensive monitoring of the tropicalisation of primary producers in the long-term.

## **7.4 Conclusion**

The Coffs Harbour coast is a region where extraordinary tropical-temperate marine biodiversity is under protection. Since the declaration of the SIMP in 1998, the region has become a symbolic and iconic monitoring location of transitional biodiversity. Monitoring, however, has been limited either to higher trophic level organisms with conservational value, or to physical oceanographic parameters to better understand the highly variable coastal oceanography and the characteristics of the EAC. This thesis provides the first ever study of phytoplankton in the region, and thus represents a strategic interface between the local biological diversity and the physical environment. From the outset, this thesis has aimed to provide a detailed survey of the phytoplankton over an annual cycle in response to the variable physical conditions typifying the Coffs Harbour region. The approach was supported through modern and traditional means of taxonomic identification. At the same time, a statistical comparison of the very different techniques microscopy and CHEMTAX was conducted to ensure that future studies understand the pros and cons of applying either approach. Such an understanding is vital to a comprehensive and realistic interpretation of collected data, especially when the purpose is to decipher long-term trends in phytoplankton communities. The backdrop to this study has been the global observations of strengthening Western Boundary Currents creating hotspots of ocean temperature warming. The EAC is one of these hotspots, thus studies both oceanographically and biologically are crucial to elucidate the impacts of a strengthening EAC on the marine environment.

This thesis has successfully achieved the aims it set out to accomplish. Combined microscopy and CHEMTAX analyses revealed a diverse phytoplankton species mix including diatoms, cyanobacteria, haptophytes, prasinophytes, chrysophytes, euglenophytes, cryptophytes, dinoflagellates and pelagophytes (in descending order of average abundance) throughout one annual cycle (2011/2012). A statistical comparison between microscopy and CHEMTAX abundance estimates showed a good agreement at the class level. While microscopy allowed a high resolution of the microphytoplankton community, CHEMTAX was greatly suited to comprehensively characterise the phytoplankton community from pico- to microphytoplankton. A distributional analysis of 137 microphytoplankton taxa revealed an equally tropical and temperate community at Coffs Harbour. The tropical species influence was elevated at times when the EAC was strong, which was expressed in the increased abundance of



tropical dinoflagellates (e.g. species of the genera *Ceratium*, *Podolampas*, *Ornithocercus* and *Oxytoxum*) and cyanobacteria (*Trichodesmium erythraeum* and *Synechococcus* sp.) offshore (~28 km away from the coast). Nutrient, especially silicate, availability was an important driving force of diatom productivity during upwelling periods, with the diatom response being greatest at the mid-shelf (~16 km away from the coast). Two blooms were identified and initiated by a current- and a wind-driven upwelling event, during December 2011 (*Pseudo-nitzschia* spp.) and September 2012 (*Leptocylindrus danicus*), respectively. Despite the high influence of the EAC on phytoplankton abundance, distribution and composition, seasonal cycles were also determined as drivers of the phytoplankton variability throughout the year. For example, *T. erythraeum* was suggested to undergo a seasonal cycle in the Coffs Harbour region. Individual phytoplankton taxa showed similar preferences for distinct combinations of cross-shelf gradients in environmental variables. Such similarities in environmental preferences were detected in two highly oceanographically distinct regions of Australia, confirming the existence of taxon-specific adaptations to marine niches across the shelf.

This thesis sets a benchmark by its multidisciplinary approach, merging oceanography, traditional, modern and statistical phytoplankton analysis. Through this approach, novel insights were gained into the dynamics of natural phytoplankton communities in the Coffs Harbour region. Without such baseline research the determination of any long-term changes in phytoplankton communities under climate change will be challenging. Considering the, here demonstrated, inseparability of the oceanographic environment and phytoplankton dynamics, we can expect that major changes in phytoplankton communities will occur along the east Australian coast as a result of the strengthening EAC. Equally, we may expect modifications in phytoplankton community structure and distribution in other WBC systems. All of these systems are currently undergoing similar changes in their physical parameters. Australia is in the unique position of being equipped with a nationally co-ordinated state of the art coastal observing system (IMOS). IMOS has opened new doors to opportunities for collaborative research between oceanographers and biologists (as evidenced by this thesis). It is the responsibility of the scientific community to proceed with such pioneering research to unravel interactions from physics to fish. Only through sharing skills and knowledge in cross-disciplinary investigations will Science achieve a global

picture of oceanic changes and their impacts on key primary producers, the phytoplankton.

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## Appendix 1

**SOLITARY ISLANDS MARINE PARK****RESEARCH PERMIT NUMBER**

SIMP 2011/002

**Marine Parks (Zoning Plan) Regulation 1999**

Part 1 Division 3. Clause 1.31: Organised Research Activity

**TO WHOM IT MAY CONCERN**

The bearer of this permit, Linda Ambrecht of Macquarie University is permitted to undertake phytoplankton sampling in the Solitary Islands Marine Park (SIMP), as part of the study "Phytoplankton characterization and survey of the Coffs Harbour region, eastern Australia, with special emphasis on changing biogeochemical parameters due to global warming". This study will involve monthly water sampling.

This permit is current until 1/7/2013.

**Permit conditions:**

- Water sampling is permitted in the Solitary Islands Marine Park
- The MPA be provided with a copy of any written publications arising from this project.
- The Marine Park Manager be informed 48 hours in advance of any work being undertaken within the Solitary Islands Marine Park
- This permit (or a copy thereof) must be produced to any Fisheries Officer or Marine Park Ranger on demand.
- Holders of this permit and their staff are expected to behave and conduct activities under a professional code of conduct that does not generate conflict and criticism from the public or other private sectors, including the fishing industries and local communities.

A handwritten signature in black ink, appearing to read "N Johnstone", with a long horizontal flourish extending to the right.

Nicola Johnstone  
Marine Park Manager  
Solitary Islands Marine Park  
11 May 2011

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## Appendix 2

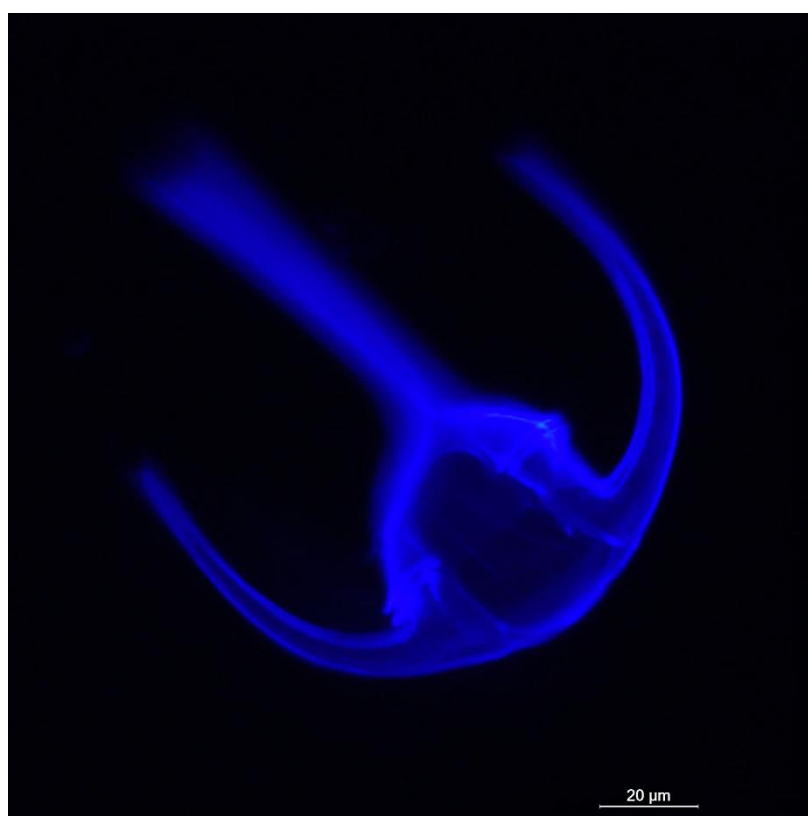
The microphytoplankton of the Coffs Harbour region (~30°S), Eastern Australia,

May 2011 – September 2012

by

Linda Hanna Armbrecht

16 June 2014



### Introduction

This identification guide provides the first compilation of marine microphytoplankton taxa identified off Coffs Harbour (~30°S), Eastern Australia, between 27 May 2011 and 12 September 2012. Within this document, a complete list of phytoplankton taxa identified by light microscopy is given. Photographs of most taxa are included, however, as photography was conducted during the analysis of field samples occasional overlying particles or mis-positioning of specimens prevented adequate photographing of some taxa.

### Methods

Phytoplankton sampling was conducted monthly off Coffs Harbour at fixed depth intervals, along two cross-shelf transects ~30 km apart and at one coastal station between the two transects. Water samples were collected with 5 L Niskin bottles (General Oceanics, USA), or with a 10 L plastic bucket (at the surface) as described in detail in Chapter 2. Two liters of seawater were fixed in plastic containers with 6 mL of Lugol's acid solution, returned to the laboratory and concentrated by sedimentation (48 hrs; Chapter 2). Identification of phytoplankton taxa preserved in 3 mL subsamples was accomplished under an inverted microscope (Leica DMI3000B) at 200x – 630x using Utermöhl counting chambers (Hydrobios Kiel, Germany). Identification was made at the lowest taxonomic level possible using appropriate taxonomic literature including Dakin & Colefax (1940), Wood (1954, 1961a, b), Crosby & Wood (1958, 1959), Wood et al. (1959), Tomas (1997), Hallegraeff et al. (2010) and further studies by Hallegraeff & Reid (1986), Ajani et al. (2001), Gómez et al. (2008), Harper et al. (2012) and Stidolph et al. (2012). A Calcofluor White Stain (Sigma Aldrich, USA) solution was added to each 3 mL sample 30 min prior to counting, at a final concentration of 20 µg mL<sup>-1</sup>, to facilitate identification of thecate dinoflagellates (Fritz & Triemer 1985). Quantitative microphytoplankton assessments undertaken are detailed in Chapter 3 – 6. The exact sampling locations and abundances of individual taxa can be accessed online at:

<http://catalogue.aodn.org.au/geonetwork/srv/en/metadata.show?uuid=f7502841-a2c7-4437-b557-20ef89e754e0> (see also Appendix 3).

## Results

A total number of 74 microphytoplankton genera including 137 taxa were determined. A complete list of taxa under their currently accepted names according to the World Register of Marine Species (WoRMS; <http://www.marinespecies.org/index.php>) and a photographic guide follow.

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## Acknowledgements

I thank Dr Penelope Ajani, Dr Tim Ingleton, Dr Leanne Armand and Dr Itsuki Suto for their help with phytoplankton identification. I also thank the Australian Biological Resources Study for funding my participation in the first Phytoplankton Identification Workshop held at the Sydney Institute of Marine Sciences, Chowder Bay, New South Wales, Australia, 30 May - 02 June 2011.

**List of taxa****Cyanophyceae**

*Trichodesmium erythraeum* Ehrenberg ex Gomont, 1893

**Bacillariophyceae (centric diatoms)**

*Anaulus minutus* Grunow, 1882

*Asteromphalus flabellatus* (Brébisson) Greville, 1859

*Asteromphalus* spp. Ehrenberg, 1844

*Bacteriastrum elongatum* Cleve, 1897

*Bacteriastrum furcatum* Shadbolt, 1854/*B. delicatulum* Cleve, 1897

*Bacteriastrum hyalinum* Lauder, 1864

*Bacteriastrum* spp. Shadbolt, 1854

*Cerataulina pelagica* (Cleve) Hendey, 1937

*Chaetoceros atlanticus* Cleve, 1873

*Chaetoceros compressus* Lauder, 1864

*Chaetoceros coronatus* Gran, 1897

*Chaetoceros curvisetus* Cleve, 1889

*Chaetoceros decipiens* Cleve, 1873

*Chaetoceros lorenzianus* Grunow, 1863

*Chaetoceros peruvianus* Brightwell, 1856

*Chaetoceros seiracanthus* Gran, 1897

*Chaetoceros socialis* H.S.Lauder, 1864

*Chaetoceros* spp. Hyalochaete (genus *Chaetoceros* Ehrenberg, 1844)

*Chaetoceros* spp. Phaeoceros (genus *Chaetoceros* Ehrenberg, 1844)

*Climacodium frauenfeldianum* Grunow, 1868

*Corethron pennatum* (Grunow) Ostensfeld, 1909

*Corethron* spp. Castracane, 1886

*Coscinodiscus* spp. Ehrenberg, 1839

*Cyclotella* spp. (Kützing) Brébisson, 1838

*Dactyliosolen fragilissimus* (Bergon) Hasle, 1996

*Detonula pumila* (Castracane) Gran, 1900

*Ditylum brightwellii* (T.West) Grunow, 1885

*Eucampia cornuta* (Cleve) Grunow, 1883

*Eucampia zodiacus* Ehrenberg, 1839

*Guinardia delicatula* (Cleve) Hasle, 1997

*Guinardia flaccida* (Castracane) H.Peragallo, 1892

*Guinardia striata* (Stolterfoth) Hasle, 1996

*Helicotheca tamesis* (Shrubsole) M.Ricard, 1987

*Hemiaulus hauckii* Grunow ex Van Heurck, 1882

*Hemiaulus membranaceus* Cleve, undated

*Lauderia annulata* Cleve, 1873/*Thalassiosira* spp. Cleve, 1873

*Leptocylindrus danicus* Cleve, 1889

*Leptocylindrus mediterraneus* (H.Peragallo) Hasle, 1975

*Odontella aurita* (Lyngbye) C.Agardh, 1832

*Odontella mobiliensis* (J.W.Bailey) Grunow, 1884

*Odontella* spp. C.Agardh, 1832

*Paralia sulcata* (Ehrenberg) Cleve, 1873

*Planktoniella sol* (C.G.Wallich) Schütt, 1892  
*Proboscia alata* (Brightwell) Sundström, 1986  
*Stephanopyxis cruciata* (Ehrenberg) Tempère & Peragallo<sup>#</sup>  
*Rhizosolenia* spp. Brightwell, 1858  
*Skeletonema* spp. Greville, 1865  
*Triceratium dubium* Brightwell, 1859  
*Triceratium obtusum*<sup>^</sup>  
*Triceratium* spp. Ehrenberg, 1839  
*Trigonium alternans* (J.W.Bailey) A.Mann, 1907  
*Undefined centric diatom*

### **Bacillariophyceae (pennate diatoms)**

*Amphora* spp. Ehrenberg ex Kützing, 1844  
*Asterionellopsis glacialis* (Castracane) Round, 1990  
*Ceratoneis closterium* Ehrenberg, 1839/*Nitzschia longissima* (Brébisson) Ralfs, 1861  
*Climacosphenia moniligera* Ehrenberg, 1843  
*Diploneis* spp. Ehrenberg ex Cleve, 1894  
*Entomoneis* spp. Ehrenberg, 1845  
*Grammatophora hamulifera* Kützing, 1844  
*Grammatophora marina* (Lyngbye) Kützing, 1844  
*Grammatophora oceanica* Ehrenberg, 1840  
cf. *Gyrosigma balticum* (Ehrenberg) Rabenhorst, 1853  
*Mastogloia rostrata* (Wallich) Hustedt, 1933  
*Meuniera membranacea* (Cleve) P.C.Silva, 1996  
*Navicula* spp. Bory de Saint-Vincent, 1822  
*Neodenticula seminae* (R.Simonsen & T.Kanaya) F.Akiba & Y.Yanagisawa, 1986  
*Nitzschia* Hassall, 1845/*Lioloma* Hasle, 1997/*Thalassiothrix* spp. Cleve & Grunow, 1880  
*Pleurosigma* spp. W.Smith, 1852  
*Pseudo-nitzschia* spp. H.Peragallo, 1900  
cf. *Pseudo-nitzschia subcurvata* (G.R.Hasle) G.A.Fryxell, 1993  
*Rhabdonema adriaticum* Kützing, 1844  
*Surirella fastuosa* (Ehrenberg) Ehrenberg, 1843  
*Synedra* spp. Ehrenberg, 1830  
*Thalassionema bacillare* (Heiden) Kolbe, 1955  
*Thalassionema nitzschioides* (Grunow) Mereschkowsky, 1902/*T. frauenfeldii* (Grunow) Hallegraeff, 1986  
*Trachyneis aspera* (Ehrenberg) Cleve, 1894  
*Undefined pennate diatom* <40µm  
*Undefined pennate diatom* >40µm

### **Dinophyceae**

cf. *Alexandrium* Halim, 1960/*Gonyaulax* Diesing, 1866/*Heterocapsa* spp. Stein, 1883  
*Amphisolenia bidentata* Schröder, 1900  
*Ceratium arietinum* Cleve, 1900  
*Ceratium candelabrum*<sup>\*</sup> (Ehrenberg) Stein, 1883  
*Ceratium concilians*<sup>\*</sup> E.G.Jørgensen, 1920  
*Ceratium extensum*<sup>\*</sup> (Gourret) Cleve, 1900

- Ceratium furca*\* (Ehrenberg) Claparède & Lachmann, 1859  
*Ceratium fusus*\* (Ehrenberg) Dujardin, 1841  
*Ceratium lineatum*\* (Ehrenberg) Cleve, 1899  
*Ceratium macroceros*\* (Ehrenberg) Cleve, 1899  
*Ceratium massiliense*\* (Gourret) E.G.Jørgensen, 1911  
*Ceratium pentagonum*\* Gourret, 1883  
*Ceratium ranipes*\* Cleve, 1900  
*Ceratium symmetricum*\* Pavillard, 1905  
*Ceratium tenue*\* Ostenfeld & Schmidt, 1901  
*Ceratium trichoceros*\* (Ehrenberg) Kofoed, 1908  
*Ceratium tripos*\* (O.F.Müller) Nitzsch, 1817  
*Ceratium* spp. Schrank, 1793  
*Ceratocorys horrida* Stein, 1883  
*Dinophysis acuminata* Claparède & Lachmann, 1859  
*Dinophysis caudata* Saville-Kent, 1881  
*Dinophysis dens* Pavillard, 1915  
*Dinophysis hastata* Stein, 1883  
*Dinophysis schuettii* Murray & Whitting, 1899  
*Dinophysis* spp. Ehrenberg, 1839  
*Dissodinium pseudolunula* Swift ex Elbrächter & Drebes, 1978  
*Gymnodinium* spp. Stein, 1878  
*Gyrodinium* spp. Kofoed & Swezy, 1921  
*Karlodinium* spp. J.Larsen, 2000  
*Noctiluca scintillans* (Macartney) Kofoed & Swezy, 1921  
*Ornithocercus magnificus* Stein, 1883  
*Ornithocercus quadratus* Schütt, 1900  
*Ornithocercus thumii* (Schmidt) Kofoed & Skogsberg, 1928  
*Ornithocercus* spp. Stein, 1883  
*Oxytoxum compressum* Kofoed (synonym: *Corythodinium compressum* (Kofoed) Taylor, 1976  
*Oxytoxum constrictum* (Stein) Bütschli, 1885  
*Oxytoxum diploconus* Stein  
*Oxytoxum laticeps* Schiller, 1937  
*Oxytoxum milneri* Murray & Whitting, 1899  
*Oxytoxum scolopax* Stein, 1883  
*Oxytoxum tessellatum* (Stein, 1883) Schütt, 1895  
cf. *Oxytoxum turbo* Kofoed, 1907  
cf. *Oxytoxum variabile* Schiller, 1937  
*Oxytoxum* spp. Stein, 1883  
*Phalacroma rotundatum* (Claparède & Lachmann) Kofoed & Michener, 1911  
*Phalacroma* spp. Stein, 1883  
*Podolampas bipes* Stein, 1883  
*Podolampas palmipes* Stein, 1883  
*Podolampas spinifera* Okamura, 1912  
*Podolampas* spp. Stein, 1883  
cf. *Pronoctiluca* spp. Fabre-Domergue, 1889  
*Prorocentrum cordatum* (Ostenfeld) Dodge, 1975  
*Prorocentrum dentatum* Stein, 1883

*Prorocentrum lima* (Ehrenberg) F.Stein, 1878  
*Prorocentrum micans* Ehrenberg, 1834  
*Prorocentrum rathymum* Loeblich, Sherley & Schmidt, 1979  
*Prorocentrum rostratum* Stein, 1883  
*Prorocentrum triestinum* J.Schiller, 1918  
*Prorocentrum* spp. Ehrenberg, 1834  
*Protoperidinium bipes* (Paulsen) Balech, 1974  
*Protoperidinium elegans* (Cleve) Balech, 1974  
*Protoperidinium* spp. Bergh, 1882  
*Schuetiella mitra* (Schütt) Balech, 1988  
*Scrippsiella trochoidea* (Stein) Balech ex Loeblich III, 1965  
*Torodinium* spp. Kofoed & Swezy, 1921 (cf. *T. robustum* Kofoed & Swezy, 1921)  
*Warnowia polyphemus* (Pouchet) J.Schiller, 1933  
*Warnowia pulchra* (Schiller) Schiller, 1933  
*Warnowia* spp. Lindemann, 1928  
*Dinoflagellate undefined*

### **Dictyochophyceae**

*Dictyocha fibula* Ehrenberg, 1839  
*Dictyocha octonaria* Ehrenberg, 1844 (synonym: *Octactis octonaria* (Ehrenberg) Hovasse, 1946)  
*Dictyocha speculum* Ehrenberg, 1839  
*Dictyocha* spp. Ehrenberg, 1837

#*Stephanopyxis cruciata* (previously *Pyxidicula cruciata* Ehrenberg, 1838) is not confirmed in WoRMS but recorded in Algaebase ([http://www.algaebase.org/search/species/detail/?species\\_id=Ce1ac6fa649e1fc6f](http://www.algaebase.org/search/species/detail/?species_id=Ce1ac6fa649e1fc6f)).

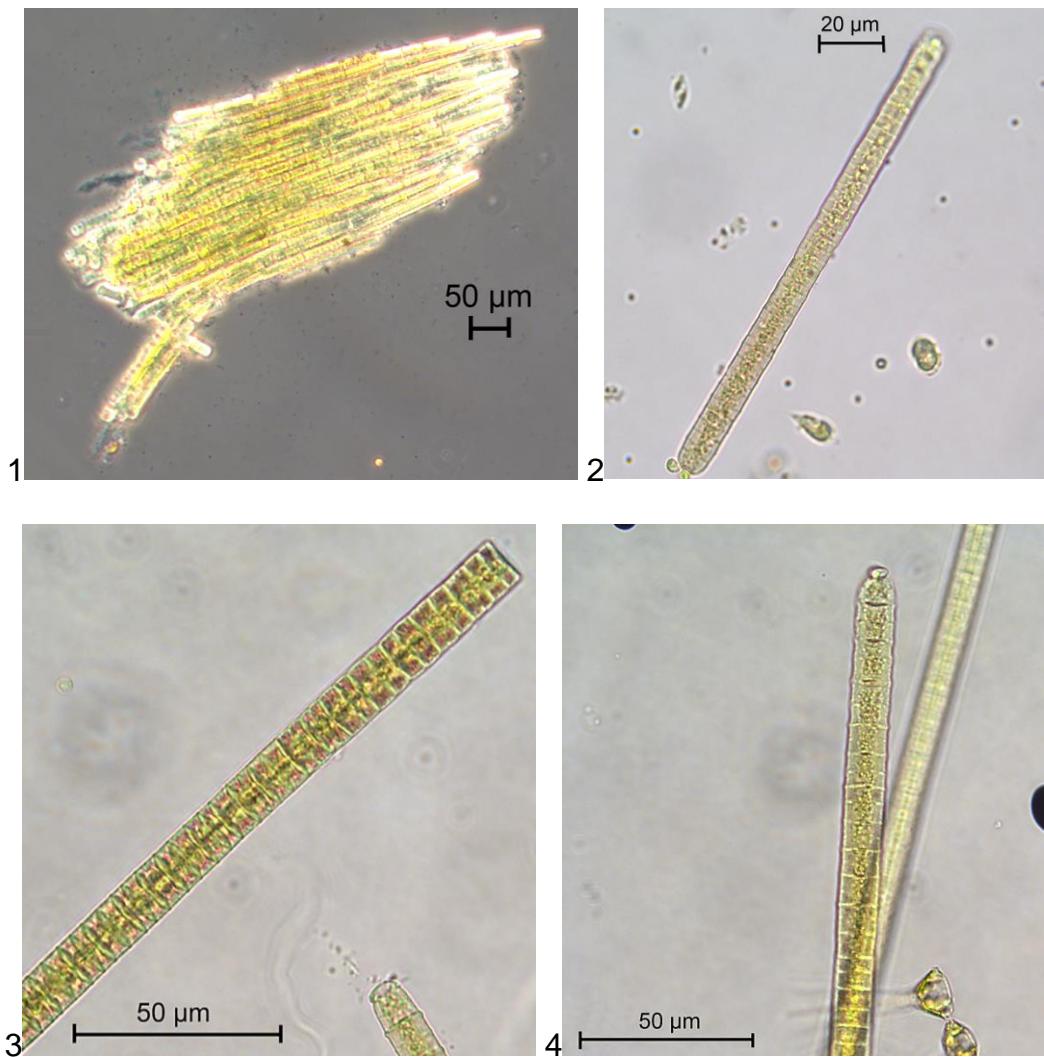
This species has been observed in one sample at CH3, 60 m, 07 November 2011, subsequently to species enumeration and is included in this identification guide for reasons of completeness. It was not included in any quantitative analysis within this thesis. Crosby & Wood (1958) have reported *S. cruciata* to occur at ~34°S (Port Hacking; in bottom mud between 60 and 120 m depth) and at ~23°S (Heron Island), Eastern Australia.

^*Triceratium obtusum* Ehrenberg is not listed in WoRMS but recorded in Algaebase ([http://www.algaebase.org/search/species/detail/?species\\_id=y91a45b6d7f4d2a63](http://www.algaebase.org/search/species/detail/?species_id=y91a45b6d7f4d2a63), MD Guiry in Guiry MD & Guiry GM (2014). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway).

\*All *Ceratium* species listed above have recently been re-classified under the new genus *Neoceratium* following Gómez et al. 2010.



**Cyanophyceae**



1 *Trichodesmium erythraeum*

30.10.2012

2 “

“

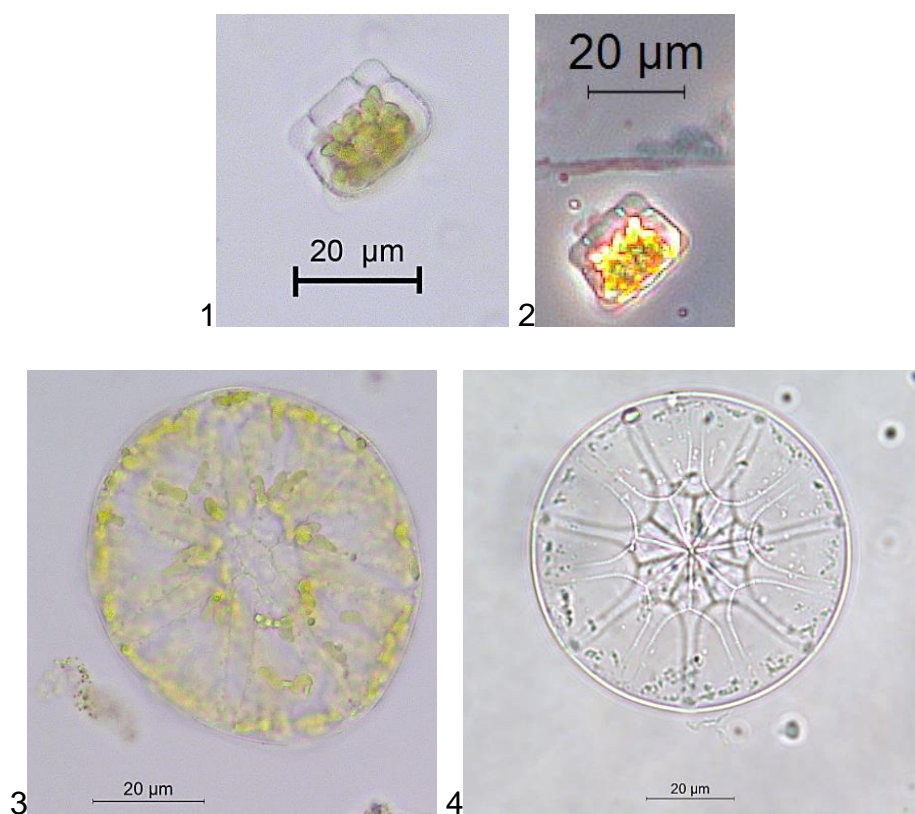
3 “

“

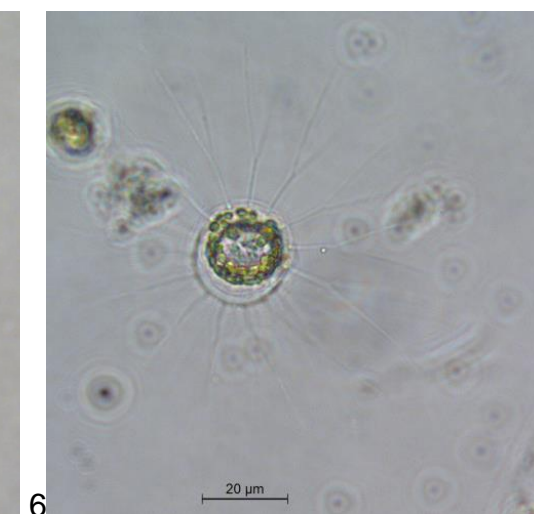
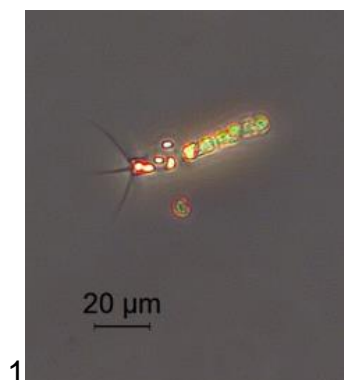
4 “

“

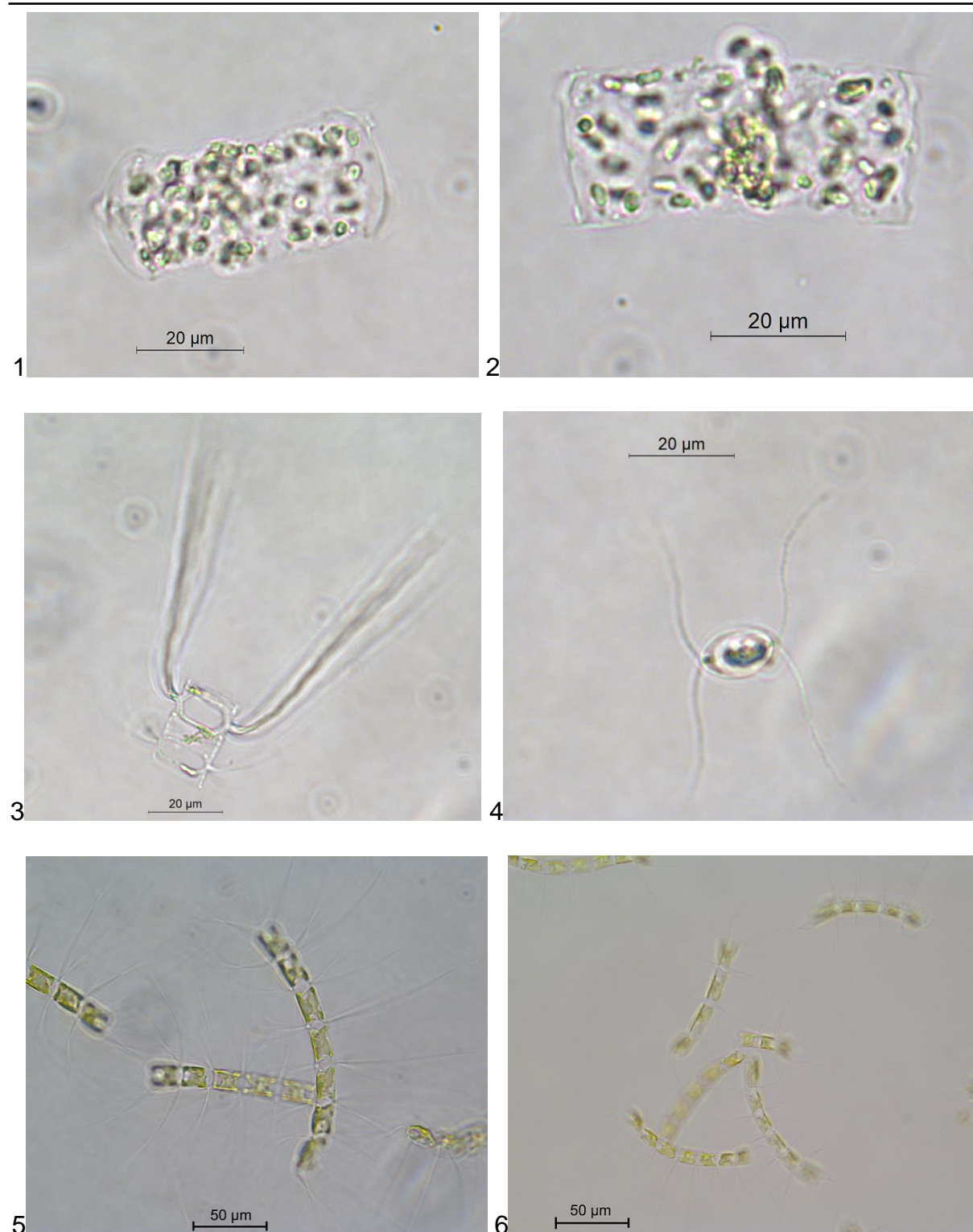
**Bacillariophyceae (centric diatoms)**



1 <i>Anaulus minutus</i>	28.05.2011
2 "	"
3 <i>Asteromphalus flabellatus</i>	07.06.2011
4 <i>Asteromphalus</i> sp.	24.01.2012

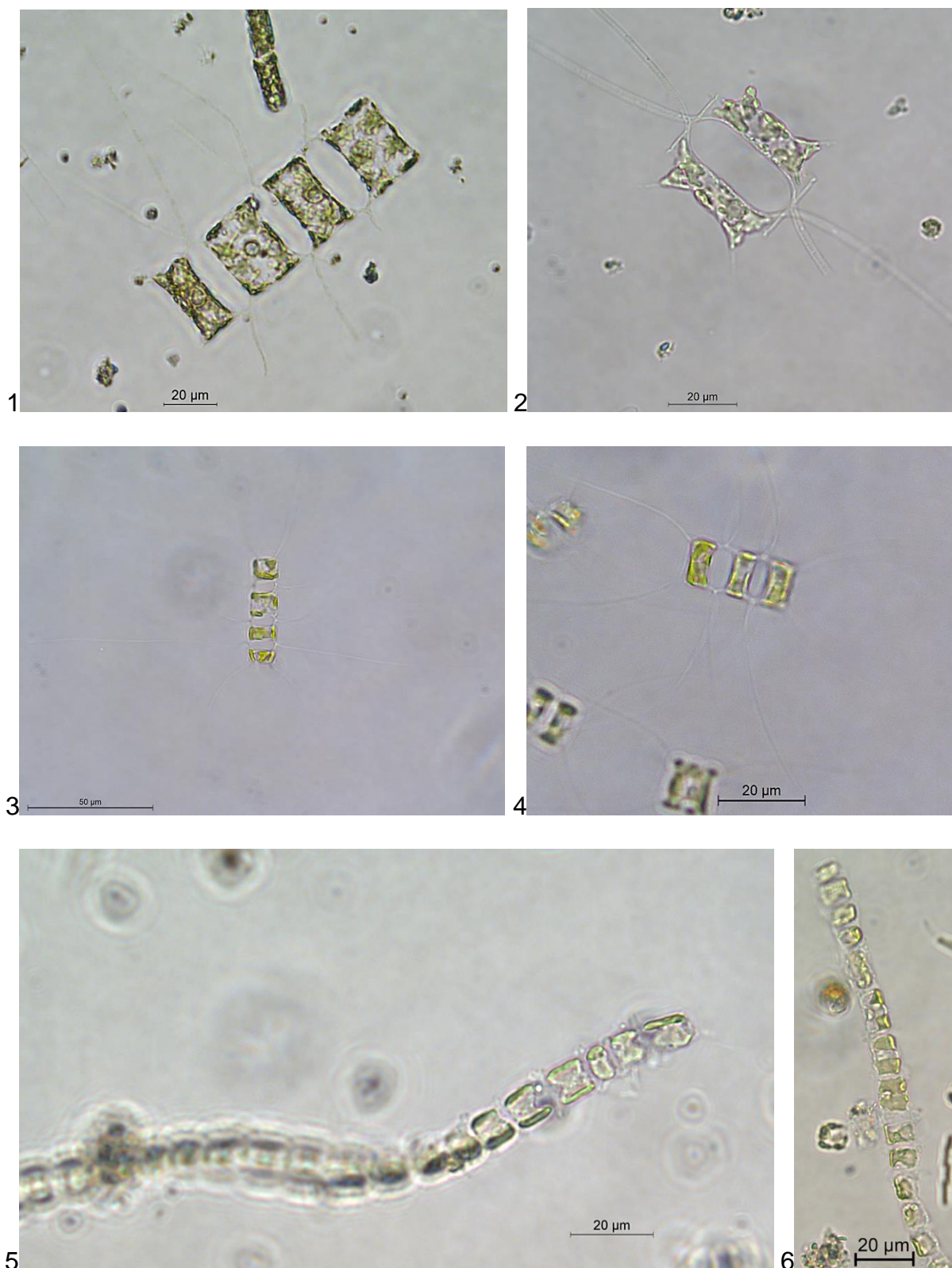


1	<i>Bacteriatrum elongatum</i>	13.07.2011
2	<i>Bacteriastrium hyalinum</i>	"
3	"	"
4	"	"
5	"	"
6	"	07.06.2011



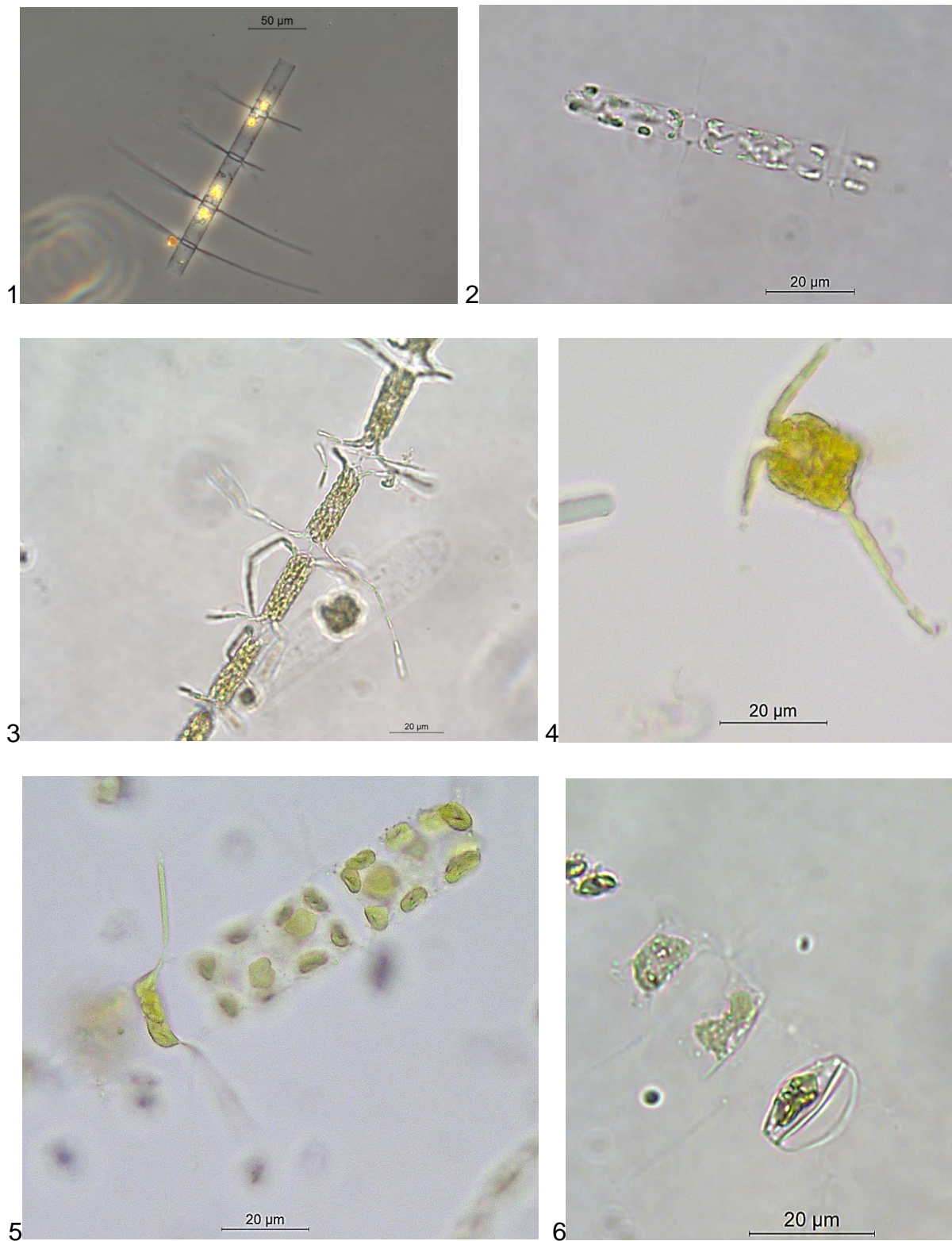
1	<i>Cerataulina pelagica</i>	07.11.2011
2	"	"
3	cf. <i>Chaetoceros compressus</i>	28.02.2012
4	"	"
5	<i>Chaetoceros curvisetus</i>	28.02.2012
6	"	"





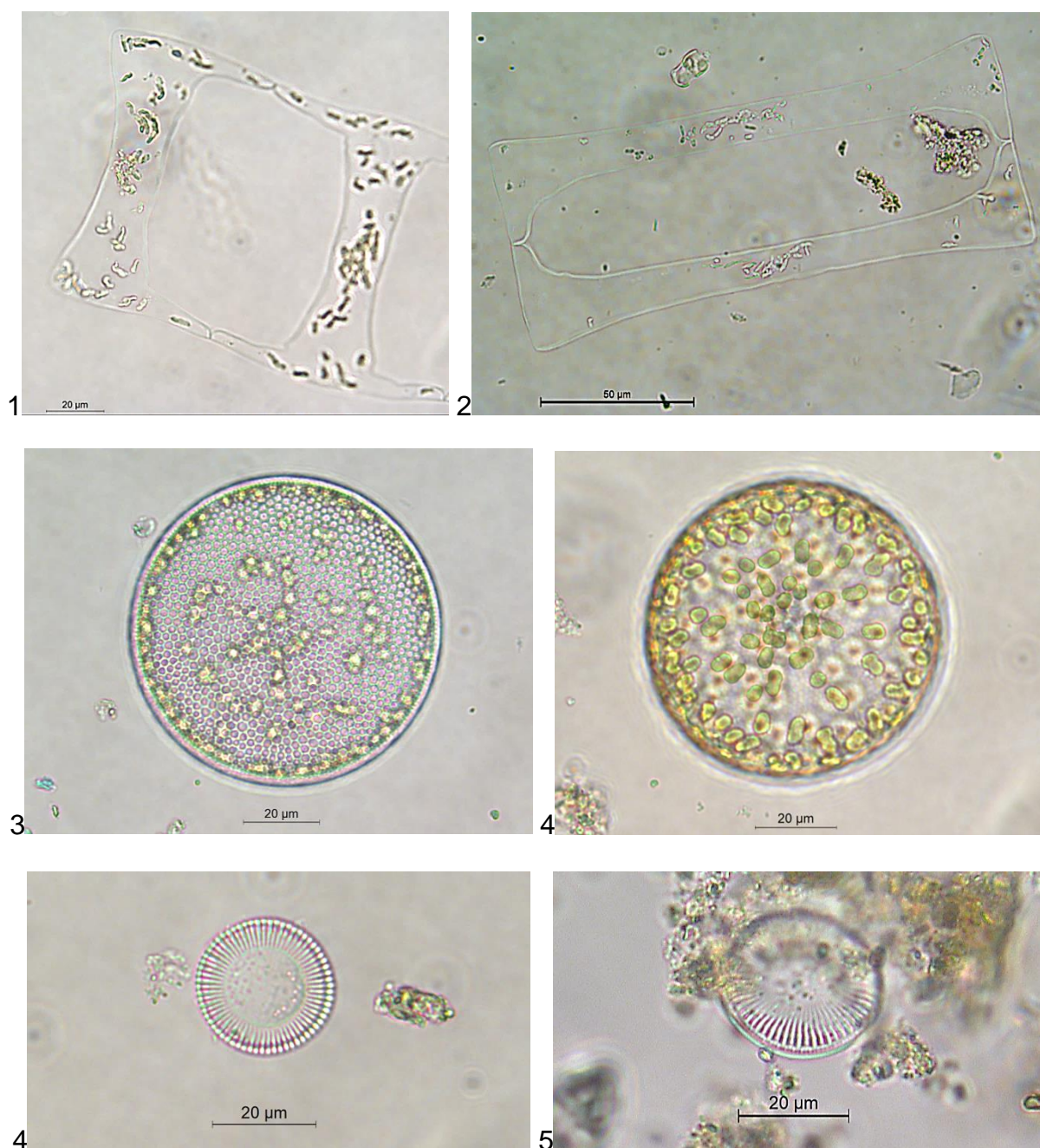
1	<i>Chaetoceros lorenzianus</i>	12.09.2012
2	"	13.07.2011
3	<i>Chaetoceros seiracanthus</i>	28.02.2012
4	"	"
5	<i>Chaetoceros socialis</i>	09.08.2011
6	"	"

## Appendix 2



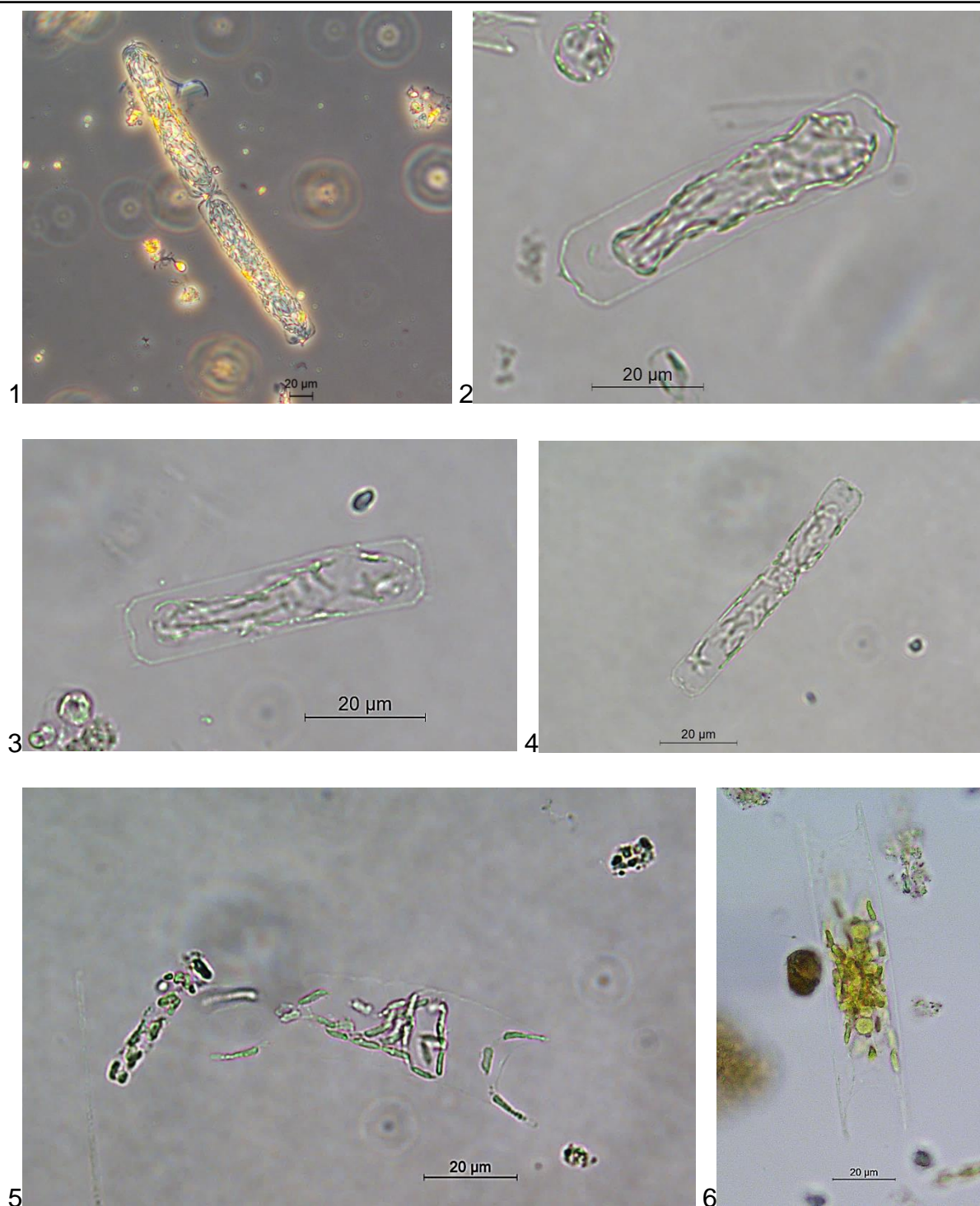
1	<i>Chaetoceros</i> sp. (Hyalochaete)	30.10.2012
2	"	13.07.2011
3	<i>Chaetoceros atlanticus</i>	12.09.2012
4	<i>Chaetoceros peruvianus</i>	28.05.2011
5	<i>Chaetoceros</i> sp. (Phaeoceros)	13.07.2011
6	<i>Chaetoceros</i> sp. resting spores	28.02.2012





1	<i>Climacodium frauenfeldianum</i>	27.05.2011
2	"	14.07.2011
3	<i>Coscinodiscus</i> sp.	07.09.2011
4	"	07.12.2011
5	<i>Cyclotella</i> sp. (empty frustule)	09.08.2011
6	"	30.05.2011

## Appendix 2



1 *Dactyliosolen fragilissimus*

2 “

3 *Detonula pumila*

4 “

5 *Eucampia cornuta*

6 “

07.06.2011

13.07.2011

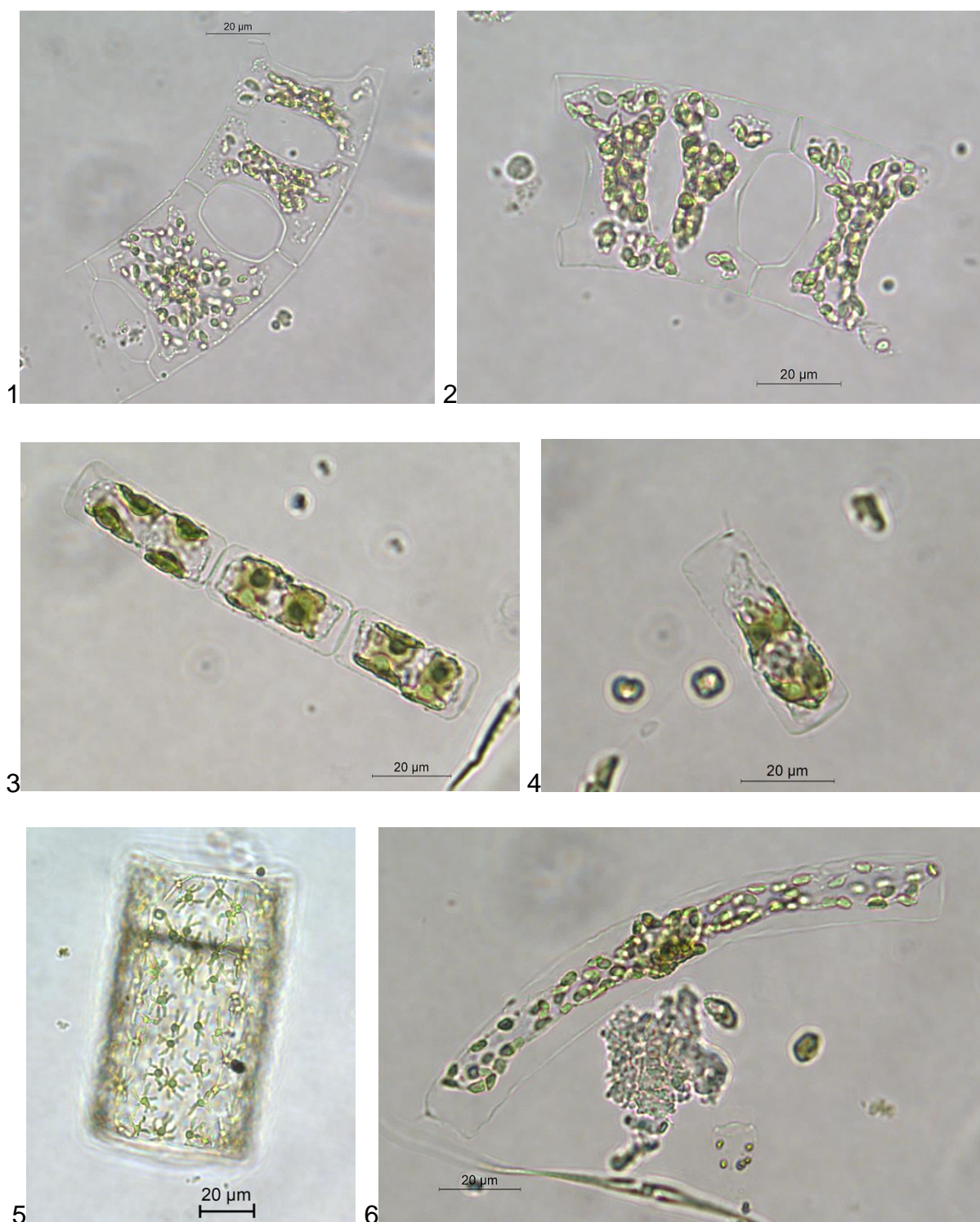
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“

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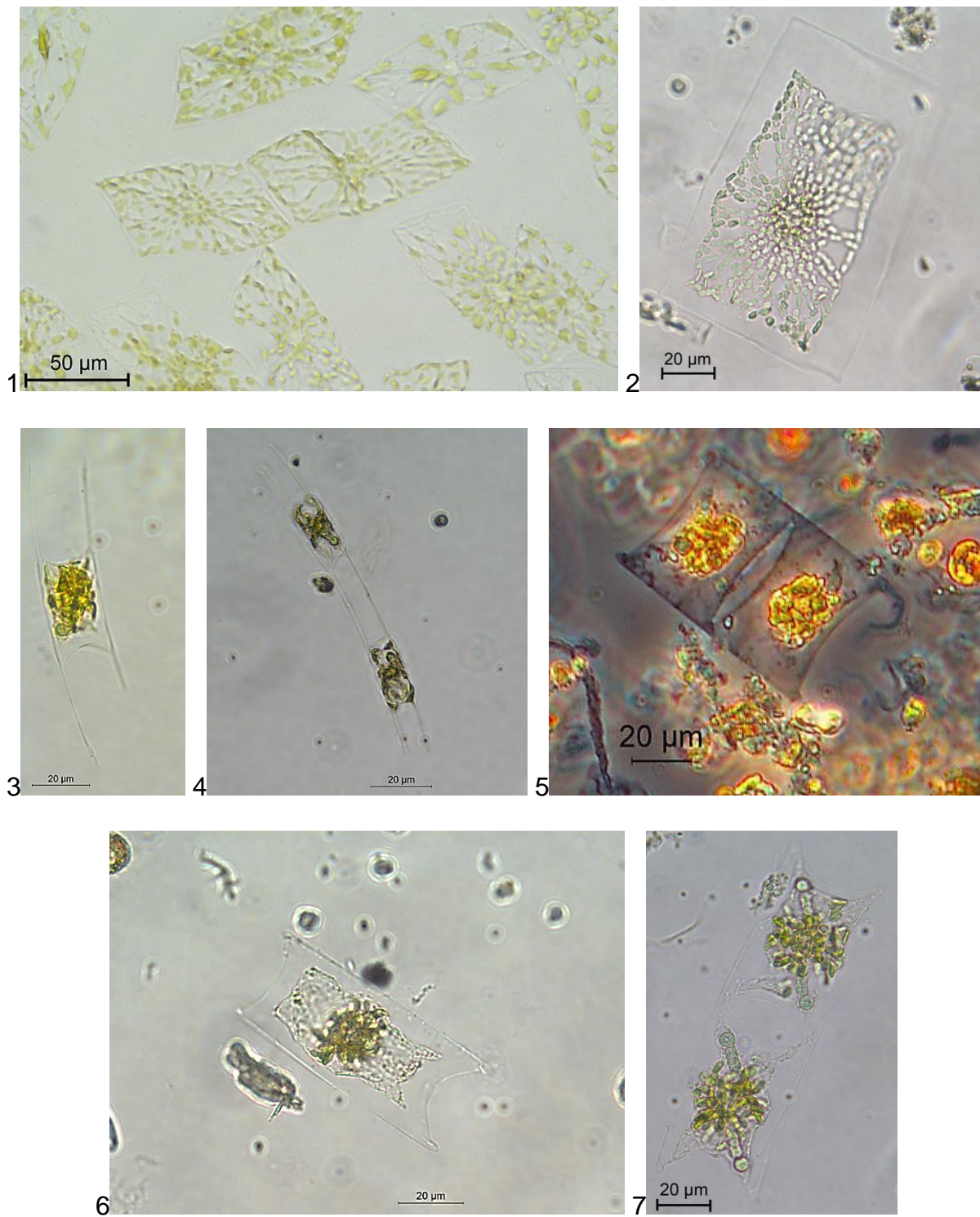
“





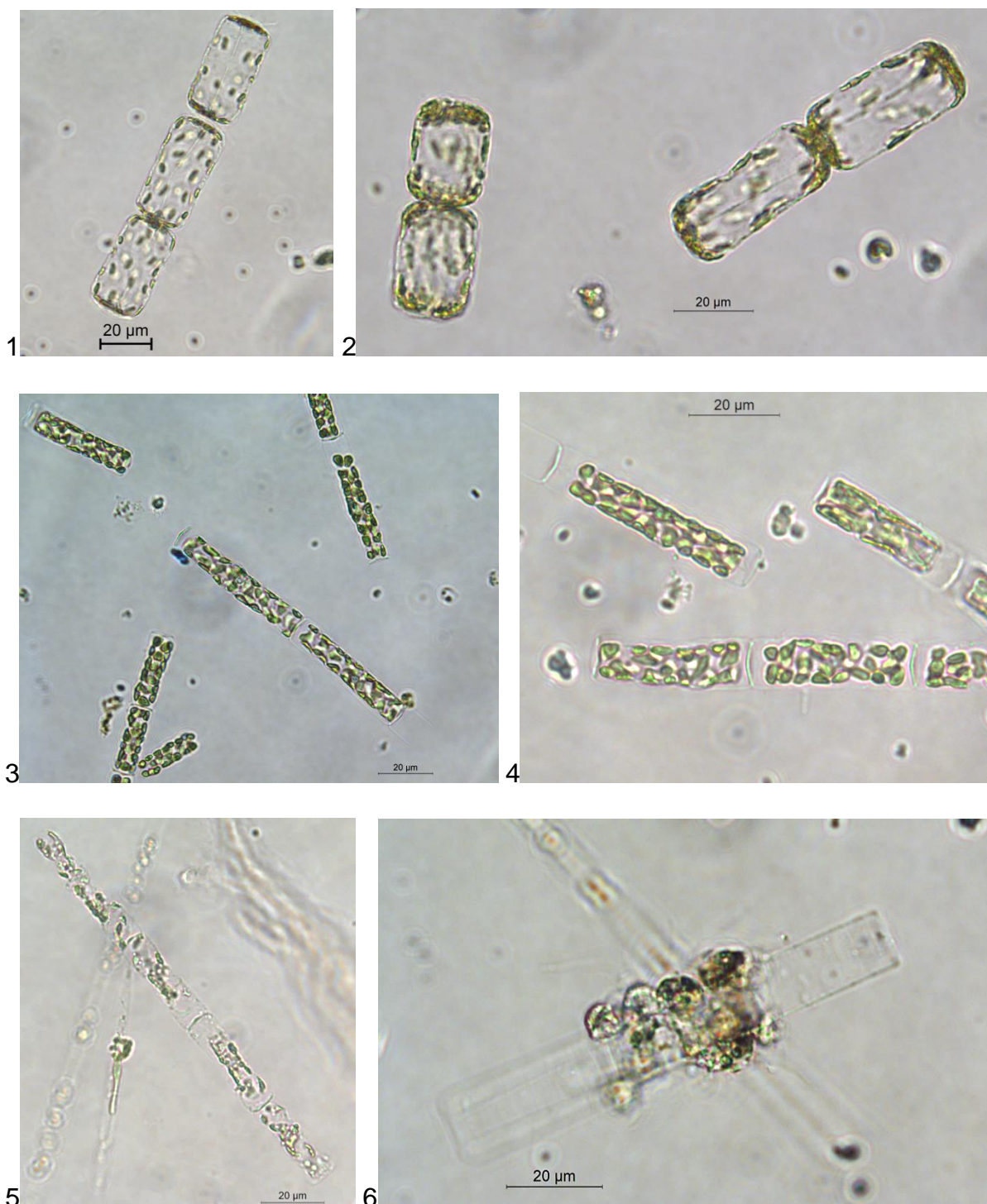
1	<i>Eucampia zodiacus</i>	07.11.2011
2	"	"
3	<i>Guinardia delicatula</i>	11.09.2012
4	"	"
5	<i>Guinardia flaccida</i>	12.09.2011
6	<i>Guinardia striata</i>	11.09.2012

## Appendix 2



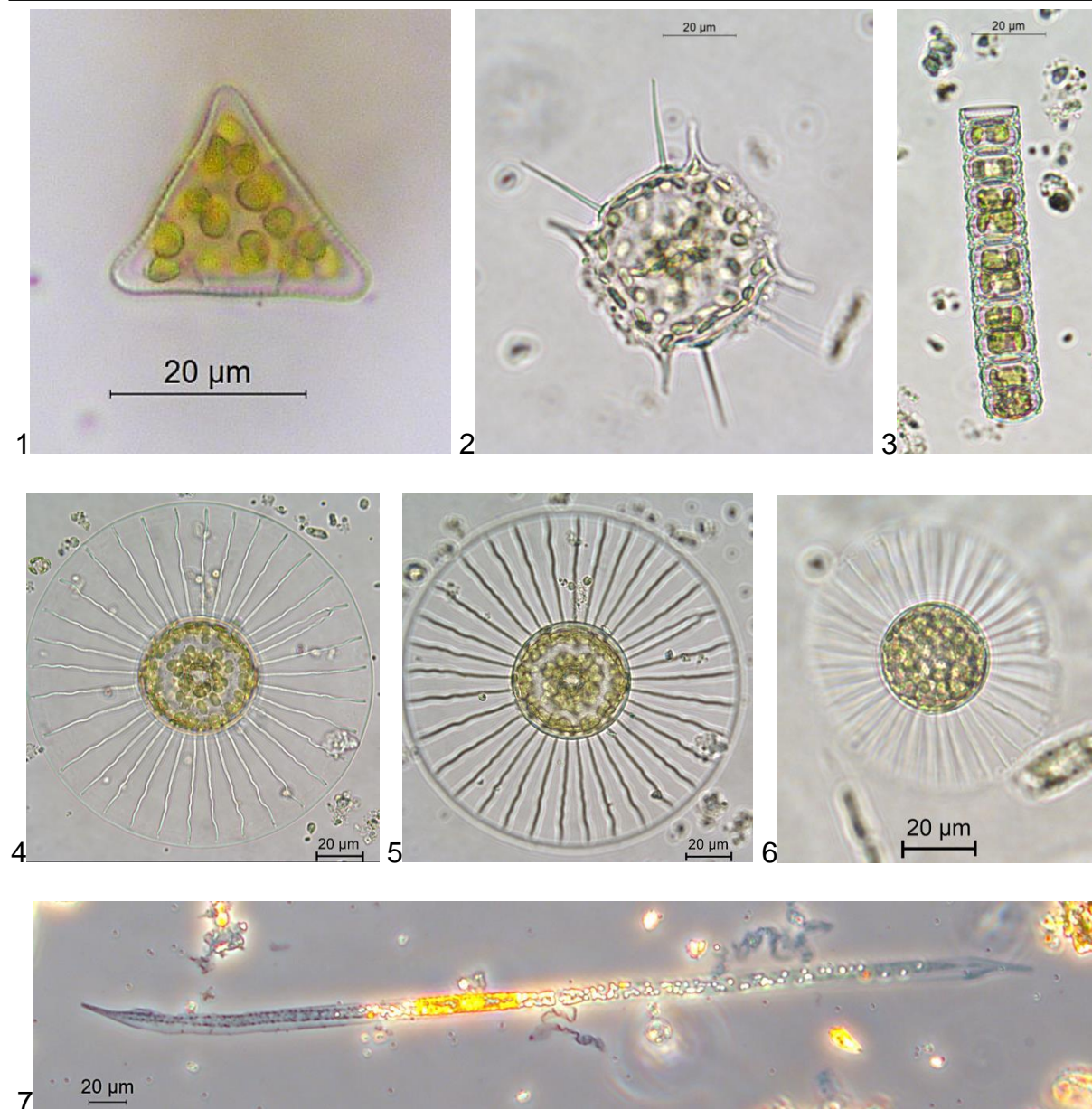
1	<i>Helicotheca tamesis</i>	28.02.2012
2	"	12.09.2012
3	<i>Hemiaulus sinensis</i>	28.05.2011
4	"	07.12.2011
5	<i>Hemiaulus membranaceus</i>	30.05.2011
6	"	27.02.2012
7	"	07.06.2011





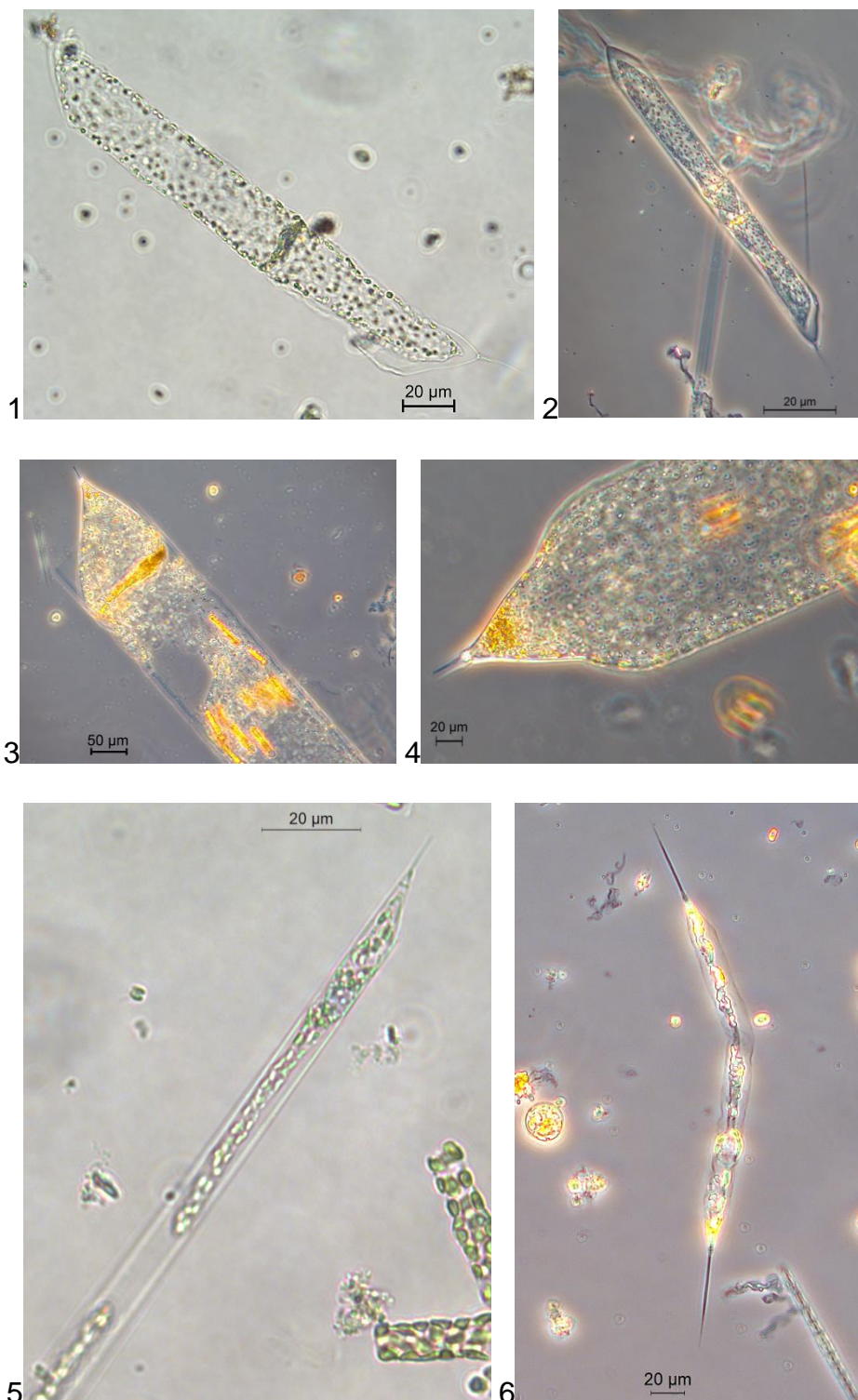
1	<i>Lauderia annulata</i>	11.09.2012
2	"	"
3	<i>Leptocylindrus danicus</i>	12.09.2012
4	"	"
5	"	"
6	<i>Leptocylindrus mediterraneus</i>	28.02.2012

## Appendix 2

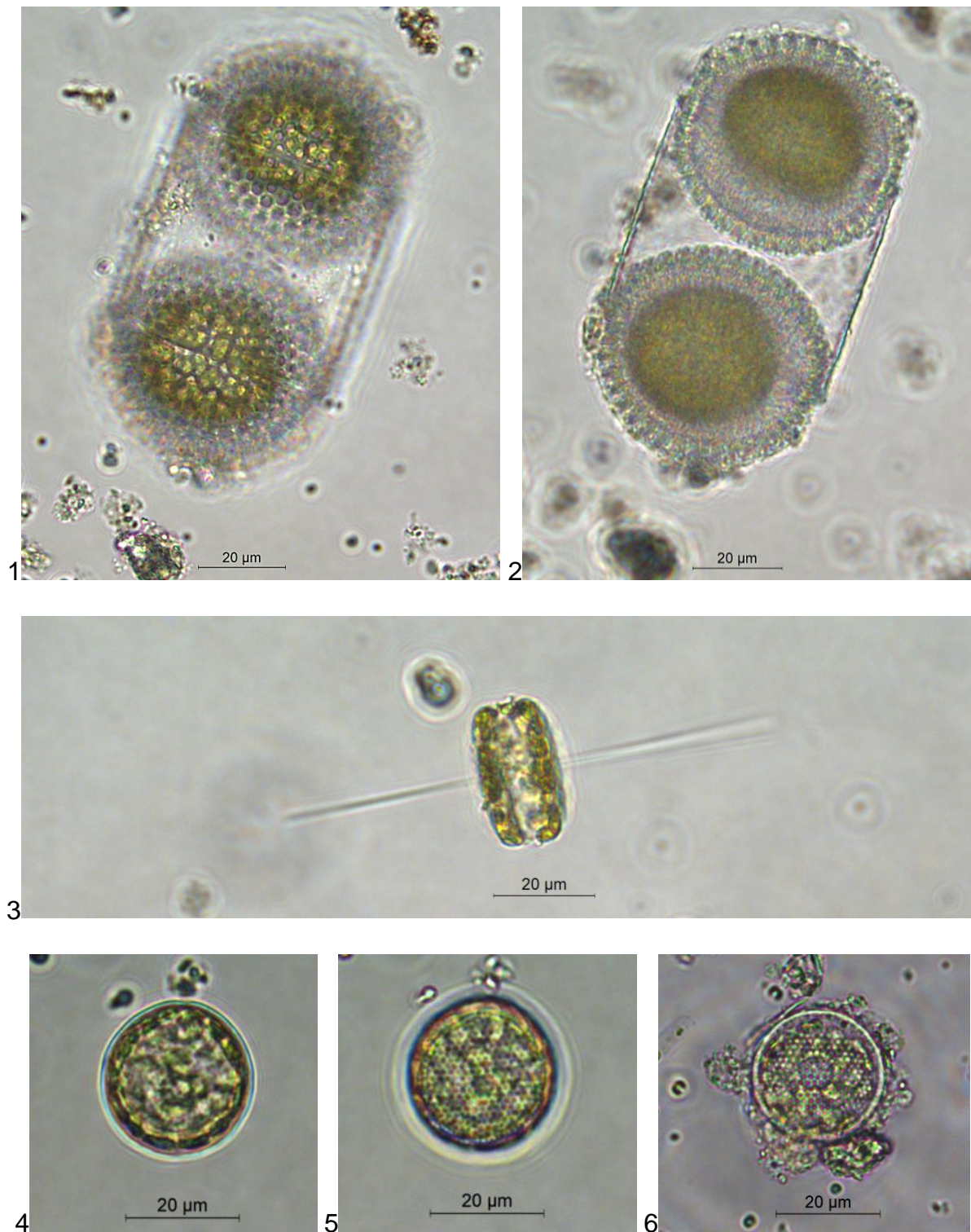


1 <i>Lithodesmium</i> sp.	28.05.2011
2 <i>Odontella mobiliensis</i>	07.11.2011
3 <i>Paralia sulcata</i>	07.09.2011
4 <i>Planktoniella sol</i>	10.10.2011
5 " (same specimen as in 4)	"
6 "	28.02.2012
7 <i>Proboscia alata</i>	28.05.2011



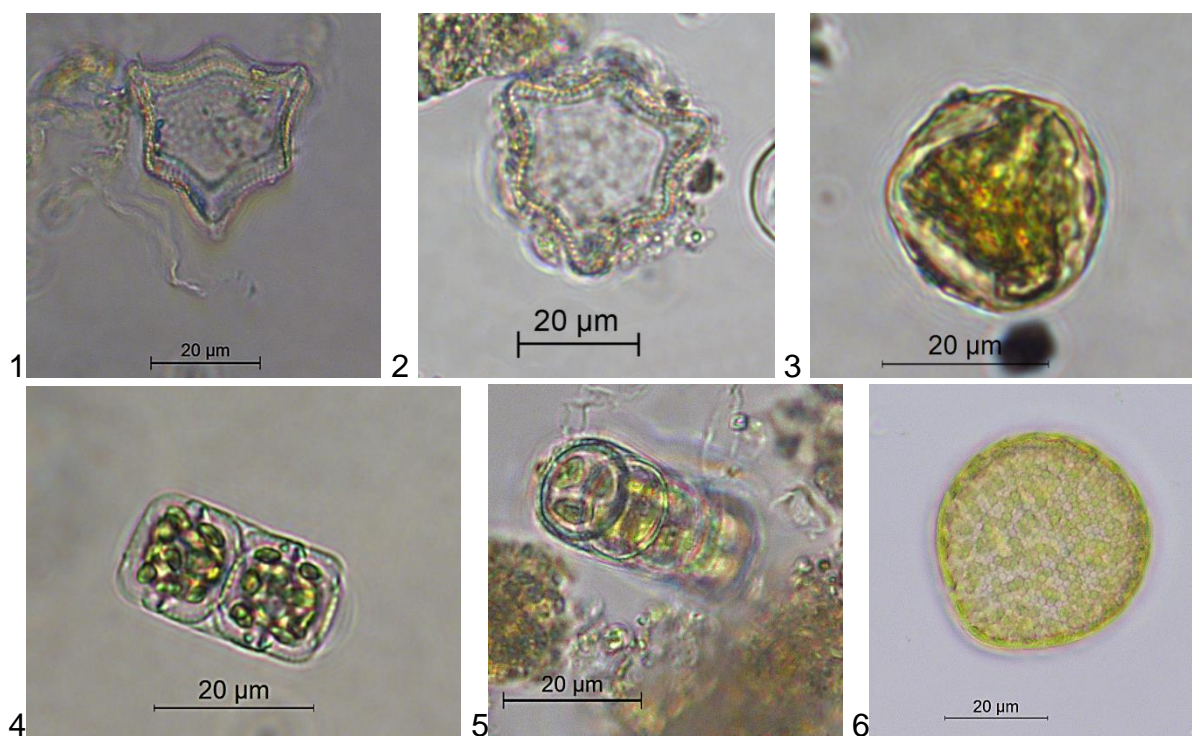


1	<i>Rhizosolenia</i> sp. ( <i>fallax</i> -type)	07.09.2011
2	"	07.06.2011
3	" (with symbionts)	"
4	"	"
5	<i>Rhizosolenia</i> sp. ( <i>fallax</i> -type)	12.09.2012
6	<i>Rhizosolenia</i> sp. ( <i>setigera</i> -type)	28.05.2011



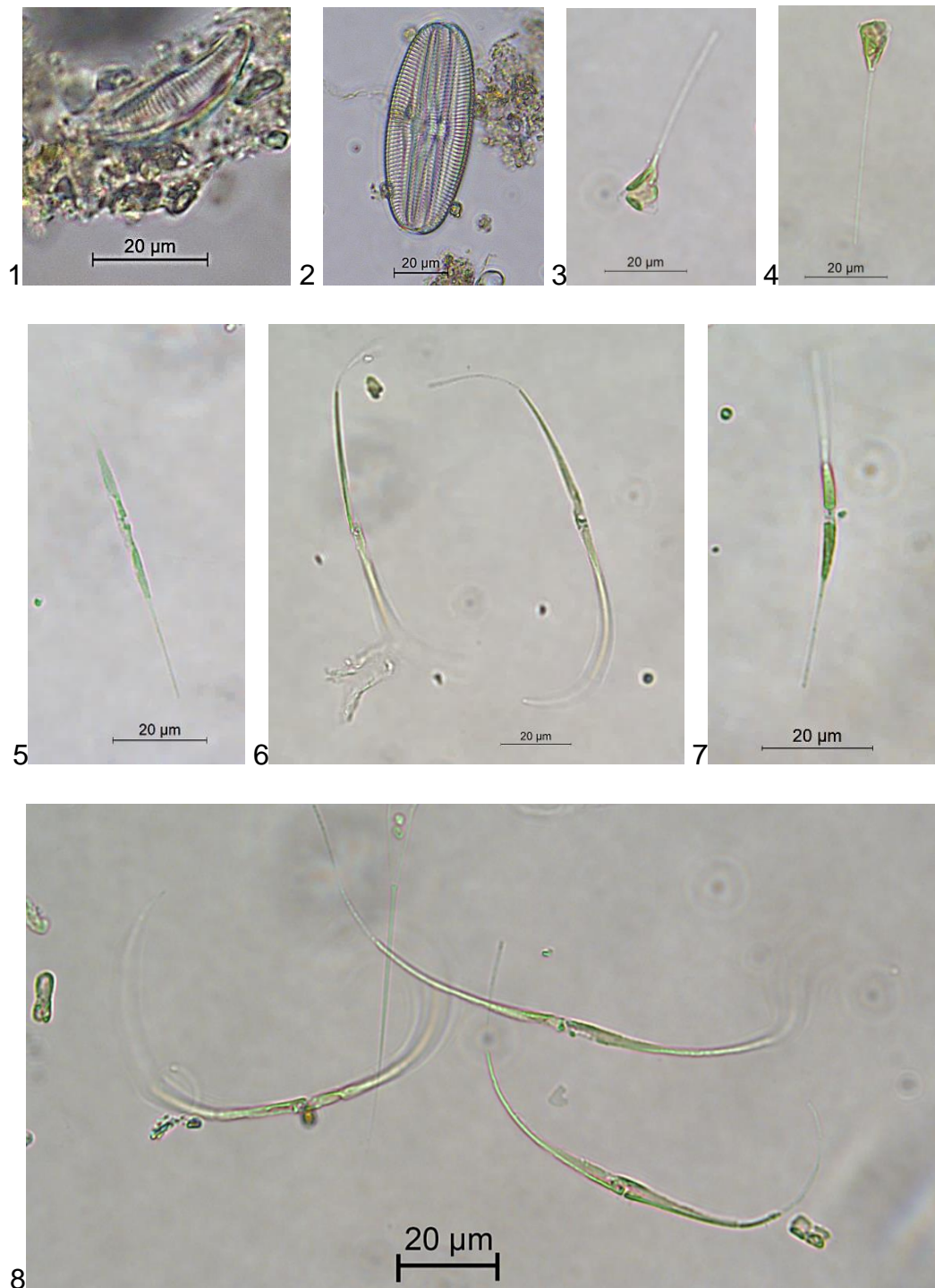
- |                                 |            |
|---------------------------------|------------|
| 1 <i>Stephanopyxis cruciata</i> | 07.11.2011 |
| 2 "(same specimen as in 1)      | "          |
| 3 <i>Thalassiosira rotula</i>   | 27.02.2012 |
| 4 <i>Thalassiosira</i> sp.      | 07.09.2011 |
| 5 " (same specimen as in 2)     | "          |
| 6 <i>Thalassiosira</i> sp.      | 10.10.2011 |





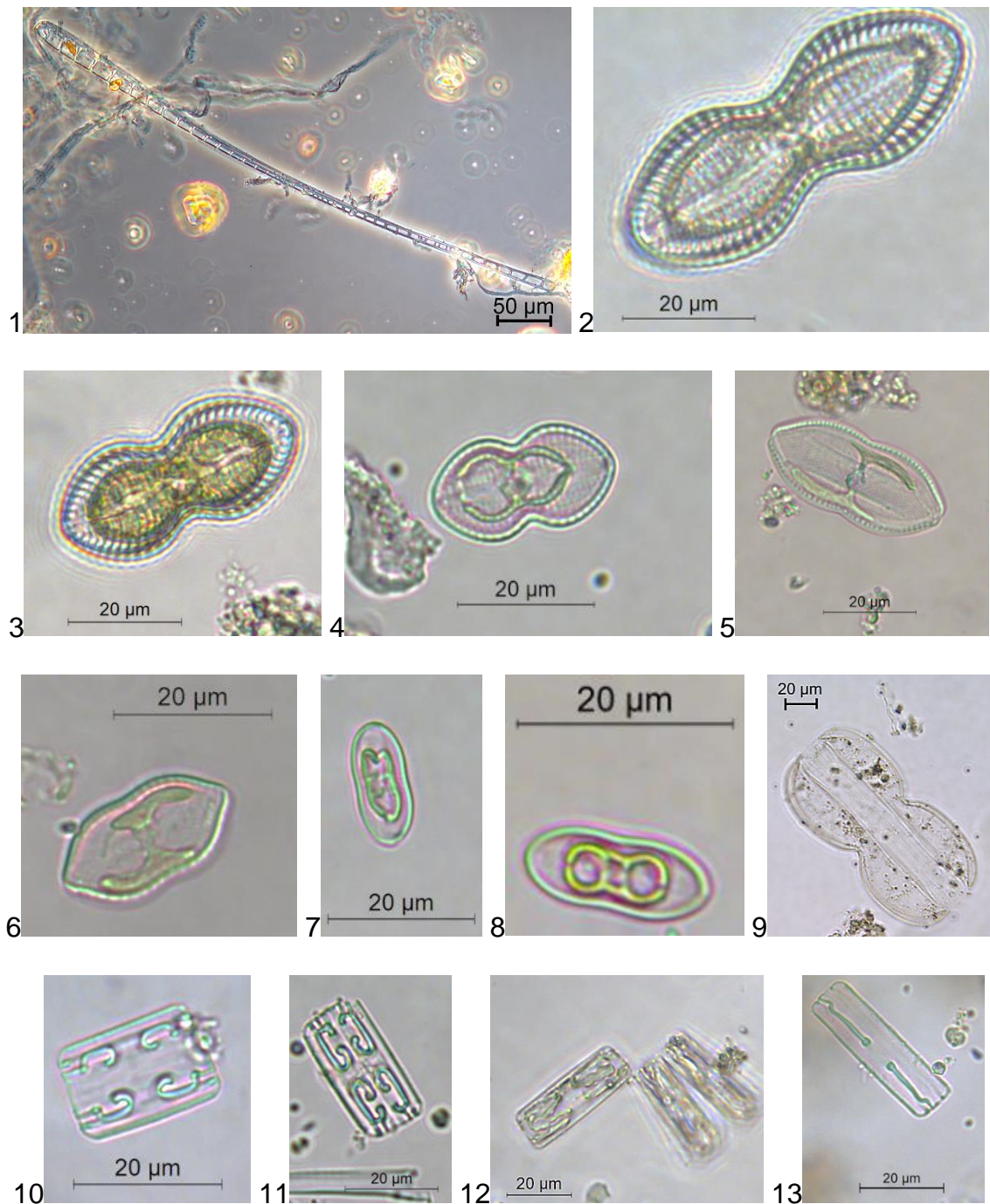
1	<i>Triceratium dubium</i> (empty frustule)	28.05.2011
2	"	06.09.2011
3	<i>Triceratium obtusum</i>	"
4	<i>Trigonium alternans</i>	07.12.2011
5	Undefined centric diatom	30.05.2011
6	"	13.07.2011

**Bacillariophyceae (pennate diatoms)**



1	<i>Amphora</i> sp. (empty frustule)	30.05.2011
2	"	13.07.2011
3	<i>Asterionellopsis glacialis</i>	11.09.2012
4	"	"
5	<i>Ceratoneis closterium/Nitzschia longissima</i>	13.07.2011
6	"	11.09.2012
7	"	12.09.2012
8	"	11.09.2012

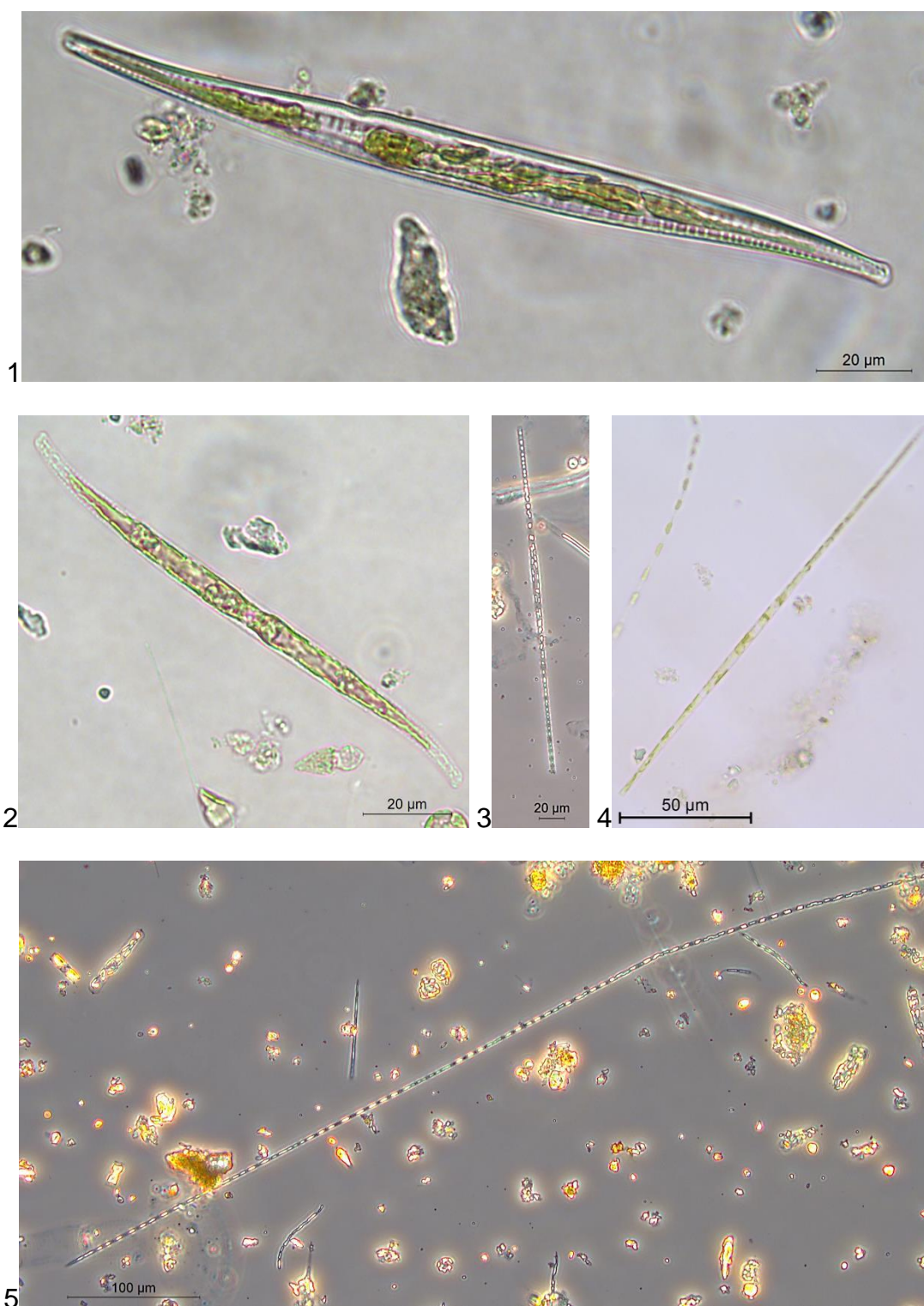




1	<i>Climacosphenia moniligera</i>	28.05.2011
2	<i>Diploneis</i> sp.	07.09.2011
3	"	"
4	"	07.11.2011
5	"	"
6	"	10.10.2011
7	"	07.09.2011
8	"	28.05.2011
9	<i>Entomoneis</i> sp. (empty frustule)	07.06.2011
10	<i>Grammatophora hamulifera</i>	07.09.2011
11	"	10.10.2011
12	<i>Grammatophora marina</i>	07.09.2011
13	<i>Grammatophora oceanica</i>	30.05.2011



1	<i>Mastogloia rostrata</i>	28.02.2012
2	"	28.05.2011
3	<i>Meuniera membranacea</i>	30.05.2011
4	<i>Navicula</i> sp.	07.09.2011
5	"	"
6	"	30.05.2011
7	"	11.09.2012

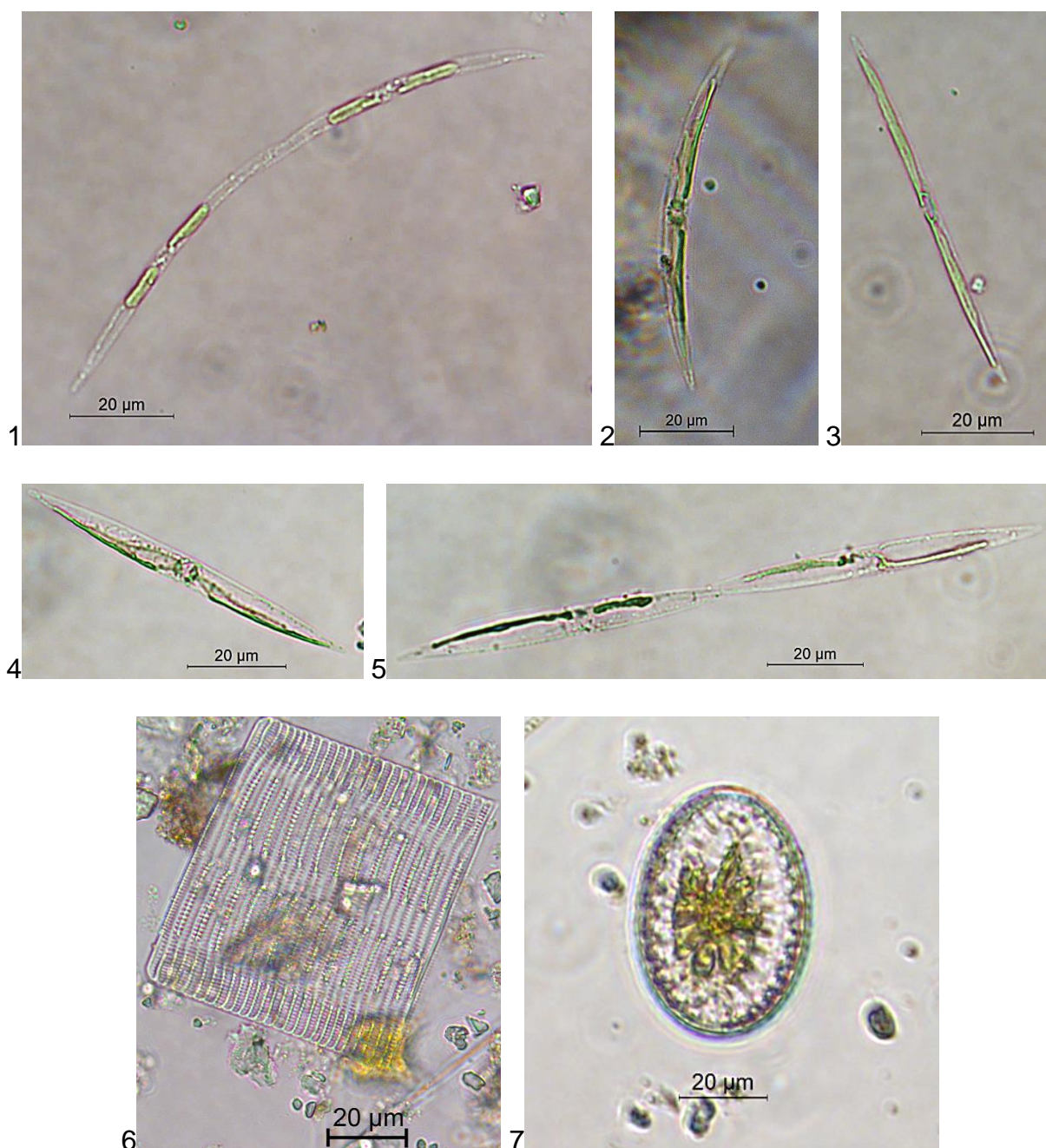


1	<i>Nitzschia</i> sp.	10.10.2011
2	"	11.09.2012
3	<i>Nitzschia/Lioloma/Thalassiothrix</i> sp.	13.07.2011
4	"	"
5	"	"



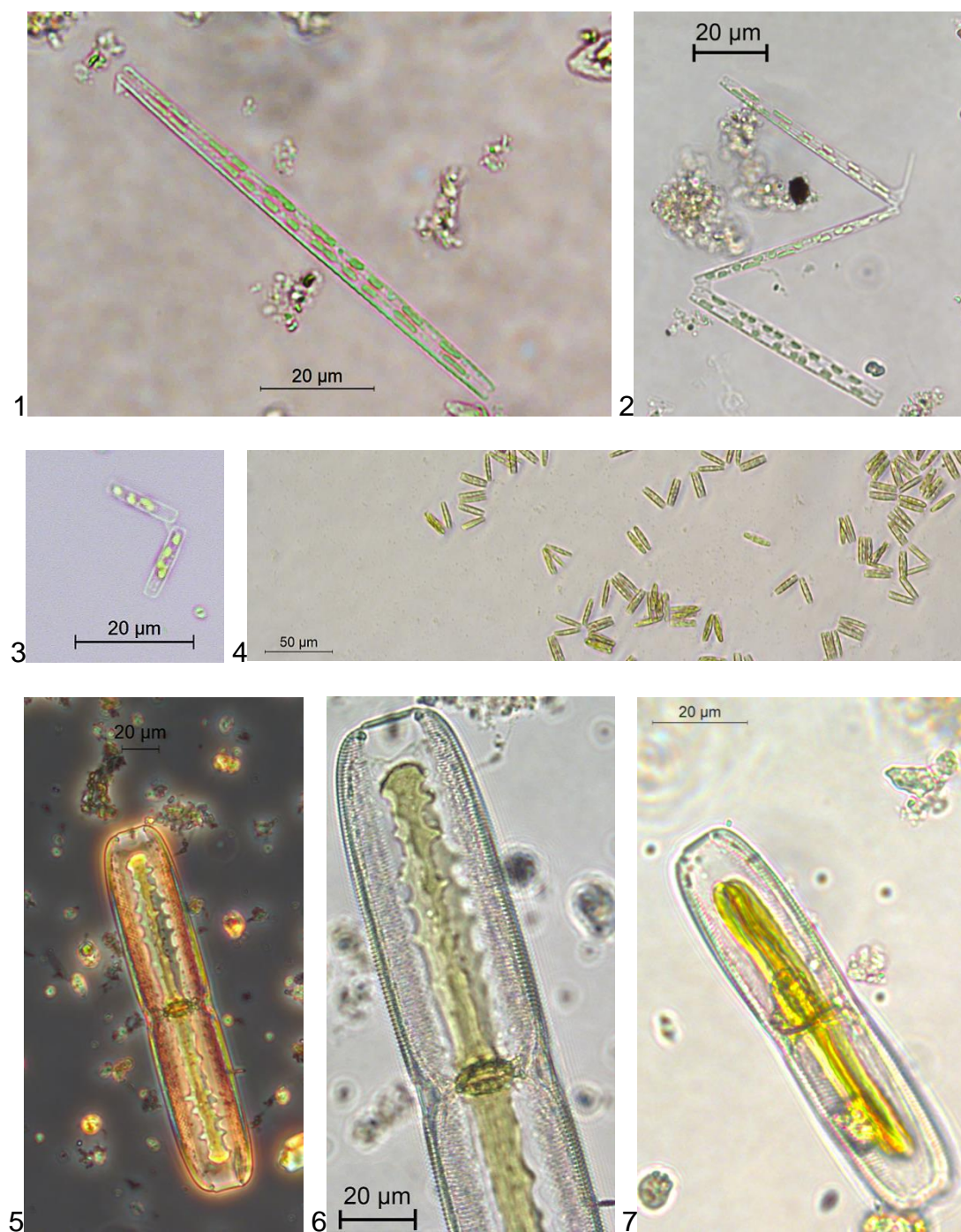


1	<i>Pleurosigma</i> sp.	13.07.2011
2	"	07.09.2011
3	"	"
4	"	"
5	"	"
6	"	10.10.2011

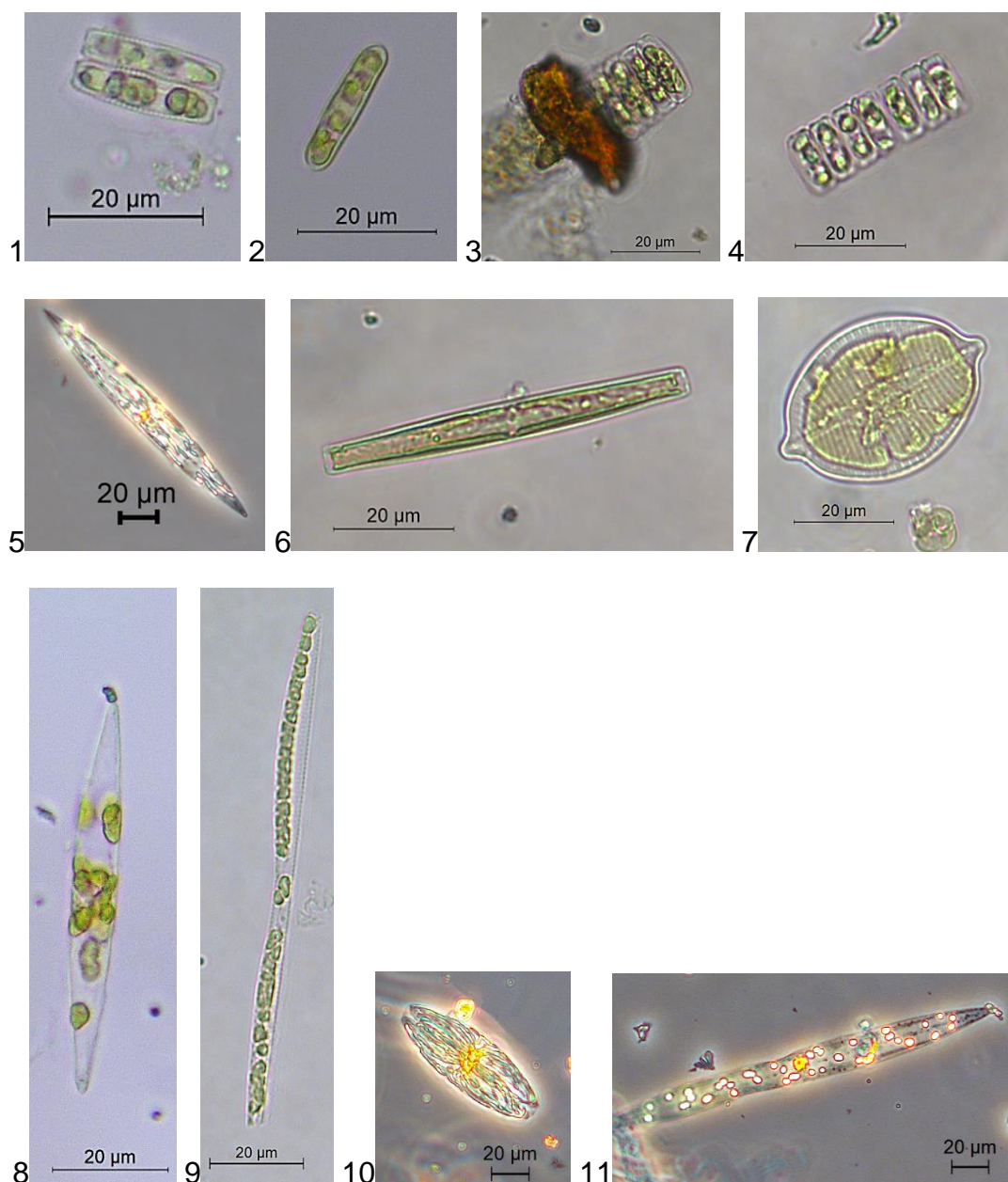


1 cf. <i>Pseudo-nitzschia subcurvata</i>	28.02.2012
2 "	28.05.2011
3 <i>Pseudo-nitzschia</i> sp.	11.09.2012
4 "	07.12.2011
5 "	"
6 <i>Striatella unipunctata</i>	28.05.2011
7 <i>Surirella fastuosa</i>	24.01.2012





1	<i>Thalassionema nitzschoides/frauenfeldii</i>	07.09.2011
2	"	28.05.2011
3	"	27.02.2012
4	<i>Thalassionema nitzschoides</i>	28.02.2012
5	<i>Trachyneis aspera</i>	07.09.2011
6	"	"
7	"	28.05.2011



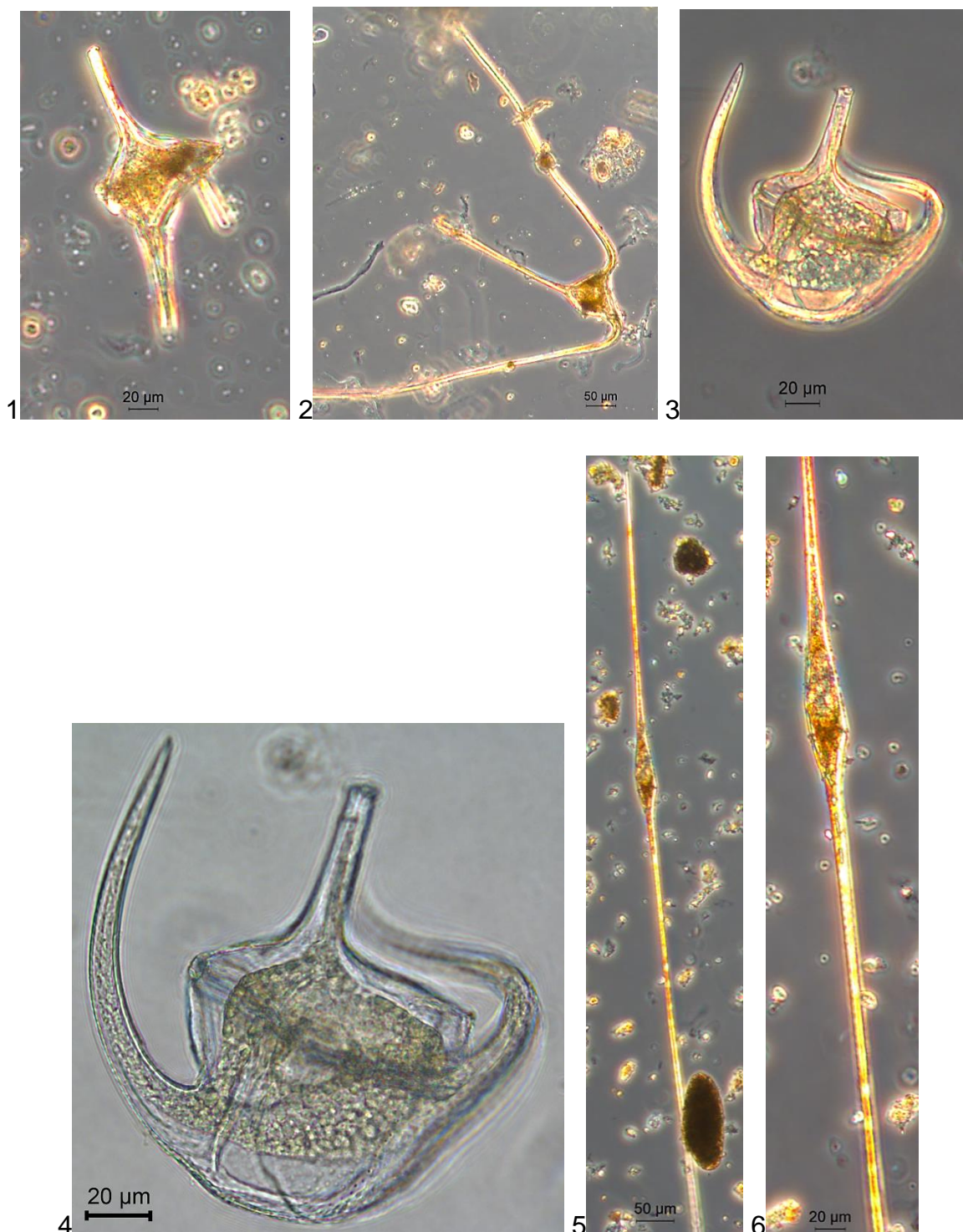
1	Undefined pennate diatom <40 µm	30.05.2011
2	"	07.06.2011
3	"	11.09.2012
4	"	12.09.2012
5	Undefined pennate diatom >40 µm	07.09.2011
6	"	07.12.2011
7	"	27.02.2012
8	"	28.05.2011
9	"	"
10	"	"
11	"	"

## Dinophyceae

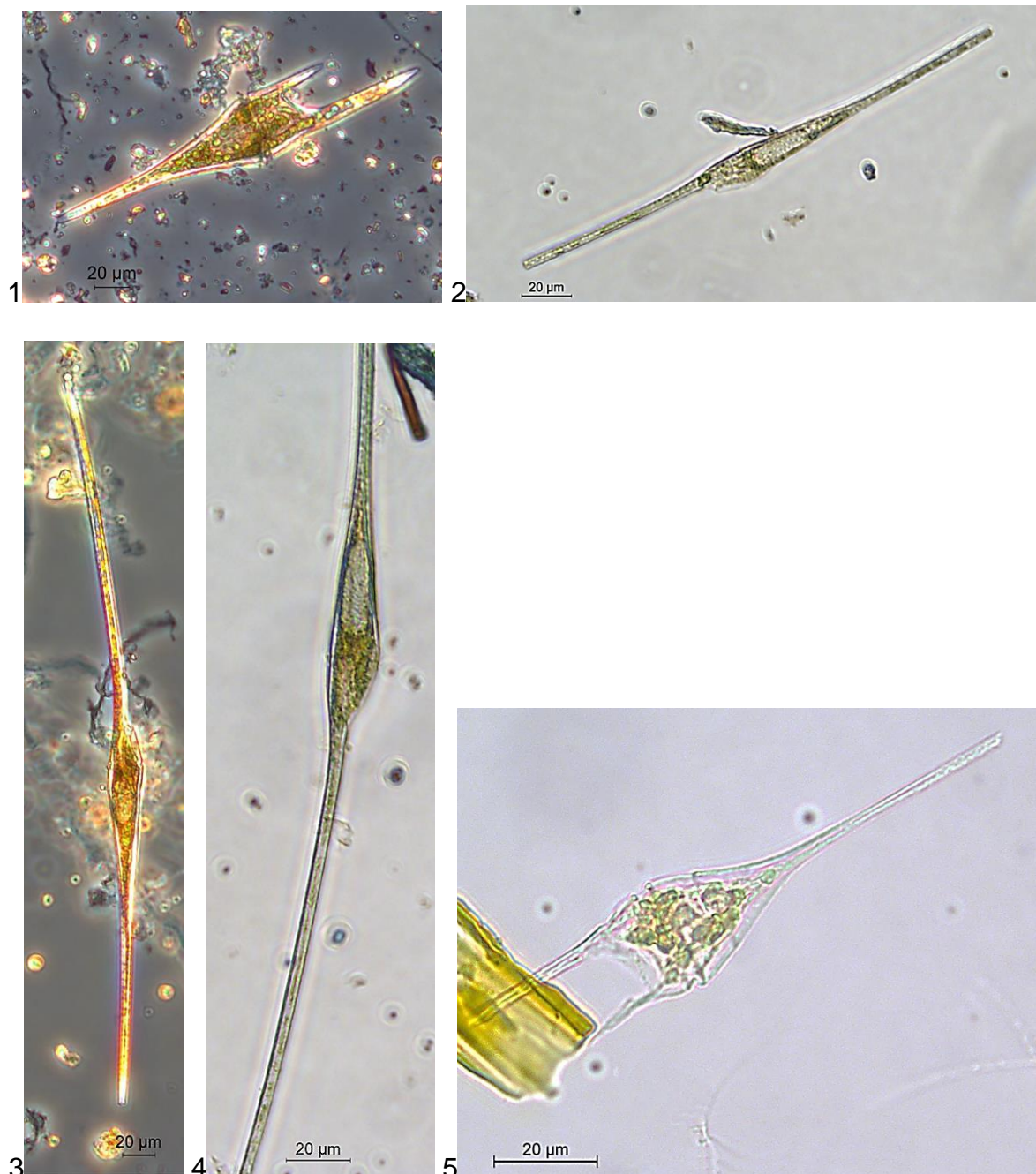


1 cf. <i>Alexandrium</i> sp.	28.05.2011
2 "	30.05.2011
3 "	30.05.2011
4 <i>Amphisolenia bidentata</i>	13.07.2011
5 " (head)	"
6 " (foot)	"
7 <i>Brachidinium capitatum</i>	07.12.2011
8 <i>Ceratium arietinum</i>	28.02.2012
9 <i>Ceratium biceps</i>	07.12.2011



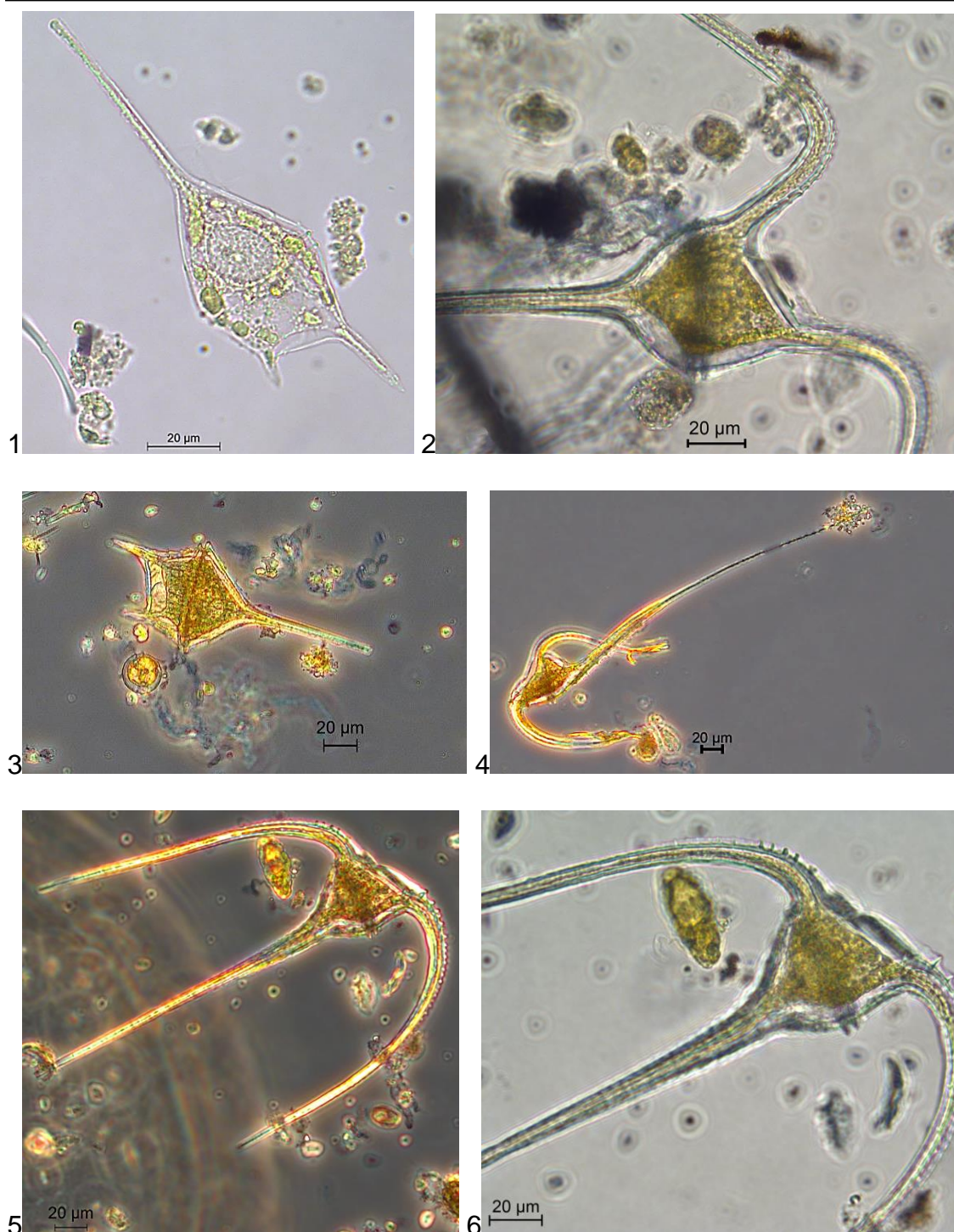


1	<i>Ceratium candelabrum</i>	12.10.2011
2	<i>Ceratium carriense</i>	28.02.2012
3	<i>Ceratium concilians</i>	14.07.2011
4	" (same specimen as in 3)	"
5	<i>Ceratium extensum</i>	07.09.2011
6	"	"

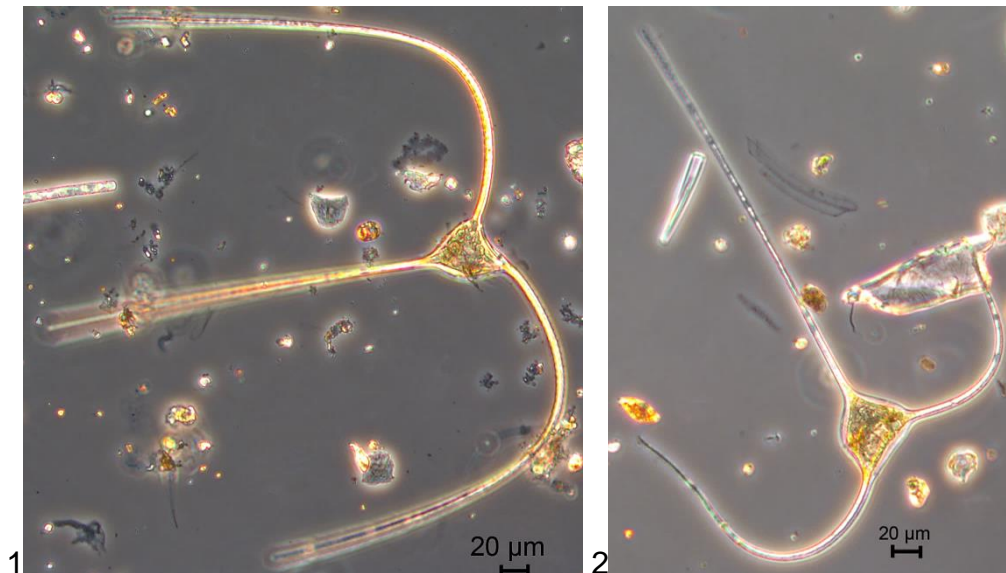


1	<i>Ceratium furca</i>	12.10.2011
2	<i>Ceratium fusus</i>	27.02.2012
3	"	07.09.2011
4	"	06.12.2011
5	<i>Ceratium kofoidii</i>	30.05.2011





1 <i>Ceratium lineatum</i>	30.05.2011
2 <i>Ceratium macroceros</i>	06.09.2010
3 <i>Ceratium pentagonum</i>	07.06.2011
4 <i>Ceratium ranipes</i>	07.06.2011
5 <i>Ceratium tenue</i>	06.09.2011
6 " (same specimen as in 5)	"



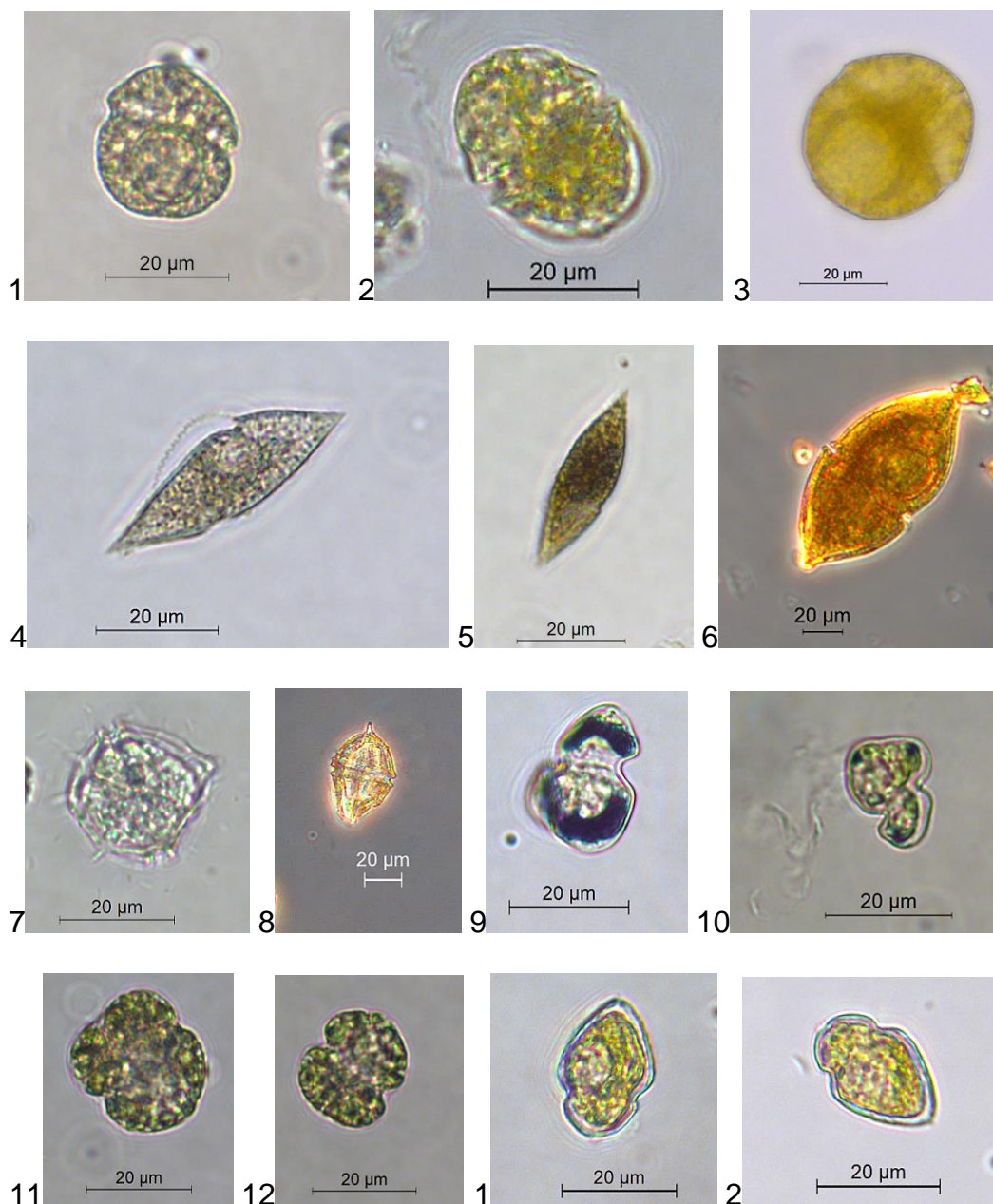
1	<i>Ceratium trichoceros</i>	07.09.2011
2	"	28.02.2012
3	"	07.09.2011
4	<i>Ceratium tripos</i>	07.06.2011
5	"	06.09.2011
6	<i>Ceratocorys horrida</i>	06.06.2011





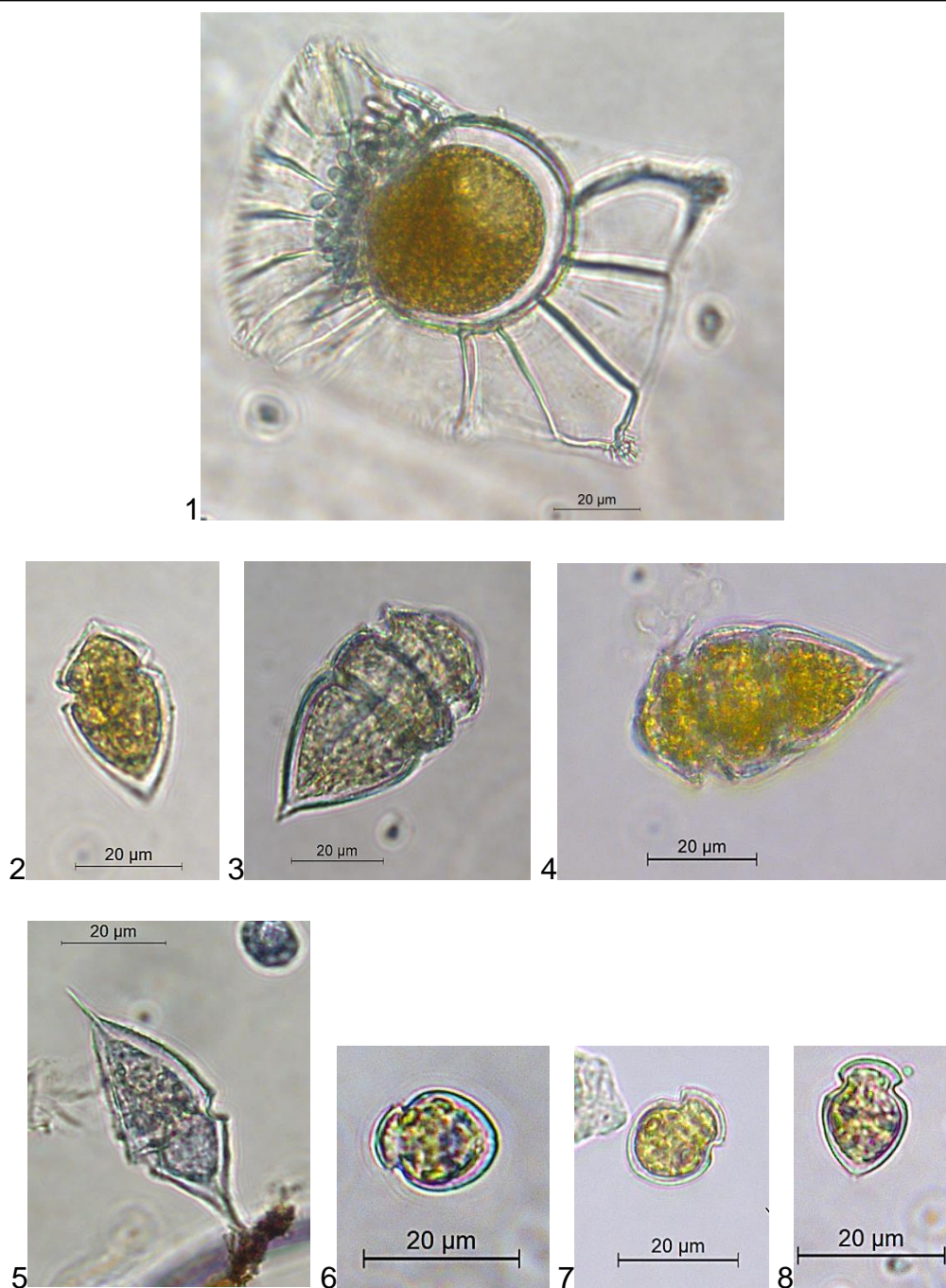
1	<i>Dinophysis acuminata</i>	06.09.2011
2	"	07.09.2011
3	"	06.09.2011
4	<i>Dinophysis caudata</i>	06.06.2011
5	"	24.01.2012
6	<i>Dinophysis hastata</i>	07.12.2011
7	<i>Dinophysis schuettii</i>	27.02.2012
8	<i>Dissodinium pseudolunula</i>	28.02.2012

## Appendix 2

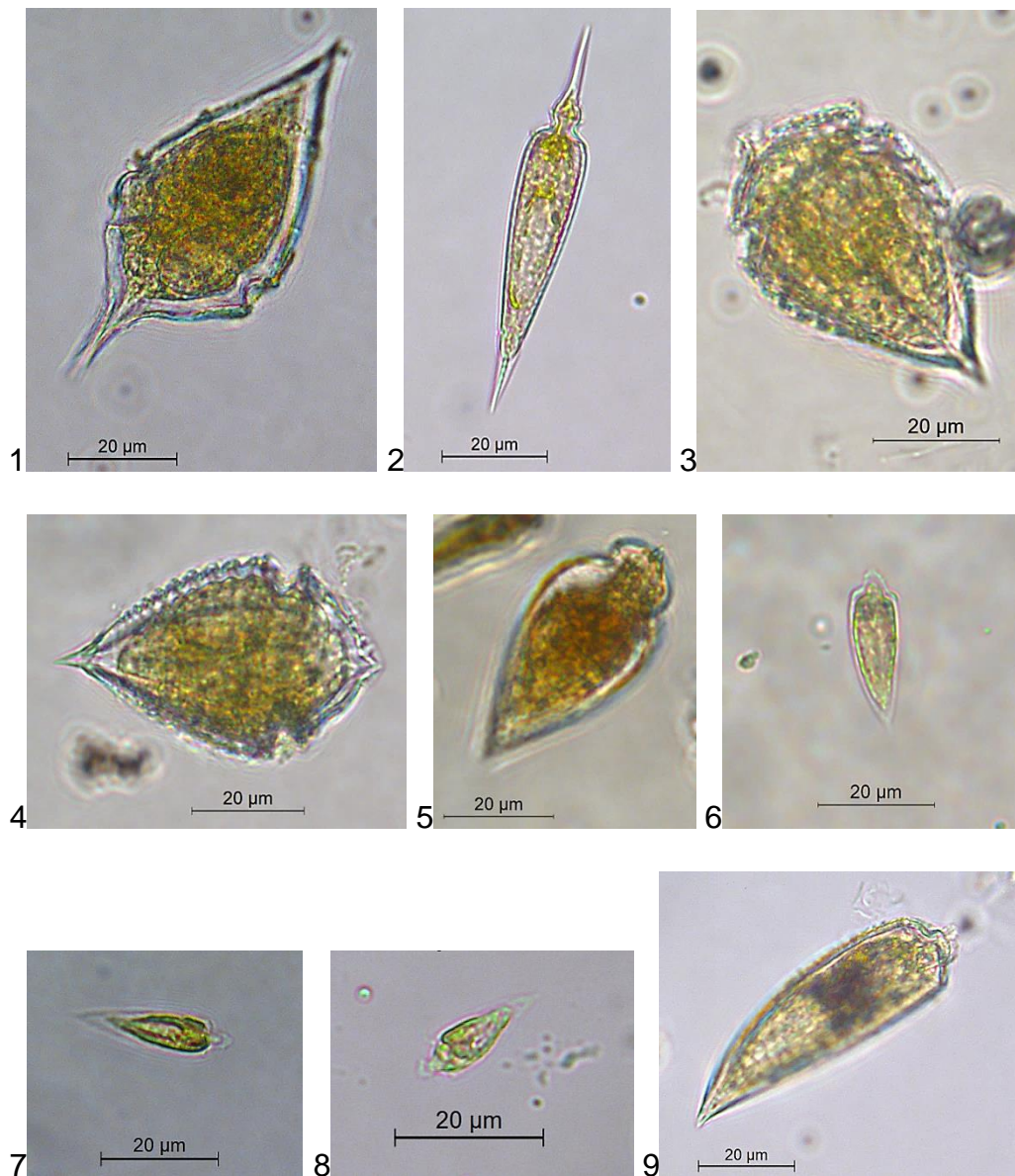


1	<i>Gymnodinium</i> sp.	07.09.2011
2	"	28.05.2011
3	"	13.07.2011
4	<i>Gyrodinium</i> sp.	"
5	"	07.12.2011
6	"	27.02.2012
7	cf. <i>Gonyaulax</i> sp.	11.09.2012
8	cf. <i>Heterocapsa</i> sp.	28.05.2011
9	<i>Karlodinium</i> sp.	"
10	"	27.02.2012
11	"	07.11.2011
12	"	"
13	cf. <i>Katodinium rotundatum</i>	28.05.2011
14	"	07.06.2011





1	<i>Ornithocercus quadratus</i>	06.06.2011
2	<i>Oxytoxum compressum</i>	06.09.2011
3	<i>Oxytoxum constrictum</i>	14.07.2011
4	"	28.05.2011
5	<i>Oxytoxum diploconus</i>	28.02.2012
6	<i>Oxytoxum laticeps</i>	28.05.2011
7	"	30.05.2011
8	"	07.06.2011



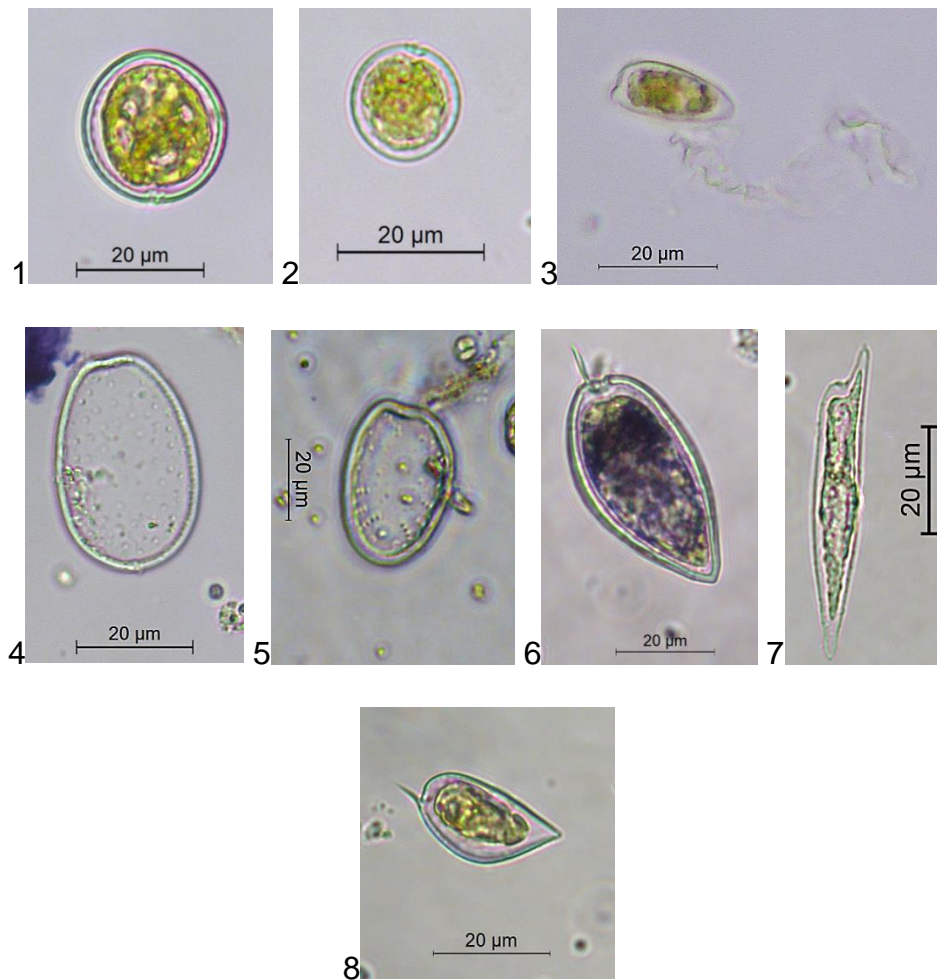
1	<i>Oxytoxum milneri</i>	07.06.2011
2	<i>Oxytoxum scolopax</i>	"
3	<i>Oxytoxum tessellatum</i>	08.11.2011
4	"	07.09.2011
5	cf. <i>Oxytoxum turbo</i>	07.12.2011
6	cf. <i>Oxytoxum variabile</i>	28.05.2011
7	"	"
8	"	30.05.2011
9	<i>Oxytoxum</i> sp.	"



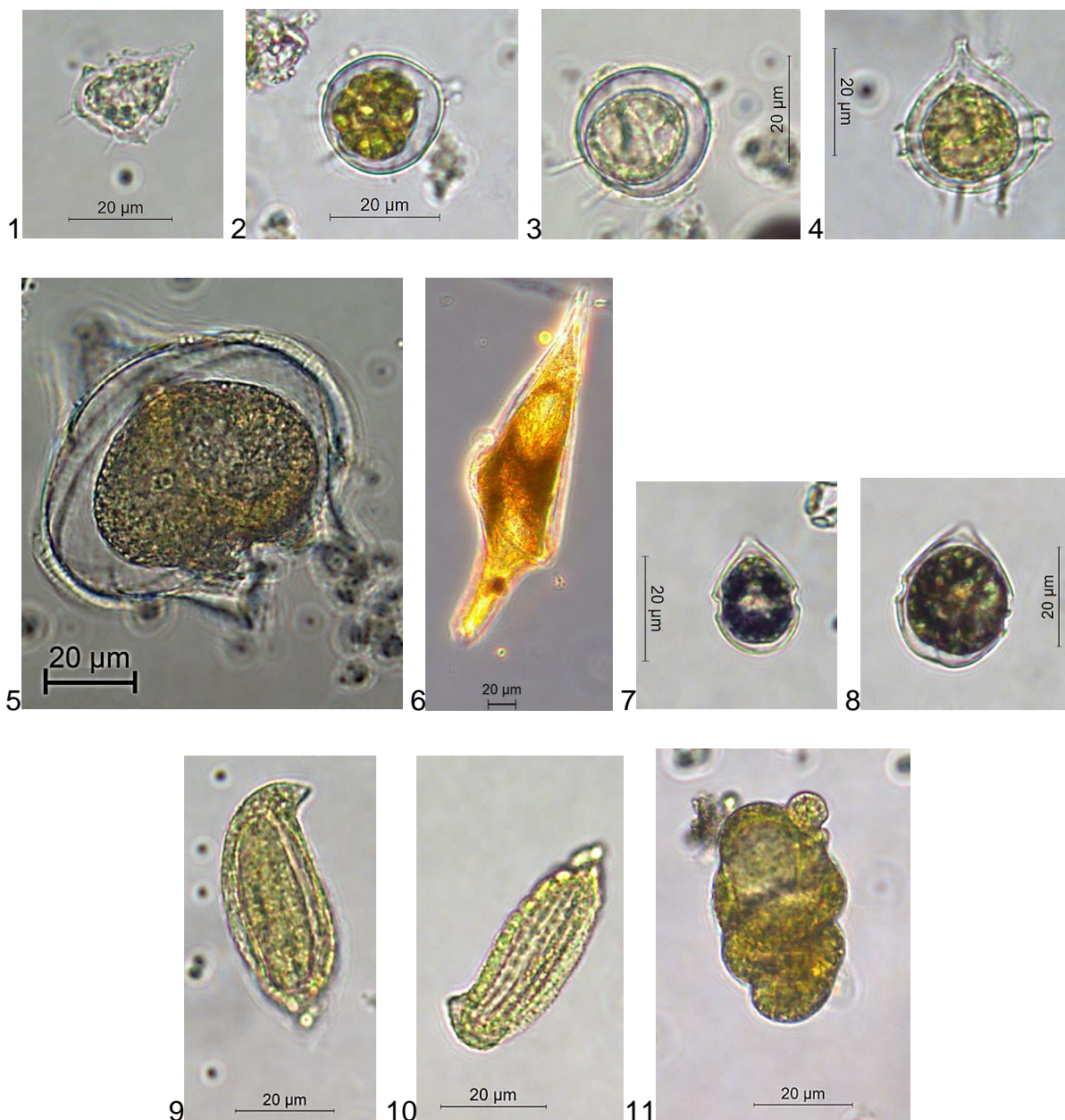


1	<i>Phalacroma rotundatum</i>	28.05.2011
2	<i>Podolampas palmipes</i>	07.06.2011
3	"	"
4	<i>Podolampas spinifera</i>	27.02.2012

## Appendix 2

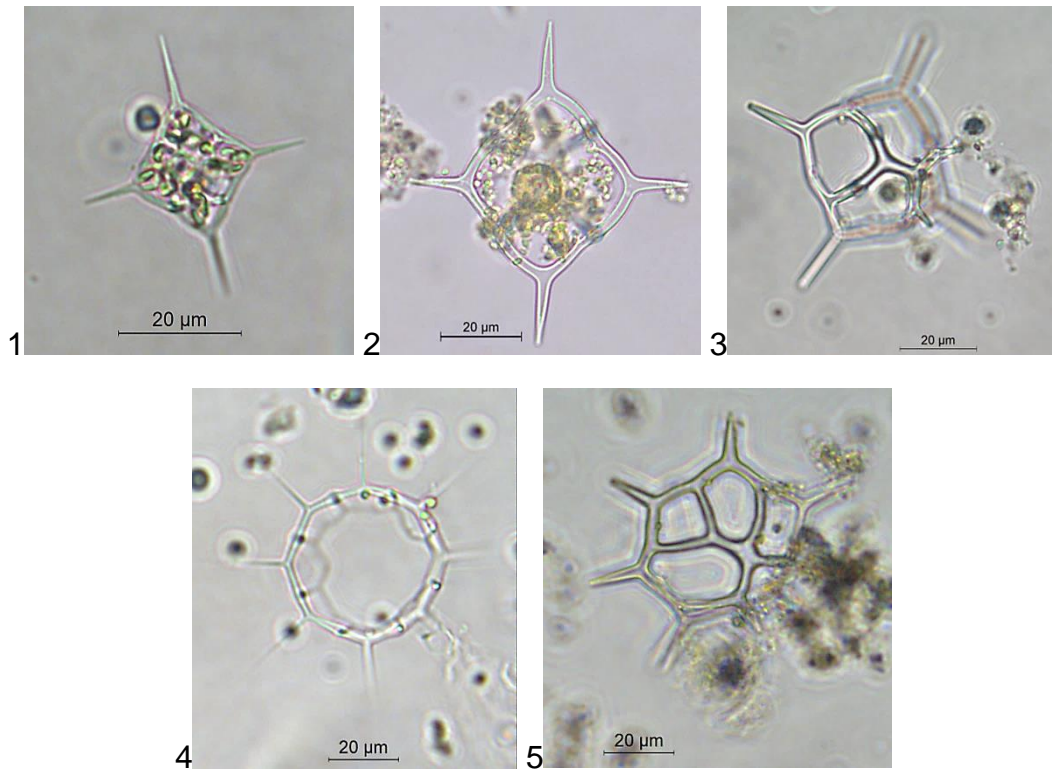


1	<i>Prorocentrum cordatum</i>	28.05.2011
2	"	07.06.2011
3	<i>Prorocentrum dentatum</i>	28.05.2011
4	<i>Prorocentrum lima</i> (empty cell)	"
5	<i>Prorocentrum rhathymum</i> (empty cell)	12.10.2011
6	<i>Prorocentrum micans</i>	09.08.2011
7	<i>Prorocentrum rostratum</i>	07.09.2011
8	<i>Prorocentrum triestinum</i>	12.10.2011



1	<i>Protoperidinium bipes</i>	10.10.2011
2	<i>Protoperidinium</i> sp.	07.09.2011
3	"	12.10.2011
4	"	06.09.2011
5	"	12.10.2011
6	<i>Schuettilia mitra</i>	28.05.2011
7	<i>Scrippsiella trochoidea</i>	09.08.2011
8	"	27.02.2012
9	cf. <i>Torodinium robustum</i>	07.09.2011
10	"	07.09.2011
11	<i>Warnowia polyphemus</i>	27.02.2012

## Dictyochophyceae

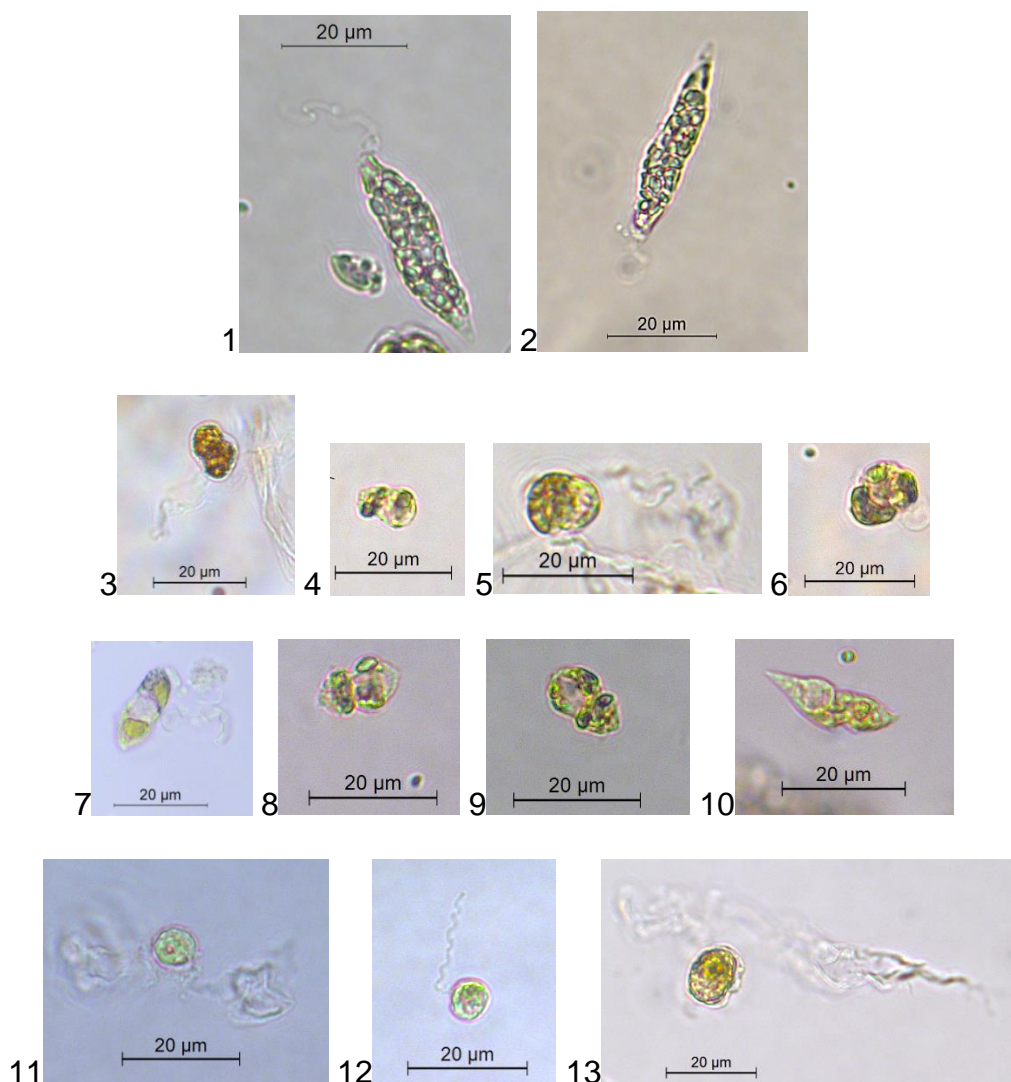



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1 <i>Dictyocha fibula</i>	07.06.2011
2 " (skeleton)	30.05.2011
3 " (skeleton)	07.09.2011
4 <i>Dictyocha octonaria</i> (skeleton)	28.02.2012
5 <i>Dictyocha speculum</i> (skeleton)	08.11.2011



## Flagellates



1	cf. <i>Eutreptiella</i> sp.	10.10.2011
2	"	
3	<i>Gymnodinium</i> -like flagellate	28.05.2011
4	"	07.06.2011
5	"	"
6	"	"
7	<i>Gyrodinium</i> -like flagellate	28.05.2011
8	"	"
9	"	"
10	"	"
11	Undefined flagellate <20 µm	28.05.2011
12	"	"
13	"	07.06.2011

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**Appendix 3**

The Australian Ocean data Network (AODN; <http://portal.aodn.org.au/aodn/>) was created by Australian Commonwealth agencies (including the Commonwealth Scientific and Industrial Research Organisation and the Integrated Marine Observing System), research institutes and universities as a central access point to share marine data. All phytoplankton and zooplankton abundance data collected throughout the time-series survey represented by this thesis was made publicly available via the AODN for archiving and distribution purposes. The data and its associated metadata can be accessed directly at this link:

<http://catalogue.aodn.org.au/geonetwork/srv/en/metadata.show?uuid=f7502841-a2c7-4437-b557-20ef89e754e0>.

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I thank Ms Katherine Tattersall, Ms Natalia Atkins and Ms Jacqui Hope (University of Tasmania) for their assistance with publishing this data through the AODN.





## *Reflection*

~

*Snow leopards have been described as ghost-cats – beautiful things that never let themselves be seen and do not ask for attention (Sean Penn, in: The Secret Life of Walter Mitty, 2013).*

*Considering their crucial role in sustaining the functioning of the Earth, going unnoticed by the majority of humanity, phytoplankton are the ghost-cats of the sea.*

*Three years of research and a 303 pages thesis, and I have still only caught a glimpse at these elusive marine ghost-cats.*

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