The effects of climate change on harmful algal blooms and plankton communities in the NE Atlantic.

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The effects of climate change on harmful algal blooms and plankton communities in the NE Atlantic

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of the requirements for the Doctor of Philosophy

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For Elsie and Olive

Mum and Dad

I hope that I've made you proud

Declaration

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Abstract

Climate change has a profound impact on the phenology and abundance of plankton in the NE Atlantic and North Sea. There is concern that harmful algal bloom (HAB) species may increase, accompanied by negative socio-economic impacts, including threats to human health and marine harvesting. We reviewed historical major UK outbreaks of poisoning and attempted to examine the epidemiology on a finer scale by linkage of hospital admissions, GP and pathology records. As expected the incidence of shellfish poisonings was very low but accurate identification of poisoning was generally unreliable. The current UK shellfish monitoring programme is the key indicator for monitoring trends in the risk of human exposure.

Using the Continuous Plankton Recorder (CPR) survey, we mapped spatial and temporal trends of various phytoplankton, including HAB species, and zooplankton (*Tintinnids* and *Calanus*). We found fundamental shifts in the relative abundance of diatoms versus dinoflagellates, with a dramatic dinoflagellate decline in recent years. Northward shifts in abundance were found for some *Tintinnid* and *Calanus* taxa. Using criteria of statistical causality, these changes were linked to climate, in particular sea surface temperature and increasingly windy conditions in the summer, with a notable non-linear interaction between these factors. Focusing on Calanus, we showed the strength of statistical links between abundance and climate variables can wax and wane as the time series lengthens. We found tentative evidence for adaptation of *Calanus* to climate change, but not at a level that could reverse overall long-term patterns.

Links with climate are often argued to be proxies for unobserved mechanisms that determine species abundance, such as stratification. We developed a new stratification index, covering the whole NE Atlantic from 1970 to 2009. We propose that this has wide applicability in marine climate change studies. Throughout, our work demonstrates the importance of consistent long-term ecological survey data.

Disclaimer

Chapter I

Stephanie Hinder (SLH) reviewed the historical data available. Caroline Brooks (CJB) and SLH obtained the HIRU (SAIL) data for Wales. Angharad Davis (APD), Mike Gravenor (MBG) and SLH examined the HIRU dataset. SLH and MBG drafted the manuscript. All authors commented upon and approved the manuscript.

Chapter II

SLH and Antony Walne (AWW) compiled the data. SLH, MBG and Graeme Hays (GCH) led the data analyses and interpretation with contributions from all authors. SLH produced all the figures. SLH, GCH and MBG wrote the paper with contributions from all authors.

Chapter III

SLH and AWW compiled the data. SLH, MBG, Jane Manning (JEM) and GCH led the data analyses and interpretation with contributions from all authors. SLH produced all the figures. SLH and GCH wrote the paper with contributions from all authors.

Chapter IV

SLH and AWW compiled the data. SLH, MBG, Clare Ostle (CO) and GCH led the analyses and interpretation of the data and the writing of the paper. SLH produced all the figures. Pat Lee (PLML) suggested the analysis of genetic structure and consideration of adaptation. MBG and SLH preformed the statistical analysis. All authors contributed to the drafts.

Chapter V

SLH obtained the climate data. SLH and Owen Bodger (OGB) developed the index with help from both GCH and MBG. SLH and OGB performed the statistical comparison methods. SLH produced all the figures. Interpretation of the methods and the data was performed by SLH, OGB, GCH and MBG. SLH led the writing of the paper with contributions from all authors.

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List of Abbreviations

ALF: Anonymous System Linking Field

ASP: Amnesic Shellfish Poisoning

AZA: Azaspiracid Poisoning

CEFAS: Centre for Environment, Fisheries and Aquaculture Science

CPR: Continuous Plankton Recorder

CTD: Conductivity Temperature Depth

DA: Domoic Acid

DSP: Diarrhetic Shellfish Poisoning

DTX: Dinophysis Toxins

EC: European Council

EFRA: Environment, Food and Rural Affairs committee

EU: European Union

FBO: Food Business Operators

FSA: Food Standards Agency

HAB: Harmful Algal Bloom

HadISST: Hadley Centre Sea Ice and Sea Surface Temperature

HIRU: Health Information Research Unit for Wales

HPLC: High Performance Liquid Chromatography

ICOADS: International Comprehensive Ocean-Atmospheric Data Set

LBMs: Live Bivalve Mollusc

LC-MS: Liquid Chromatography – Mass Spectrometry

LC-UV:	Liquid Chromatography – UV detection
LT:	Lipophilic Toxin
MBAs:	Mouse Bioassay
MLD:	Mixed Layer Depth
NAOI:	North Atlantic Oscillation Index
NHS:	National Health Service
NRL:	National Reference Laboratory
OA:	Okadaic Acid
OSD:	Ocean Station Data
PCI:	Phytoplankton Colour Index
PSP:	Paralytic Shellfish Poisoning
PTX:	Pectenotoxin
SAIL:	Secure Anonymised Information Linkage
SAMS:	Scottish Association for Marine Science
SI:	Stratification Index
SSSWS:	Surface Summer Scalar Wind Speed
SST:	Sea Surface Temperature
STX:	Saxitoxin
TPO:	Temporary Prohibition Order
UK:	United Kingdom
VCA:	Voluntary Closure Agreement
WW:	Westerly Wind

XBT: eXpendable BathyThermographs

YTX: Yessotoxin

Foreword

The main theme of this thesis is the importance of long-term ecological surveys in monitoring trends in a range of plankton populations, including some key Harmful Algal Bloom (HAB) taxa, in the NE Atlantic Ocean and the North Sea. Using a range of statistical tools, we investigated the link between changes in spatial and temporal plankton abundance and climate factors. We highlight the key synergistic effects of these climate variables, which must be considered to help develop our understanding of the mechanistic processes underlying biological changes in the ocean. The thesis is comprised of five main results chapters, as outlined below, that address discrete research questions. The main results of each chapter are presented separately, with the key themes of the thesis brought together in a final chapter where the conclusions are discussed.

Chapter I

The current epidemiology of toxic microalgae poisoning around the UK was examined in two ways: firstly we described the key toxic syndromes that present a potential risk in UK waters and attempted to gather together, in one report, the disparate data on their epidemiology, using UK records and monitoring procedures. Secondly, using NHS hospital admissions and GP records, we attempted to quantify the incidents of shellfish poisoning using an independent data source.

Chapter II

Marine diatoms and dinoflagellates play a variety of key ecosystem roles as important primary producers and grazers. Additionally some are HAB species and there is widespread concern that HAB species may be increasing accompanied by major negative socioeconomic impacts. Using the Continuous Plankton Recorder (CPR) survey data, we generated a 50 year time series (1960-2009) of diatom and dinoflagellate occurrence in the NE Atlantic and North Sea. We undertook the most comprehensive analysis, to date, of extended spatio-temporal patterns of abundance by examining 23 taxa, including those HAB taxa most relevant to north Atlantic waters. We employed a range of statistical methods to examine the links between abundance, sea surface temperature (SST) and summer surface scalar wind speed. These methods were chosen to avoid effects of spurious association between variables commonly encountered when dealing with time series, and to strengthen indications of causal links.

Chapter III

Microzooplankton are often a neglected component of the plankton community, despite their role as a vital link in marine food webs. Tintinnids (Ciliophora, Protista) are important grazers which may be able to significantly regulate phytoplankton, including some harmful algae, in the initial stages of a bloom. The group are themselves also preyed upon by protozoan and metazoan plankton including copepods. The phenology, distribution and abundance of tintinnids were examined using the CPR survey data for the NE Atlantic and North Sea. Total tintinnid abundance was measured using presence / absence data for the long time series over the period 1960-2009, and the abundance of 5 individual taxa were examined in more detail for the period 1996-2009. From these long-term changes, we attempted to identify and discuss possible climate drivers for these populations, in particular SST and the North Atlantic Oscillation Index (NAOI).

Chapter IV

The copepod crustacean *Calanus finmarchicus* plays a key trophic role in the NE Atlantic, providing food for a number of commercially important fish such as cod and other gadoids. Declines in *C. finmarchicus* have been reported previously as the species range contracts to colder more northerly waters, and this species has been widely used as an important indicator of the impacts of climate change on individual populations and hence ecosystems. Using the extended CPR survey data for the NE Atlantic and North Sea between 1960-2010, the abundance, spatial and temporal distributions of *C. finmarchicus* and a congeneric species that favours rather warmer waters, *C. helgolandicus,* were examined. These abundance changes were linked, using a range of statistical techniques, to SST, westerly wind speed and the NAOI. The use of a long and detailed time series allowed us to examine how conclusions over links with climate can change depending on the study time point. The possibility of species adaptation to changes in climate, based on the changes in the strength of the statistical links with climate throughout the time series, was also examined.

Chapter V

Many studies have managed to find correlations between climate variables and changes in the distribution of species, including plankton communities. It is often the case, however, that the specific climate variable is assumed to be a proxy for the actual mechanism that is driving population change. For plankton, links with SST could have direct effects (on, say, metabolism) but temperature could also drive indirect effects of great importance to plankton communities via effects on the environment such as the timing and degree of ocean temperature stratification. However, in most large scale (temporal and spatial) plankton distribution studies, the level of stratification is rarely directly measured. Here, a new method to examine the structure of temperature depth profiles was created. We used over 55,000 temperature depth profiles, obtained from Conductivity Temperature Depth profiles, Ocean Station Data and Expendable Bathythermographs, to generate a novel spatio-temporal stratification index. We used the index to describe how the seasonal thermocline and temperature has changed in the NE Atlantic between 1970 and 2009.

Conclusions

Lastly, the main themes and results of the thesis are brought together and discussed in a short Conclusions chapter. We also outline how the work presented here can be used to guide future quantitative studies on the distribution of plankton species.

Chapter I

Toxic marine microalgae and shellfish poisoning in the British Isles: history, review of epidemiology, and future implications

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SLH reviewed the historical data available. CJB and SLH obtained the HIRU (SAIL) data for Wales. APD, MBG and SLH examined the HIRU dataset. SLH and MBG drafted the manuscript. All authors commented upon and approved the manuscript.

Abstract

The relationship between toxic marine microalgae species and climate change has become a high profile and well discussed topic in recent years, with research focusing on the possible future impacts of changing hydrological conditions on Harmful Algal Bloom (HAB) species around the world. However, there is very little literature concerning the epidemiology of these species on marine organisms and human health. Here, we examine the current state of toxic microalgae species around the UK, in two ways: first we describe the key toxic syndromes and gather together the disparate reported data on their epidemiology from UK records and monitoring procedures. Secondly, using NHS hospital admissions and GP records from Wales, we attempt to quantify the incidence of shellfish poisoning from an independent source. We show that within the UK, outbreaks of shellfish poisoning are rare but occur on a yearly basis in different regions and affect a diverse range of molluscan shellfish and other marine organisms. We also show that the abundance of a species does not necessarily correlate to the rate of toxic events. Based on routine hospital records, the numbers of shellfish poisonings in the UK are very low, but the identification of the toxin involved, or even a confirmation of a poisoning event is extremely difficult to diagnose. An effective shellfish monitoring system, which shuts down aquaculture sites when toxins exceed regularity limits, has clearly prevented serious impact to human health, and remains the only viable means of monitoring the potential threat to human health. However, the closure of these sites has an adverse economic impact, and the monitoring system does not include all toxic plankton. The possible geographic spreading of toxic microalgae species is therefore a concern, as warmer waters in the Atlantic could suit several species with southern biogeographical affinities enabling them to occupy the coastal regions of the UK, but which are not yet monitored or considered to be detrimental.

Introduction

Within the UK, several toxic marine microalgae species, where some species are also known as Harmful Algal Bloom (HAB) species are present throughout the coastal regions. These species produce biotoxins, which are ingested by filter feeding organisms, accumulating within their flesh (Wang, 2008). These toxins gradually get transferred to the higher trophic levels within the food web posing a threat to human health if the shellfish is consumed (Davidson and Bresnan, 2009; Moore et al., 2008; Shumway et al., 2003).

In UK waters, an effective shellfish monitoring system acts as a sentinel, and has prevented serious impact on human health but this has led to long-term closures of fisheries, with severe economic consequences (Fehling et al., 2006). The shellfish industry is an important aspect of the economy in the UK; with shellfish contributing to 42% of UK landings, and with the shellfish industry worth £267.1 million in 2008 (Irwin and Padia, 2009). There have been several in-depth reviews concerning the toxins produced by these toxic microalgae species, and the future implication of climate change on these species composition and potential alterations in locations (Hallegraeff, 1993; Moore et al., 2008). However, despite many closures of fisheries there is very little literature concerning the epidemiology of adverse events on human health in the UK. Generally, information regarding the acute manifestations of these illnesses is greatly under reported, and species involved not even identified. With the changing hydrological conditions and the general idea that toxic microalgae species are increasing in their geographical location and frequency (Fraga and Bakun, 1993; Kirkpatrick et al., 2004), it is important to determine the possible future threat in the UK.

Here, we address the problem in two ways: first we describe the key toxic syndromes and gather together the disparate data on their epidemiology from sporadic UK records and monitoring procedures. We describe the ecology of the main species, including their seasonal patterns, global distribution and how these might be affected by climate change. Second, using the Health Information Research Unit for Wales (HIRU), all NHS hospital admissions and GP records involving shellfish toxins were identified within Wales, and the implication for monitoring shellfish poisoning in the UK are discussed.

Toxic syndromes

There are around 60 to 80 toxic marine microalgae species throughout the world, with dinoflagellates accounting for 75% of all such species (Smayda, 1997). Seven major classes of marine biotoxins have been identified: Domoic Acid (DA) group, responsible for amnesic shellfish poisoning (ASP); Saxitoxin (STX) group, responsible for paralytic shellfish poisoning (PSP); Lipophilic toxins (LT) group, including the Okadaic Acid (OA) and Dinophysis Toxins (DTXs) responsible for diarrhetic shellfish poisoning (DSP), Azaspiracids (AZA), Pectenotoxins (PTXs) and Yessotoxins (YTXs). In the European Union (EU) (including the UK), PSP, ASP and LTs are the three major shellfish biotoxin groups which are subject to statutory monitoring to protect human health.

ASP is produced by marine diatoms of the genus *Pseudo-nitzschia* (Van Dolah, 2000), while PSP toxins are produced by dinoflagellate algae of the genera *Alexandrium, Gymnodinium* and *Pyrodinium* (Smart, 1995; Wang, 2008). Of the LTs, OA and DTXs are produced by dinoflagellate genera *Dinophysis* and *Prorocentrum* (Miles et al., 2004; Wang, 2008), and the newly identified AZA toxins are produced by the dinoflagellate *Azadinium spinosum* (Magdalena et al., 2003). PTXs, are also produced by the dinoflagellate *Dinophysis* (Dominguez et al., 2010). However, there is great debate to whether PTXs should be included within the DSP group, as some studies have found evidence of diarrhoeic effects (Ishige et al., 1988), while others did not (Miles et al., 2004). Nearly all information regarding the toxicology of PTXs have been obtained from controlled *in vitro* and *in vivo* experiments (Dominguez et al., 2010). Under EC Regulation 853/2004 the maximum permitted levels of PTXs are set together with the DSP toxin group (EC, 2004). YTXs and its analogues are produced by the dinoflagellates *Protoceratium reticulatum* (Satake et al., 1997), *Lingulodinium polyedrum* (Paz et al., 2004) and *Gonyaulax spinifera* (Rhodes et al., 2006). To date there have been no recordings of human poisoning induced by YTXs, although contaminated shellfish have been reported worldwide (Tubaro et al., 2010). Initially, YTXs and its analogues were included into the DSP toxin group, mainly as they were detected with OA and other LTs during shellfish extraction procedure for DSP using mouse bioassays (Tubaro et al., 2010). Since then, YTXs have shown not to cause diarrhoea or share the same mode of action (inhibition of protein phosphatases (Tubaro et al., 2010)), and have been removed from the DSP group (Dominguez et al., 2010; Tubaro et al., 2010). Under EU Regulation 853/2004, YTXs are now classified and regulated separately from DSP (EC, 2004).

For each toxin syndrome, there is a different lethal dose, onset and duration time, and a range of symptoms. A review of each toxin and symptoms involved can be found in Table 1.1.

Table 1.1. Toxin syndromes and symptoms within UK waters. (PTXs and YTXs are not included as there are no known human cases of poisoning and only limited information exists from experimental animal studies).

	Toxin	Causal species	Symptoms	References
PSP	Saxitoxin and Gonyautoxin	*Alexandrium spp.* *Gymnodinium spp.* *Pyrodinium spp.*	Tingling and numbness Drowsiness Incoherence **In high doses** – respiratory arrest or cardiovascular shock or death	(Schantz, 1984; Smart, 1995; Van Dolah, 2000; Wang, 2008)
DSP	Okadaic acid and Dinophysis toxin (1,2 and 3)	*Dinophysis spp.* *Prorocentrum spp.*	Nausea Vomiting Diarrhoea Abdominal cramps **In high doses** – dehydration and shock	(Miles et al., 2004; Vale and Sampayo, 2002; Van Dolah, 2000; Wang, 2008)
AZA	Azaspiracids	*Azadinium spinosum*	Nausea Vomiting Diarrhoea Abdominal cramps	(Magdalena et al., 2003)
ASP	Domoic acid	*Pseudo- nitzschia*	Nausea Vomiting Diarrhoea Abdominal cramps Loss of short term memory	(Smart, 1995; Todd, 1993; Van Dolah, 2000)

UK monitoring procedures

Legislative requirements are implemented to monitor shellfish to ensure human consumer protection and to control the risk of shellfish poisoning (Morris et al., 2007). Within the UK, the Food Standards Agency (FSA) is the competent authority responsible for the implementation of these regulations. The FSA funds the work of a group of National Reference Laboratories (NRL), including an NRL on Biotoxins in Live Bivalve Molluscs (LBMs), which is currently undertaken by the Agri-Food and Bioscience Institute in Belfast (Agri-Food and Bioscience Institute, 2009).

Within the UK, monitoring of marine biotoxins are divided into two elements, flesh monitoring, where samples of shellfish are tested, and water monitoring, to check for the presence of potentially toxin-producing plankton. Within England and Wales, the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) is the contracted laboratory responsible for the analysis of both flesh and water samples (FSA England and Wales, 2009). In Scotland, water samples are monitored by the Scottish Association for Marine Science (SAMS), while testing of shellfish for the presence of biotoxins are carried out by CEFAS (FSA Scotland, 2009). In Northern Ireland, the Agri-Food and Biosciences Institute conducts the testing program (FSA Northern Ireland, 2009). Biotoxin monitoring programmes in England and Wales, Scotland and Northern Ireland apply different frequencies of sampling of biotoxin testing. The number of water samples, shellfish flesh sampled and the number of contributing harvesting areas in England Wales and from 1999-2009 are shown in Table 1.2.

Maximum regulative limits have been set for both the toxin level and the action limit in water samples (Table 1.3) (EFRA, 2004). If toxins are shown to exceed these maximum regulatory limits, under European Union procedures, a Temporary Prohibition Order (TPO) is placed on the relevant infected bed. Closure of the bed is maintained until two consecutive samples, taken at least 48 hours apart, are tested below maximum limits (EFRA, 2004). To ensure the safety of the shellfish placed on the market, the Food Business Operators (FBOs) are required under regulation (EC) No. 853/2004 to monitor the levels of biotoxins and ensure they do not exceed regulatory limits (McElhiney and Kennington, 2007).

Table 1.2. The number of shellfish flesh samples, water samples and the number of active classified shellfish production and relaying areas collected by CEFAS from 1999-2009 in England and Wales.

	Shellfish Testing		**Water Samples**		**Reference**
	No. of Samples	**No. of active classified shellfish production and relaying areas**	**No. of Samples**	**No. of active classified shellfish production and relaying areas**	
April 1999 - March 2000	1017	25	320	19	(Higman and Milligan, 2000)
April 2000 - March 2001	703	34	350	20	(Higman et al., 2001)
April 2001 - March 2002	1326	67	195	23	(Gubbins and Higman, 2002)
April 2002 - March 2003	1529	64	1529	23	(CEFAS, 2003)
April 2003 - March 2004	1326	66	388	23	(CEFAS, 2004)
April 2004 - March 2005	949	64	314	21	(Stubbs et al., 2005)
April 2005 - March 2006	1143	64	737	61	(Stubbs et al., 2006)
April 2006 - March 2007	941	64	879	54	(Stubbs et al., 2007)
April 2007 - March 2008	1163	64	1122	54	(Stubbs et al., 2008)
April 2008 - March 2009	1059	66	1079	56	(Coates et al., 2009)

Table 1.3. Regulative limits of the maximum toxin levels of the major seafood poisoning categories that is allowed to be present and the action limit of four genera in water samples within the UK. (Toxins are tested in LBMs, live echinoderms, live tunicates and live marine gastropods, for the whole body or any part edible separately).

Regulative limits of maximum toxin level within the UK		
Type of Shellfish Poisoning	**Toxin**	**Maximum Level of Toxin**
PSP	STX	800 µg STX equivalents/kg (EC, 2004)
DSP	OA, DTXs and PTXs	160 µg OA equivalents/kg (EC, 2004)
YTX	YTXs	1 mg YTX equivalents/kg (EC, 2004)
AZA	AZA	160 µg/kg (EC, 2004)
ASP	DA	20 mg/kg (EC, 2004)

Water Sampling, maximum species abundance in the UK		
	Species	**Action Limit (cells/litre)**
PSP	*Alexandrium spp.*	Present (Stubbs et al., 2008)
DSP	*Dinophysis spp.*	100 (Stubbs et al., 2008)
	Prorocentrum spp.	100 (Stubbs et al., 2008)
ASP	*Pseudo-nitzschia spp.*	150,000 (Stubbs et al., 2008)

The method currently specified by European Food Safety legislation for Official Control testing for PSP and DSP are mouse bioassays (MBAs) based on the protocol of Yasumoto et al., (1978), whereby shellfish extract is injected into mice, followed by observation of the survival time (EFRA, 2004). The bioassay results are compared to the threshold (maximum) limits (Table 1.3) to determine if the toxin level is exceeded, which could result in the closure of the bed (Morris et al., 2007). However, with the discovery of a range of novel lipophilic compounds, PTXs and YTXs, which give a positive result in the MBAs, this approach is considered inadequate in scale for these toxins (EC) No 225/2002 (Stobo et al., 2008). This and the fact that the MBA method has considerable ethical objections, has lead to a demand to use alternative approaches (Chapela et al., 2008; Morris et al., 2007). Commission Regulation (EC) 2074/2005 was revised to allow other detection approaches to be used, as alternative or supplementary methods to the MBAs, as long as they are as effective and EC 853/2004 valid (EFSA, 2009). Since 2006, a high performance liquid chromatography (HPLC) method has been used as a qualitative screen with the MBA used to provide a quantitative result from HPLC positive samples (Turrell et al., 2007). AZA toxins are harder to detect, as the toxins are not confined to the digestive glands but are distributed throughout all tissues, rendering the MBAs method inefficient

(James et al., 2002; Magdalena et al., 2003). Liquid Chromatography–Mass Spectrometry (LC–MS), under the EU regulation 15/2011 (EU Regulation 15/2011), and Liquid Chromatography- UV detection (LC-UV) are used as references methods for the detection of lipophilic toxins (PTXs, YTXs, and AZAs) (Christian and Luckas, 2008; Magdalena et al., 2003; Ofuji et al., 1999). The success of the toxin detection and quantification of the LC-MS method is due to its efficient toxin separation, high sensitivity (lower limits of detection than MBA), high selectivity, and accurate and precise quantification (Chapela et al., 2008). However, LC-MS cannot detect mixtures of two different toxins, unlike the MBA method. Figure 1.1, shows the locations of samples collected in England and Wales from April 2008 to March 2009, and Tables 1.4 and 1.5 show the CEFAS shellfish testing and water sample results from April 1990- March 2009.

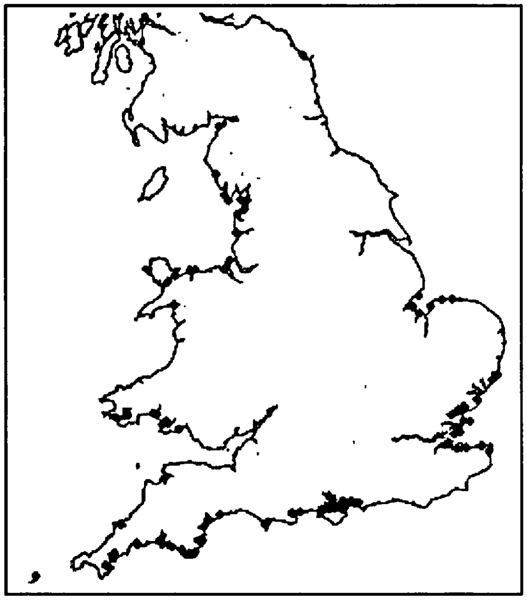

Figure 1.1. English and Welsh flesh sampling locations - FSA Biotoxin monitoring programme 1st April 2008 to 31st March 2009 (adapted with permission from CEFAS (Coates et al., 2009)).

Table 1.4. CEFAS shellfish flesh results for the toxins, DSP, PSP and ASP from 1999-2009 in England and Wales. The number of samples tested for each toxin, with the % presence of each toxin and the number of areas each toxin has affected are recorded. In 2006, LTs were detected, which included OA/DTXs, AZAs, YTXs and PTXs.

	DSP			**PSP**			**ASP**			
	No. samples tested	**% Present**	**No. areas effected**	**No. samples tested**	**% Present**	**No. areas effected**	**No. samples tested**	**% Present**	**No. areas effected**	**Reference**
April 1999 - March 2000	302	15 (5.0 %)	5	370	5 (1.4%)	3	345	21 (6.1%)	21	(Higman and Milligan, 2000)
April 2000 - March 2001	579	115 (19.9%)	35	438	8 (1.8%)	3	365	32 (8.8%)	32	(Higman et al., 2001)
April 2001 - March 2002	1173	166 (14.2%)	5	774	5 (0.6%)	4	768	10 (1.3%)	3	(Gubbins and Higman, 2002)
April 2002 - March 2003	1342	203 (15.1%)	20	981	12 (1.2%)	2	902	10 (1.1%)	7	(CEFAS, 2003)
April 2003 - March 2004	1128	148 (13.1%)	15	873	1 (0.1%)	1	818	11 (1.3%)	7	(CEFAS, 2004)
April 2004 - March 2005	847	14 (1.6%)	8	876	2 (0.2%)	2	841	8 (1.0%)	5	(Stubbs et al., 2005)
April 2005 - March 2006	1023	4 (0.4%)	3	1085	4 (0.4%)	2	1004	9 (0.9%)	2	(Stubbs et al., 2006)
	LTs			**PSP**			**ASP**			
April 2006 - March 2007	821	5 (0.6%)	3	892	8 (0.9%)	3	823	21 (2.6%)	7	(Stubbs et al., 2007)
April 2007 - March 2008	1024	9 (0.9%)	5	1099	3 (0.3%)	1	985	6 (0.6%)	5	(Stubbs et al., 2008)
April 2008 - March 2009	926	66 (7.1%)	5	991	4 (0.4%)	1	904	2 (0.2%)	1	(Coates et al., 2009)

Table 1.5. CEFAS water sample results for four toxic marine microalgae species from 1999-2009 in England and Wales. The number of water samples tested, with the species % present in each sample, and the % of samples exceeding the action limit have been recorded. (NA = no results during that time period).

		Dinophysis spp.		*Prorocentrum spp.*		*Alexandrium spp.*	*Pseudo-nitzschia*		
	No. water samples tested	**% Present**	**%> limit**	**% Present**	**%> limit**	**% Present**	**% Present**	**%> limit**	**Reference**
April 1999 - March 2000	320	NA	1 (0.3%)	NA	NA	4 (1.3%)	NA	0	(Higman and Milligan, 2000)
April 2000 - March 2001	350	25 (7.1%)	2 (0.6%)	NA	NA	NA	15 (4.3%)	0	(Higman et al., 2001)
April 2001 - March 2002	195	NA	0	NA	NA	NA	14 (7.2%)	0	(Gubbins and Higman, 2002)
April 2002 - March 2003	1529	23 (1.5%)	1 (0.1%)	NA	NA	NA	50 (3.3%)	0	(CEFAS, 2003)
April 2003 - March 2004	388	0	0	NA	NA	13 (3.4%)	18 (4.6%)	0	(CEFAS, 2004)
April 2004 - March 2005	314	6 (1.9%)	1 (0.3%)	6 (1.9%)	1 (1.9%)	6 (1.9%)	28 (8.9%)	0	(Stubbs et al., 2005)
April 2005 - March 2006	737	23 (3.1%)	7 (0.9%)	3 (0.4%)	1 (0.1%)	80 (10.9%)	277 (37.6%)	3 (0.4%)	(Stubbs et al., 2006)
April 2006 - March 2007	879	83 (9.4%)	31 (3.5%)	10 (1.1%)	5 (0.6%)	150 (17.1%)	374 (42.5%)	32 (3.6%)	(Stubbs et al., 2007)
April 2007 - March 2008	1122	18 (1.6%)	4 (0.4%)	3 (0.3%)	0	139 (12.4%)	570 (50.8%)	4 (0.4%)	(Stubbs et al., 2008)
April 2008 - March 2009	1079	25 (2.3%)	4 (0.4%)	11 (1.0%)	5 (0.5%)	75 (6.9%)	438 (40.6%)	21 (1.9%)	(Coates et al., 2009)

UK incidents

Although the potential adverse effects of toxic marine microalgae are well documented, there are very few epidemiological studies designed to thoroughly assess these effects (Moore et al., 2008). Within the UK, there are few published records of shellfish poisonings, and we suspect that there is a high level of underreporting, as there is no set database which systematically records the number or frequency of incidents.

Epidemiology of shellfish poisoning in the UK, 1960-2009

PSP

The first reliably reported case of PSP in the UK was on the east coast in 1969, where high concentrations of *Alexandrium tamarense* were monitored up to 15 miles offshore (Joint et al., 1997; Shumway et al., 2003). This outbreak caused illness in 78 humans and was responsible for the death of numerous birds and other marine mammals in the region (Coulson et al., 1968; Shumway et al., 2003; Van Dolah, 2000) (Table 1.6). In the UK, it is thought that PSP toxin is regional in nature, occurring in particular hotspots mainly in Scotland, e.g. Orkney and Shetland Islands (Davidson and Bresnan, 2009). There appear to be, however, few reported cases of PSP toxin affecting fisheries throughout the UK, with the longest closure occurring in Scotland during 2000-2001, with aquaculture and scallop fisheries affected by PSP toxin throughout the year (Table 1.6).

Throughout 1999-2009, the PSP toxin has remained at a low level in routine testing (average 0.73%), affecting between 1-4 sampling locations, within England and Wales (Table 1.4). The PSP toxin producing species *Alexandrium* has also maintained a low abundance throughout England and Wales during 2005-2009 (Table 1.5).

Table 1.6. The history and consequences of toxic events of dinoflagellate PSP toxin seafood poisoning within the UK from 1969-2007. (TPO = Temporary Prohibition Order. VCA = Voluntary Closure Agreement).

Year	Event	Reference
1969	Toxins monitored up to 15 miles offshore on the East Coast of UK. Caused illness to 78 humans and was responsible for the death of numerous birds and marine mammals. Species responsible: *Alexandrium tamarense*.	(Coulson et al., 1968; Joint et al., 1997; Shumway et al., 2003; Van Dolah, 2000)
1990	On the NE English coast in May high levels of toxins detected in mussels and scallops. Commercial fisheries were closed.	(Joint et al., 1997)
2000	TPO was placed in Falmouth in July, as PSP was above action limit. Species responsible was *Alexandrium.*	(Higman et al., 2001)
2000-2001	In Scotland, toxins detected throughout the year in aquaculture sites along the west coast, and in scallop fisheries grounds in Orkney and East coast Scotland. Led to restrictions in Fishing.	(FAO, 2004)
2001	Toxins found in scallops in the sea adjacent to Northern Ireland. Led to a ban on scallop fishing.	(FAO, 2004)
2002	Warning notices and VCA were placed in Salcombe estuary, Devon, from July to November, and Holy Island, Northumberland, in May, when cockles and mussels returned positive results.	(CEFAS, 2003)
2002	Loch Eishort, in Skye and Lock Hourn, were closed in June and July where toxins were detected in mainly mussels.	(Howard et al., 2003)
2002-2003	PSP was detected in scallops in Orkney, Moray Firth, and the North Minch from May to September. Fisheries closures were implemented.	(Howard et al., 2003)
2005	Mussels from Pont Pill, Fowey found PSP toxins above the regulatory limit. The site was subjected to temporary harvesting restrictions.	(Stubbs et al., 2006)
2006-2007	Toxins were detected in two areas of the Fal, Cornwall. Temporary harvest restrictions in June and July.	(Stubbs et al., 2007)

DSP

DSP toxin was first reliably recorded in the UK in 1997, when 49 patients showed symptoms 30 minutes after consuming mussels in two London restaurants (Durborow, 1999). Since then the incident and the presence of DSP toxin appear to be more frequent and prolonged (EU-NRL, 2000), which may be partly due to increased knowledge and surveillance programmes. Table 1.7 shows 19 incidents from 1999-2009, over a wide range of areas throughout the UK. Temporary closures and voluntary closures lasted between a few weeks up to seven months.

During 1999-2004, there was a high percentage of DSP toxin detected in shellfish samples (average of 13.5%) (Table 1.4). However, the level of LTs detected, which include newly identified toxins, has dramatically decreased, only averaging 2.1% from 2004-2009, within England and Wales. *Dinophysis spp.* has been detected in UK waters, from 2004-2009 (Table 1.5) and it is thought that *D. acuminata* and *D. acuta* are the main species that dominate, especially in Scottish waters (Davidson and Bresnan, 2009). During 2006-2007, 3.5% of *Dinophysis spp.* samples returned a greater than the action limit throughout England and Wales. The genus *Prorocentrum* is also associated with DSP, and has been detected since 2004, but due to its epiphytic and epibenthic nature, it may be under-represented in sampling programmes (Davidson and Bresnan, 2009; Foden et al., 2005).

Table 1.7. The history and consequences of toxic events of DSP within the UK from 1997-2009.

Year	Event	Reference
1997	49 patients showed symptoms 30 minutes after consuming mussels in two London restaurants.	(Durborow, 1999)
1999	Voluntary closure and warning signs for the general public was undertaken in Holy Island, Northumberland for 6 weeks as Pacific oyster returned a positive DSP result.	(Higman and Milligan, 2000)
2000	TPO was induced from early February to March on the Northern side of the Solent, as oysters returned positive results.	(Higman et al., 2001)
2000	Toxins detected in mussels from Cornwall, cockles from southeast England and from south Wales, led to harvesting restrictions.	(EU-NRL, 2001)
2000	DSP was detected in England and Wales, with large scale closures (TPO and VCA) with the Solent shellfisheries from June - November, and the Thames shellfisheries from July - September.	(Higman et al., 2001)
2000	In July, toxins detected in Fleet Lagoon, Dorset. Harvesting was closed until 4th September. Species responsible: *Prorocentrum lima.*	(Foden et al., 2005)
2000	DSP affected a large number of areas in Scotland. East coast between July-September. Orkney between July-August. 10 sites within Shetlands between July-October. Outer Hebrides in May, and July-October, and 28 locations in Clyde between May–December. Long-term closures of shellfish farming in Scotland lasted up to 24 weeks.	(Howard et al., 2001)
2000-2001	Camel Estuary, Devon was closed from August until February as mussels returned positive DSP results.	(Higman et al., 2001)
2000-2001	Toxins detected in mussels and scallops and re-appeared at several sites throughout the year. Restrictions on harvesting at affected sites.	(EU-NRL, 2001)
2001	DSP was detected in the Thames from June-September, and Blyth Northumberland in July, leading to TPO and VCA within shellfisheries.	(Gubbins and Higman, 2002)
2001	The Wash shellfisheries were subjected to closures over winter as DSP was detected in cockles.	(Gubbins and Higman, 2002)
2001-2002	Cockles from Burry Inlet, Wales produced positive DSP results from June 2001 to March 2002. Leading to a long-term closures of shellfisheries.	(Gubbins and Higman, 2002)
2001	Toxins found in scallops in the sea adjacent to Northern Ireland. Led to a ban on scallop fishing.	(FAO, 2004)
2002-2003	Burry Inlet, Thames Estuary and the Wash were subjected to harvest closures every month except May and November for Burry Inlet, September for the Thames, and September, October and February for the Wash.	(CEFAS, 2003)
2002	DSP toxins were detected throughout Scotland, where VCAs were placed in several regions. Majority of closures lasted for periods of four to six weeks, but some closures lasted up to seven months.	(Howard et al., 2003)

Continues on next page

Year	Event	Reference
2005	DSP were detected in three areas, East of Ajax, Plymouth, The Wash, and Clamerkin Creek, Newtown. All were subject to temporary harvesting restrictions.	(Stubbs et al., 2006)
2006-2007	Toxins were detected in three regions in Cornwall, which led to temporary harvest restriction.	(Stubbs et al., 2007)
2007-2008	Toxins detected in Southampton Water, Fal River: Cornwall, Salcombe, Devon, which led to temporary harvesting restrictions.	(Stubbs et al., 2008)
2008	Shetland Islands were affected by a large outbreak, closing 13 areas intermittently between April-October 2008.	(Stubbs, 2009)
2008-2009	Seven regions in Argyll and Bute, Scotland suffered temporary closures. With the West Loch Tarbert being closed intermittently between April 2008-Feburary 2009.	(Stubbs, 2009)

AZA

AZA toxins have been identified in mussels within the UK (Ito et al., 2000; James et al., 2002), although there have been no reported incidents of poisoning. However, mussels cultivated in Killary Harbour, Ireland, were responsible for the intoxication of at least eight people in the Netherlands in November 1995 (Ito et al., 2000) (Table 1.8). Since 1996, several other human intoxications have been reported in Ireland around the Arranmore Island region on Donegal, Northwest Ireland (Mc Mahon and Silke, 1998), and in 1997 AZA toxin persisted in this region for seven to eight months (James et al., 2002). In 2000, a number of food poisoning incidents occurred in the UK after the consumption of processed mussels which originated from the SW coast of Ireland. These mussels were initially deemed safe for human consumption following negative MBAs, but it was later identified that AZA was the causative toxin (Furey et al., 2010).

Table 1.8. The history and consequences of toxic events of dinoflagellate AZA toxin seafood poisoning within the UK from 1995-2000.

Year	Event	Reference
1995	Mussels cultivated in Killary Harbour, Ireland, were responsible for the intoxication of at least eight people in the Netherlands in November.	(Ito et al., 2000)
1996	Human intoxications have been reported in Ireland around the Arranmore Island region on Donegal, Northwest Ireland.	(Mc Mahon and Silke, 1998)
2000	In August, a number of incidents of food poisoning occurred in Sheffield, Warrington, Alyesbury and the Isle of Wight after the consumption of processed mussels originating from the SW coast of Ireland. These mussels were deemed safe-for-human consumption following negative mouse bioassays. Later identified that AZA was the causative toxin.	(Furey et al., 2010)

ASP

ASP was first detected in the UK in Scotland (Shetland) in 1997, when traces of DA were detected (EU-NRL, 1998). Since then, there have been several ASP toxin outbreaks throughout the UK, causing temporary fisheries closures. There have been two very large outbreaks; in July 1999, when a scallop fishing area of 8,000 square miles was closed in the north west of Scotland following the discovery of ASP toxin over the regulatory limit. In 2002 ASP toxin was detected at offshore scallop grounds throughout the year, leading to fisheries closures (Table 1.9).

During 1999-2000 and 2000-2001, tests for ASP toxin returned a positive result in 6.1% of samples affecting 21 areas, and 8.8% affecting 32 areas respectively (Table 1.4). Since then, the percentage of positive results has remained between 0.2 - 2.6 %. However, *Pseudo-nitzschia* has showed a dramatic increase in abundance since 2005, with an average of 42.9% of water samples returning a positive result (Table 1.5, though note that not all species are toxic). During 2006-2007, and 2008-2009, 3.6%, and 1.9% samples respectively were greater than the regulatory limit (>150,000 cells/Litre) (Table 1.5).

Despite the increase in *Pseudo-nitzschia* in recent years, which has been shown in both the CEFAS water samples and the Continuous Plankton Recorder (CPR) survey, the level of toxin is not representative. This suggests that toxin production might not be directly determined by the abundance of the species alone. Water samples showed ASP toxin was at its highest when the percentage of *Pseudo-nitzschia* was at its lowest (Table 1.4 and Table 1.5), implying that stressful conditions could cause a greater increase in toxin presence.

Table 1.9. The history and consequences of toxic events of diatom ASP toxin seafood poisoning within the UK from 1999-2003.

Year	Event	Reference
1999	In July, a scallop fishing area of 8,000 square miles was closed in the north west of Scotland following the discovery of ASP toxins over the regulatory limit. Species responsible *Pseudo-nitzschia australis.*	(Bates, 2000; Campbell et al., 2001)
1999	A TPO was taken in Poole Harbour on the 6 March. One mussel sample returned over 20µm of DA.	(Higman and Milligan, 2000)
2000	VCA of four aquaculture sites in Scotland as scallops returned positive results for ASP toxin.	(Howard et al., 2001)
2000-2001	Toxins detected in scallops above the regulatory limit. Restrictions on fishing activities were placed on affected regions in Scotland.	(EU-NRL, 2001)
2002	ASP was detected at Dale Voe, Shetlands in September, in Loch Moidart during July, and Broadford Bay in July. Harvesting restrictions were imposed as necessary.	(Howard et al., 2003)
2002-2003	Offshore scallop grounds in Scotland, continually detected ASP throughout 2002 and early 2003. Fisheries closures were implemented in affected shellfisheries.	(Howard et al., 2003)

Routine clinical records (NHS) survey, 1998-2009

The reporting of shellfish poisoning incidents to date has been sporadic. To attempt a systematic review, we accessed hospital records in Wales using the Secure Anonymised Information Linkage (SAIL) databank (Lyons et al., 2009). The important step here is the anonymous linkage of hospital events to a very wide set of health information to the individual, including demographics, mortality statistics, GP records and laboratory tests. This potentially allows a very detailed assessment of the hospital event, and confirmation of its cause and longer term effects. In brief, a split-file approach is used to ensure anonymisation. The datasets are prepared by Health Solutions Wales and separated into clinical and demographic data. An anonymous system linking field (ALF) is assigned to ensure that the data can be re-connected later at the analysis stage (Ford et al., 2009). Clinical data include information on diagnostic tests, therapeutic tests and interventions. Demographic data are comprised of person based variables, such as gender and age.

First, all hospital episodes that had any mention of "Toxic effects of noxious substances eaten as seafood (ICD10 code T61)" for all Welsh NHS Trusts during April 1998 – 31st August 2009 were identified. From 1998-2009, there were 61 hospital episodes within Wales, with 5 of those being re-admission following an initial visit. Out of the 56 individual hospital patients, 51 were successfully allocated an ALF, which enables the anonymous linkage of person level data within and across all national datasets. From the 51 ALF admissions, 7 detailed pathology reports, and 6 mortality records from 2003-2009, were identified.

GP clinical information recorded within +/- 30 days of the indexed hospital episode was identified for 10 of the patients.

Interpretation of routine clinical records

Within Wales, 56 individual patients were identified with "Toxic effects of noxious substances eaten as seafood" from 1998-2009, with an age range of 5-94 years. The length of stay in hospital varied between 1 and 11 days, with an average stay of 2.5 days (significantly related to age). The majority of incidents occurred during the summer months (June-August). Six patients were noted to have died, with a delay of between 7 months and 9 years after their incident, suggesting that there have been no deaths in Wales directly resulting from shellfish poisoning over the survey period. The population of Wales is approximately 2.9 million, hence assuming these patterns are representative, these results imply an estimated incidence of shellfish poisoning of 100 cases per year in the UK (or 16 per million per year).

These estimates, however, must be treated with caution. The clinical presentation may be non-specific, since viral infections, particularly norovirus, or allergy, can cause similar gastrointestinal symptoms to those of shellfish toxin poisonings. A definitive diagnosis is only possible where samples of suspect food are available and tested for toxin, which happens rarely as the food has usually been consumed or discarded before a formal investigation can begin, unless part of a wider epidemiological investigation. Timing of symptom onset is the most helpful factor in distinguishing toxin-induced (very rapid onset) from viral (several hours) causation. Our dataset does not contain this information, although negative results were noted for all bacteriology/virology tests, where they had been performed. Two patients were also tested for the antibody Immunoglobulin E (IgE) to Chub Mackerel, where low traces were detected, potentially indicating that the patients suffered from an allergic reaction to Chub Mackerel. It has been shown that Chub Mackerel are lethal vectors for PSP toxins (STX, and gonyautoxins) all year around (Castonguay et al., 1997), suggesting a possible toxin involvement in these two cases.

Therefore, in many cases, it is difficult to be sure whether a case coded as 'toxin poisoning' was not in fact viral gastroenteritis or allergy - where a medical diagnosis of "food poisoning following shellfish" has been made this may or may not be inputted as toxin-related by the final coder. Furthermore, mild cases are likely to go unreported.

We conclude that although the incidence of shellfish poisoning is very low, the current data are insufficient to allow an accurate estimate. This raises the important question of how changes in the incidence rate, that might be associated with the distribution of toxic microalgae, would be detected? At the human level, although it is a notifiable event, reporting is not likely to be accurate enough to quickly identify trends over time. In contrast, routine testing at the fisheries level is much more likely to be able to identify changes in the rate of toxic events and remains the key surveillance system in the UK, acting as a sentinel for potential human impact.

The future of key indicator toxic microalgae species in UK waters

Recent studies suggest that some toxic microalgae species are increasing in frequency and geographical location on a global scale (Moore et al., 2008). It is thought that human assistance has spread some species through a variety of mechanisms e.g. ballast water transfer, increase in eutrophication, and aquaculture development (Heil et al., 2005; Van Dolah, 2000). However, the establishment and reoccurrence of blooms cannot occur without a hospitable environment (Omori et al., 1994). There have been several suggestions that an alternative or additional explanations for the spreading of some species are as a result of the effects of changing currents, weather patterns, and changing ocean temperatures associated with global warming (Fleming et al., 2006; Kirkpatrick et al., 2004), allowing species to occupy regions in which they would not normally survive.

There is increasing evidence in the literature of the effects of climate change on the phenology of marine organisms. Estimates show that British winters now end 11 days earlier on average than in the mid 1970s (Thackeray et al., 2008). These changes in phenology have caused dramatic shifts in the timing and occurrence of species during the year with a pole-ward shift in latitudinal distribution range (Edwards and Richardson, 2004), in response to the changing environmental conditions. The UK could gain species from an equatorial direction, and lose existing species as conditions become too warm (Edwards and Richardson, 2004). In the North Sea, total species abundance has remained relatively stable but the species composition has changed (Beaugrand, 2004). Warm-water species have increased, while colder-water species have decreased, e.g. euphausiid crustaceans (Kempf et al., 2006) owing to the warming sea temperatures and changes in climate indices such as the North Atlantic Oscillation Index (Heath, 2005). From the CEFAS water sample results; we have already noticed a change in species abundance with *Pseudo-nitzschia* now being present in 37-51% of samples during 2005-2009 (Table 1.5). This increase in *Pseudo-nitzschia* has also been observed (personal observation), using the CPR survey. There has been an expansion of the geographic range and a lengthened seasonal window of *Pseudo-nitzschia seriata* in the NE Atlantic and North Sea during 2000-2009. We note, however, that toxicity of the species cannot be inferred from these data.

Although there have been few human related illnesses from ingestion of toxins from shellfish in the UK, there remains a threat that this risk will increase over time. The potential future change in species composition due to climate change could bring new toxic species into the surrounding UK waters. Unless these potential toxic marine species are monitored in their distribution and frequency, these new species could go unnoticed by the current monitoring system. If that is the case there could be several more human related incidents within the UK, whether it is medical related or indirect, via the closure of aquaculture sites. In contrast, in the USA, estimates of the economic impact of HABs are averaged at $75 million/year over the period 1987-2000, which includes impacts from public health, commercial fishing, recreation and tourism, and monitoring and management costs (Hoagland and Scatasta, 2006). PSP is the most severe of the toxin syndromes, with a total of 500 cases and 30 deaths reported in California, since 1927 (a mortality rate ranging between 1-12%, (Trainer, 2002)), caused in part by poor access to advanced life support capabilities (Trainer, 2002).

While the geographic distributions of species are clearly important, we also note that the abundance of a species does not necessarily correlate strongly to the amount of toxin produced. There have been several CEFAS reports, where there was high toxin content detected, but water samples showed a low species abundance (Higman and Milligan, 2000; Stubbs et al., 2005). This was especially noticeable for the toxin causing ASP, where the highest levels of toxin detected was when *Pseudo-nitzschia* was at its lowest abundance. The mechanisms behind toxin production are not fully understood, and it is thought that stress could be an initial trigger. If that is the case, toxin production within the UK could potentially increase as the current UK HAB species have to adapt to the changing hydrological conditions.

Conclusions

We have shown that within UK waters, outbreaks of shellfish poisoning are occurring on a regular basis in different regions and affect a diverse range of molluscan shellfish and other marine organisms. Every year several aquaculture sites and shellfisheries are closed, due to shellfish toxins, with closures ranging from weeks to several months. The toxin syndrome DSP has been shown to be the most problematic toxin within the UK, with 19 records during the period 1999-2009, which resulted in several shellfish harvesting closures. However, despite the regular occurrence of shellfish poisonings, data on human epidemiology of poisoning linked to toxic marine microalgae species is sparse. An extensive literature review of shellfish poisoning has shown that only disparate records are available for incidents and episodes within the UK, with only the large outbreaks related to shellfish and aquaculture industries being recorded. However, the accuracy of the UK data must be treated with caution. Despite obtaining data for hospital admissions, GP records, and pathology records, it remains extremely difficult to determine the precise number of patients who have been affected by toxic shellfish poisoning.

In the absence of a suitable epidemiological surveillance system, the routine fisheries testing regimes remain the key indicator of any potential change in exposure of the human population. From this routine testing we have already seen an increase in abundance of *Pseudo-nitzschia* in the last 5 years with the species now being present in over 50% of the water samples. In addition, predicted warmer waters in the UK could suit several species with southern biogeographical affinities enabling them to occupy the coastal regions of the UK, but which are not yet considered to be detrimental. To ensure the UK monitoring systems are up to date with the increasing number of new toxic species and the changing seasonal and geographic distribution patterns, research into the environmental conditions that lead to bloom development and toxin production would help predict bloom events. Long-term time series of toxic marine microalgae need to be monitored to assess and determine if species are indeed spreading in their geographical location or increasing in frequency.

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Web resources

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www.food.gov.uk/foodindustry/farmingfood/shellfish/
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www.food.gov.uk/foodindustry/farmingfood/shellfish/algaltoxin
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Chapter II

Changes in marine dinoflagellate and diatom abundance under climate change

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SLH and AWW compiled the data. SLH, MBG and GCH led the data analyses and interpretation with contributions from all authors. SLH produced all the figures. SLH, GCH and MBG wrote the paper with contributions from all authors.

Abstract

Marine diatoms and dinoflagellates play a variety of key ecosystem roles as important primary producers (diatoms and some dinoflagellates) and grazers (some dinoflagellates). Additionally some are Harmful Algal Bloom (HAB) species and there is widespread concern that HAB species may be increasing accompanied by major negative socioeconomic impacts, including threats to human health and marine harvesting (Anderson, 1989; Hallegraeff, 1993). Using 92,263 samples from the Continuous Plankton Recorder (CPR) survey we generated a 50 year (1960-2009) time series of diatom and dinoflagellate occurrence in the NE Atlantic and North Sea. Dinoflagellates, including both HAB taxa (e.g. *Prorocentrum spp.*) and non-HAB taxa (e.g. *Ceratium furca*), have declined in abundance, particularly since 2006. In contrast, diatom abundance has not shown this decline with some common diatoms, including both HAB (e.g. *Pseudo-nitzschia spp.*) and non-HAB (e.g. *Thalassiosira spp.*) taxa, increasing in abundance. Overall these changes have led to a dramatic increase in the relative abundance of diatoms versus dinoflagellates. Our analyses, including Granger tests to identify criteria of causality, suggest that this switch is driven by an interaction effect of both increasing sea surface temperatures combined with increasingly windy conditions in summer.

Changing hydrological conditions, associated with regional climate warming, are thought to underlie changes in the abundance and distribution of diatoms and dinoflagellates (Reid et al., 1998) and be one of the possible mechanisms for the increase in the geographical location and frequency of Harmful Algal Bloom (HAB) species (Hallegraeff, 2010; Moore et al., 2009). Other factors implicated in HAB species increases include ballast water transport, aquaculture development, transport of seed stock, and eutrophication of coastal waters due to human activities (Anderson, 1989; Hallegraeff, 1993). Here we use data from the Continuous Plankton Recorder (CPR) survey (1241-2253 per year since 1960-present), which is the longest multi-decadal plankton monitoring programme in the world (Richardson et al., 2006), to undertake the most comprehensive analysis to date of extended spatio-temporal patterns of abundance for dinoflagellates and diatoms, including key HAB taxa, in the NE Atlantic region. The CPR does not sample very close (<10km) to the coast and hence will not reveal locally specific patterns of plankton change (e.g. caused by local eutrophication, local changes in circulation due to coast development), but rather provides a view of broader-scale regional changes. As such the CPR data reveals changes that are often caused by climate impacts operating over large areas (Richardson et al., 2006). Integrated abundance of twelve diatom taxa and nine dinoflagellate taxa were examined in the NE Atlantic region (45-60^{o}N; 15^{o}W-10^{o}E) (see Methods for taxa selection and data analysis details). Included within these analyses was the spatio-temporal variability of the diatom *Pseudo-nitzschia seriata spp.* an important HAB taxa and several abundant non-HAB taxa including the diatom *Thalassiosira spp.* and the dinoflagellate *Protoperidinium spp.* In addition to these 21 diatom and dinoflagellate taxa, for some other taxa for which there were shorter time series we also examined trends in abundance. These shorter time series were produced because the taxa were not identified in the earlier years of the CPR survey. These taxa were selected because they have been shown to be HAB species: the dinoflagellates *Prorocentrum spp.* (identified since 1981) and *Dinophysis spp.* (a mixotrophic species identified since 1971). *Noctiluca scintillans* was selected as an example of a heterotrophic species (identified since 1997) which is not typically considered a HAB species, but may act as a vector for toxigenic phytoplankton (Escalera et al., 2007) and have other harmful effects (Okaichi and Nishio, 1976). In these ways we addressed the questions of how diatom and dinoflagellate abundance has changed over the last 50 years and whether HAB species have been increasing.

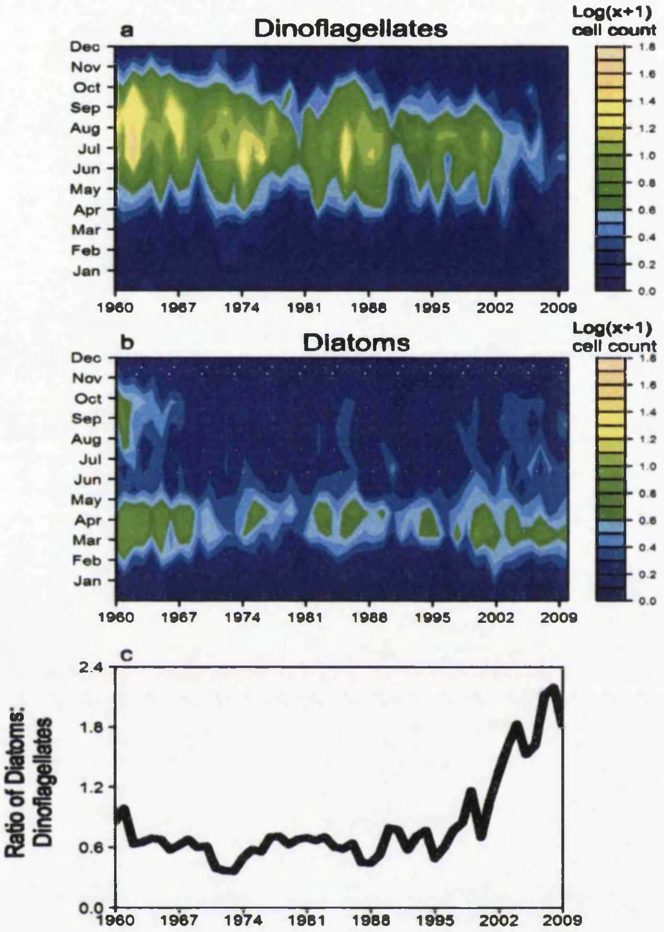

Figure 2.1. Abundance of dinoflagellates and diatoms in the northeast Atlantic region from 1960 to 2009 based on twelve diatom taxa and nine dinoflagellate taxa routinely identified in the CPR samples. Monthly mean cell numbers per sample of (a) dinoflagellates and (b) diatoms. (c) The relative abundance of diatoms/dinoflagellates, highlighting the shift from dinoflagellates to diatoms during the past 50 years.

To examine long-term changes in distribution, we spatially interpolated data for each of the six species every 5 years by ordinary kriging, using R (R, 2009) and packages “sp” (Pebesma and Bivand, 2005) and “gstat” (Pebesma, 2004). Our results reveal a dramatic change in the relative abundance of diatoms versus dinoflagellates (Figure 2.1). For example, in the 1960s-1980s dinoflagellates were relatively more abundant than diatoms in the CPR samples, but this pattern has been reversed in the last decade. These broad taxonomic changes are also reflected within individual taxa albeit with some level of variability in patterns across taxa. As such our results challenge the view that HAB species have increased in frequency and intensity and have become more widespread in recent decades (Fraga and Bakun, 1993; Hallegraeff, 1993) and instead point to a broader switch from dinoflagellate to diatom dominance. For example, previously abundant dinoflagellates such as *Ceratium furca* and *Protoperidinium spp.* are now increasingly rare compared to previous levels (Figure 2.2 and 2.3) and similarly the HAB dinoflagellate *Prorocentrum spp.* has been very rare since 2007 (Figure 2.2 and Figure 2.3). Conversely both non-HAB (e.g. *Thalassiosira spp.*) and HAB diatoms (e.g. *P. seriata*) have become more abundant and widespread in the last decade (Figure 2.2 and 2.3). In some cases this increase in diatom abundance extends back through the 50 year times series (e.g. *Thalassiosira spp.*) while for others the current increase spans only two or three decades increase (e.g. *Rhizosolenia imbricata shrubsolei* and *P. seriata*) (Figure 2.3). In addition, some diatoms (such as *P. seriata*) also now show extended high abundance throughout the summer in between the spring and autumn peaks (Figure 2.2e). This increase in *Pseudo-nitzschia* is already having a profound effect within UK waters. Since 1999, there have been frequent closures of shellfish harvesting sites around the Scottish west coast and islands due to *Pseudo-nitzschia* (Fehling et al., 2006; Fehling et al., 2004). Our results support those from experimentation with *P. seriata* in Scottish waters, where enhanced growth rates and toxin production occur when temperatures reach 15°C and there is a long photoperiod (Fehling et al., 2005; Fehling et al., 2004). A caveat of our study is that changes in coastal ecosystems might be taking place in ways that are not observed by the CPR sampling (i.e. areas within 10 km of land).

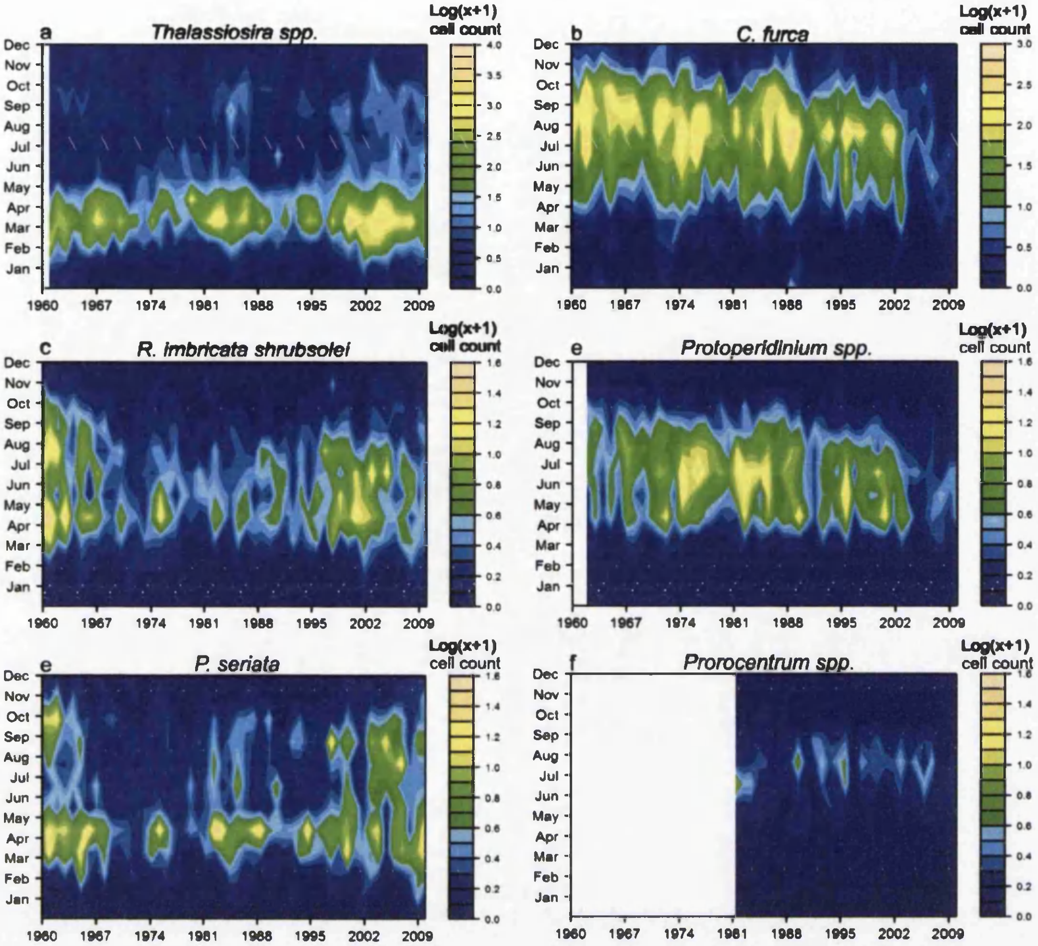

Figure 2.2. Abundance of selected taxa in the northeast Atlantic region to illustrate the shift from dinoflagellates to diatoms during the past 50 years. The panels are ordered to show diatoms (left panel) and dinoflagellates (right panel). *P. seriata* and *Prorocentrum* spp. are HAB taxa (shown in bottom panels). Colour scale represents monthly mean cell numbers per sample (log (x+1)).

The current warming of the NE Atlantic region is favouring warmer-water species at the expense of cold-water species, with consequent range extensions and contractions respectively (Beaugrand et al., 2002). We investigated the links with climate by analysing Sea Surface Temperature (SST) (data supplied by Hadley Centre of the UK Met Office), and summer surface scalar wind speed (SSSWS) (data supplied by The International Comprehensive Ocean Atmospheric Data Set) in the NE Atlantic from 1960-2009. We employed a range of statistical methods to address issues of potential spurious correlation (correcting for non-stationarity by de-trending and correcting for serial autocorrelation) and to highlight potential causal and modifying mechanisms (examining Granger causality criteria and regression interaction terms).

We examined the observed yearly average abundances for all the abundant diatom taxa (n=12) and dinoflagellate taxa (n=9) for which long time series existed (see Methods) as well as integrated diatom and dinoflagellate abundance. In addition we considered *Dinophysis spp.* as an example of a mixotrophic HAB dinoflagellate and *N. scintillans* as an example of a heterotrophic dinoflagellate even though shorter time series existed for these taxa. After adjusting for serial autocorrelation using the modified Chelton method (Pyper and Peterman, 1998), we found significant correlations between mean annual abundance for many taxa against either SST or SSSWS (or both) (Table 2.1). For the dinoflagellate taxa, abundance tended to decrease with increasing temperature and/or wind. This effect was seen for 10 of the 12 individual dinoflagellate taxa, with the reverse pattern tending to be found for diatoms. In addition the integrated dinoflagellate abundance showed a significant negative correlation with both SST and SSSWS (Table 2.1 and Figure S2.1). The pattern of long-term change was therefore more consistent across dinoflagellates, which have largely showed a long-term decline, and more variable across diatoms, many of which have shown a long-term increase although this pattern is not present across all taxa (Figure S2.2 and Figure S2.3).

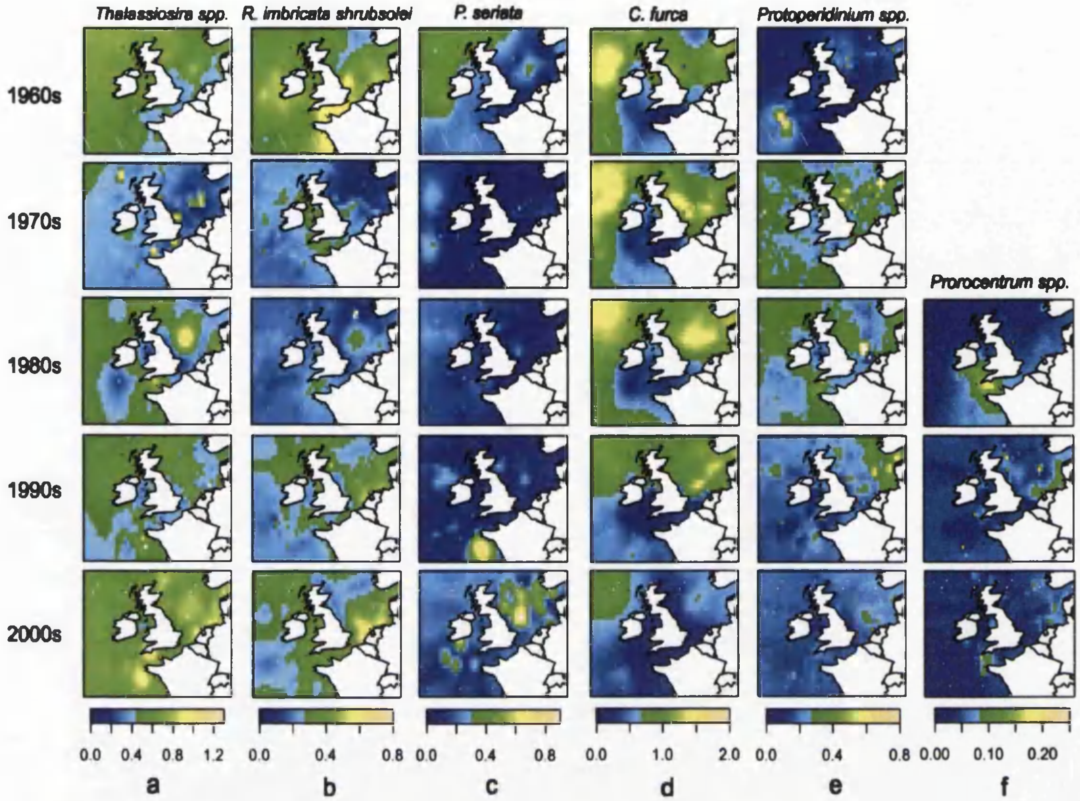

Figure 2.3. Decadal spatio-temporal changes in abundances of selected dinoflagellates and diatoms in the northeast Atlantic. (a–c) Diatoms (*Thalassiosira spp.*, *R. imbricata shrubsolei* and *P. seriata*). (d–f) Dinoflagellates (*C. furca*, *Protoperidinium spp.* and *Prorocentrum* spp.). Colour scale represents decadal mean cell numbers per sample (log (x+1)).

Potential causal pathways in a time series can be further investigated with a Granger test (Thurman and Fisher, 1988), where past values of a putative causal environmental variable (SST or SSSWS) are used in a regression model to predict future values of plankton abundance, after adjusting for past values of plankton abundance (and de-trending for stationarity). This approach therefore represents a measure of forecasting over and above that provided simply by past temporal correlations, and helps point towards a causal link (Thurman and Fisher, 1988). We found evidence for significant Granger-causality in the relationship between either SST or SSSWS for five of the dinoflagellate taxa (*Protoperidinium spp.*, *C. furca*, *C. tripos*, *Dinophysis spp.* and *N. scintillans*), and for five of the diatom taxa (*Thalassiothrix longissima, Skeletonema costatum*, *Pseudo-nitzschia delicatissima*, *R. hebetata semispina and P. seriata*) as well as the combined “diatom” group (at

10% level), with the effect of SST and SSSWS appearing over a lag of 1-3 years (Table S2.1).

Table 2.1. The significance of modified Chelton correlation for diatoms and dinoflagellates against Sea Surface Temperature (SST) and Surface Summer Scalar Wind (* p<0.05). The sign of the affect of SST or SSSWS on abundance is indicated (+ or -).

	Modified Chelton p-value for correlation coefficient			
	SST		**Surface Summer Scalar Wind**	
Integrated Diatoms	0.12		0.40	
P. seriata	0.008*	+	0.02*	+
Thalassiosira spp.	0.005*	+	0.02*	+
R. imbricata shrubsolei	0.009*	+	0.50	
T. longissima	0.56		0.005*	-
T. nitzschioides	0.07		0.06	
Eucampia zodiacus	0.12		0.63	
Ditylum brightwellii	0.005*	+	0.006*	+
Skeletonema costatum	0.20		0.33	
P. delicatissima	0.39		0.50	
R. styliformis	0.001*	-	0.42	
R. hebetata semispina	0.03*	-	0.02*	-
R. alata indica	0.19		0.02*	-
Integrated Dinoflagellates	0.001*	-	0.001*	-
Prorocentrum spp.	0.81		0.03*	-
Protoperidinium spp.	0.006*	-	0.007*	-
C. furca	0.002*	-	0.002*	-
C. fusus	0.0004*	-	0.004*	-
C. lineatum	0.0002*	-	0.005*	-
C. tripos	0.001*	-	0.01*	-
C. macroceros	0.11		0.003*	-
C. longipes	0.005*	-	0.0005*	-
C. minutum	0.02*	+	0.48	
C. hexacanthum	0.06		0.002*	+
Dinophysis spp.	0.02*	-	0.01*	-
N. scintillans	0.87		0.008*	-

Lastly, we investigated whether there was any interaction between the two climate variables in a regression model predicting the abundance of our diatom and dinoflagellate taxa, that would indicate a more complex relationship between abundance and climate change, and shed any light on the possible mechanisms involved. We focused on the North Sea (51-60°N; 3°W-10°E), which has shown the largest regional change in plankton abundance. Highly significant interaction terms, between SST and SSSWS, were found in the linear regression models for several taxa. For diatoms the significant interaction terms were all showing a synergistic positive effect on abundance (*P. seriata*, p = 0.003; *Ditylum brightwelli*, p = 0.0002, *P. delicatissima*, p = 0.0004; *S. costatum*, p = 0.0002; integrated diatom abundance, p = 0.01). For dinoflagellates the significant interaction terms were negatively correlated with abundance (*C. furca*, p=0.02; *C. macroceros*, p=0.02; with 2 species significant at the 10% level). This means that the effect of each climate variable cannot be considered in isolation. For example, the diatom, *P. seriata* shows high abundance only when both SST and SSSWS are at a high level (Figure S2.4). When either SST or SSSWS levels are low, *P. seriata* occurs at low abundance. In clear contrast, the opposite interaction effect was evident in the dinoflagellate taxa where low abundance occurred at high SST, but only when accompanied by high SSSWS conditions. Such interactions could explain why dinoflagellates have become relatively less abundant compared to diatoms.

Since 1960, there has been both a 1°C rise in SST and a significant increase in SSSWS in the NE Atlantic region (Figure S2.1). This increase in wind introduces surface kinetic energy, which will stimulate vertical mixing leading to increased turbulence (Mann and Lazierm, 1996). As turbulent environments are associated with diatoms (Thurman and Fisher, 1988) and calm conditions with dinoflagellates (Margalef, 1978), the recent switch from dinoflagellate to diatom dominance with increasing SSSWS is perhaps not surprising. What is less clear cut are the mechanisms behind this relationship. For those species that are either entirely or mainly dependent on photosynthesis to obtain energy, diatoms are generally considered superior competitors to dinoflagellates (Ross and Sharples, 2007). Diatoms have numerous physiological advantages over dinoflagellates, including higher growth rates, higher photosynthetic rates and an improved ability to compete for nitrogen at low concentrations (Ross and Sharples, 2007). As a consequence, providing there is sufficient turbulence to enable diatoms to remain suspended within the surface mixed layer, diatoms typically out compete dinoflagellates. Interestingly, we found that the recent increased windiness has caused a decline in abundance of all dinoflagellates regardless of trophic status (i.e. phototrophic, mixotrophic and heterotrophic species), even including heterotrophic species which predominantly feed on diatoms (e.g. *Protoperidinium*), suggesting additional mechanisms at play. Both field and laboratory studies indicate that dinoflagellates are especially sensitive to small-scale turbulence (Berdalet et al., 2007). Although species specific differences exist, in general, small-scale turbulence appears to impact negatively on dinoflagellate cell-division and life-cycle processes (Berdalet et al., 2007). The ability of some common, cosmopolitan dinoflagellate species to bloom in turbulent upwelling regions provides an exception to this rule (Smayda, 2010) and indicates that further research is required in order to explain large scale shifts in plankton composition presented within this study. The interactive effect of temperature is presumably linked to the general impact of higher temperatures leading to increased vital rates such as cell division and photosynthesis.

Our study shows a fundamental shift in the relative abundance of diatoms versus dinoflagellates in the NE Atlantic and North Sea, with a dramatic decline in dinoflagellates in recent years. Using a range of statistical treatments we have shown that both sea temperature and summer windiness are important climate drivers of the biological changes and we highlight the key synergistic effects of these climate variables. Given the many potential changes in climate variables, it is unlikely that simple linear relationships will exist with species abundance. Synergistic effects, such as those described here, must be considered to help develop our understanding of the mechanistic processes underlying biological changes in the oceans.

Acknowledgments

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Methods

Integrated indices of diatoms and dinoflagellates abundance

To create integrated indices of "diatom" and "dinoflagellate" abundance, from the CPR database twelve diatom taxa (*Thalassiothrix longissima*, *Thalassionema nitzschioides*, *Eucampia zodiacus*, *Ditylum brightwellii*, *Skeletonema costatum*, *Pseudo-nitzschia delicatissima*, *Pseudo-nitzschia seriata*, *Thalassiosira spp*., *Rhizosolenia imbricata shrubsolei*, *Rhizosolenia styliformis*, *Rhizosolenia hebetata semispina,* and *Rhizosolenia alata indica*) and nine dinoflagellate taxa (*Ceratium furca*, *C. fusus*, *C. lineatum*, *C. tripos*, *C. macroceros*, *C. longipes*, *C. hexacanthum*, *C. minutum*, and *Protoperidinium spp*.) were selected based on three criteria. Firstly, only taxa abundant enough to reach a threshold maximum abundance value were included so that rarer taxa were excluded. Secondly, species needed to be present on > 4% of all samples. Thirdly, we only included taxa which were first identified between 1960-1963 and were persistently present until 2009, i.e. we only included taxa for which there was a long time series.

Pseudo-nitzschia seriata (complex)

Identification of *P. seriata* complex is achieved using the method of Tomas, (1997), splitting *P. seriata* complex and *P. delicatissima* complex, based on their cell transapical axis. However, accurate identification of *P. seriata* complex at the species level is not possible in routine CPR analysis and requires further investigation (Richardson et al., 2006). In this paper we refer to the complex as "*P. seriata*".

Large scale hydro climatic parameters

Monthly mean gridded (1°x1°) Sea Surface Temperature (SST), from 1960-2009 were taken from Hadley Centre of the UK Met Office (HadISST). Annual SST means were calculated for the NE Atlantic region (45-60°N; 15°W-10°E), and a sub-set of this region corresponding to the North Sea (51-60°N; 3°W-10°E) for the months May, June, July and August.

Monthly mean Surface Scalar Wind Speed (SSWS) (m/s), was obtained from The International Comprehensive Ocean-Atmosphere Data Set (ICOADS) for 1960-2009 (http://www.ncdc.noaa.gov/oa/climate/coads/). Data are summarized for a given latitude and longitude. Yearly scalar wind was averaged over the months May, June, July and August for the NE Atlantic region (45-60°N; 15°W-10°E) and a sub-set of this region corresponding to the North Sea (51-60°N; 3°W-10°E).

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Chapter II

Supplementary Material

Changes in marine dinoflagellate and diatom abundance under climate change

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Supplementary methods

Long-term spatial changes

The CPR data was firstly separated into decadal datasets (1960s, 1970s, 1980s, 1990s, and 2000s) and then into monthly datasets for each decade. A spatial grid resolution of 0.5° longitude by 0.5° latitude was used, and interpolated values for the grid obtained using ordinary kriging. Spatial dependence was based on an estimated variogram, from the best fit from 5 types of model available in the fit.variogram function from the gstat package (Pebesma, 2004). Ordinary kriging estimates the spatially varying local means as opposed to the global constant means, implying only data closest to the location have a strong influence on the estimation (Saito et al., 2005). One of the assumptions of kriging is that spatial structures are stable in time, at least for the duration of the sampling period (Simard et al., 1992). However, this is clearly not the case for the CPR data, with a sampling period of 50 years. We address this issue by partitioning the datasets into shorter temporal periods, and treating each temporally partitioned dataset individually. The data was initially sectioned into 10 shorter time periods of generally 5 years (1960-1964, 1965-1969, 1970-1974, 1975-1979, 1980-1984, 1985-1989, 1990-1994, 1995-1999, 2000-2004, 2005-2009). Next, 12 monthly averages maps were created for each of the 5 years intervals using the kriging method described above. To produce decadal maps, the corresponding months for each two 5 year intervals e.g. January 1960-1964 and 1965-1969, were averaged at each grid node to produce decadal monthly composite maps. These 12 monthly composite maps for each decade were then averaged at each grid node to produce decadal composite maps (Figure S2.5). The justification for this approach is described in detail in Edwards, (2000). Briefly, for each kriging map we aimed to increase the accuracy of estimating a spatial signal in the data (by including a large number of observations; average 775, range 426 to 1416) whilst reducing the temporal influence. This was achieved firstly by avoiding averaging over different months (where most of the ecological temporal variation is found) and secondly by averaging over a relatively short time period. The 5-year window was chosen as the period over which temporal autocorrelations could potentially be detected, and this approach has been tested extensively over the scale of the North East Atlantic. All CPR abundance values were log (x+1) transformed prior to analysis.

Relationship between abundance and environmental variables

The average yearly abundance over a given geographical area (NE Atlantic or North Sea) was compared to the corresponding (average) changes in the SST and SSSWS in a set of correlation and regression analyses. It is well known in the ecological field that spurious correlations can arise in time series analysis, and that simple correlation can arise from a range of unrelated and non-causal mechanisms. We therefore employ a range of statistical methods that address different sources of spurious correlation. Together, they provide a robust set of tools for describing the link between abundance and environmental variables. It is recognised that when there is a time trend (when the time series are not stationary), temporal associations between two such variables inevitably arise, but are not necessarily causal and may be due to another, unmeasured factor, which also changes over time. We control for temporal trends (non-stationarity) by fitting first or (as appropriate) second order linear regressions and basing our analysis on the residuals (Votier et al., 2008). A separate problem is one of serial autocorrelation (that is often present in ecological time series), which can lead to high type I error rates in correlation analysis (Yule, 1929). We further control for this in our correlation analysis using the 'modified Chelton method' (Pyper and Peterman, 1998). Our next step was to build on the correlation analysis by inspecting the criteria of Granger causality. Since changes in causal factors must precede effects on a putative dependent variable this analysis focuses on lagged time series. Furthermore, there is a stringent criteria for testing the significance of the regression coefficient, which must remain significant after adjusting for past values of the abundance variable. That is we test whether lagged values of SST or SSSWS were significant predictors of plankton abundance, over and above the information already provided simply by past values of plankton abundance. This approach is very well used in econometrics (and neuroscience), and has considerable potential for investigating links between large numbers of climate and ecological variables (Bressler and Seth, 2011; Granger, 1969; Thurman and Fisher, 1988). Finally, we investigated the potential interaction between SST and SSSWS, which is also useful for interpretation of potential causal mechanisms. We fitted linear regressions, with the two environmental covariates as the independent variables and abundance as the dependent variable, and examined the significance of the interaction term with a likelihood ratio test. In this analysis, the data was not de-trended, as we were not focusing on the simple correlation between the environmental variable and abundance but rather whether the relationship between SST and abundance was modified by the level of SSSWS. This term in the model is not, in general, susceptible to the simple influence of parallel time trends, as discussed above. Taxa which were absent for large proportions of the time series (> 90%) were excluded from the interaction analysis. All analyses were performed using R (base package and lmtest (Zeileis and Hothorn, 2002)).

Relative abundance was calculated directly from the yearly average abundance for each taxa, and then creating an overall yearly average for each "diatom" and "dinoflagellate" group. Relative abundance was then calculated by dividing the "diatom" yearly abundance by the "dinoflagellate" abundance.

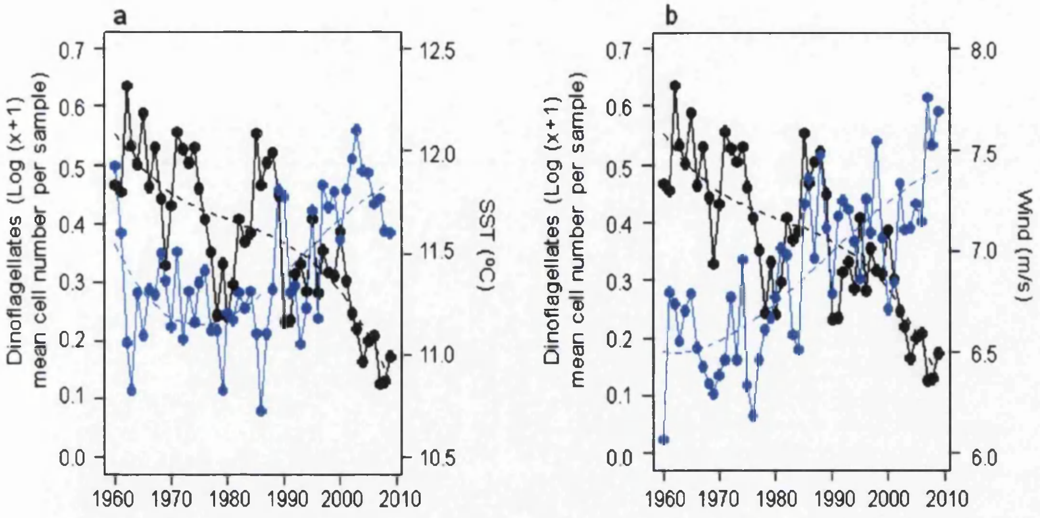

Figure S2.1. Chelton corrected correlation analysis for the integrated abundance of dinoflagellates in the NE Atlantic. (a) sea surface temperature (solid blue line and closed blue circles), and (b) summer surface scalar wind (solid blue line and closed blue circles). Solid black line and solid circles = dinoflagellate annual mean cell numbers per sample (log (*x*+1)). Dashed blue and black lines represent a line of best fit using a 3rd order polynomial curve, to give a indication of the trend.

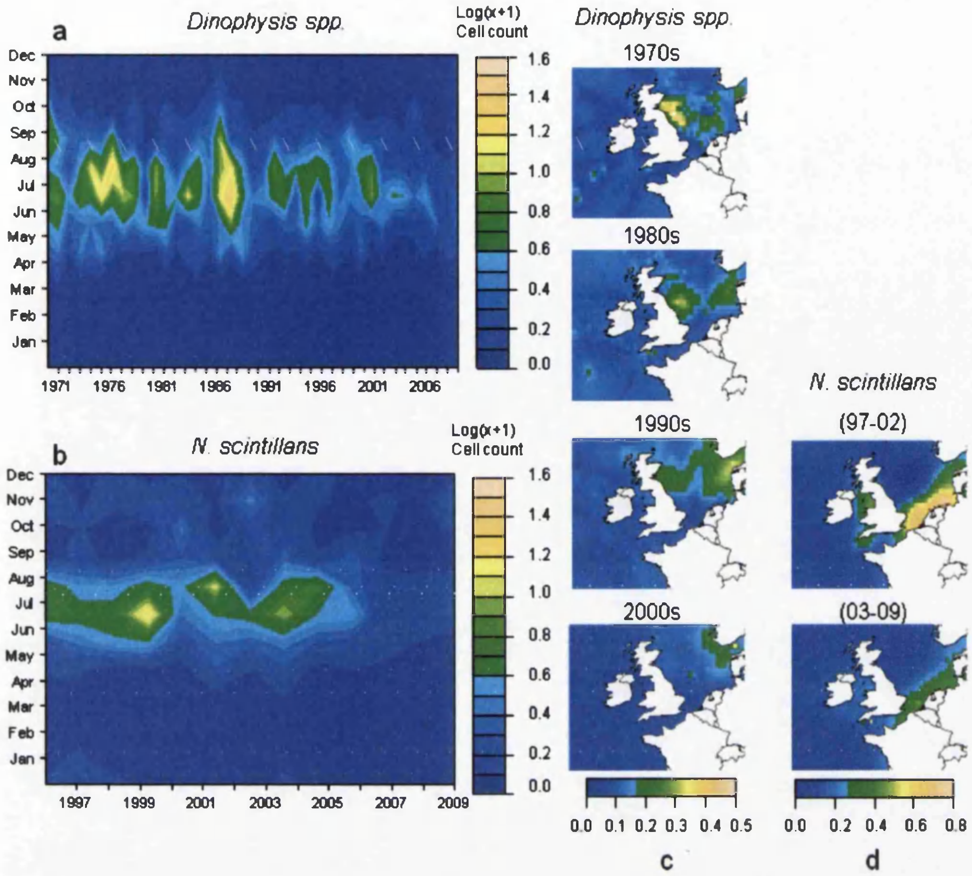

Figure S2.2. Abundance of a heterotrophic dinoflagellate *Noctiluca scintillans* and the mixotrophic HAB dinoflagellate *Dinophysis spp.* Colour scale represents monthly mean cell numbers per sample (log (x+1)). *N. scintillans* was relatively abundant in the late 1990s and early 2000s with a range centred on the southern North Sea, confirming the observations by Edwards et al., (2006) and Miyaguchi et al., (2008). However, this relatively high abundance lasted for only approximately one decade (1997-2006), after which *N. scintillans* has again declined to very low levels. Since 2006, *N. scintillans* has been virtually absent throughout the NE Atlantic and North Sea. *Dinophysis spp.* was relatively abundant in the North Sea in the 1970s, 1980s and 1990s. However in recent years this taxa has declined to very low levels in common with other dinoflagellates.

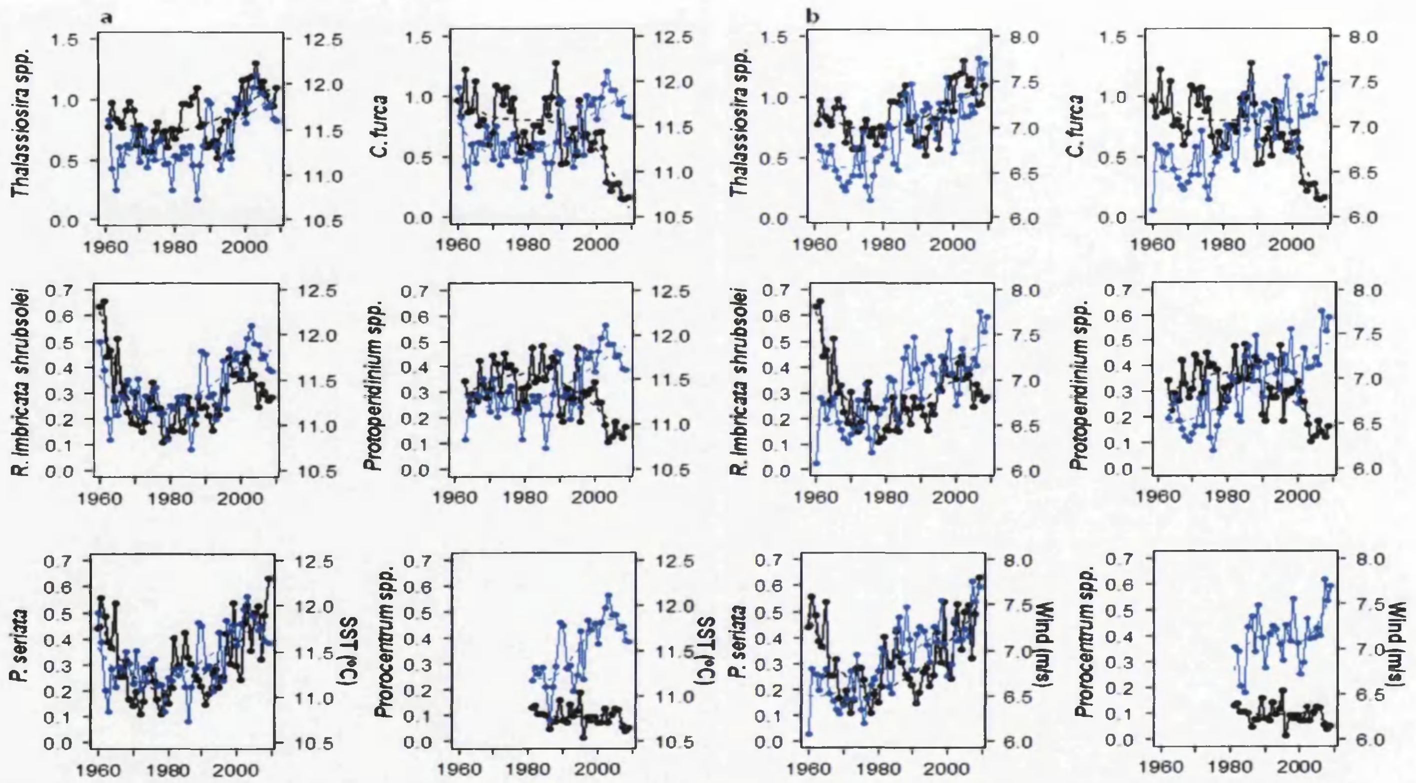

Figure S2.3. Relationships between the abundance of six example taxa (solid black lines with closed black circles) in the NE Atlantic region against (a) sea surface temperature (solid blue line with closed blue circles), and (b) summer surface scalar wind (solid blue line with closed blue circles). Taxa abundance is the annual mean cell numbers per sample (log $(x+1)$). Dashed blue and black lines represent a line of best fit using a 3rd order polynomial curve, to give a indication of the trend. These plots illustrate the general pattern of the relationships seen across groups with dinoflagellates (here represented by *Ceratium furca*, *Protoperidinium spp.*, and *Prorocentrum spp.*) generally being less abundant with high sea temperature and high windiness with the reverse pattern generally seen in diatoms (here represented by *Thalassiosira spp.*, *R. imbricata shrubsolei* and *Pseudo-nitzschia seriata*).

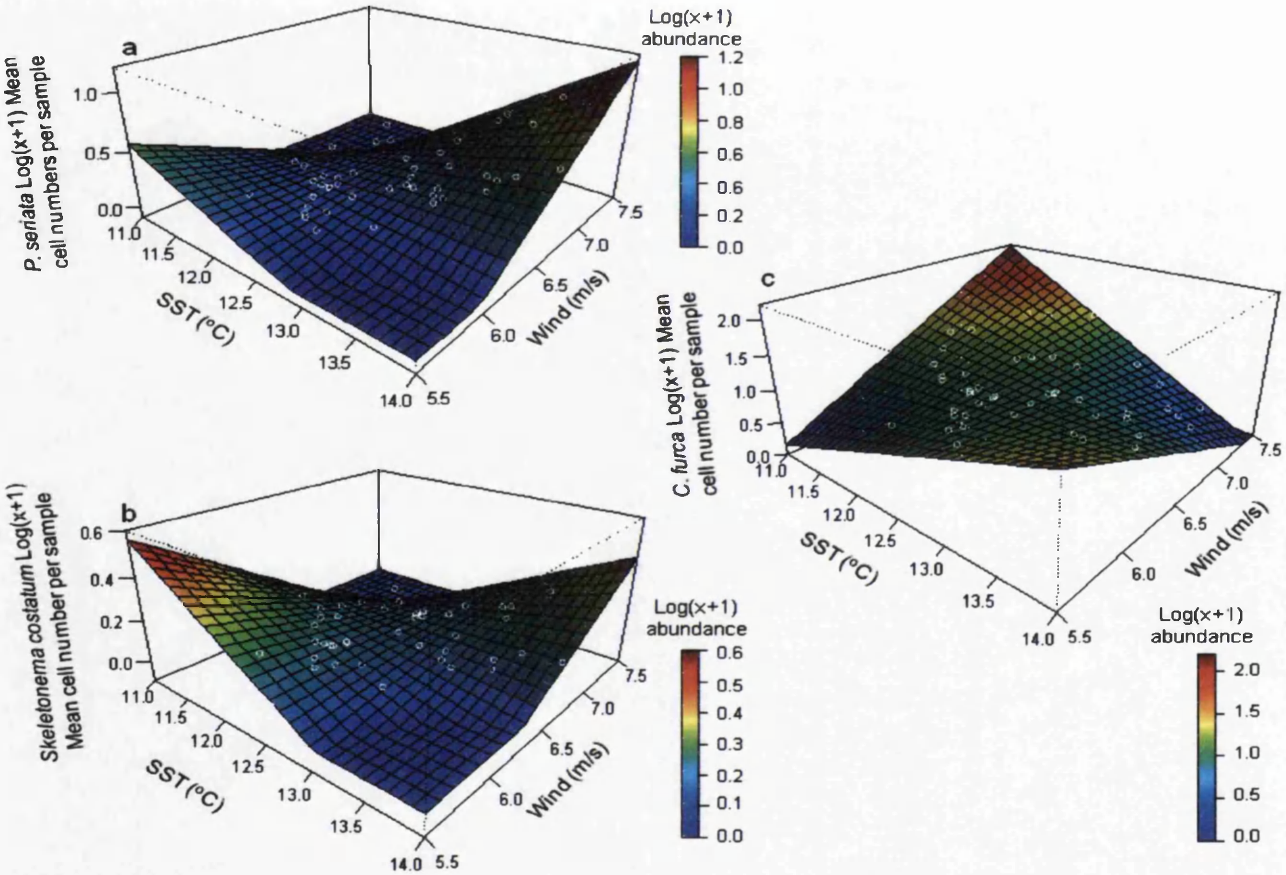

Figure S2.4. Examples of the interaction regression model for (a) *P. seriata* (p = 0.003), (b) *Skeletonema costatum* (p = 0.0002), and (c) *Ceratium furca* (p = 0.02) in the North Sea (51-60°C, 3°W-10°E). The 3D plane is a graphical representation of the interaction model, where the p values represent the significance level of the interaction model for each taxa. Colour scale signifies the abundance of taxa (log (*x*+1)) at SST and wind levels suggested by the interaction model. Open white circles represent annual mean abundance of each taxa (log (*x*+1)).

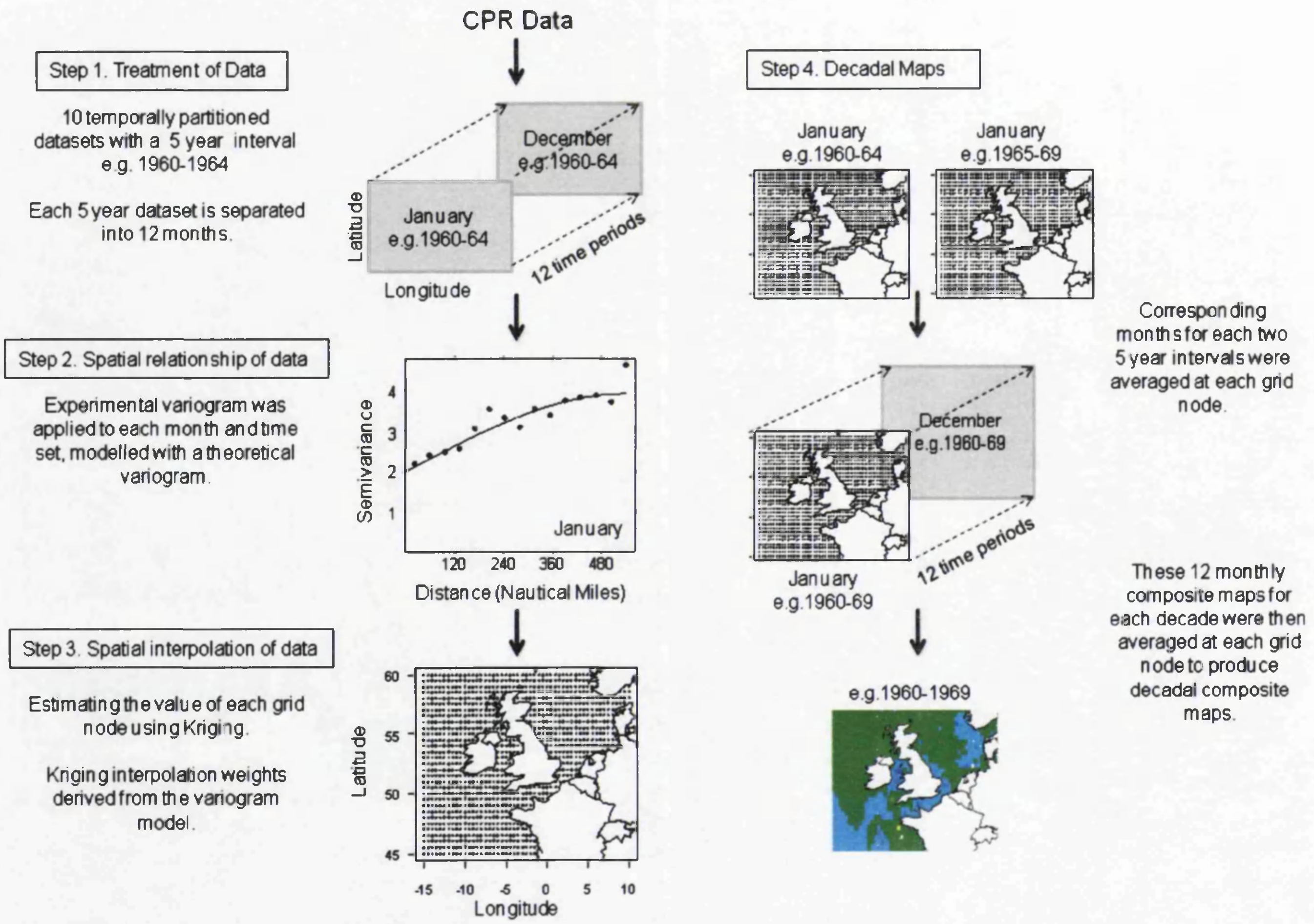

Figure S2.5. Schematic of data organisation and kriging methods. Based on Edwards, (2000).

Table S2.1. Granger-causality in the relationships between diatom and dinoflagellate abundance versus Sea Surface Temperature (SST) and Surface Summer Scalar Wind Speed (SSSWS). Significant relationship ($p<0.05$) indicated by *. Lag time scale represents years.

	Granger-Causality					
	SST			Surface Summer Scalar Wind		
	Lag 1	Lag 2	Lag 3	Lag 1	Lag 2	Lag 3
Integrated Diatoms	0.07	0.58	0.75	0.93	0.14	0.25
P. seriata	0.32	0.88	0.89	0.15	0.03*	0.07
Thalassiosira spp.	0.54	0.83	0.82	0.55	0.62	0.68
R. imbricata shrubsolei	0.13	0.54	0.44	0.70	0.99	0.79
T. longissima	0.01*	0.12	0.27	0.67	0.72	0.57
T. nitzschioides	0.15	0.81	0.93	0.68	0.56	0.80
Eucampia zodiacus	0.91	0.52	0.56	0.91	0.57	0.45
Ditylum brightwellii	0.96	0.33	0.62	0.29	0.36	0.53
Skeletonema costatum	0.08	0.05*	0.10	0.78	0.92	0.69
P. delicatissima	0.005*	0.15	0.32	0.36	0.40	0.81
R. styliformis	0.51	0.37	0.10	0.69	0.93	0.76
R. hebetata semispina	0.59	0.23	0.19	0.67	0.01*	0.03*
R. alata indica	0.29	0.52	0.90	0.58	0.15	0.35
Integrated Dinoflagellates	0.20	0.52	0.11	0.19	0.48	0.78
Prorocentrum spp.	0.91	0.83	0.90	0.87	0.74	0.68
Protoperidinium spp.	0.03*	0.004*	0.01*	0.85	0.71	0.81
C. furca	0.05*	0.08	0.002*	0.70	0.95	0.82
C. fusus	0.27	0.66	0.26	0.16	0.52	0.85
C. lineatum	0.84	0.43	0.48	0.32	0.59	0.68
C. tripos	0.04*	0.10	0.02*	0.04*	0.12	0.27
C. macroceros	0.89	0.17	0.70	0.07	0.21	0.57
C. longipes	0.86	0.95	0.68	0.30	0.51	0.57
C. minutum	0.99	0.94	0.45	0.52	0.76	0.41
C. hexacanthum	0.32	0.64	0.61	0.14	0.15	0.24
Dinophysis spp.	0.03*	0.04*	0.12	0.81	0.37	0.44
N. scintillans	0.04*	0.18	0.23	0.39	0.53	0.65

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Long-term changes in abundance and distribution of microzooplankton in the NE Atlantic and North Sea

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SLH and AWW compiled the data. SLH, MBG, JEM and GCH led the data analyses and interpretation with contributions from all authors. SLH produced all the figures. SLH and GCH wrote the paper with contributions from all authors.

Abstract

Long-term changes among mesozooplankton and phytoplankton have been well documented in the North Atlantic region whereas there has been a scarcity of data for microzooplankton. This neglected component of the plankton is a vital link in marine food-webs, grazing on smaller flagellates and cyanobacteria and in turn providing food for the larger mesozooplankton. We use the latest tintinnid (Ciliophora, Protista) data from the Continuous Plankton Recorder (CPR) survey in the NE Atlantic and North Sea to examine the phenology, distribution and abundance of this important group of ciliates. Presence/absence data came from 167,122 CPR samples collected between 1960 - 2009 and abundance data from 49,662 samples collected between 1996 - 2009. In the North Atlantic the genus *Dictyocysta spp.* dominated and *Parafavella gigantea* showed an increase in abundance around Iceland and Greenland. In the North Sea higher densities of *Tintinnopsis spp.*, *Favella serrata* and *Ptychocylis spp.* were found. The presence of tintinnids in CPR samples collected in the North Atlantic has increased over the last 50 years and the seasonal window of high abundance has lengthened. Conversely in the North Sea there has been an overall reduction in abundance. We discuss possible drivers for these long-term changes and point the way forward to more holistic studies that examine how ecosystems, rather than just selected taxa, are responding to climate change.

Introduction

It has been widely reported that there have been profound changes in the biology of the oceans over the last 50 years linked to climate (Edwards et al., 2002; Parmesan and Yohe, 2003; Walther et al., 2002). Changes have been detected at all levels of ecological organization and have included life history changes, phenology changes, shifts in geographic range, changing species composition and local population increases and declines (Barnard et al., 2004). Marine plankton communities are particularly sensitive to climate change (Edwards and Richardson, 2004) and some of the clearest evidence of ecological change comes from plankton data particularly data supplied by the Continuous Plankton Recorder (CPR) survey (Beaugrand et al., 2002; Hays et al., 2005; Reid et al., 1998). The CPR survey is the largest multi-decadal plankton monitoring programme in the world (Richardson et al., 2006). Copepods, diatoms and dinoflagellates are the groups most commonly recorded by the CPR and most long-term climate related studies have focussed on these mesozooplankton (large plankton >200 μm in diameter) and phytoplankton species. However, the CPR survey also generates data on microzooplankton (20-200 μm) and historically this group has been under-investigated.

Tintinnids are a group of loricate ciliates that make up just 7 of the 436 taxa counted in routine CPR analysis. Records of tintinnids exist in almost all marine waters and the distribution of taxa is thought to be related to water temperature, with cosmopolitan, neritic, boreal, austral and warm water taxa having been identified (Pierce and Turner 1993). The tintinnids are important grazers (Admiraal and Venekamp, 1986) which may be able to significantly regulate phytoplankton including some harmful algal species in the initial stages of a bloom (Kamiyama, 1997). The group are exploited at higher trophic levels by protozoan and metazoan plankton including foraminiferans (Thompson, 2004), copepods (Stoecker and Sanders, 1985), crustacean larvae (Thompson, 2004) and fish (Shaheen et al., 2004). Tintinnids therefore play important trophic roles and so changes in distribution and phenology of tintinnid taxa could cause mismatches between successive trophic levels. Tintinnid CPR data has been mapped only twice before in the literature (Barnard et al., 2004; Lindley, 1975). We used geostatistical approaches and include the latest CPR data to redress the lack of information about the biogeography and long-term changes within this ecologically relevant group.

Method

CPR survey

CPR samples are collected from ships-of-opportunity that tow a robust plankton filtering instrument at a depth of 6-7m. The water passes through a moving filter band of silk (270 µm mesh size) which collects plankton from the ambient water. For further information regarding the technical background, consistency and comparability of CPR sampling see Richardson et al., (2006). As tintinnids are generally smaller than the silk mesh their retention is deemed semi-quantitative. Using the CPR to collect samples is a less gentle method compared to collection of whole water samples and although tintinnids are sturdier than aloricate ciliates, they are still more fragile than dinoflagellates and copepods which are sampled by the CPR with less distortion (Pierce and Turner 1993). However, data between tows are comparable as the sampling methodology has remained broadly consistent over time and patterns in the data are said to reflect true changes in abundance, distribution and community composition (Robinson, 1970). Over small spatial scales, the CPR survey may provide limited samples which constrains the value of the data (e.g. Hays et al., (1993)), but over large scales the consistency of the sampling methodology means that the data are thought to be one of the best available reflections of changes in abundance, distribution and community composition of plankton (Hays et al., 1993).

CPR identification numbers for tintinnid taxa

Presence and absence data for 1960-2009 were used to measure the spatial and temporal variability of total tintinnids (n=167,122 samples). Relative abundance data were examined for five sub-categories of tintinnid identified by the CPR survey since 1996: *Dictyocysta spp.*, *Favella serrata*, *Parafavella gigantea*, *Ptychocylis spp.* and *Tintinnopsis spp.* In addition tintinnids of other taxa were pooled into an "unknown taxa" category in the CPR analysis since 1996.

Spatial maps of individual taxa

Data derived from 49,662 CPR samples were used to create maps showing the general spatial distribution and relative abundance of five tintinnid taxa (*Dictyocysta spp.*, *Favella serrata*, *Parafavella gigantea*, *Ptychocylis spp.* and *Tintinnopsis spp.*) between 1996 and 2009 in an area 55°W-10°E and 40-65°N. Maps were calculated using an ordinary kriging method, which was carried out using the packages "sp" (Pebesma and Bivand, 2005) and "gstat" (Pebesma, 2004) within R (R, 2009). One of the assumptions of kriging is that spatial structures are stable over the duration of the sampling period (Simard et al., 1992) which was clearly not the case for CPR data collected over a period of 14 years. To address this issue, when producing spatial maps the datasets were partitioned into shorter temporal periods (Edwards, 2000). For each individual taxa the abundance data were firstly split into two seven-year intervals (1996-2002 and 2003-2009). For each time period, data was split into months where kriging was applied to produce twelve maps. Finally, for each taxa and time period, a singular composite map was produced by averaging each grid node over the twelve months. The justification for this approach is described in detail in Edwards, (2000). Briefly, for each kriging map we aimed to increase the accuracy of estimating a spatial signal in the data (by including a large number of observations; average 1911, range 1716 to 2525) whilst reducing the temporal influence. This was achieved firstly by avoiding averaging over different months (where most of the ecological temporal variation is found) and secondly by averaging over a seven year period, which was the highest practical resolution of division possible.

Relative proportions of individual taxa

Relative abundance data for each individual taxa (*Dictyocysta spp.*, *Favella serrata*, *Parafavella gigantea*, *Ptychocylis spp.* and *Tintinnopsis spp.*) were examined across years (1996-2009) in two regions: NE Atlantic and UK Shelf Seas (Figure 3.1). The percentage of total tintinnid occurrences accounted for by each individual taxon within each category was calculated.

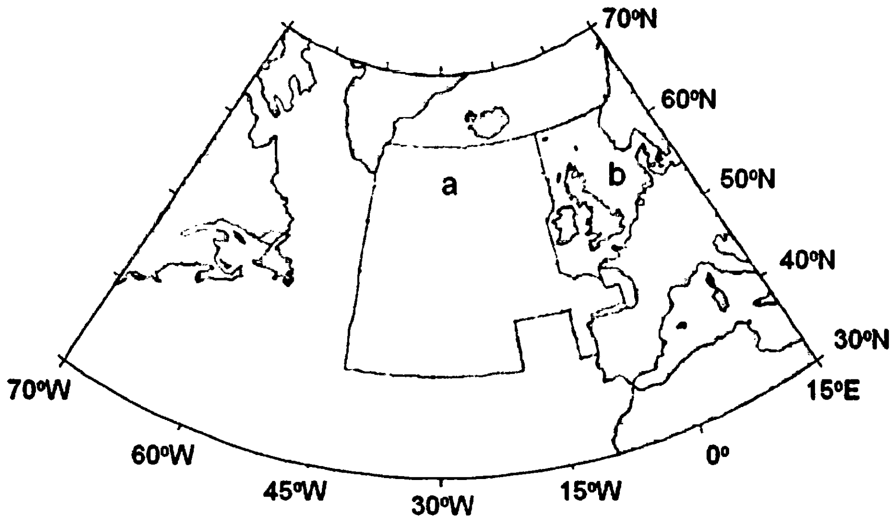

Figure 3.1. CPR tintinnid data separated into two regions, (a) NE Atlantic and (b) UK Shelf Seas.

Phenological and annual changes in total tintinnid occurrence

CPR samples were used to look for monthly and yearly changes in the occurrence of tintinnids between 1960-2009, in the NE Atlantic and UK Shelf Seas (Figure 3.1). Data were interpolated using R (version 2.12) to create plots of percentage occurrence (defined as the percentage of CPR samples in which tintinnids were present). The mean annual peak in percentage occurrence was calculated based on the central tendency method (Edwards and Richardson, 2004). Linear regression analysis was used to identify the change in timing of peak of the seasonal cycle. The length of the seasonal window was estimated in two ways; firstly, the number of months per year where the percentage of occurrence was over 20% was calculated and its trend over time examined. Secondly, linear regression analysis was used to determine whether, for each month, there was a significant increase in abundance over the 50 year time period.

Yearly abundance of individual taxa

For each individual tintinnid taxa, we retained a 5-year period for generating kriging estimates, but defined the abundance values for each year as the estimate obtained by kriging the 5-year window for which the year is the centroid. Abundance was averaged over the NE Atlantic and the UK Shelf Seas regions (Figure 3.1).

Results

Individual taxa distribution

The five tintinnid taxa had different patterns of distribution (Figure 3.2). *Dictyocysta spp.* was the most abundant and extensively distributed taxa occurring widely in the NE Atlantic. *Tintinnopsis spp.* was relatively abundant in the shelf seas around the UK, while *Favella serrata* and *Ptychocylis spp.* showed generally low levels of abundance with fragmented small areas of higher abundance in the North Atlantic. *Parafavella gigantea* showed maximum abundance in the coastal waters off Iceland and Nova Scotia (Canada).

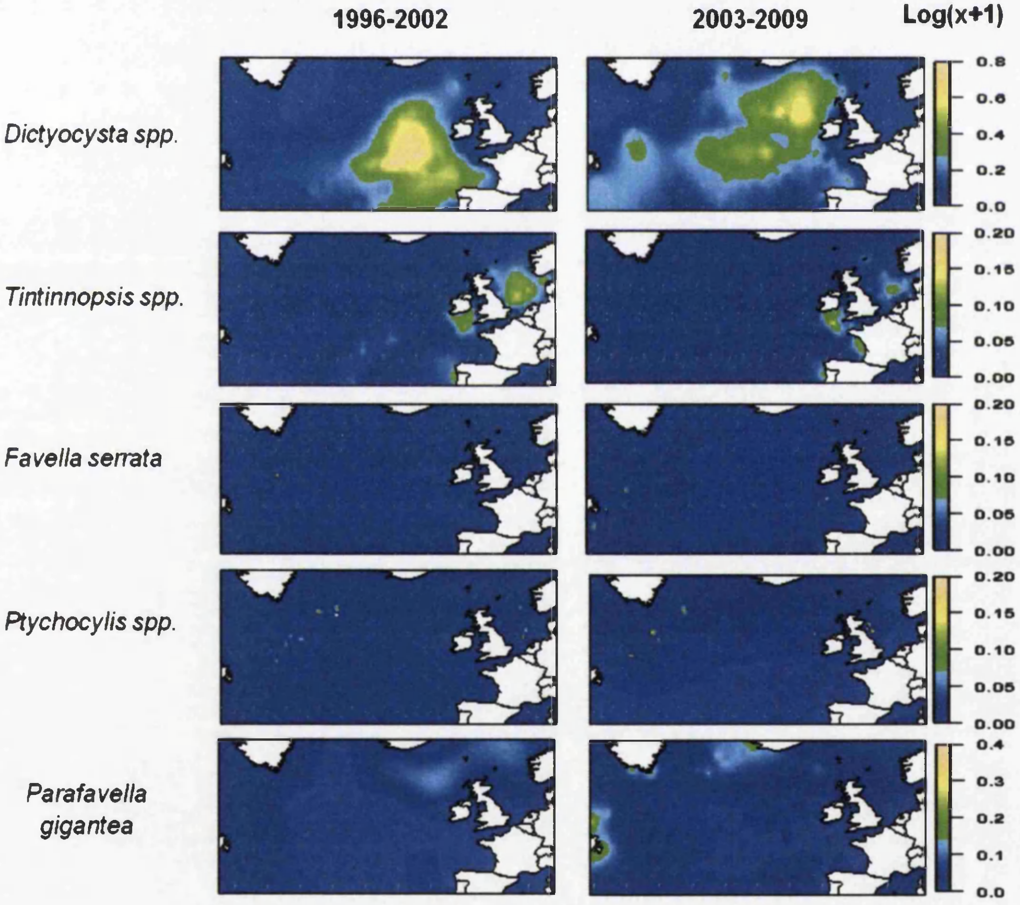

Figure 3.2. Abundance and distribution of individual tintinnid taxa over two time periods. The scale shows mean numbers per sample (x) that have been log (x+1) transformed.

In the NE Atlantic, *Dictyocysta* accounted for the highest percentage of occurrence during 1996-2009, with a range between 40% - 76% of all tintinnid occurrences in the CPR samples (Figure 3.3a). While, all other taxa examined (*Parafavella gigantea*, *Favella serrata*, *Ptychocylis spp.* and *Tintinnopsis spp.*) only contributed between 1.5% - 6.5% of all tintinnid occurrences. The UK Shelf Seas tintinnid population consisted of more equal proportions of all five taxa (Figure 3.3b). *Tintinnopsis spp.* and *Dictyocysta spp.* percentage occurrence ranged between 15% - 37% and 17% - 36% respectively of all tintinnid occurrences, while, *Favella serrata*, *Parafavella gigantea* and *Ptychocylis spp.* contributed each with a range of 2.5-10% from 1996-2009.

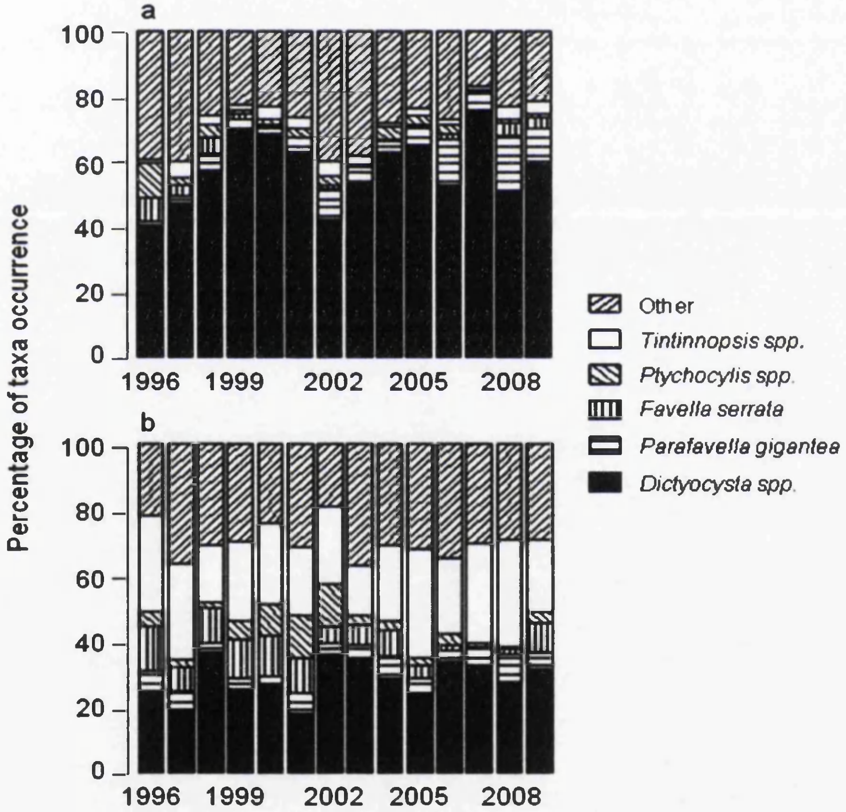

Figure 3.3. Contribution of different taxa to the total number of tintinnids recorded each year in the CPR survey. Data start from 1996 as this is when individual tintinnid taxa started to be identified in the CPR survey. (a) NE Atlantic and (b) UK Shelf Seas. *Dictyocysta spp.* (Black), *Parafavella gigantea* (Horizontal), *Favella serrata* (Vertical), *Ptychocylis spp.* (Left Diagonal), *Tintinnopsis spp.* (White), and Unidentified tintinnids (Right Diagonal).

Individual taxa abundance

For each individual tintinnid taxa, long-term trends in abundance were similar for both the NE Atlantic and the UK Shelf Sea regions. *Dictyocysta spp.* and *Tintinnopsis spp.* showed a relatively stable yearly abundance throughout 1998 to 2007, while *Parafavella gigantea* has considerably increased in abundance, especially in the NE Atlantic since 2000 (Figure 3.4). *Ptychocylis spp.* and *Favella serrata* have both decreased in yearly abundance since 2004 and 1998 respectively (Figure 3.4).

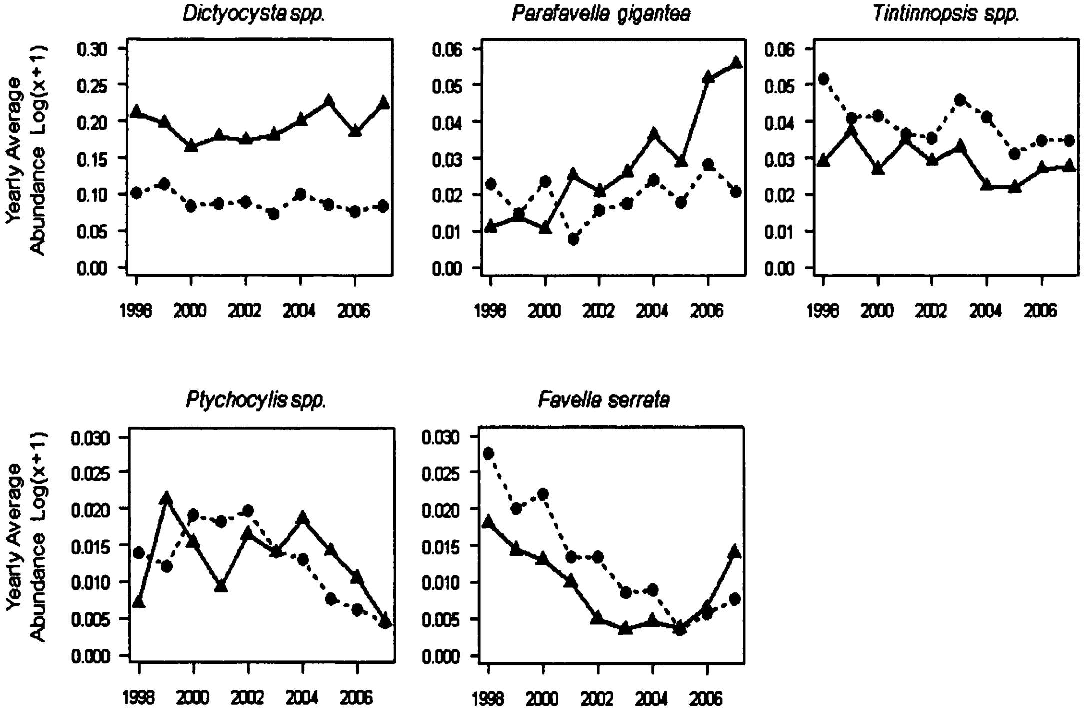

Figure 3.4. Annual average abundance of individual tintinnid taxa for NE Atlantic (solid line with closed triangles) and the UK Shelf Seas (dashed line with closed circles) (using 1996-2009 data). The scale shows mean numbers per sample (x) that have been log (x+1) transformed.

Phenology and annual patterns of tintinnid occurrence

In the NE Atlantic the absolute occurrence of tintinnids in CPR samples increased between 1960-2009 (Figure 3.5a). In 1960, tintinnids occurred in less than 20% of samples whereas in 2007-2009 tintinnids were present in > 50% of samples for part of the year. In the UK Shelf Sea waters, tintinnids occurred in less than 50% of CPR samples in all years, i.e. levels of occurrence were much lower than in the NE Atlantic (Figure 3.5b). Although tintinnid occurrence in UK Shelf Seas increased from 1960 until the early 2000s, there was subsequently a marked drop in occurrence from 2003 onwards with tintinnids occurring in less than 10% of samples in all months. Peak abundance tended to be uni-modal in this region and occurred within the months May to September for most of the 50 year period of the time series.

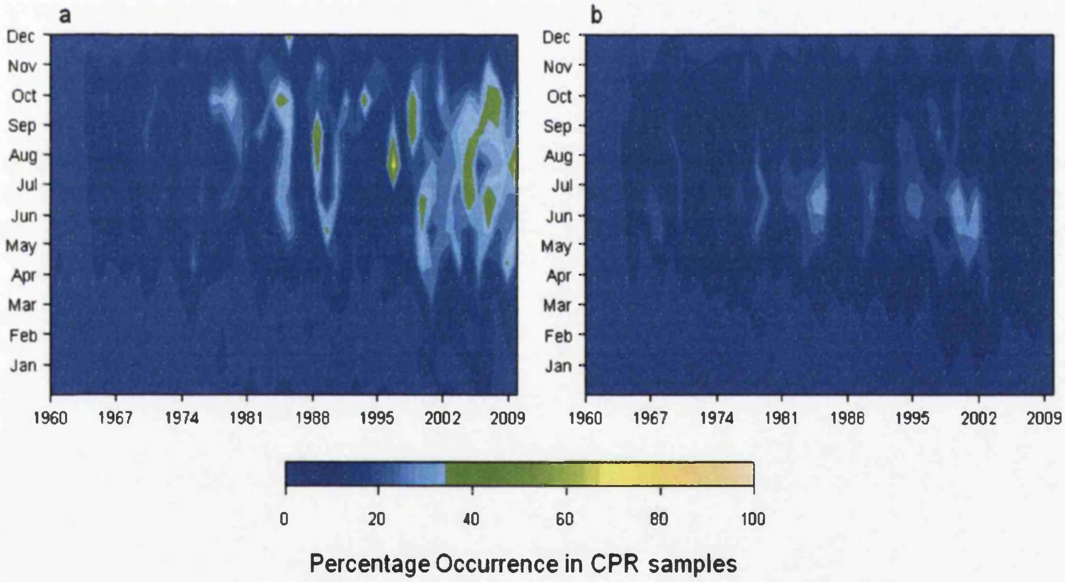

Figure 3.5. Total tintinnid occurrence (%) by year and month in (a) NE Atlantic, (b) UK Shelf Seas.

In both the NE Atlantic and the UK Shelf Seas waters, no monotonic long-term changes in the seasonal timing of peak tintinnid occurrence were detected, suggesting there has been no systematic modification in the phenology of tintinnids in either region. However, in the NE Atlantic and in the UK Shelf Seas, we found significant increases in the number of months that had over 20% occurrence

(Figure S3.1) (NE Atlantic: $p<0.001$, $r^2 = 0.65$, UK Shelf Seas: $p = 0.002$, $r^2 = 0.18$). Additionally, over the 49 year period the months February to December had significant increases in the percentage of samples that contained tintinnids, (Table S3.1). e.g. In February 1960 there were no tintinnids present in samples, while in February 2009, 11% of the samples contained tintinnids. These results suggest that there has been an expansion in the length of the seasonal window of high abundance since 1960, with tintinnids now being present in relatively high levels throughout the February to December period.

Discussion

It is important to identify how plankton functional groups respond to climate. Our results extend previous work focussing on mesozooplankton and phytoplankton e.g. Hays et al., (2005) by showing important changes in abundance and distribution for a key component of the microzooplankton, the tintinnids. While there have been many site-specific studies of the community composition of tintinnids, there have been few sampling programmes that have looked at distribution across large spatial scales. In our study we have shown a long-term increase in the occurrence of tintinnids in the North Atlantic as well as a northerly shift in their distribution. Since CPR sampling will not detect all cells of a particular tintinnid species, it is possible that some cells were already present, but undetected, at more northerly locations at the beginning of the study period. So the northerly shift in CPR records should be viewed as an indication of the relative abundance of taxa and resulting range changes, not the absolute presence/absence of taxa in certain areas at certain time periods.

In the North Atlantic, the tintinnids identified in the CPR survey are dominated by *Dictyocysta spp.* We can use this more recent data on species abundance (1996-present) to help inform about the longer time series for which simply tintinnids presence/absence has been recorded (1960-present). The lack of high levels of occurrence for tintinnids recorded in the earlier part of the presence/absence time series suggests that the increase in the occurrence of tintinnids in the last 50 years is mainly caused by *Dictyocysta spp.* Our results support previous conclusions of Lindley, (1975) and Fileman and Leakey, (2005), who showed that *Dictyocysta spp.* was the most abundant tintinnid in the NE Atlantic. Overall it is thought that *Dictyocysta spp.* is a cosmopolitan species recorded broadly in temperate and boreal waters (Pierce and Turner, 1993). These observed northerly shifts in the relative abundance of tintinnid occurrence in the North Atlantic, identified by the CPR survey, parallel northerly shifts observed for other zooplankton and have been linked to warming sea temperatures (Beaugrand, 2004). Accompanying the range change and a slight increased occurrence, there has been an extension to the seasonal window of high tintinnid occurrence in the NE Atlantic with tintinnids now being present in appreciable numbers (over 20%) for six months of the year. Taken together all these long-term changes point to conditions (presumably prey availability, predation pressure and/or hydrological conditions) now being more favourable for this group in the NE Atlantic. Using the modified Chelton method we looked for trends between individual taxa, against Sea Surface Temperature (SST, (Hadley Centre of the UK Met Office)), the winter North Atlantic Oscillation Index (NAOI), and against the CPR Phytoplankton Colour Index (PCI) for both the NE Atlantic and the UK Shelf Sea regions. Annual average abundance values for individual taxa were calculated from the 5 year moving window average (1998-2007). The NE Atlantic and the UK Shelf Seas, PCI and SST have both risen since the 1980s (Figure 3.6a, b), which is consistent with findings by McQuatters-Gollop et al., (2011), and Beaugrand, (2009). While the NAOI has been dominated by a positive phase during the 1990s and early 2000s, there has been a recent switch to a negative phase during the winters of 2003 to 2006 and the winter of 2008/2009 (Figure 3.6c). However, no significant correlations were detected between taxa and climate variables or PCI for either region, but this could be due to the small time scale involved (1998-2007) which reduces the power of this analysis. As the CPR time series on tintinnid abundance extends, it will become easier to resolve if there are climate drivers underlying the changes in abundance and distribution. Another important factor to be considered is the size of the lorica oral diameter of each tintinnid taxa. Dolan, (2010), has shown that the differences in lorica oral diameter could reflect adaptation to distinct ecological niches. Several other studies have shown that the size spectrum of phytoplankton prey might be the cause of seasonal shifts in tintinnid community, with the size of the lorica affecting the feeding activities of tintinnids (Admiraal and Venekamp, 1986; Middlebrook et al., 1987). Of the species we examined, *Dictyocysta spp.* has the smallest oral diameter and cell length, while *Favella serrata*, *Parafavella gigantea* and *Ptychocylis spp.* show a distinctive larger cell length and oral diameter size (Table 3.1). These differences could suggest that size of prey is influencing tintinnid taxa distribution and abundance.

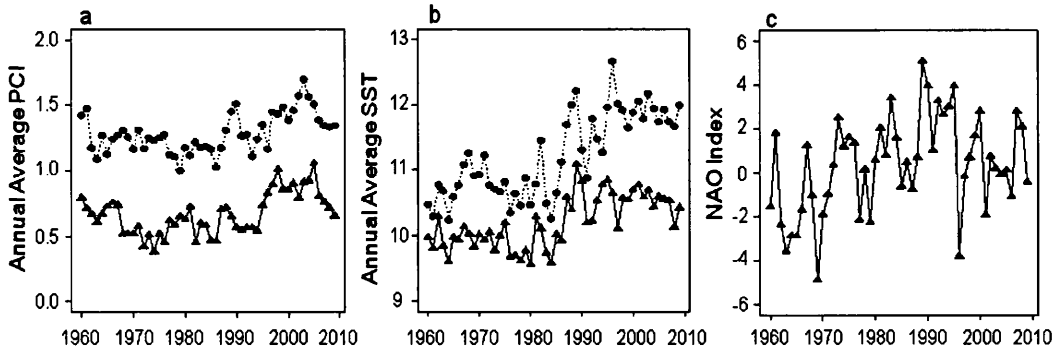

Figure 3.6. Annual average values for (a) CPR Phytoplankton Colour Index (PCI), (b) Sea Surface Temperature (SST) and (c) Winter North Atlantic Oscillation Index, for both the NE Atlantic (solid line with closed triangles) and the UK Shelf Seas region (dashed line with closed circles) (1960-2009).

Contrasting long-term patterns of tintinnids occurrence were found in the UK Shelf Seas with a recent decrease in occurrence. These contrasting long-term trends in tintinnids occurrence between the NE Atlantic and UK Shelf Seas suggest that large scale climate drivers (e.g. the NAOI) are not having a common impact on tintinnids, but rather that regional specific factors, which could be influenced by large scale climate drivers, are driving the long-term changes. This decline in tintinnid occurrence in the North Sea may be linked with the large changes in other components of the plankton observed in this area in recent years (Beaugrand, 2004; Beaugrand et al., 2002; Edwards and Richardson, 2004; Reid et al., 2001). Plankton taxa in the North Sea have shown biogeographical shifts and phenology changes over the last two decades (Beaugrand, 2009; Reid et al., 1998). These responses are thought to be species dependent. Several studies have shown that the recent warming in the North Sea has resulted in dinoflagellates and copepods showing peak abundance, on average, 23 days and 10 days earlier (Edwards and Richardson, 2004). While diatom bloom duration and seasonal timing has remained largely unchanged, and it has been suggested that diatom growth is dependent upon daylight duration and intensity rather than diatoms being physiologically temperature mediated (Edwards and Richardson, 2004). These species-dependent phenology responses could cause trophic mismatches or abrupt ecosystem shifts (Beaugrand et al., 2008; Edwards and Richardson, 2004). In addition to phenology changes, Beaugrand, (2009) has shown that the diversity of calanoid copepods in the North Sea has increased. This diversity is associated with a reduction in the number of cold water species, while there has been an increase in the number of warmer water species. Hatun et al., (2005) suggested these diversity changes could be attributable to a negative period of the NAOI as it allows the subarctic gyre to contract, enabling more warmer water species to penetrate north and west. Taken together, our findings and those of others point to the need for a more holistic understanding of long-term changes in ecosystems that include all trophic levels and take account of species interactions.

Table 3.1. Cell length (µm) and oral diameter (µm) estimates of the five taxa examined.

Taxa	Cell Length (µm)	Oral Diameter (µm)	Reference
Dictyocysta spp.	60-70	35-40	(Kofoid and Campbell, 1939)
Tintinnopsis spp.	40-200	26-86	(Hada, 1939; Kofoid and Campbell, 1939)
Ptychocylis spp.	65-190	60-100	(Hada, 1939; Kofoid and Campbell, 1939; Marshall, 1969)
Favella serrata	130-350	87-145	(Marshall, 1969)
Parafavella gigantea	200-750	66-71	(Hada, 1939; Kofoid and Campbell, 1939)

In the UK shelf seas area, while *Dictyocysta spp.* was still an important component of the tintinnids identified in the CPR samples, other species also occurred in relatively high abundance, particularly *Tintinnopsis spp.* This taxon is generally regarded as a neritic species (Pierce and Turner, 1993; Pilling et al., 1992) and this is supported by the CPR records. In one of the few previous studies that have shown patterns of abundance for tintinnids over a large spatial scale, Corderio et al., (1997) showed that in the North Sea the groups *Tintinnopsis spp.*, *Favella spp.* and *Ptychocylis urnula* were abundant in late spring 1986. Our results are broadly consistent with these findings. However, Corderio et al., (1997) also reported high levels of abundance for some tintinnid taxa that are not counted by the CPR survey because they are too fragile and break up on the CPR meshes. Examples of these taxa include *Stenosemella nivalis*, *Helicostomella subulata*, and *Acanthostomella norvegica*. The CPR method only measures plankton at a depth of 6-7m, therefore in our analysis; we note that we are not getting an unbiased view of all tintinnid abundance, but rather only the levels of abundance for the robust taxa that are present in the near surface waters. Nevertheless, despite these caveats the CPR data confirms that changes have occurred within the microzooplankton of the North Atlantic and North Sea over the last few decades.

In addition to *Dictyocysta spp.* and *Tintinnopsis spp.* other groups of tintinnids were found in low levels in the CPR samples. *Favella serrata* and *Ptychocylis spp.* were found locally within the North Atlantic and both taxa have shown a recent decrease in yearly abundance (Figure 3.4). *Favella serrata* has been described as a neritic species, and although low numbers were detected in the North Sea, the several isolated fragments of occurrence within the North Atlantic could be attributable to the type of available prey. Several studies have shown that *Favella* may be a specialised predator on dinoflagellates and therefore a sharp peak in abundance could be from *Favella serrata* locating a particular dinoflagellate prey (Bernard and Rassoulzadegan, 1993; Gold, 1969; Stoecker et al., 1981). *Ptychocylis spp.* is considered to be restricted to cold waters in the Northern Hemisphere, and therefore classified as a boreal species (Pierce and Turner, 1993). Although we have seen *Ptychocylis spp.* abundance as fragmented pockets, similar spatial patterns were also detected by Lindley, (1975). It is thought that Cape Hatteras, on the American east coast, appears to be the southern limit of *Ptychocylis spp.* in the western Atlantic (Pierce and Turner, 1993). *Parafavella gigantea* was generally located north of the UK, but since 1998 this species has shown a considerable increase in abundance, and illustrates a more northwest movement towards Iceland and Greenland since 2003. As a boreal species (Pierce and Turner, 1993), this shift could be linked to cold sea surface temperatures.

Phenological changes have been widely reported in the plankton (Edwards and Richardson, 2004). For some plankton taxa it has been found that maximal abundance is occurring progressively earlier in the year, while for other taxa the reverse pattern is evident (Edwards and Richardson, 2004). This lack of consistent across-taxa change has led to the suggestion that new trophic matches and mismatches may be produced (Edwards and Richardson, 2004). Set against this we found no systematic shift in the seasonal timing of peak tintinnids occurrence. Hence there is no qualitative change in the seasonal timing of abundance, but rather the pattern reflects a rise in abundance throughout almost the entire year, resulting in the extended seasonal window. This suggests that, while overall abundance is changing dramatically, the actual seasonal timing is not driven simply as a response to a changing climate parameter such as SST. Rather some invariant environmental feature (e.g. photoperiod) or direct responses to prey availability (bottom-up control) may be controlling tintinnid seasonal occurrence. It should be noted that this conjecture is based on combined trends for all tintinnids, and clearly individual species might differ.

As discussed above, it is the consistency and large scale of the CPR data collection that allows semi-quantitative estimates of abundance for such species that are generally smaller than the CPR mesh size. However it is possible that additional factors such as high abundance of other species of zooplankton or phytoplankton could lead to clogging of the mesh and increase the chances of microzooplankton capture, and hence confound apparent trends in the tintinnid time series. Here, we argue that such factors are unlikely to be responsible for the broad trends we have described. First, our conclusions on phenology are based solely on presence/absence data (of any taxa). Second, we see contrasting trends across different sized taxa, with the smallest taxa (*Dictyocysta spp.*) increasing in the North Atlantic while larger taxa (*Favella serrata* and *Ptychocylis spp.*) have declined. This pattern is unlikely to be explained via clogging alone. Third, any large temporal patterns in mesh clogging would likely be reflected in the PCI, which shows small changes and is less pronounced in the North Atlantic.

In summary we have observed changes in tintinnid composition in recent decades with an increase in tintinnid occurrence in the North Atlantic and a decrease in the North Sea. The wider trophic implications of these changes are unknown and point the way forward to more holistic studies that examine ecosystem changes across trophic levels.

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Supplementary Material

Long-term changes in abundance and distribution of microzooplankton in the NE Atlantic and North Sea

Hinder, S.L., Manning, J.E., Gravenor, M.B., Edwards, M., Walne, A.W., Burkill, P.H. and Hays G.C.

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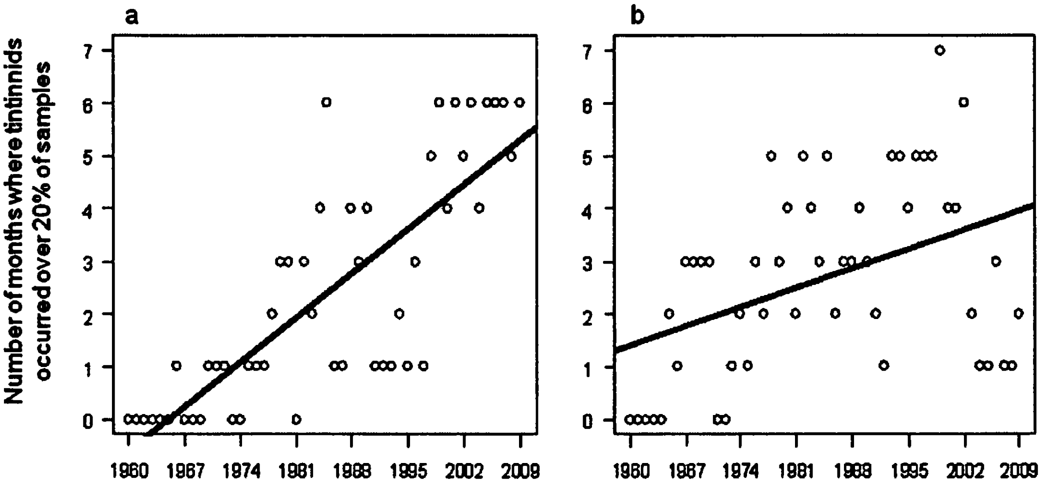

Figure S3.1. The number of months over 20% occurrence from 1960-2009, for (a) NE Atlantic and (b) UK Shelf Seas.

Table S3.1. Linear regression analysis of tintinnid occurrence for each month over 1960-2009 for the NE Atlantic and the UK Shelf Seas region (* p<0.05).

	NE Atlantic		**UK Shelf Seas**	
	p	r^2	p	r^2
January	0.08	0.06	0.07	0.07
February	0.004*	0.16	0.01*	0.13
March	<0.001*	0.28	<0.001*	0.35
April	<0.001*	0.35	<0.001*	0.63
May	<0.001*	0.37	<0.001*	0.28
June	<0.001*	0.41	<0.001*	0.23
July	<0.001*	0.36	0.005*	0.15
August	<0.001*	0.42	0.05*	0.07
September	<0.001*	0.38	<0.001*	0.21
October	<0.001*	0.32	<0.001*	0.37
November	<0.001*	0.29	0.009*	0.13
December	0.06	0.07	0.002*	0.17

Chapter IV

A 50 year time series for ocean plankton in the face of climate change

Hinder, S.L., Gravenor, M.B., Edwards, M., Ostle, C., Lee, P.L.M., Walne, A.W. and Hays G.C.

SLH and AWW compiled the data. SLH, MBG, CO and GCH led the analyses and interpretation of the data and the writing of the paper. SLH produced all the figures. PLML suggested the analysis of genetic structure and consideration of adaptation. MBG and SLH preformed the statistical analysis. All authors contributed to the drafts.

Abstract

For many decades there has been interest in patterns of abundance for *Calanus finmarchicus* as this species provides important food for commercially exploited fish such as cod and other gadoids. Declines in *C. finmarchicus* abundance in the NE Atlantic region have been reported previously as the species range contracts to colder more northerly waters. We use the latest data from the Continuous Plankton Recorder (CPR) survey to show this decline has continued unabated with protracted (>50 year) declines in the abundance of *C. finmarchicus* in the North Sea and NE Atlantic. Over the same period the congeneric *C. helgolandicus* has increased in abundance in the same areas. Changes in abundance of both *Calanus* species are linked to sea surface temperatures (SST), westerly wind speed and the North Atlantic Oscillation Index although the strength of these links has changed through the time series, with correlations to SST getting stronger. Granger causality tests point to an absence of lag effects, i.e. abundance in each year is primarily driven by conditions that year rather than carry-over effects from the previous year.

Introduction

Climate change is having profound impacts on the phenology, abundance and distribution of a broad range of taxa including marine plankton. For example, in response to rising water temperatures, warm water species are showing an expanded range in the NE Atlantic and colder-water species a corresponding range contraction (Beaugrand et al., 2009; Beaugrand et al., 2002). The pace of these range changes often considerably exceed those seen in terrestrial systems, with a range extension of 23.16 km per year reported for some NE Atlantic zooplankton (Beaugrand et al., 2009). These amplified marine biological responses to climate change seem to reflect environmental conditions changing more rapidly in marine verses terrestrial systems (Brown et al., 2011). Limiting our understanding of these changes is the relatively short length of available biological time series. However as time series lengthen, so our understanding of the processes that drive abundance and distribution improve.

In this regard some of the most instructive time series are the longest. The Continuous Plankton Recorder (CPR) survey is widely recognized as one of the longest and most valuable marine biological time series in existence (Edwards et al., 2010; Reid et al., 2003a) and has underpinned a number of key studies on natural patterns of biological change (Beaugrand et al., 2002; Edwards and Richardson, 2004; Reid et al., 2001). For example *Calanus finmarchicus* plays a key trophic role in the North Atlantic, providing food for a number of commercially important fish such as cod (*Gadus morhua*) (Dickson and Brander, 1993). Declines in the abundance of *C. finmarchicus* in the southern parts of its range in the NE Atlantic were noted at least 20 years ago (e.g. Planque and Fromentin, (1996)) using CPR data and were initially linked to the North Atlantic Oscillation Index (NAOI), although strong correlations with westerly wind stress were also noted at that time (Table 1 from Fromentin and Planque, (1996)). However, data subsequently collected in the 1990s seemed to indicate that this relationship between *C. finmarchicus* abundance and the NAOI has broken down (Planque and Reid, 1998) and continued declines in *C. finmarchicus* abundance were linked to sea surface temperature (SST) (Beaugrand, 2004; Beaugrand, 2009; Beaugrand et al., 2002; Helaouet and Beaugrand, 2007; Kamenos, 2010). Against a general decline in abundance in the North Sea, higher levels of *C. finmarchicus* abundance for a few years in the 1970s were linked to much better than average recruitment of a number of gadoid species (cod, hake, haddock and whiting) a phenomenon termed the gadoid outburst (Cushing, 1984). While *C. finmarchicus* has declined in abundance in the North Sea and Atlantic areas west of the UK, the congeneric species *C. helgolandicus*, which has a generally warmer-water more southerly distribution, has increased in abundance (Beaugrand et al., 2002; Fromentin and Planque, 1996; Reygondeau and Beaugrand, 2011). These contrasting trends in abundance for *C. finmarchicus* and *C. helgolandicus* using the long-term CPR data were last reported together using data extending up until 1999 (Beaugrand et al., 2002), although the time series for *C. finmarchicus* has been reported more recently (Beaugrand et al., 2009).

In recent years SSTs have continued to increase in the NE Atlantic region. One scenario, if links between *C. finmarchicus* abundance and SST have been maintained in recent years, is that abundance will have continued to decline towards the southern limits of the species range. However, an oft cited criticism of links between biological time series and environmental parameters is that “correlation does not equal causality” and the literature is littered with strong correlations that have broken down as more data has been collected (Planque and Reid, 1998). One possibility is that species sometimes show adaptation to changing conditions and this might be linked to a change in the expected abundance trajectory based on past relationships with climate indices. We therefore used the latest CPR data, extending up to 2010, to examine trends in abundance of *C. finmarchicus* and the warmer water congeneric species *C. helgolandicus* in the NE Atlantic region. Furthermore we use Granger causality tests to shed further light on the possible mechanistic links between *Calanus* abundance and environmental conditions.

Methods

CPR survey

CPR samples are collected from ships-of-opportunity that tow a robust plankton filtering instrument at a depth of 6-7m (Hays, 1994), and due to the mixing effect of the ship the CPR samples the 0-20m surface layer (Hunt, 1969). Therefore changes in the abundance of *Calanus* are based on the subsurface distribution of the species. For further information regarding the technical background, consistency and comparability of CPR sampling see Richardson et al., (2006).

CPR data for *C. finmarchicus* and *C. helgolandicus* from 1960-2010 (n=117,056) (Johns, 2012) were examined within seven areas of the North Atlantic: NE Atlantic (20°W-8°E, 45-64°N), southern North Sea, northern North Sea, north west Scotland sector, north west Atlantic approaches, Irish Sea, and the south west Atlantic approaches (Figure 4.1). These regions were chosen based on Planque and Batten (2000), who concluded that long-term patterns of *C. finmarchicus* variability are region specific and Beaugrand, (2009) who suggested the need for more localized analysis of ecosystem shifts.

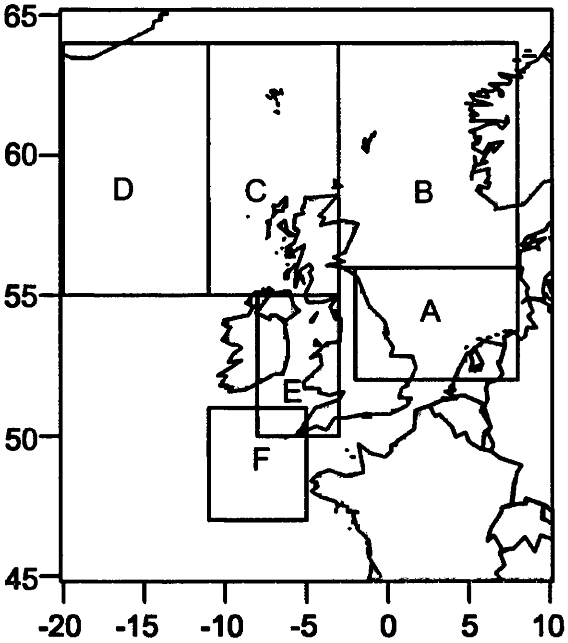

Figure 4.1. North Atlantic CPR data separated into six regions: (a) S. North Sea (52-56°N, 2°W-8°E); (b) N. North Sea (56-64°N, 3°W-8°E); (c) N.W. Scotland sector (55-64°N, 11-3°W); (d) N.W. Atlantic approaches (55-64°N, 20-11°W); (e) Irish Sea (50-55°N, 8-3°W); (f) S.W. Atlantic approaches (47-51°N, 11-5°W).

Large scale hydro climatic parameters

The winter NAOI for the period 1960-2010 was used, where the 1960 value is represented by December of 1959 and January to March of 1960. The NAOI is calculated as the normalized difference in winter sea-level pressures between Stykkisholmur in Iceland and Lisbon in Portugal (Hurrell et al., 2001) (http://www.cgd.ucar.edu/cas/jhurrel/).

Monthly mean gridded (1°x1°) SST and westerly wind speed (m/s) data from 1960-2010, was obtained from the Hadley Centre of the UK Met Office (HadISST) (http://www.metoffice.gov.uk/hadobs/hadisst/) (Rayner et al., 2003) and The International Comprehensive Ocean-Atmosphere Data Set (ICOADS) (http://www.ncdc.noaa.gov/oa/climate/coads/) respectively. Data are summarized for a given latitude and longitude. Annual SST and westerly wind speed averages were calculated for the NE Atlantic region (45-64°N; 20°W-8°E), and for the areas A-F (Figure 4.1).

Spatial maps of individual taxa

Data derived from 117,056 CPR samples were used to create maps showing the general spatial distribution and relative abundance of *C. finmarchicus* and *C. helgolandicus* between 1960 and 2010 in an area 20°W-8°E and 45-64°N. Maps were calculated using an ordinary kriging method, which was carried out using the packages "sp" (Pebesma and Bivand, 2005) and "gstat" (Pebesma, 2004) within R (R, 2009). One of the assumptions of kriging is that spatial structures are stable over the duration of the sampling period (Simard et al., 1992) which was clearly not the case for CPR data collected over a period of 51 years. To address this issue, when producing spatial maps the datasets were partitioned into shorter temporal periods (Edwards, 2000). For each individual taxa the abundance data were firstly split into approximately 5-year intervals (1960-1964, 1965-1969, 1970-1974, 1975-1979, 1980-1984, 1985-1989, 1990-1994, 1995-1999, 2000-2004, 2005-2010). For each time period, data was split into months where kriging was applied to produce twelve maps. Finally, for each taxa and decadal time period, a singular composite map was produced by averaging each grid node over the twelve months (see Hinder et al., (2012) for further description of methodology).

Relationship between abundance and environmental variables

The average yearly abundance for *Calanus* over a given geographical area (NE Atlantic or areas A-F (Figure 4.1)) was compared to the corresponding (average) changes in SST, westerly wind speed and the NAOI in a set of correlation and regression analyses. We employed a range of statistical methods that address different sources of spurious correlation that can arise in ecology time series analysis. We initially used Pearson correlation to test the relationships between the three climate variables and *Calanus* taxa. However, a problem with long-term data is one of serial autocorrelation (that is often present in ecological time series), which can lead to high type I error rates in correlation analysis (Yule, 1929). We controlled for this autocorrelation by using the 'modified Chelton method' (Pyper and Peterman, 1998).

We further tested for long-term links between variables with ‘causal’ methods borrowed from econometrics. The criteria for Granger causality is that forecasting of future values of a target variable is significantly improved by past observations of a causal variable after adjustment for past values of the target variable. This can be achieved in a standard regression framework, using lagged values of the target and putative causal factor as predictors (Thurman and Fisher, 1988). All variables were time de-trended initially, by extracting the residuals from regression models, as non-stationary time series can lead to spurious results. A range of plausible lags were investigated and statistical significance was assessed by comparing models with / without the putative causal factor using Akaike Information Criteria (Burnham and Anderson, 2002). This represents a test of the link between environmental and distribution variables over and above that provided by correlation alone (since lagged values of the target variable are included in the regression themselves).

Similarly, cointegration can be used to tease out genuine links between time series beyond the simple correlation that is often induced by common short term time trends, and hence be used as a cross check on the Granger models. If, for time series processes of a given order of integration, there exists a linear combination of the series that has a lower order of integration, the two series are considered to be cointegrated. As such, cointegrated time series have trajectories that are intimately linked and unlikely to diverge (Phillips and Ouliaris 1990). The presence of cointegration indicates a true long-term relationship is more likely, and also directly implies Granger causality in one or other direction (though the converse is not true). Cointegration models were fitted using the R function po.test (Phillips and Ouliaris 1990).

Results

SST, the NAOI and westerly wind speed varied systematically through the time series. SST initially decreased in the early 1960s before showing a long-term increase that has been maintained until present, with temperatures in the NE Atlantic around 1°C higher in 2010 compared to the 1980s (Figure 4.2a). Westerly wind speed has increased since the early 1960s; however there was a dip in 1996 and 2010 (Figure 4.2b). The NAOI has been dominated by a positive phase during the 1990s and early 2000s, while there has been a recent switch to a negative phase during the winters of 2003 to 2006 and the winters of 2009-2010 (Figure 4.2c).

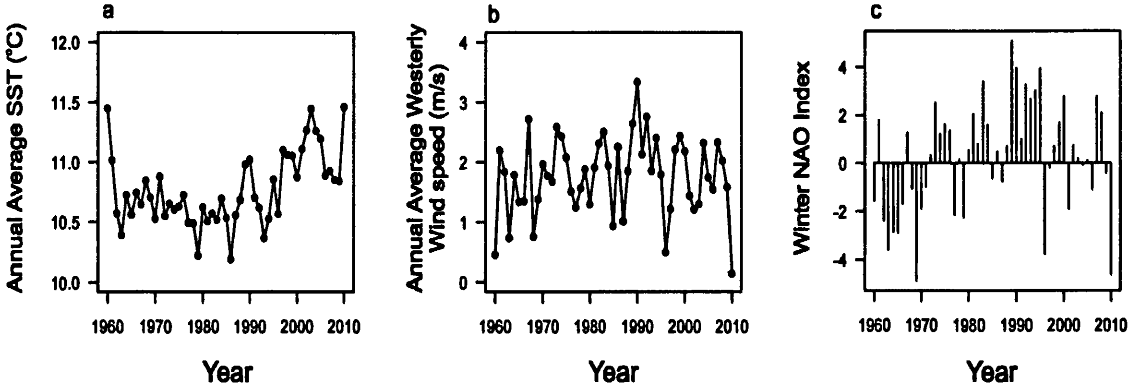

Figure 4.2. Annual average values for (a) Sea Surface Temperature (SST) (°C), (b) westerly wind speed (m/s) and (c) winter North Atlantic Oscillation Index (NAOI) for the NE Atlantic (1960-2010).

There have been very clear changes in the distribution and abundance of *Calanus* in the NE Atlantic region (Figure 4.3). For example, *C. finmarchicus* was maximally abundant in the northern North Sea and off north west Scotland in the 1960s-1980s, but there have been dramatic declines in abundance in these areas in the last two decades (Figure 4.3 and 4.4). The abundance of *C. helgolandicus* has generally followed the reverse pattern (Figure 4.3 and 4.4). In some areas *C. helgolandicus* has been relatively abundant throughout the time series (e.g. the south west approaches), while in other areas (generally further north) there has been a progressive increase in abundance such as off north-west Scotland and in the northern North Sea (Figure 4.3 and 4.4). In most areas both the decrease in abundance of *C. finmarchicus* and the increase in abundance of *C. helgolandicus* were significantly correlated to SST. Fewer regions had significant correlations with the NAOI and abundance changes were generally not related to westerly wind speed (Table 4.1).

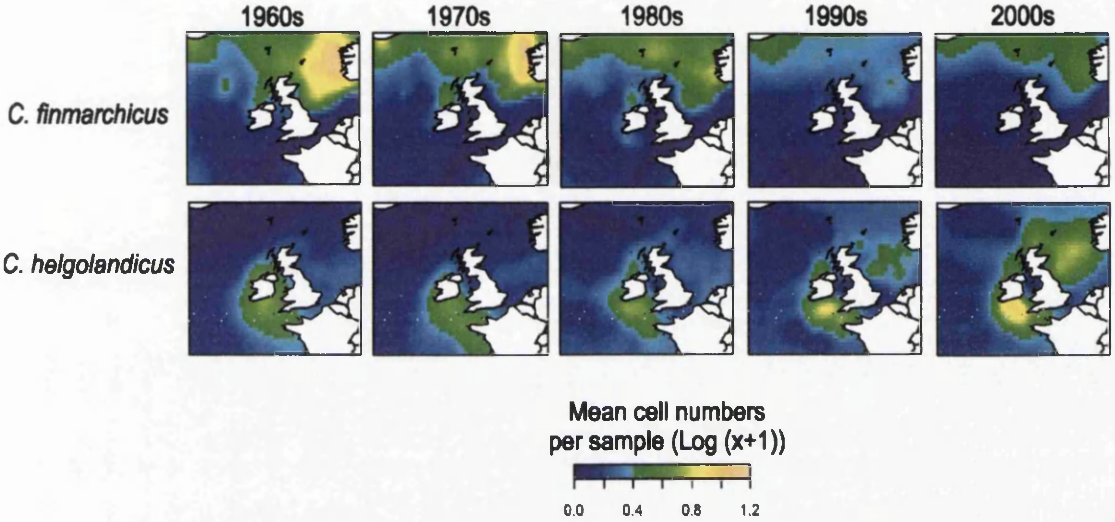

Figure 4.3. Decadal spatio-temporal changes in the abundance of *C. finmarchicus* and *C. helgolandicus*. Colour scale represents decadal mean cell numbers per sample (log (x+1)).

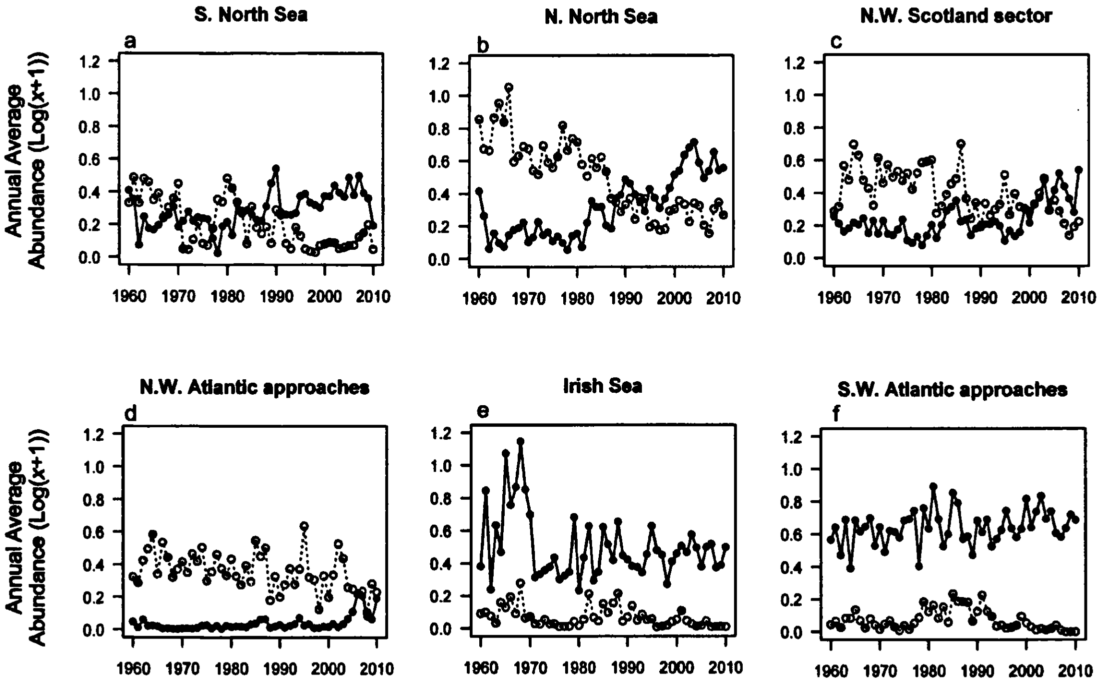

Figure 4.4. Annual average abundance of *C. finmarchicus* (dashed lines with open circles) and *C. helgolandicus* (solid line with solid circles) in six regions of the NE Atlantic between 1960-2010. The scale shows mean number per sample (x) that have been log (x +1) transformed.

Table 4.1. The significance of modified Chelton correlation for *C. finmarchicus* and *C. helgolandicus* against annual SST, annual Westerly Wind speed (WW), and the winter NAOI (* $p<0.05$). The sign of the affect of SST, WW or NAOI on abundance is indicated (+ or -).

	Modified Chelton p-value for correlation coefficient					
			C. finmarchicus			
	SST		**WW**		**NAOI**	
NE Atlantic	0.001*	-	0.11		0.002*	-
S. North Sea	0.0001*	-	0.43		0.03*	-
N. North Sea	0.002*	-	0.04*	-	0.003*	-
N.W. Scotland	0.09		0.29		0.01*	-
N.W. Atlantic	0.46		0.21		0.12	
Irish Sea	0.10		0.46		0.22	
S.W. Atlantic	0.002*	-	0.37		0.72	

			C. helgolandicus			
	SST		**WW**		**NAOI**	
NE Atlantic	0.0004*	+	0.50		0.12	
S. North Sea	0.000003*	+	0.74		0.01*	+
N. North Sea	0.0001*	+	0.53		0.11	
N.W. Scotland	0.004*	+	0.29		0.25	
N.W. Atlantic	0.67		0.12		0.23	
Irish Sea	0.24		0.23		0.02*	-
S.W. Atlantic	0.34		0.71		0.45	

We examined how these plankton relationships with SST, the NAOI and westerly wind speed varied through the time series. To do this we first considered the years 1960-1992, (based on Fromentin and Planque, (1996)), and tested for significant relationships between *Calanus* abundance and SST, the NAOI and westerly wind speed using Pearson's correlation and after adjusting for serial autocorrelation using the modified Chelton method (Pyper and Peterman, 1998). The time series was progressively extended by one year until the full time series (1960-2010) was analysed.

This analysis showed how initially when the *Calanus* abundance time series was 32 years long, the abundance of *C. finmarchicus* and *C. helgolandicus* were both significantly correlated with both westerly wind speed and the NAOI (negatively correlated for *C. finmarchicus* and positively correlated with *C. helgolandicus*) (Figure 4.5 and Figure S4.1). Only *C. helgolandicus* had a significant correlation with SST. However, as the time series lengthened, the significant correlations with the NAOI and westerly wind speed were reduced, with a clear step wise decrease in the correlation in 1996 (Figure 4.5) and the relationship between *C. helgolandicus* and westerly wind speed disappeared. The relationship with SST was enhanced with both *C. finmarchicus* and *C. helgolandicus* showing an increasing significant correlation (Figure 4.5 and Figure S4.1). These changing relationships reflect the patterns of the NAOI and SST, with both variables initially co-varying until the mid 1980s, after which their trends differed.

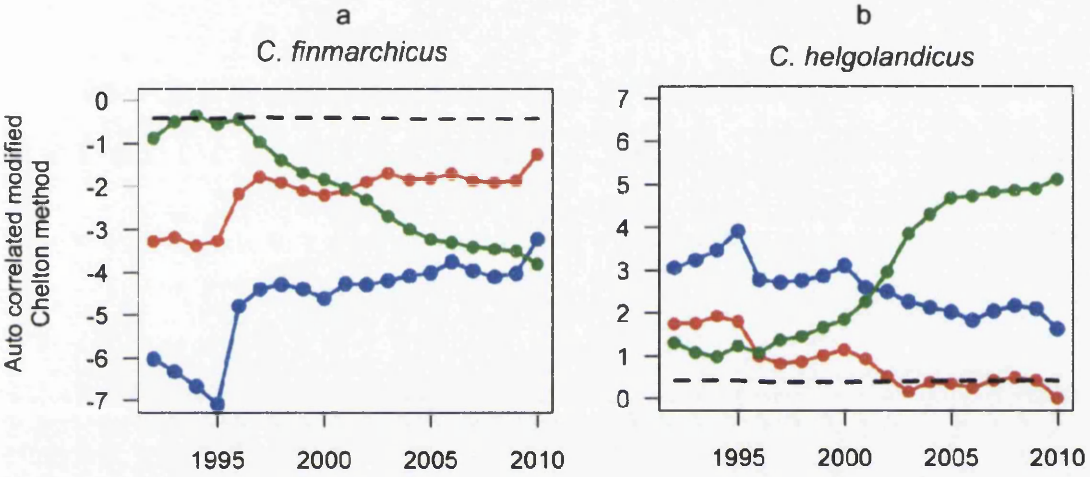

Figure 4.5. Correlation analysis by the modified Chelton method, measuring the changing significance values of the correlation coefficient for the relationships between the NAOI (solid blue line and closed blue circles), annual SST (solid green line with closed green circles), and annual westerly wind speed (solid red line with closed red circles), for the abundance of (a) *C. finmarchicus* and (b) *C. helgolandicus* for the NE Atlantic region. Correlation was first calculated for 1960-1992. Subsequently, one year was added, and at each step a new correlation coefficient calculated until 1960-2010. Black dashed line represents the 5 % significance level (due to the use of the modified Chelton method, a guide to significance is given by the average significance level over the three time series: NAOI, SST, westerly wind speed. Note that in all analyses, specific significance levels are used). The sign of the Chelton method coefficient represents the direction of correlation: negative correlation for *C. finmarchicus* and positive correlation for *C. helgolandicus*.

We tested for Granger causality, where past values of a putative causal environmental variable (SST, westerly wind speed, and the NAOI) are used in a regression model to predict future values of *Calanus* abundance after adjusting for past values of *Calanus* abundance (Thurman and Fisher, 1988). This approach represents a measure of forecasting over and above that provided simply by past temporal changes, and helps point towards a causal link (Thurman and Fisher, 1988). This analysis showed little evidence for Granger causality for either species against SST, the NAOI and westerly wind speed (Table S4.1). Although there was evidence for a significant Granger causality in the NE Atlantic and north west Atlantic approaches for *C. helgolandicus* against SST appearing over a lag of 1 year and 2 years respectively (p=0.03 and p=0.03, Table S4.1). This result was consistent with estimates of cointegration, which was significant at the 10% level (p = 0.076). No significant Granger causality was found for *C. finmarchicus* against SST, although there was some small indication of cointegration (p = 0.15). No evidence for cointegration or granger causality was found for the NAOI or wind.

Although the decline of *C. finmarchicus* and the increase in *C. helgolandicus* appear to have continued unabated (as seen in Figure 4.3 and Figure 4.4) and the long-term relationship with SST appears to have strengthened (Figure 4.5) we looked carefully for any evidence of adaptation within the decadal data. For each decade, we generated a SST/abundance response curve by plotting mean observations across the 6 regions defined in Figure 4.1. Due to the varying latitude, the expected abundance for each species can be shown across a wide range of SST levels. We tested for adaptation by comparing the fit of a model that assumes a constant relationship between SST and abundance with one that fits each decade separately. We used linear regression models, with quadratic terms as required. The fit of competing models was compared with likelihood ratio tests, and the regression slopes investigated for evidence of changes in the relationship between abundance and SST over time. All analyses were performed using the statistical environment R (R, 2009).

The decadal inter-regional temperature response relationships between *C. finmarchicus* abundance and SST is shown in Figure S4.2. There is a clear non-linear relationship, with the greatest declines in *C. finmarchicus* expected across the lower temperatures. A likelihood ratio test shows a highly significant change in the relationship between the decades (p = 0.00067). The decadal pattern is one of a steady, and generally consistent decline in the slope of the relationship between the 1960s and 2000s (see Table S4.2. for regression equations).

The pattern for *C. helgolandicus* is not nearly as strong. First, the relationship between SST and abundance is (on this scale) linear. Second, the constrained model, with a common slope for the whole time period is favoured in the likelihood ratio test, i.e. there is no statistically significant decade effect as might be expected from adaptation (p = 0.54). However, as can be seen in Figure S4.2, if individual decade models are fitted, they again display a steady and consistent flattening of the abundance/SST relationship over time.

Discussion

The central finding from our study is that decreases in the abundance of *C. finmarchicus* and increases in the abundance of *C. helgolandicus* in the NE Atlantic region have been maintained in recent years in the CPR records and that these long-term (51-year) decreases and increases in abundance are strongly linked to SST.

There were particularly prominent changes in *Calanus* abundance in certain areas. For example, in the northern North Sea, *C. finmarchicus* has shown a dramatic decrease in abundance compared to previous years, while *C. helgolandicus* has shown over a 3 fold increase in abundance since the 1960s. This huge reduction in *C. finmarchicus* abundance may partially reflect a movement to a greater depth. The CPR samples in the upper 10m of the water column, but recent net sampling has shown that appreciable numbers of *C. finmarchicus* continue to reside at greater depths in the North Sea (Jonasdottir and Koski, 2011). So as well as a northerly contraction of range, *C. finmarchicus* may also be shifting its depth distribution. Similarly it has been suggested that those North Sea fish that prefer cooler water, have shifted their distribution to greater depths over recent years (Dulvy et al., 2008).

Aspects of the life-history of *Calanus* species are well known. In particular in the NE Atlantic region *C. finmarchicus* is known to overwinter in deep water (>500m) off the shelf as a juvenile stage (copepodite IV), surviving on stored lipid (Heath et al., 2004). Then at the end of the winter the population ascends to the surface and metamorphoses into the adult stage and reproduces at the surface. In this way egg production is timed so that the developing next generation is present when maximal food abundance is occurring during the spring plankton bloom (Niehoff et al., 1999).

The temporal correlations (Figure 4.5 and Figure S4.1), consistent decadal trends (Figure 4.3), and causal models, all point towards SST as a strong driver of changes in *C. helgolandicus* abundance. Granger causality was not detected for *C. finmarchicus*. This may indicate a process that is primarily driven by variables other than temperature (though correlated with SST), but could also be due to other factors. Our time series, despite their unusual length, may still be too short to identify a causal link. The use of de-trended data, while important to avoid false positives, can also serve to reduce important signals in the data, making our Granger models potentially very conservative (see Kang, (1985)). Finally, we note that the Granger model is designed to detect linear causality. This may be particularly appropriate for *C. helgolandicus*, however Figure S4.2 shows that the response of *C. finmarchicus* to temperature may be more severe at the lower temperature ranges. We consider our time series too short for new methods designed for non-linear Granger causality (see Marinazzo et al., (2011)).

Whilst performing the granger causality tests we noted the strong autocorrelation over the period of 1 year for both species. i.e. abundance at year *t* was a very good predictor of abundance at year *t+1*. This implies that the number of eggs, as well as their survival rate to adulthood may be important in determining adult abundance in any year. The processes that drive the survival of *Calanus* from egg through to nauplius and copepodite stages are not well known but are presumably linked to prey availability, which in turn may be driven by environmental conditions. In addition, advection of individuals may be important in driving the levels of abundance recorded at sites on the shelf (Beare et al., 2002; Reid et al., 2003b). Sea temperature is clearly strongly related to *Calanus* abundance across years and this link may be related to a direct causal link, i.e. species thermal preferences, or an indirect effect of SST being linked to other conditions such as food availability.

It was suggested that there may have been a fundamental regime shift in the NE Atlantic in 1996 as evidenced by the break-down of a previously significant relationship between *Calanus* abundance and the NAOI (Planque and Reid, 1998). Our analysis of extended time series shows how links between plankton abundance and environmental parameters change over time, with the strength of the correlation between *Calanus* abundance and the NAOI and westerly wind speed reducing as the time series has extended from 30 to 50 years. However, the extended time series has shown that the relationship between *Calanus* abundance and SST has increased in strength. Hence the regime shift might simply reflect a change in the relationship between various environmental indices, only some of which are casually related to *Calanus* abundance.

Given the generally NE flows of the North Atlantic Current it might be argued that the abundance of *Calanus* in the NE Atlantic might also be related to the abundance in distant sites in the western Atlantic. However, two lines of evidence argue against this scenario. First for both *C. finmarchicus* and *C. helgolandicus* there is evidence for genetic differentiation between populations across the North Atlantic region (Bucklin et al., 2000; Unal and Bucklin, 2010; Yebra et al., 2011) which suggests there is limited gene flow across very broad scales. Second the trans-Atlantic drift time is fairly slow. For example, drift times from Newfoundland to northern Europe estimated from satellite tracked Lagrangian drifter buoys and oceanographic particle tracking models are of the order of several years (Scott et al., 2012); likewise the Great Salinity Anomaly took several years to travel from the NW Atlantic to Northern Europe (Dickson et al., 1988). Hence levels of *Calanus* abundance in the NW Atlantic in any particular year, will not be able to directly influence levels of abundance in that year in the NE Atlantic.

In summary, our results show the continued decline in abundance in *C. finmarchicus* in the NE Atlantic and point to the lack of adaptation to changing conditions and a corresponding continued 51 year increase in *C. helgolandicus* abundance. The increasingly strong links of these plankton changes to SST point to these trends being maintained if predicted increases in SST occur over further decades.

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Supplementary Material

A 50 year time series for ocean plankton in the face of climate change

Hinder, S.L., Gravenor, M.B., Edwards, M., Ostle, C., Lee, P.L.M., Walne, A.W. and Hays G.C.

Table S4.1. Granger-causality in the relationships between *C. finmarchicus* and *C. helgolandicus* abundance versus annual SST and annual westerly wind speed (WW) and the winter NAOI. Significant relationship (p<0.05) indicated by *. Lag time scale represents years.

		Granger-Causality					
		C. finmarchicus			***C. helgolandicus***		
	Lag	**SST**	**WW**	**NAOI**	**SST**	**WW**	**NAOI**
North Atlantic	1	0.52	0.73	0.52	0.03*	0.77	0.34
	2	0.34	0.51	0.31	0.10	0.50	0.60
	3	0.61	0.24	0.24	0.18	0.49	0.16
S. North Sea	1	0.63	0.37	0.47	0.44	0.47	0.64
	2	0.49	0.59	0.23	0.39	0.20	0.70
	3	0.75	0.87	0.11	0.17	0.13	0.17
N. North Sea	1	0.83	0.97	0.80	0.27	0.43	0.63
	2	0.42	0.11	0.70	0.42	0.59	0.70
	3	0.19	0.08	0.66	0.46	0.08	0.06
N.W. Scotland	1	0.64	0.08	0.12	0.48	0.83	0.39
	2	0.94	0.02*	0.11	0.23	0.91	0.53
	3	0.97	0.03*	0.08	0.20	0.74	0.78
N.W. Atlantic	1	0.56	0.87	0.47	0.48	0.52	0.67
	2	0.90	0.25	0.07	0.03*	0.62	0.77
	3	0.68	0.29	0.15	0.23	0.43	0.56
Irish Sea	1	0.17	0.13	0.37	0.53	0.78	0.32
	2	0.21	0.41	0.71	0.08	0.97	0.90
	3	0.08	0.57	0.62	0.50	0.98	0.66
S.W. Atlantic	1	0.60	0.35	0.46	0.35	0.64	0.59
	2	0.63	0.79	0.71	0.68	0.20	0.76
	3	0.61	0.88	0.81	0.56	0.25	0.83

Table S4.2. Coefficients of the regression models for the temperature / abundance response curves for *C. finmarchicus* and *C. helgolandicus*.

	C. finmarchicus		
Model	**Intercept**	**Linear Coefficient**	**Quadratic Coefficient**
All data	*5.49*	*-0.87*	*0.04*
1960s	7.40	-1.16	0.05
1970s	8.97	-1.51	0.06
1980s	5.60	-0.92	0.04
1990s	3.85	-0.62	0.03
2000s	3.56	-0.54	0.02

	C. helgolandicus		
Model	**Intercept**	**Linear Coefficient**	**Quadratic Coefficient**
All data	*-0.92*	*0.12*	-
1960s	-1.24	0.15	-
1970s	-1.16	0.14	-
1980s	-0.93	0.12	-
1990s	-0.74	0.10	-
2000s	-0.41	0.08	-

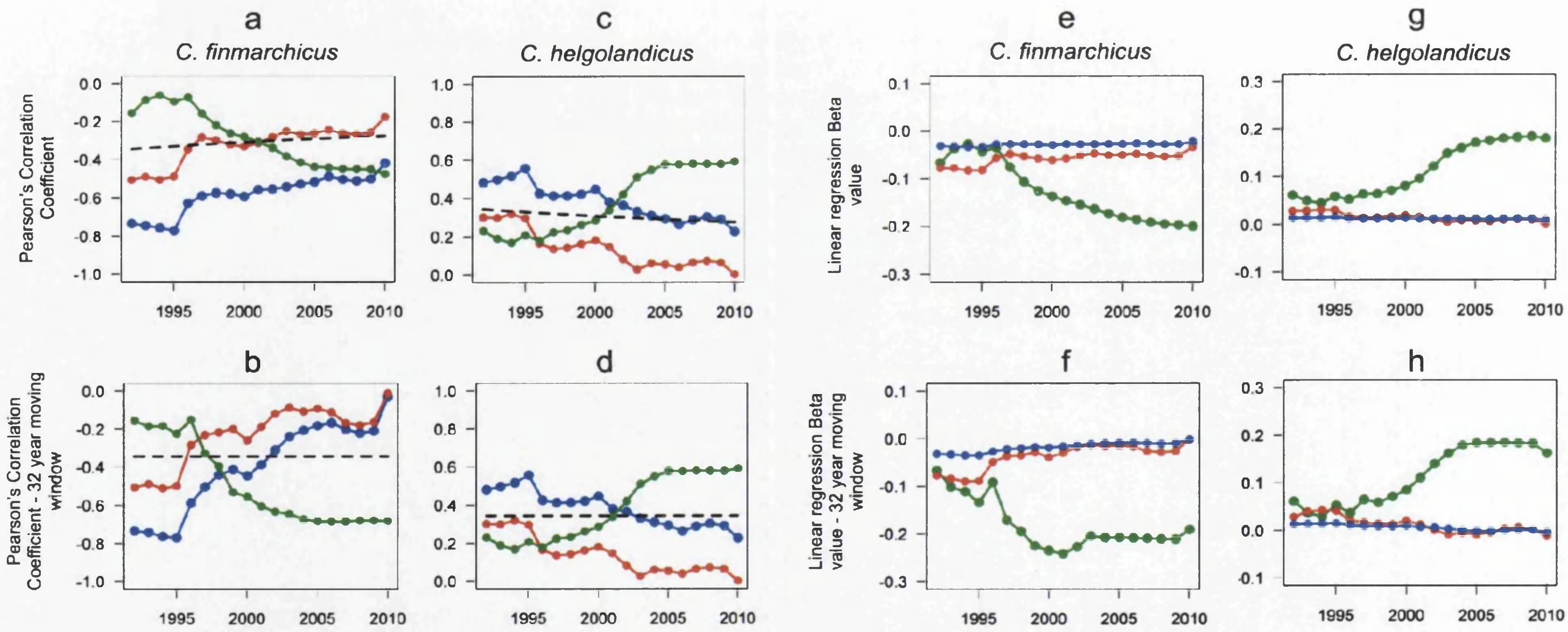

Figure S4.1. Correlation analysis by (a, c) Pearson's correlation, (b, d) a 32-year moving window Pearson's correlation, (e, g) linear regression coefficient (beta value), and (f, h) a 32-year moving window of linear regression coefficient (beta value). Here we monitor the changing significance values of the relationships between the NAOI (solid blue line and closed blue circles), annual SST (solid green line with closed green circles), and annual westerly wind speed (solid red line with closed red circles), for the abundance of both *C. finmarchicus* and *C. helgolandicus* for the NE Atlantic region. (a-d) Black dashed line represents the line of 5% significance. (a-h) Values indicate a general negative correlation for *C. finmarchicus* and positive correlation for *C. helgolandicus*.

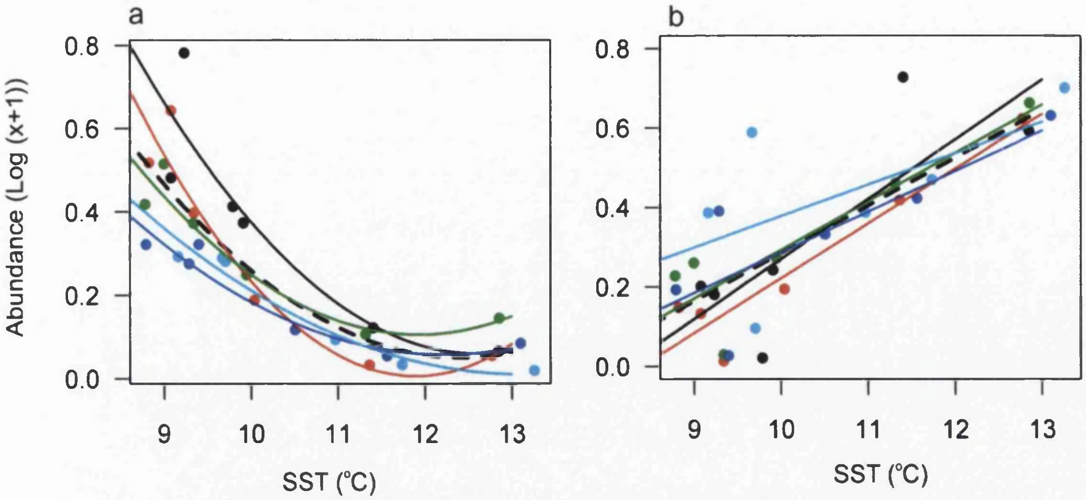

Figure S4.2. Decadal inter-regional temperature response relationships for (a) *C. finmarchicus* and (b) *C. helgolandicus* abundance. The mean values were used from six regions (A-F) in each decade. C. *finmarchicus* was best fitted with a quadratic function and C. *helgolandicus* with a linear regression. 1960s: solid black circle and solid black lines; 1970s: solid red circles and solid red lines; 1980s: solid green circles and solid green line; 1990s: solid dark blue circles and solid dark blue line; 2000s: solid light blue circles and solid light blue line. Black dashed line is the (a) quadratic or (b) linear regression for all data. The model for decadal changes in the regression coefficients was statistically significant for *C. finmarchicus* only.

Has stratification changed in the NE Atlantic?

Hinder, S.L., Bodger, O.G., Gravenor, M.B., Edwards, M., Walne, A.W. and Hays, G.C.

SLH obtained the climate data. SLH and OGB developed the index with help from both GCH and MBG. SLH and OGB performed the statistical comparison methods. SLH produced all the figures. Interpretation of the methods and the data was performed by SLH, OGB, GCH and MBG. SLH led the writing of the paper with contributions from all authors.

Abstract

The NE Atlantic Ocean has experienced dramatic alterations in the biological community in recent years, with large spatial and temporal changes observed for plankton taxa. A number of southern and pseudo-oceanic temperate plankton taxa have shifted, by 10° of latitude in the NE Atlantic. Conversely, the diversity of colder-temperate, subarctic and arctic plankton taxa has shown a similar range contraction. These changes have often been associated with sea surface temperature (SST). However studies have shown that the temperature isotherms have not moved as far as the plankton. SST could have an indirect effect on the environment such as the timing and degree of the thermocline (summer stratification). However, in most large scale plankton distribution studies, the level of stratification is rarely directly measured. Using over 55,000 temperature depth profiles from 1970-2009, we developed a new technique to estimate the level of stratification by modelling the whole available temperature profile. We found relatively stable seasonal patterns throughout the NE Atlantic, with no significant shifts in the onset of stratification over the four decades. Significant changes in the intensity of the thermocline were found, however these changes were not uniformly shifting northwards, but show a more variable pattern. We propose that the index has wide applicability for use in the study of the complex interaction between climate and changing marine ecosystems.

Introduction

The NE Atlantic Ocean has experienced dramatic alterations in the biological community in recent years, with large spatial and temporal changes observed for plankton taxa. The most substantial latitudinal changes were observed by Beaugrand et al., (2002) and (2009), who demonstrated that a number of southern and pseudo-oceanic temperate plankton taxa have shifted, by 10° of latitude in the NE Atlantic. Conversely, the diversity of colder-temperate, subarctic and arctic plankton taxa has shown a similar range contraction (Beaugrand et al., 2002).

The causes of these dramatic spatial changes are not fully understood. Temperature is often the main factor considered when investigating latitudinal gradients in plankton biodiversity (Beaugrand et al., 2010; Rombouts et al., 2009), with sea surface temperature (SST) as one of the best predictors of the spatial distribution of some marine taxa in the NE Atlantic (Beaugrand, 2009; Beaugrand et al., 2010). Changes in temperature in the NE Atlantic Ocean have occurred at both a local scale and a basin-wide scale over the last few decades. Levitus et al., (2000) has shown that the heat content of the world oceans (top 300m) has markedly increased since the mid 1950s. The local SST in the North Sea has shown a mean increase of about 1.1°C, shifting it from a boreal into a temperate system (Beaugrand et al., 2008). Despite these warming conditions, these changes actually do not directly account for the large 10° shift in latitudinal distribution of the key indicator species. Hobson et al., (2008) has shown that the present warming conditions are no more persistent than those encountered 150 years ago, and despite some isotherms showing a more northerly tendency in recent years, they have not strayed beyond the extremities of temperature in previous years. i.e. The temperature isotherms have not moved as far north as the plankton.

An alternative explanation for these pole-ward shifts, which are still statistically linked to temperature, could be a change in the intensity and location of the seasonal thermocline (summer stratification), which is defined as a vertical zone of rapid temperature change in the water column located below the surface layer of rapid mixing (Kaiser et al., 2005; Reygondeau and Beaugrand, 2011). At high latitudes temperature profiles vary seasonally, with a shallow, strong, seasonal thermocline during spring and summer, a deep thermocline in autumn, and no evidence of stratification in winter. The structure of the water column is thought to have a considerable influence on the spatial, vertical and horizontal distribution of plankton (Reygondeau and Beaugrand, 2011) and therefore could potentially explain the dramatic pole-ward shifts. Several studies have examined the structure of the water column using oceanic profile data, and Reygondeau and Beaugrand, (2011) have classified these threshold methods of estimating the thermocline into two broad categories: (i) depth-to-depth temperature or density difference (Defant, 1961; Kara et al., 2000) and (ii) temperature or density difference between a reference depth and other depths in the profile (Levitus, 1982). However, most of these studies focus on the Mixed Layer Depth (MLD), which is categorized as a separate hydro-dynamical feature to the thermocline (Sprintall and Roemmich, 1999). These methods are also sensitive to instrument variability, with problems of resolution of temperature depth profiles, and rapid environmental variation over short depth ranges.

Taking these issues into account, a new technique of examining the structure of the temperature depth profiles has been created, based on modelling the whole temperature profile. This new method was applied to over 55,000 temperature depth profiles, with the aim of determining if the seasonal thermocline has changed, spatially and temporally, in the NE Atlantic between 1970-2009.

Methods

Physical data

Temperature depth profiles were sourced from the World Atlas Ocean database for the NE Atlantic (35°N-65°N, 20°W-10°E). This area was chosen based on the pole-wards shift in species composition observed by Beaugrand et al., (2002) and the sea surface isotherms examined by Hobson et al., (2008). These profiles consisted of three sources of temperature profiles: Ocean Station Data (OSD), Conductivity Temperature Depth profilers (CTD), and eXpendable BathyThermographs (XBT) for the period 1970-2009.

Monthly mean gridded (1° x 1°) summer surface scalar wind speed (m/s) for 1970-2009 was obtained from The International Comprehensive Ocean Atmospheric Dataset (http://www.ncdc.noaa.gov/oa/climate/coads/). Data are summarized for a given latitude and longitude for the summer months: June, July, August and September, for the NE Atlantic (35°N-65°N, 20°W-10°E).

Calculation of a stratification index

To allow for comparisons across regions, and to minimize any potential bias in the profile data due to noise from instrument deployment or diurnal fluctuations of water temperature (de Boyer Montégut et al., 2004; Reygondeau and Beaugrand, 2011), all temperature depth profiles were taken from 2 to 200m. If a profile did not have a value at the 2 or 200m point, the value was interpolated (with a linear function) based on the preceding and succeeding value. If the profile did not fit our specification i.e. allow for interpolation, the profile was removed. Additionally, only profiles which had a maximum gap, between observations, of no more than 50m were selected (maximum gap interval). This was done, after trial and error, to reduce estimated bias caused by large gap intervals (see Figure S5.6, and Supplementary material on Resolution of data).

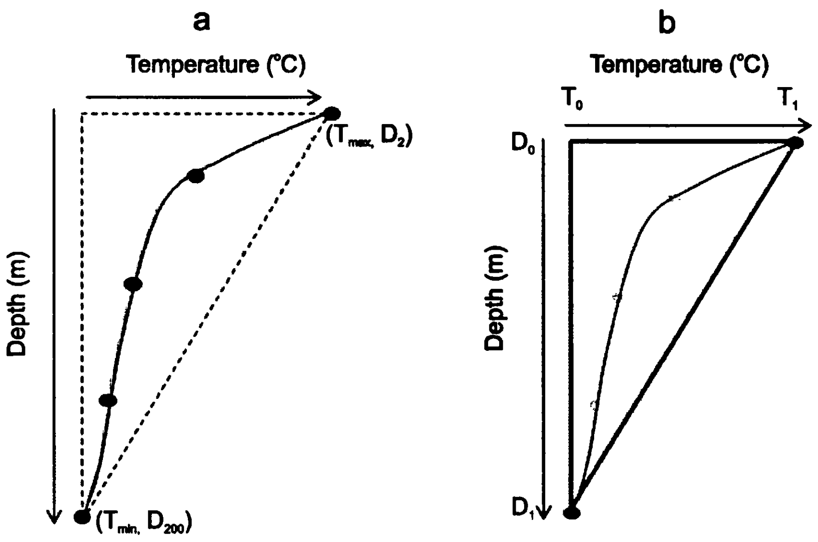

Figure 5.1. Schematic of the stratification index method. (a) theoretical profile with linear function from maximum temperature (T_{max}) to minimum temperature (T_{min}). The proportion of the triangle given by the grey shading represents the degree of stratification that we wish to calculate. (b) Example of triangle scaled over a depth 0-1, and temperature 0-1, with a stratification index of 0.5 (shaded area). This represents perfect stratification.

The stratification index for each profile is determined by measuring the area produced when the individual points deviate from a straight line, created by the maximum and minimum temperature values (Figure 5.1a, Equation 1-3). With the assumption that the maximum temperature value occurs at 2m, and the minimum temperature occurs at 200m.

Equation 1. $$Area\ 1 = \sum_{i=2}^{200} 0.5 \times \left(T_i + T_{(i+1)}\right) \times \left(D_{(i+1)} - D_i\right)$$

Equation 2. $$Area\ 2 = Area\ 1 - \left(T_{min} \times \left(D_{200} - D_2\right)\right)$$

Equation 3. $$SI = 0.5 - \left(\frac{Area\ 2}{\left(D_{200} - D_2\right) \times \left(T_{max} - T_{min}\right)}\right)$$

One of the problems with using temperature depth profiles over a large spatial location and seasonal time period is the range of temperatures at which these profiles occur. For example, water around Iceland and the North Sea would be colder than the Spanish Coast and profiles taken in the winter months would be cooler than profiles taken from summer months. We found, while exploring a wide range of stratification indices that unless these effects can be controlled for then each index was strongly confounded by latitude and longitude, a fatal flaw for any index that is to be used to explain geographic trends. To correct for this problem and enable our method to compare profiles from the different temperature ranges, the temperature and depth values for all profiles were rescaled to 0-1 (Figure 5.1b, Equation 2). Some of the previous methods developed to estimate the thermocline or MLD, used a threshold value to distinguish between different water types (stratified or mixed) (Defant, 1961; Levitus, 1982; Reygondeau and Beaugrand, 2011). This was not needed for the method given here. Re-scaling the temperature and depth values to 0-1, meant that the stratification index had a range of -0.5 to 0.5. All positive values implied that the water column shows stratified water, with a value of 0.5 being the most stratified (Profile 1, Figure S5.5b). Values near zero represent a homogenous water column, and negative values suggest the profiles have a surface temperature inversion or a dicothermal layer (Profile 6, Figure S5.5b).

Temperature profile patterns

During the process of developing the method, the 2m and 200m temperature values were used to estimate the area for each profile. However, this caused problems on occasions when the maximum and minimum temperature values did not actually occur at the 2 and/or 200m value, i.e. a temperature inversion at the surface or a dicothermal layer (Figure 5.2). If the maximum / minimum temperature values are greater than the 2m / 200m value, then the area produced can be greater than 0.5, outside the scale of our proposed index. We therefore tested for these anomalies in the temperature profile and adjusted the calculation of the index to use the true maximum and minimum temperature values instead.

We found that a significant number of profiles (7,161 and 6,032) had their maximum values exceed the 2m observation, or their minimum values differ from the 200m observation >1°C. These profiles were referred to as "volatile profiles" and the spatial location of these profiles was examined. These profiles were also separated into two groups; those with a maximum value that differs by over 1°C in comparison with the 2m value (surface profiles), and those with a minimum value that varies over 1°C from the 200m value (depth profiles). It should be noted some of these patterns occurred within both groups.

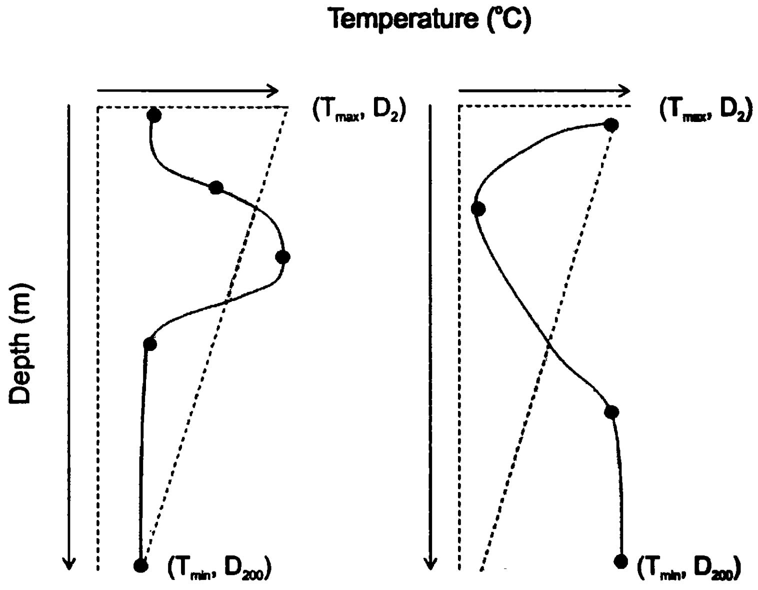

Figure 5.2. Two example profiles where the maximum and minimum temperature values are not at 2 and 200m. Grey shading shows the area that is now used to calculate the stratification index.

Seasonal structure

To estimate the summer seasonal structure of the water column for each decade, only the months April-October were examined. Profiles and their estimated stratification index values were separated into six 5° latitudinal bands (35-40°N, 40-45°N, 45-50°N, 50-55°N, 55-60°N and 60-65°N) and due to sample size restrictions only measured from 20-5°W. The separation of data was to reduce estimated bias caused by latitudinal effects.

Statistical analysis

All profiles were grouped into 5°/5° latitude and longitude grid cells (Figure S5.1, see Supplementary material on Correction bias). A Z-score was calculated for the maximum and minimum temperature values for each cell and for each decade. To remove extreme outliers, caused by large temperature fluctuations within a profile, a Z-score greater or less than 3 was removed (645 profiles) (Figure S5.2)

To estimate if the thermocline has changed temporally, comparisons were made across two seasonal time periods: annual and summer (months: June, July, August and September). Each seasonal time period was corrected for the bias introduced from the method (see Supplementary material on Correction bias), and weighted so that the relevant months contributed equally. This eliminated any possible error caused by a variable number of profiles within each month. A Mann-Whitney U test in SPSS (version 16.0.2) was used to compare the stratification index and the temperature at 2 and 200m for the 1970s against the 2000s data.

As a large number of tests are performed, to control for false positive rates, we assume the global null hypothesis “all significant results are false positives”. A bootstrapping of 10,000 simulations of 25 trials was applied to assess the probability of any given number of significant results.

Monthly stratification index

The structure of the water column shows clear seasonal patterns from April to October. The expected pattern giving some confidence as to the validity of the proposed index. The onset of stratification starts to occur in May, peaking during the summer (June to August) (stratification value above 0.15), and de-stratifying during October (Figure 5.3). All four decades show a similar pattern with no clear indication that stratification index is occurring earlier or later during the season over time.

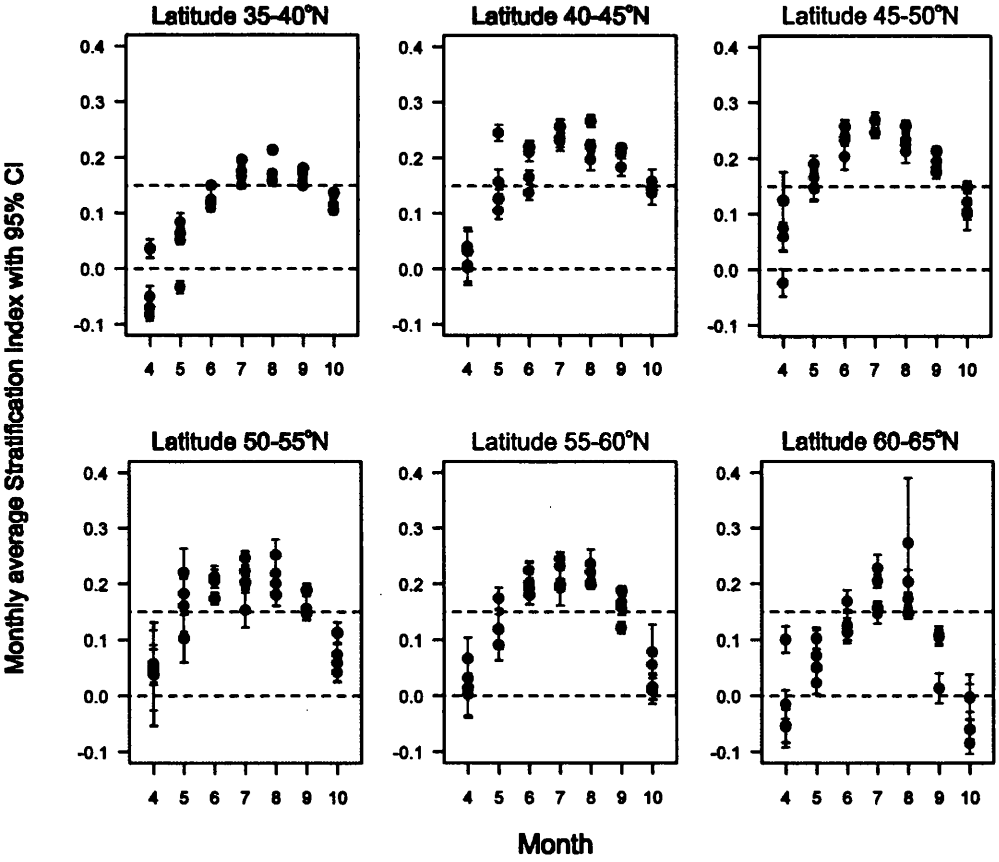

Figure 5.3. Monthly average stratification index with 95% Confident Limits for six latitude and for four decades in the NE Atlantic (35-65°N, 20-5°W). 1970s: black closed circles and black lines; 1980s: green closed circles with green lines; 1990s: blue closed circles and blue lines and 2000s: purple closed circles and purple lines. Dashed black lines represent the stratification index at 0 and 0.15, with 0 representing a homogeneous water column and water above 0.15 having a notably stratified water column.

Spatial distribution

The spatial coverage of the temperature depth profiles is relatively similar throughout the four decades, except for a large area (55-65°N, 20-5°W) of missing profile data in the 2000s (Figure 5.4). The stratification index, during the summer months, ranges between 0.1 and 0.3 (Figure 5.4), with lower stratification index values found in the southern part of the NE Atlantic and the water just above Scotland. Summer temperature values at 2 and 200m, in the NE Atlantic, show a clear latitudinal gradient, with water near the coast of Portugal with the highest temperatures (35-40°N, 20-5°W) and the water near Iceland and Norway being the coldest. The water temperature at 2m is warmer throughout the NE Atlantic compared to the temperature at 200m.

In the NE Atlantic, summer surface scalar wind speed ranges between 6-8 m/s and does not show a latitudinal gradient pattern. A noticeable increase in scalar wind has occurred since the 1970s, with some regions (50-55°N, 20-10°W) in the 2000 decades showing a 10-12 m/s wind speed.

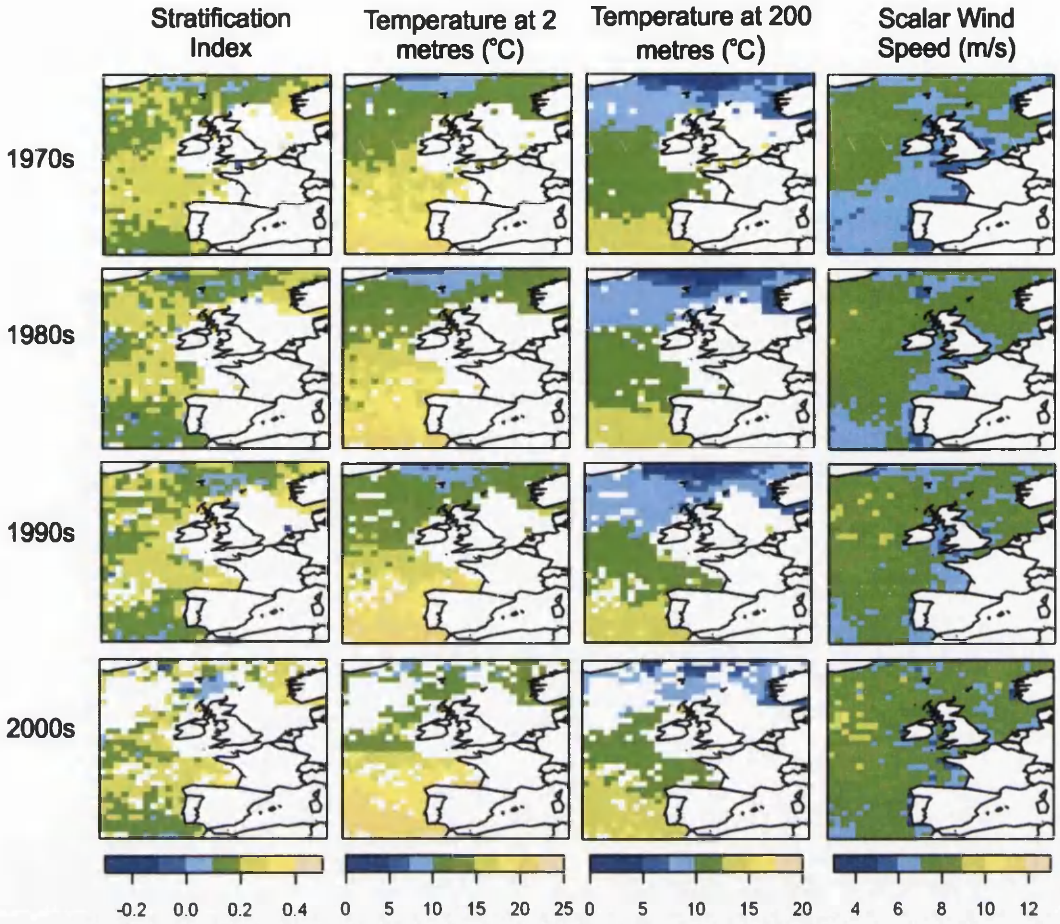

Figure 5.4. Summer stratification index, summer temperature at 2 and 200m (°C), and summer surface scalar wind speed (m/s) for profiles in four decades (1970s, 1980s, 1990s and 2000s) in the NE Atlantic (35-65°N, 20°W-10°E). White spaces represent no profiles.

Spatial correlations

The stratification index and temperature at 2 and 200m have significantly changed, since the 1970s, in the NE Atlantic (13-24 cells) (Figure 5.5). Using the estimated distributions calculated from bootstrapping, the probability of our number of significant results being due to false positives are highly unlikely ($p<0.001$ for 13 significant cells), therefore the global null hypothesis can be rejected and our results are not simply a result of multiple testing.

The annual data indicates that the relationship between the change in stratification index and location is not clear, with cells showing irregular and disparate patterns (9 cells decreasing, 7 cells increasing) (Figure 5.5). The region, 45-60°N, 20-10°W, shows a large decline in stratification, while the area, 35-50°N, 10-5°W, demonstrates a significant increase. The differences in summer show a more uniform pattern with the waters around 35-55°N, 20-10°W, declining in stratification, while the more coastal waters around southern England and west of France, signify an increase in stratification. There are disagreements, for both the summer and annual results, with the water around 60-65°N, 10°W-10°E, large significant changes in both directions within the area are observed, which could be down to the number of profiles within this region.

The water temperature at 2 and 200m demonstrates a relatively constant increase for both the annual and summer data. There is a region in the west Atlantic, 45-60°N, 20-10°W, which has shown no significant change in water temperature at 2m for summer data, and a decline in water temperature for the 2m annual data (Figure 5.5).

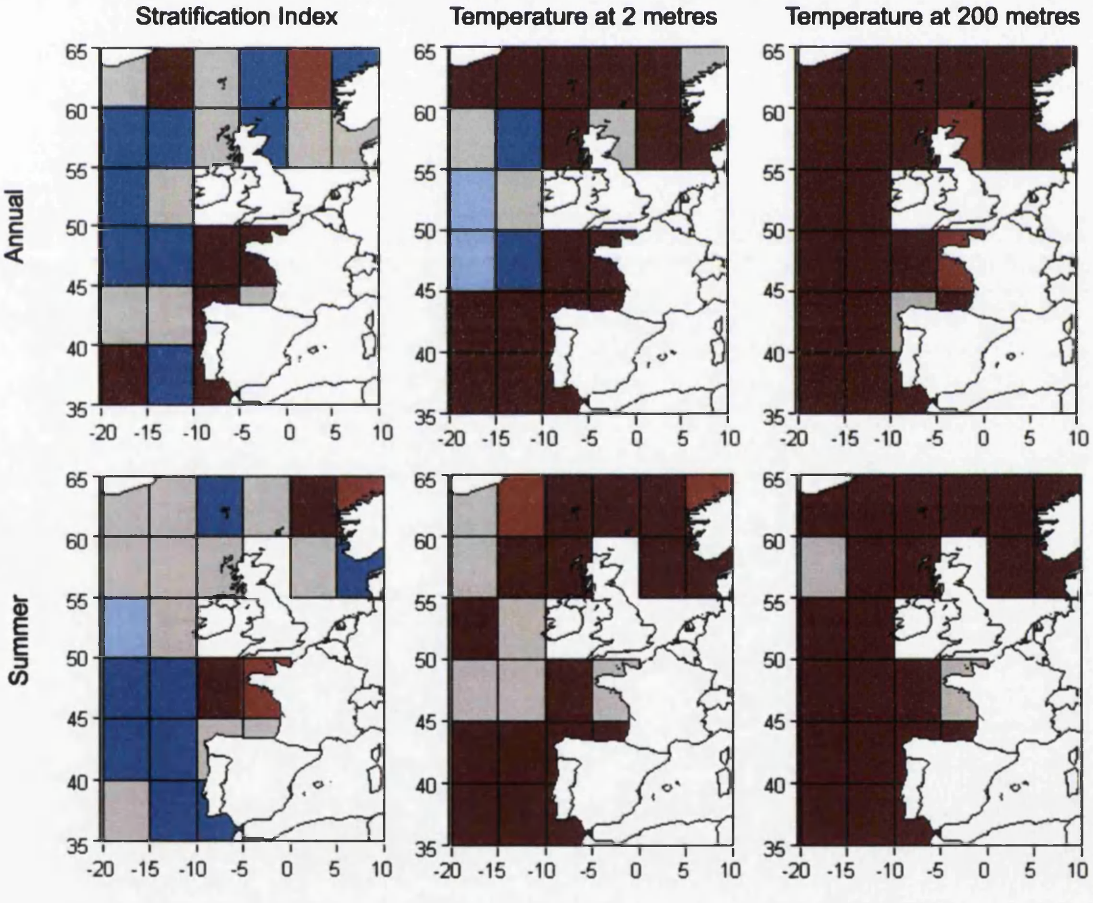

Figure 5.5. Changes between the 1970s and 2000s data, using a Mann Whitney U test, for stratification index, temperature at 2 and 200m for both annual and summer data for each 5° latitude and longitude cell. White zones = no profiles, dark blue = decline in 2000s ($p<0.01$), light blue = decline in 2000s ($p<0.05$), grey = no significant change, light red = increase in 2000s ($p<0.05$), dark red = increase in 2000s ($p<0.01$).

Volatile profiles

Profiles that have been classified as volatile, with maximum and/or minimum values varying by over 1°C compared to the 2 and/or 200 m value, mainly occurred in two locations: the opening water around the Mediterranean Sea (35-45°N, 20-6°W) and the coastal waters off Norway (Norwegian Trench) (55-65°N, 0-10°E) (Figure 5.6).

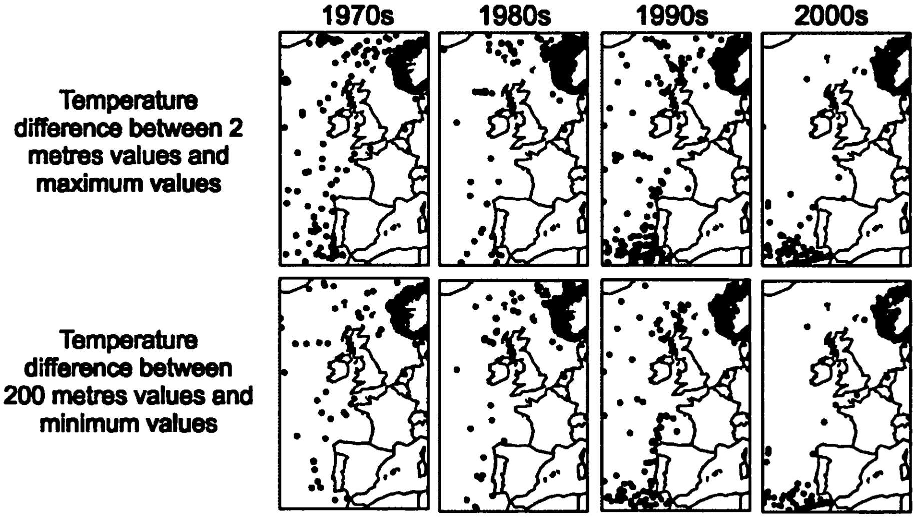

Figure 5.6. Location of the volatile profiles with a minimum temperature difference of 1°C at the 2 and 200m depths for each of the four decades.

Norwegian coastal waters

The great majority (> 94%) of volatile profiles occurred in the Norwegian coastal region (Table S5.1), with a decline in the number of profiles since the 2000s for both surface and depth regions. The peak frequency of these profiles occurred from November to May for the surface profiles and from January to May for the depth profiles (Figure S5.3). When examining a sample of these profiles, only the top few metres of the water column changed with an approximately 5-10°C temperature difference, while the rest of the profile remains relatively constant. This is known as surface thermal inversion (Kara et al., 2000) (Figure S5.4a, b). Fewer of these profiles occurred at the 200m depth.

Outflow from the Mediterranean Sea

The changes in the area around the outflow of the Mediterranean Sea seemed to be more of a recent effect, with a significant increase in the number of profiles since the 1990s (Table S5.1). The volatile profiles occurred throughout the year but a sharp peak in June for the 2000 decade was present (Figure S5.3) for both surface and depth regions. When this sharp peak was examined, it was found that all profiles were taken from the same boat transect, suggesting faulty equipment during sampling. No clear pattern can be observed in the example profiles, in contrast to the Norwegian coast. Temperature differences varied greatly, with some profiles showing over a 20°C sharp increase (Figure S5.4c, d).

Discussion

The technique to estimate the index of stratification was achieved by modelling the standardised complete temperature profile. Our aim with this approach was to avoid bias associated with instrument noise and variations in sampling intervals and absolute temperature values, all of which were found to affect simpler indices. The index is therefore appropriate for making comparisons between locations and time periods. In comparison with other methods, we do not rely on smoothing techniques, interpolation of points, or an *a priori* threshold value to distinguish between water temperature profiles.

From this method, the seasonal distribution of the water column stratification showed a relatively stable pattern throughout the NE Atlantic. The re-stratification period was detected in April-May, which is in agreement with Levitus, (1982), Defant, (1961) and Reygondeau and Beaugrand, (2011) when measuring oceanic water using a threshold method of 0.15° $5m^{-1}$. The peak stratification was observed in June-September, which is also shown by Reygondeau and Beaugrand, (2011) using a threshold method of $0.4^{\circ}C$ $5m^{-1}$. Our index detected de-stratification of the water column in October, which is in agreement with Levitus, (1982). However, Reygondeau and Beaugrand, (2011) described the start of the de-stratification a month earlier (September) for both nertic and oceanic water types.

It has been suggested that the seasonal timing of the establishment of the thermocline is shifting (Walther et al., 2002). This type of change has already been observed in plankton taxa, with alterations in the phenology of taxa in the North Sea over the last two decades (Beaugrand, 2009; Reid et al., 1998). These responses are thought to be species dependant, but several studies have shown that within the North Sea, dinoflagellates and copepods showing peak abundance, on average, 23 and 10 days earlier (Edwards and Richardson, 2004). While diatom bloom duration and seasonal timing have remained largely unchanged (Edwards and Richardson, 2004). However, from our index, no significant shifts in the onset of the thermocline over the four decades were observed, and the changes in plankton phenology did not track the stratification index.

In the NE Atlantic, large spatial and temporal changes have been observed for both dinoflagellates and copepods, with a dramatic 10° latitudinal shift for some taxa. Although, significant changes in the intensity of the thermocline have been found, these changes do not appear to be uniformly shifting northwards, but demonstrate a more variable pattern. The southwest region of the NE Atlantic has shown a considerable decrease in the stratification index for both annual and summer seasons, while the water around the French and English coast have shown an increase. Therefore simple assumptions that the thermocline intensity is the cause of species shifting northwards no longer apply, and a more complex mechanism underlies these patterns. Hinder et al., (2012) demonstrated that within the North Sea, some plankton taxa were affected by an interaction of SST and wind combined. For example, *Pseudo-nitzschia seriata* showed high abundance only when both SST and wind were high; if either variable was low, *P. seriata* abundance was also low.

An increase in summer surface scalar wind speed has occurred since the 1970s throughout the NE Atlantic. Windier conditions could have a negative impact on stratification that overrides effects of rising temperature. An increase in wind introduces turbulent kinetic energy, which will stimulate vertical mixing (Mann and Lazierm, 1996) and potentially the breakdown of the stratification layer. Turbulence would also lead to a nutrient injection into surface euphotic layers (Falkowski and Oliver, 2007). In some regions, our results have shown stratification has significantly decreased since the 1970s, with the decreasing regions occurring where there has been the greatest change in surface wind speed: 50-60°N, 20-10°W for annual stratification and 35-50°N, 20-5°W for summer stratification. However, there are some areas where changes in stratification have not been detected. For example the region 50-60°N, 20-10°W in the summer months. This could be down to the disparate regions of missing data, especially for the 2000s.

These changing conditions, with decreasing stratification irrespective of temperature and winder conditions in the NE Atlantic, could have large effects on plankton species distribution and abundance. Experimental studies have shown that flagellates are more sensitive to turbulence and associated shear-stress conditions, compared to diatoms (Estrada and Berdalet, 1998). These more turbulent conditions are expected to negatively impact dinoflagellates, as laboratory experiments have shown that excess turbulence can alter dinoflagellates cells by physical damage, physiological impairment and behavioral modification (Berdalet and Estrada, 1993; Smayda, 2002). In addition to increased turbulence, nutrient injection into surface euphotic layers is generally expected to favor diatoms since, unlike dinoflagellates, most taxa have a non-mobile nature, which means they cannot move vertically to acquire nutrients from sub-thermocline depths when surface conditions become more oligotrophic (Falkowski et al., 2004; Margalef, 1978). Essentially, wind-induced summer nutrient injections will re-establish the early spring (nutrient rich) conditions that favour diatoms over dinoflagellates. Hinder et al., (2012) have observed this type of change in the NE Atlantic. Using 21 species from the Continuous Plankton Recorder survey data, a fundamental switch in the relative abundance of diatoms versus dinoflagellates, with a dramatic decline in dinoflagellates in recent years has been detected. Such changes could have knock-on effects at subsequent trophic levels.

During the development of the method, a large number of volatile profiles (>1°C) were detected within the NE Atlantic. The majority of these occurred within the Norwegian Coastal waters (Norwegian trench) where profiles exhibited temperature inversion at the surface. It is likely that such profiles are formed by precipitation or ice sheet effects. However, we also note a decline in the frequency of these profiles in the region since the 2000s, which could be linked to a significant increase in water temperature at 2 and 200m. This could imply that around the Norwegian Coast less ice is forming in the warming conditions.

A second region of temperature profile volatility is the outflow of the Mediterranean Sea. An increase in the frequency of such profiles has occurred since the 1990s, with some profiles exhibiting a sharp peak (20°C) in temperature along the profile. Given that there is no consistent pattern within these profiles, it is more likely that the majority are due to sampling / equipment error or down to atmospheric conditions at the time.

Our index provides a simple, but consistent description of the structure of the water column, reducing the problems of resolution of profiles and temperature differences. However, there are several issues that need to be taken into account when using this method or for future development. Firstly, one of the main problems of our data is the loss of over 300,000 profiles due to our rigorous selection process of profiles, a decision that we regard as important in the avoidance of bias. Consequently, however, some regions in the NE Atlantic and North Sea are not covered by the index. Secondly, when the maximum and minimum temperature values were used instead of the true values at 2 and 200m, an under or over estimate of the index could occur. Thirdly, the time of day / weather conditions at the time of sampling were not considered in the process. These factors could be a cause of some volatile profiles. Fourthly, when applying the bias correction factor we assumed a linear relationship between the error and our variables. This might not be the case and a more complex function could improve our method. Despite these caveats, the index provides a comprehensive description of the spatial and temporal structure of the NE Atlantic water column. Significant changes have been detected for both stratification and temperature and we propose that the index has wide applicability for mapping against biological and climate variables for a better understanding of ecological change in this region.

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Chapter V

Supplementary Material

Has stratification changed in the NE Atlantic?

Hinder, S.L., Bodger, O.G., Gravenor, M.B., Edwards, M., Walne, A.W. and Hays, G.C.

Table S5.1. The number of profiles, with a >1°C temperature difference at either 2m or 200m for each of four decades.

	>1°C Temperature difference between 2 metres and maximum value		**>1°C Temperature difference between 200 metres and minimum value**	
	Out flow of Mediterranean Sea	**Norwegian Coast**	**Out flow of Mediterranean Sea**	**Norwegian Coast**
1970s	42	1220	11	1100
1980s	13	1428	12	1192
1990s	96	1463	60	928
2000s	96	1017	66	830

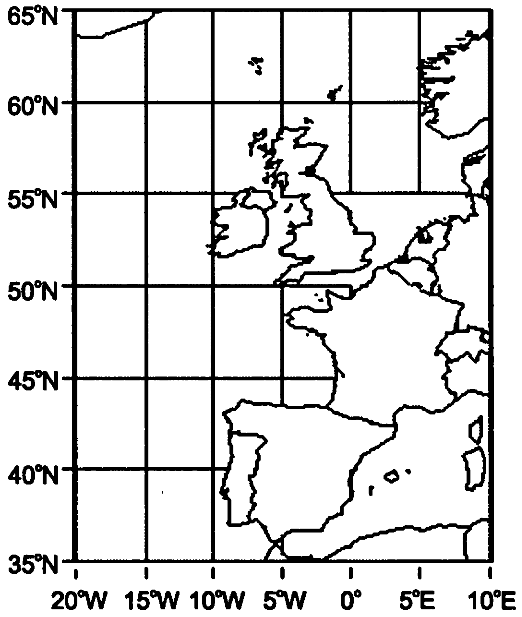

Figure S5.1. NE Atlantic and North Sea (20°W-10°E, 35-65°N) separated into 5°/5° latitude and longitude grid cells.

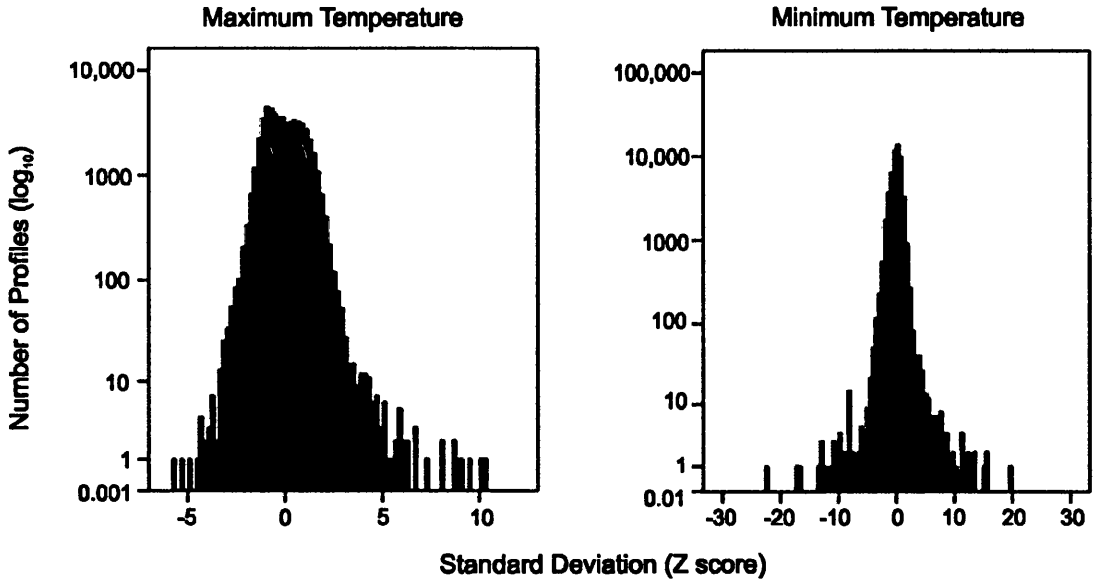

Figure S5.2. Maximum and minimum temperature Z score values for all profiles (1970-2009). Number of profiles are $\log_{10}$ transformed.

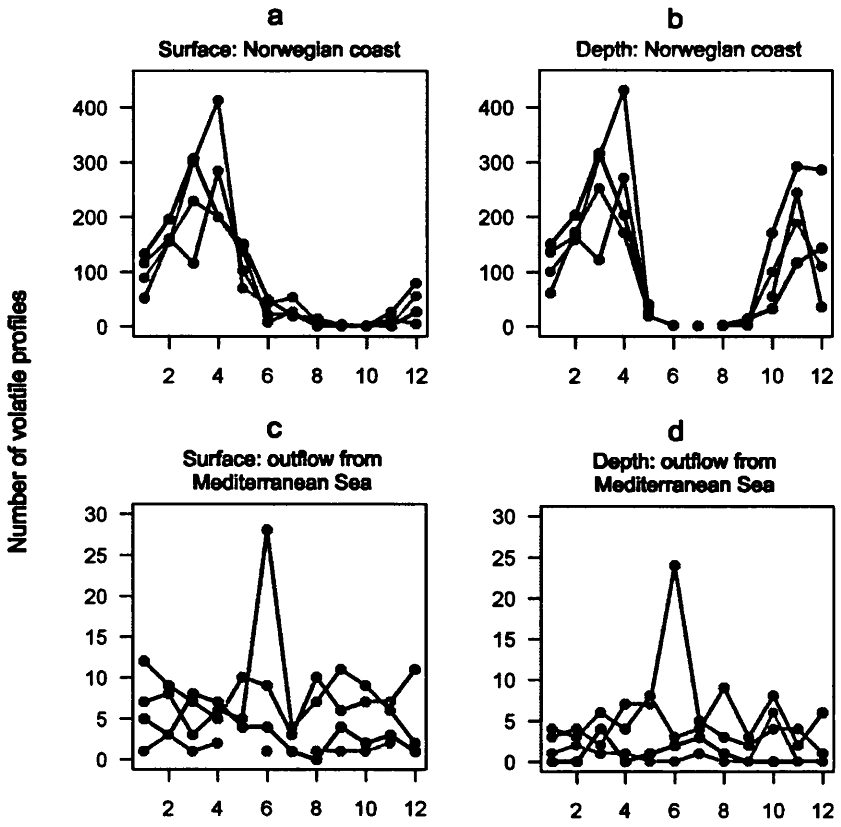

Figure S5.3. Monthly frequencies of volatile profiles, having a minimum temperature difference of 1°C in comparison with the 2m or 200m values, occurring in the Norwegian coastal waters (55-65°N, 0-10°E) for the (a) surface waters and (b) waters at depth, and the outflow from the Mediterranean Sea (35-45°N, 20-6°W) for the (c) surface waters and (d) water at depth, for four decades, 1970s (black line closed circles), 1980s (green line closed circles), 1990s (blue line closed circles) and 2000s (purple line closed circles).

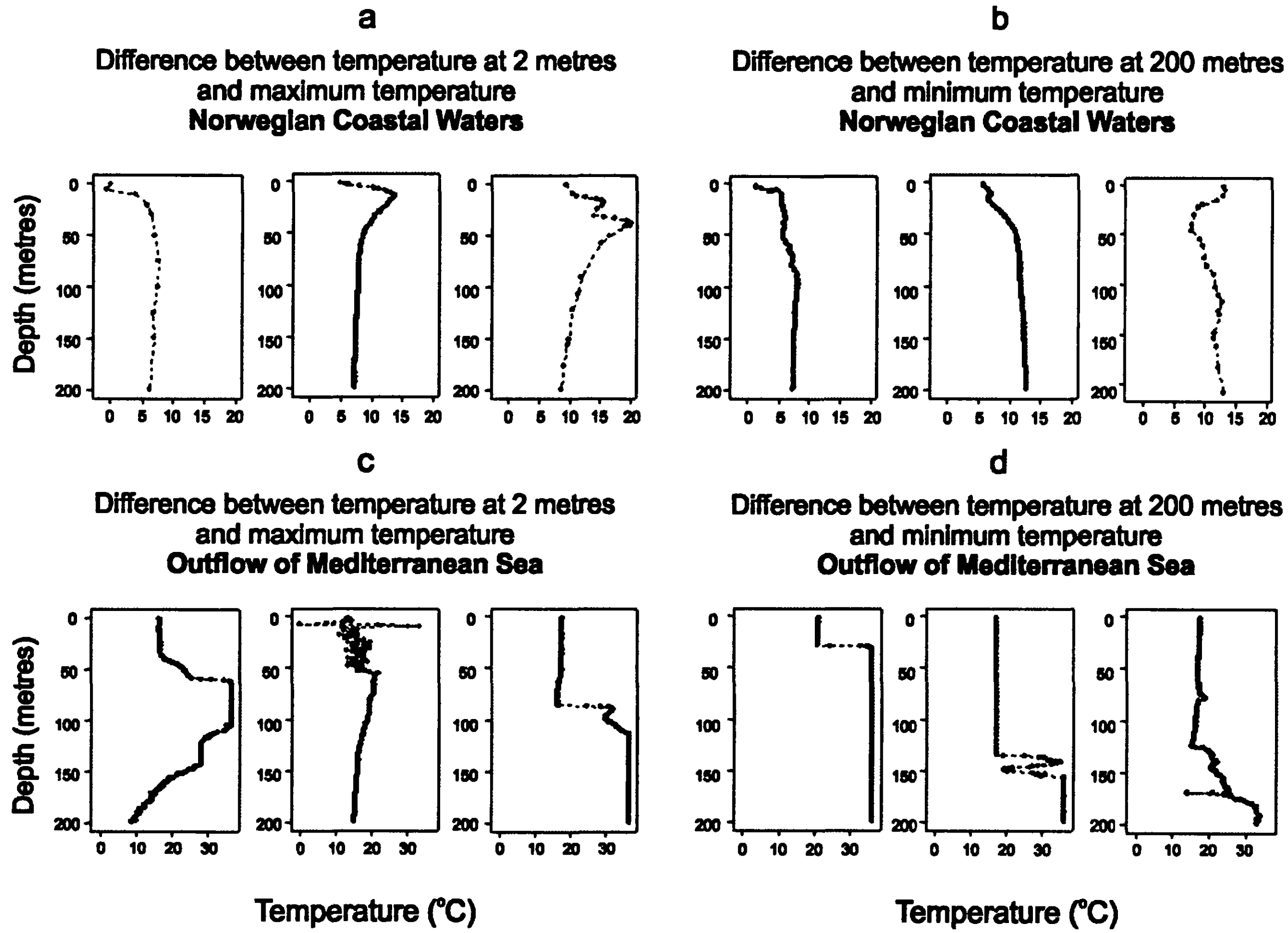

Figure S5.4. Examples of volatile profiles, defined as having a minimum temperature difference of 1°C, at (a) 2m and (b) at 200m at Norwegian coastal waters (55-65°N, 0-10°E) (c) at 2m and (d) at 200m for the outflow water around the Mediterranean Sea (35-45°N, 20-6°W).

This supplementary information was included to provide not only a more detailed description of the methods, but also a description of the process by which we developed the method. Although a range of measurements can be used to describe the presence and degree of stratification, most notably the definition, and detection, of a specified threshold drop in temperature, we encountered a range of biases whereby potential indices were confounded with important factors such as absolute temperature or latitude. The final index evolved over a process of trial and error during which we attempted to meet the requirements of a robust measure of stratification.

Initial stratification method: first attempt

This initial attempt of calculating the stratification index gave us a great insight into the profile data and demonstrated large flaws which needed to be rectified in the final method. Therefore, we felt it was important to include this work to explain why and how we progressed to the final stratification index.

Firstly, the resolution of profiles was restricted by only selecting those which had a minimum of 20 points per profile and had a maximum gap interval of 5m between successional points. If the profile did not meet these requirements, no estimation of stratification was made. After this step, a total of 99,377 profiles from 1970-2009 remained. The stratification index was then calculated using a step wise moving window, where the rate of change between successional depths was determined using equation 4.

Equation 4. $$S_i = \frac{T_{(i-1)} - T_{(i+1)}}{D_{(i+1)} - D_{(i-1)}} \quad (i = 3 \; to \; 199)$$

Where S_i is the rate of change at depth (D) i, and T_i is the temperature at depth i. The stratification index was calculated where the absolute S_i value was at its maximum at D_i and T_i for each profile. So for each profile we obtained data for maximum rate of change (Index ^{o}C m^{-1}) and the depth and temperature where the maximum rate of change occurred. This is the essence of a 'threshold' approach for identifying stratification that has been used in the literature.

Unfortunately, this method of calculating the stratification index is fraught with problems and biases. We considered this method as inadequate due to four major issues. Firstly, although the stratification index was good at classifying profiles that had a “perfect” stratification profile (see for example Figure S5.5a, profile 1) it was not appropriate for profiles that varied slightly from this, even if a stratification pattern was fairly clear ‘by eye’. Given the nature of the data, some profiles can be quite volatile with large variations in temperature over both a small or large depth (Figure S5.5a, profile 5 and 6). Using the initial stratification method, we would incorrectly assign the depth of stratification, the temperature, and the degree (index) of stratification. As this method measures the maximum index value from the profile based on individual points, any minor deviation in these points, due to sampling for example, and the calculated index would be magnified at that point, giving a faulty indication of the stratification intensity and location. Secondly, the resolution of the data was a problem. Although we reduced the level of error created by the number of points per profile and the maximum gap interval between points, there would still have been a temporal difference brought about by the 2000s providing more accurate data (having a higher number of 1m maximum gap interval data) than the 1970s decade. Due to this bias there is no way of comparing indices calculated in the 1970s with those in the 2000s. This introduced a systematic effect, as the earlier data was effectively ‘smoothed’. Such temporal confounding is crucial to avoid when trying to explain temporal changes in biological populations. Thirdly, the stratification index value was an arbitrary number with no natural scale, therefore comparisons within a range of index values is only valid when the profiles are similar or collected under very comparable circumstances. i.e. a value of 0.5 can result from very different profiles. Fourthly, a similar issue is that surface temperature alone can affect the index (even for similar shaped profiles) so that no comparisons can be made across geographic regions.

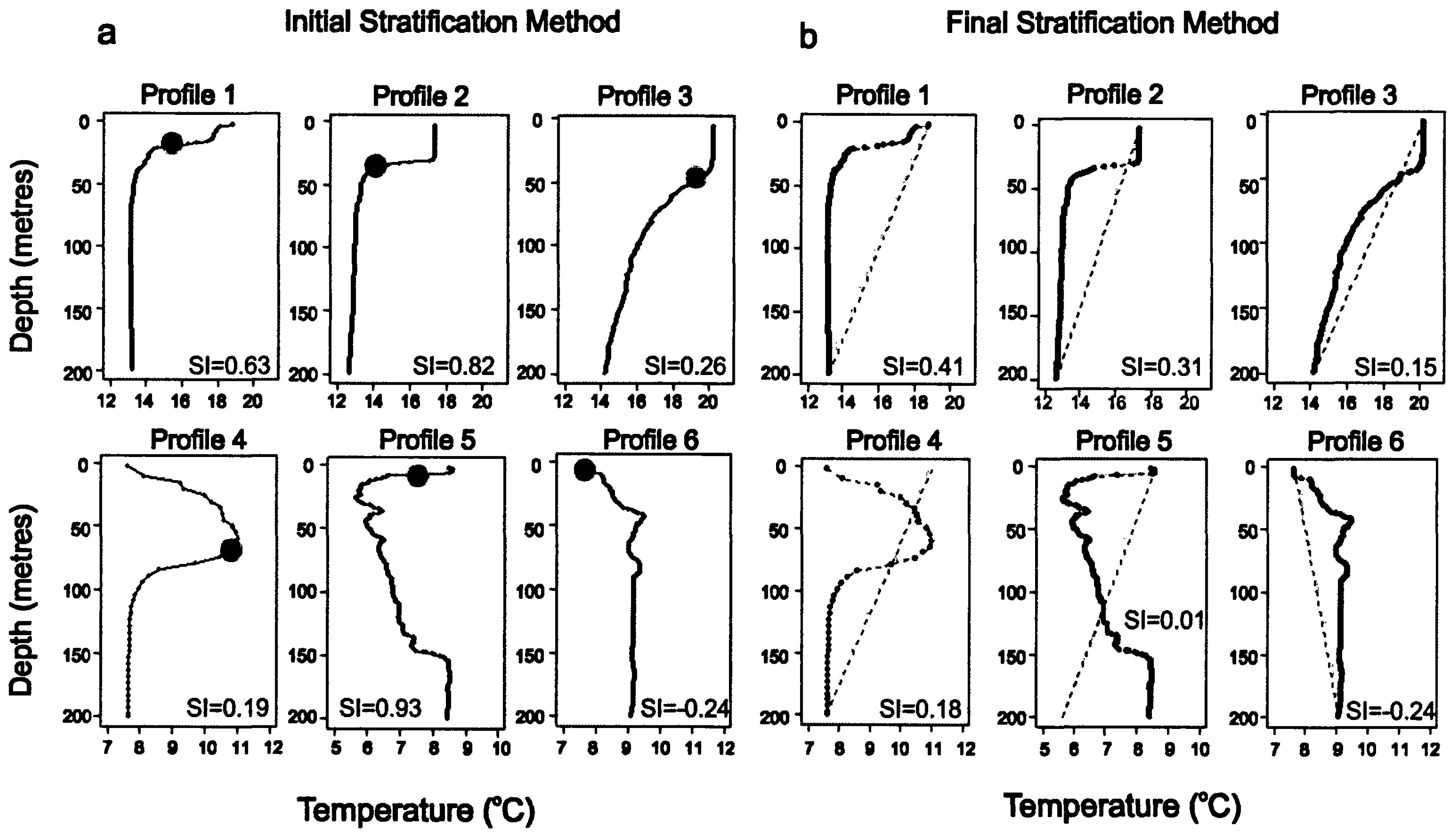

Figure S5.5. Six example profiles with the respective stratification index value (SI) calculated using both (a) the first attempt method, and (b) the final method. Grey circles represent the location at which stratification index was calculated using the first attempt method, and the grey shaded regions shows the area that was calculated using the final method. See text for full details.

Final stratification index

After taking into account the problems we encountered with the resolution of our data and the volatility of profiles, the final stratification index for each profile was determined by measuring the area produced when the individual points deviate from a straight line, created by the maximum and minimum temperature values (Figure 5.1a, Equation 1-3). With the assumption that the maximum temperature value occurs at 2m, and the minimum temperature occurs at 200m. The central idea being that the greater the stratification the larger the area that is produced. Furthermore the area is standardised to allow comparisons across regions and time points that naturally differ by temperature or depth.

This reduced previous problems of dealing with individual points, by using the profile as a whole. Figure S5.5 demonstrates the differences using both methods. Profile 1, 4 and 6 from Figure S5.5a, b, shows that a similar stratification value is achieved using both methods. However Profile 2, 3 and 5 are greatly over estimated using the initial method. Using the final method also allowed us to control and correct for the differences in resolution between profiles (see Resolution of data) allowing comparisons between decades.

Resolution of data

After obtaining the stratification index value for each profile, the next step was to correct for the differences in profile resolution, which could lead to temporal biases. Due to the duration of data collection, technological changes have resulted in more accurate and higher resolution data in the latter part of the time series. During the 2000s, 62% of the profiles had a 1m resolution, while in the 1970s there was <1% of data at this resolution (Figure S5.6). To reduce this bias we aimed to correct the low resolution data to a 1m scale, this was achieved by examining the maximum gap interval between points. Using the maximum gap interval implies there would be no differences between unevenly and evenly spaced data.

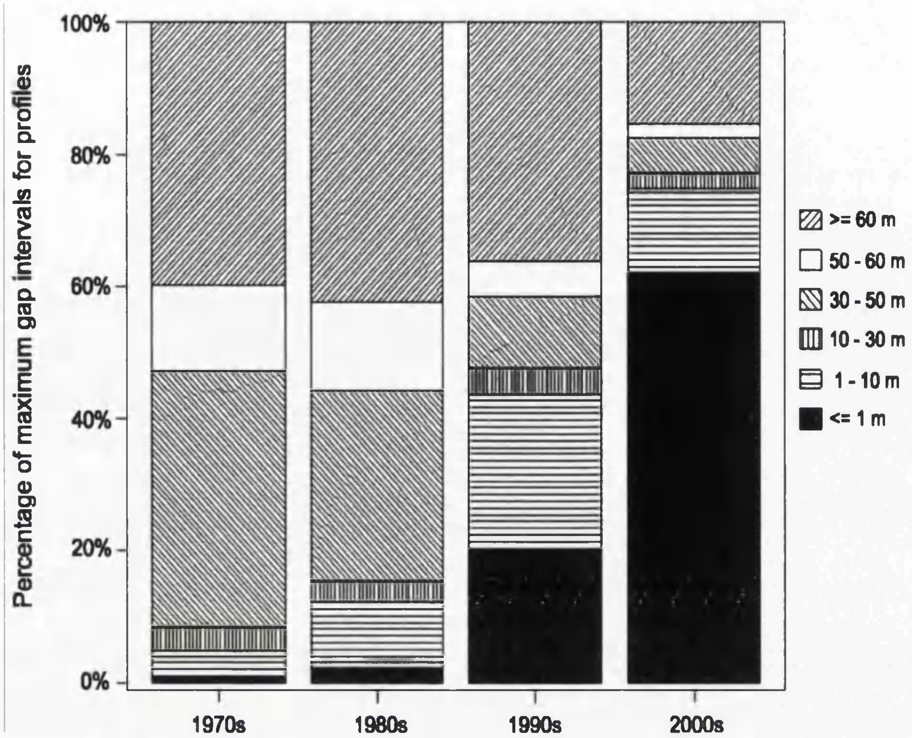

Figure S5.6. Contributions of different resolution of data for each of the four decades. Equal or less than 1m maximum gap interval (black), between 1 and 10m (horizontal), between 10 and 30m (vertical), between 30 and 50m (left diagonal), between 50 and 60m (white) and equal or over 60m maximum gap interval (right diagonal).

To determine how the maximum gap interval affected our data, we used a sample of 26 profiles from a range of time periods, both seasonal and yearly, which had a 1m gap interval from 2-200m. Bootstrapping, without replacement, was used to measure introduced bias as a result of deliberate degradation of data by limiting the number of points per profile. The stratification index was calculated for each profile at 1m intervals (198 points per profile), and subsequently the stratification index was calculated for 5, 7, 10, 20, 25, 40, 50 and 100 points per profile. Each bootstrapping was replicated 50 times for each chosen number of points and was performed using the statistical programming environment R. Before sampling, the 2 and 200m values were kept constant, and the random values were chosen, without replacement, between these points. i.e. if we were calculating 10 points, two values would be the 2 and 200m values, and the other eight values would be randomly chosen from the profile.

This produced a wide spectrum of maximum gap intervals and allowed us to understand the relationship between the stratification index and the resolution of profiles at all scales of the index. A maximum gap interval of 50m was chosen to give a good compromise between providing an accurate stratification index value with a low bias and error, whilst allowing between 45% and 85% of the profiles to be kept for analysis in each decade (Figure S5.6). The error for each maximum gap interval produced is very small (Figure S5.7), with a ± 0.04 error at a maximum gap interval of 50m and with very little bias (Figure S5.7a). Using linear regression analysis, a significant relationship between the actual stratification index for the 1m profiles against the estimated bootstrapped sampling stratification index values was found ($p<0.01$, $r^2=0.99$) (Figure S5.7b), and there was only a weak relationship between calculated random sampling stratification index values against the grouped maximum gap interval ($p=0.13$, $r^2=0.07$), suggesting that our calculation of stratification index is accurate and unbiased using profiles up to a maximum gap of 50m.

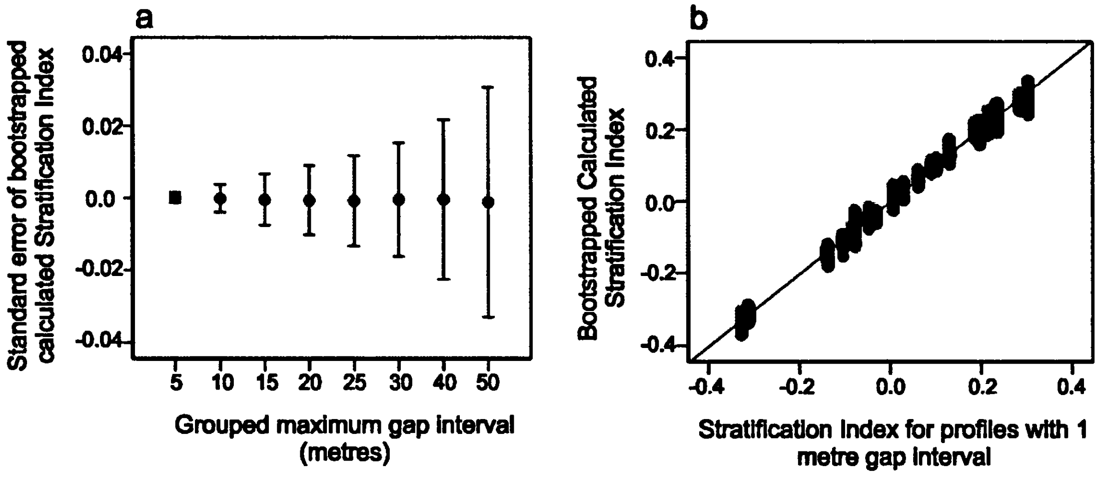

Figure S5.7. Using the 26 example profiles and a maximum gap interval of 50m, (a) the standard error for each grouped maximum gap interval when using the calculated bootstrapped sampling stratification index. (b) the relationship between the actual value of stratification index at 1m gap intervals, against the bootstrapped sampling calculated stratification index with linear regression line ($r^2=0.99$).

Correction bias

The stratification index we have chosen is robust to changes in the sampling framework. To explore this problem a set of sample profiles were degraded by randomly removing a proportion of the measurements in a single profile. From this bootstrapping approach we were able to demonstrate that while the mean size of the error rises with increased spacing of the observations no significant bias is introduced in the estimated index. As a consequence no correction was necessary to compensate for the limitations of the coarser profiles (as shown in Figure S5.7).

While the errors arising from our degraded profiles are not affected by the sampling frequency, they are significantly correlated with certain environmental variables. Table S5.2 shows that there are small, but significant, correlations with latitude, longitude and temperature. If these variables were stable, exhibiting little variation, this would not cause a problem. In practice as our profiles are taken from a large region of the NE Atlantic all three variables vary enormously. For this reason we must attempt to ensure that these correlations do not impact on our conclusions on the changing level of stratification.

The first way in which we can achieve this is to perform a paired-samples test in which we break the NE Atlantic and North Sea into a 5 degree grid (Figure S5.1). As long as all comparisons are made within a grid cell we can ensure that the bias attributable to latitude and longitude are minimised. A second step was to ensure that each annual set of profiles was distributed equally by month, by suitable weighting. This reduced the impact of seasonal variations in sampling. Finally, we looked at the variation of these confounding variables (latitude, longitude and temperature) for the cells and calculated, for each, the maximum bias in the index that could be attributed to it based on our bootstrapping results. Summing all sources of potential bias gives us an upper limit on the true bias. In practice these variables show high levels of correlation and as such our 'maximum bias' almost certainly overestimates the true scale of the bias. We then remove this 'maximum bias' from any observed difference between samples that we wish to test statistically, so we can ensure that our conclusions are highly resistant to the effect of confounding factors.

Table S5.2. Pearson correlation and linear regression coefficient analysis for nine variables against the bias produced from the bootstrapped stratification index value (* p<0.01).

	Pearson correlation		Linear regression coefficient
	coefficient	p value	
Latitude	-0.15	0.001*	0.0001
Longitude	-0.15	0.001*	0.0003
Year	0.01	0.39	
Temperature at 2 metres	0.18	0.001*	0.0003
Temperature at 200 metres	0.13	0.001*	0.0004
Maximum gap interval	-0.01	0.38	
Minimum temperature	0.13	0.001*	0.0003
Maximum temperature	0.18	0.001*	0.0004
Correct stratification index at 1 metre intervals	0.18	0.001*	0.009

Conclusions

Conclusions

Climate change is having profound impacts on the phenology, abundance and distribution of marine plankton in the NE Atlantic and North Sea (Beaugrand et al., 2009; Beaugrand et al., 2002). Throughout this thesis we have aimed to show the importance of long-term ecological surveys and how they can be used to monitor trends in plankton ecosystems. Using a range of statistical analyses we also aimed to identify relationships between plankton populations and several climate variables.

Our aim in Chapter I was to examine the epidemiology of Harmful Algal Bloom (HAB) poisoning within the UK by two methods: firstly by describing the key toxin syndromes and gathering together reported data on their epidemiology within the UK. This reported data focuses largely on outbreaks of the toxins in shellfish populations. Secondly, by attempting to quantify the incidents of shellfish poisoning directly, for the first time, using linked NHS hospital admission data and GP records from Wales. Most data on shellfish toxins are recorded in a wide range of records contained in industry reports and government websites, rather than together in accessible published and standardised studies. These sites are often out of date, and with changes in not only individual website formats, but in the governmental structure for gathering and recording the information (for example changes in the reporting laboratories), it is difficult to form a clear historical picture of the impact of shellfish toxins in the UK, and any trends over time. Our review of the disparate data showed that, within UK waters, outbreaks of toxins in shellfish are occurring on an infrequent but regular basis and affect a diverse range of molluscan shellfish and other marine organisms. Aquaculture sites and fisheries are closed on a regular basis around the coastline, with closures ranging from weeks to several months. In our review we aimed to gather together the often confusing literature on outbreaks, the UK reporting structure, regulations for safe levels of toxins, and regulations for fishery closure.

Direct evidence for the impact of shellfish poisoning on the UK population tends to come from rare, but dramatic outbreaks, traced to food suppliers or restaurants. We aimed to quantify the incidence using individual clinical records. The opportunity for this arose from the Secure Anonymised Information Linkage (SAIL) databank (Ford et al., 2009). This represents a state-of-the-art system for linking the full range of clinical data in the UK, which is contained in independent databases, on an individual patient level, whilst retaining patient anonymity that is essential for the exploitation of patient data. Thus we were able to perform the unique linkage between hospital admission records of shellfish poisoning, with prior and subsequent visits to the GP, and laboratory records of confirmed pathologies. This 'patient journey' represents an extremely powerful epidemiological tool, allowing electronic follow up of anonymous patients on an individual level for the first time. At present, this system is only available in Wales and hence any conclusions would need to be extrapolated from a relatively small population. However, there are unlikely to be very large demographic differences, with respect to exposure, in Wales as compared to the rest of the UK.

We found 51 hospital episodes recorded as "Toxic effects of noxious substances eaten as seafood (ICD10 code T61)" for all Welsh NHS Trusts from April 1998-August 2009. The follow up information, however, was disappointing. Hospital coding of events can be inaccurate, and are often very broadly defined. Clinical presentation may be non-specific and viral infections, particularly norovirus, or allergies can cause similar gastrointestinal symptoms to those of shellfish poisoning. This illustrates the great importance of SAIL for linking to additional clinical records for any real confirmation of toxin involvement. We were able to link the hospital admission records to 7 detailed pathology reports, 6 mortality records, and GP clinical information recorded +/- 30 days of indexed hospital episodes for 10 patients. Each record was examined in detail by a clinical microbiologist, and unfortunately no diagnosis could be made. Although symptoms were described in detail, along with all medical interventions, these can both be very general. There were no reports of food being identified and tested for toxin. This would provide the definitive diagnosis, but tends to be rarely performed as the food is either consumed or has been discarded. The timing of onset can also be used to distinguish between a viral and toxin cause, but was also unclear in the data. Therefore in many, or even all, cases the episodes coded as "toxin poisoning" could be viral gastroenteritis or be due to an allergic reaction. Although we were able to link hospital admissions to pathology, clinical and GP records, there was insufficient information within these electronic records to distinguish and estimate if "toxic poisoning due to shellfish" has actually occurred. We propose, however, that as electronic records become more sophisticated, our approach demonstrates the potential for electronic records to reveal a much more accurate picture of the true burden of marine toxins on human health in the UK. At present, the basic hospital data simply shows, as expected, that the incidence of shellfish poisoning within the UK is very low, with insufficient data to allow for a more accurate estimate.

In the absence of a routine human health epidemiological surveillance system, the fisheries testing regime remains the key robust indicator of any potential change in exposure of the human population. From this routine testing we have already seen an increase in the abundance of *Pseudo-nitzschia* in the last 5 years. This increase reflects the changes we have observed in the Continuous Plankton Recorder (CPR) survey data in Chapter II (see below), where a fundamental shift in relative abundance of diatoms versus dinoflagellates has occurred. Throughout the thesis our results have shown large changes in the abundance, phenology and spatial distribution of other plankton taxa. Additionally, an increase in Sea Surface Temperature (SST) in the NE Atlantic and North Sea has occurred over the last 50 years. This warming could suit species with a more southern biogeographical affinity enabling them to potentially occupy UK waters (Edwards and Richardson, 2004), and which are not yet considered indicator species in the UK testing regime. These results highlight the importance of ensuring the UK monitoring systems are up to date with the potential for new species and the changing seasonal and geographic distribution patterns of other plankton. Continuing assessment and measuring of plankton abundance, via long-term records, is essential.

Using the longest multi-decadal plankton monitoring system in the world, the CPR, our aim in the next three chapters of the thesis was to examine how, and why, various plankton populations have changed in the NE Atlantic and North Sea. In Chapter II, we focused on HAB species, but also examined their changes in abundance and distribution within a wider group of 21 phytoplankton taxa, from 1960-2009, and 3 taxa of shorter time periods (1971-2009, 1981-2009 and 1997-2009), to undertake the most comprehensive analysis to date of extended spatio-temporal patterns of abundance in the NE Atlantic. Marine diatoms and dinoflagellates play a variety of key ecosystem roles as important primary producers and grazers. Some are known as HAB species, in particular species of *Pseudo-nitzschia seriata*, *Dinophysis spp.*, and *Prorocentrum spp.* There is widespread concern that these HAB species may be increasing in abundance accompanied by the associated major negative socioeconomic impacts, including threats to human health and marine harvesting (Hallegraeff, 2010; Heil et al., 2005; Moore et al., 2009).

Our results from Chapter II imply a fundamental shift in relative abundance of diatoms versus dinoflagellates in the NE Atlantic, with a dramatic decline in dinoflagellates in recent years. These broad changes were also reflected within individual taxa albeit with some level of variability in patterns across taxa. As such, our results challenge the over simplified view that blooms of HAB species have increased in frequency and intensity in recent decades and instead point to a broader switch from dinoflagellate to diatom dominance. Using a range of statistical analyses we have shown that both SST and summer scalar wind may be important climate drivers of these biological changes. Correlations between ecological and climatic variables can occur for a variety of reasons, and it is well known that such a link does not necessarily imply a causal or mechanistic relationship. Apparently significant correlations can arise from issues of multiple testing (which increase the false positive rate), a particular risk when dealing with high dimensional climate data sets that are now readily available. In addition, when analysing time series, spurious correlations can also arise due to the presence of autocorrelation in the time series, and when long-term trends exist in the data (for example when unrelated time series are both increasing over time). We tackled these substantial issues in two ways, firstly by introducing statistical methods from economics that are rarely used in the ecological literature and secondly by concentrating on likely mechanistic relationships that have a biological interpretation.

Statistical methods in biology and medicine are largely based on the classic frameworks developed in the 1930s by R.A. Fisher. However, these methods were initially designed for controlled experiments or trials (Fisher et al., 1990). Many of the methods were based on the need to avoid bias in the interpretation of data and to stress the central role of randomisation. In contrast, ecology relies heavily on 'observational studies' where repetition and randomisation are impossible and bias is often present. In such studies, causal relationships are inherently difficult to establish. It is even common in the medical literature to refer to an 'ecological study', meaning a set of uncontrolled observations on the health of populations (Coggon et al., 1997), and to post such a study near the bottom of the 'hierarchy of evidence', at the opposite end of the spectrum from double-blind randomised controlled trials, or formal cohort or case control studies. This does not mean that observational studies are less important, but that the interpretation can often be much more difficult (Barton, 2000).

In contrast, the development of statistics in the field of economic analysis (econometrics) has always tended to deal primarily with observational data alone, and possibly as a consequence of this, since the 1960s a range of methods for revealing true or causal links between time series of economic variables have been developed (Granger, 1969). These methods culminated in the award of the Nobel Prize for Granger and Engle in 2003 (Granger, 2004; Engle III, 2004), a recognition for advances in statistical methods that is notably absent from the medical field (Grieve, 2005). The methods are surprisingly straightforward (described in detail in Chapter II), based on the simple idea that evidence for 'Granger' causality is present if past values of x significantly improve the forecast of future values of y, over and above the forecast provided by past values of y alone. The adjustment for past values of y avoids the issues caused by simple temporal correlation that may exist between unrelated variables (see Thurman and Fisher, (1988) for a simple explanation). These methods should therefore be ripe for exploitation in ecology and in particular climate studies, but do not seem to have filtered into ecology. At the time of writing we could not find an example in the ecological literature, although the methods are clearly becoming popular in a few areas of biology, notably neuroscience (Ding et al., 2006). Here, we advocate a much more thorough investigation of the potential for econometrics models in ecology time series studies.

The significant correlations, and subsequent Granger causality criteria, found for SST and surface wind conditions were investigated in tandem with regression models that included interaction terms. The significant interactions detected point towards a particular mechanistic interpretation. Since 1960, there has been both a rise in SST and a significant increase in summer surface scalar wind in the NE Atlantic region. SST may favour warmer water species, but in addition we propose that this increase in wind introduces surface kinetic energy, which will stimulate vertical mixing leading to increased turbulence, which is the environment generally favoured by diatoms (Thurman and Fisher, 1988). The presence of an interaction indicates that the effect of each climate variable cannot be considered in isolation. In order to answer the question: what is the impact of a rise in SST? One must, by the definition of an interaction term, supply information on the corresponding changes in surface wind. Although species specific differences exist, in general, small scale turbulence and an increase in SST appear to impact negatively on dinoflagellates cell division and life cycle processes (Berdalet et al., 2007). Given the many potential changes in climate variables, it is unlikely that a simple linear relationship exists with species abundance. Synergistic effects, as shown in this study, must be considered alongside advances in statistical methods to help to develop our understanding of the mechanistic processes underlying biological changes in the oceans.

In Chapter II, we demonstrated how phytoplankton abundance and distribution has changed over the last 50 years and it is therefore important to examine the next stage in the trophic level by looking at some important zooplankton taxa. Firstly, in

Chapter III we examined tintinnids, which are a group of loricate ciliates and often a neglected component of the plankton. Using the CPR data we found a long-term increase in the occurrence of tintinnids in the NE Atlantic as well as a northerly shift in their distribution. In the North Atlantic the genus *Dictyocysta spp.* dominated and *Parafavella gigantea* showed an increase in abundance around Iceland and Greenland. Conversely, in the North Sea higher densities of *Tintinnopsis spp.*, *Favella serrata* and *Ptychocylis spp.* were found, but there has been an overall reduction in abundance of tintinnids in this region.

These long-term contrasting trends in tintinnids occurrence between the NE Atlantic and North Sea suggest that conditions e.g. prey availability, predation pressure and / or hydrological conditions are more favourable for the taxa in the NE Atlantic. Tintinnids play an important trophic role, as the group are grazers (Admiraal and Venekamp, 1986) which may significantly regulate phytoplankton (Pierce and Turner, 1993), including some HAB species in the initial stages of a bloom (Kamiyama, 1997). The group are also exploited by protozoan and metazoan plankton, including copepods (Stoecker and Sanders, 1985). Therefore, this decline in tintinnid occurrence in the North Sea may be linked with large changes in other components of the plankton observed in recent years. Plankton taxa have shown biogeographical shifts and phenology changes over the last two decades, found in notable studies (Beaugrand, 2009; Reid et al., 1998) in addition to our work presented in Chapters II and IV (diatoms, dinoflagellates and see discussion of copepods below). We also found some indication that the size of the lorica oral diameter may be influencing tintinnid taxa distribution and abundance. Of the taxa examined, *Dictyocysta spp.*, has the smallest oral diameter and cell length, while *F. serrata, P. gigantea* and *Ptychocylis spp.* show a distinct larger cell length and oral diameter, suggesting that size of prey is an influence of tintinnid taxa (Admiraal and Venekamp, 1986; Dolan, 2010), which could relate to changing phytoplankton distributions.

We considered the importance of climate drivers in our investigation of tintinnid abundance by looking for associations between the individual tintinnid taxa, SST and the North Atlantic Oscillation Index (NAOI). No significant correlations were detected, but this could represent the relatively low power of the analysis due to the short time scale involved. This time scale is likely to preclude an extensive Granger causality investigation of the trends for some time, as the adjustment for past values means the criteria for causality can be conservative, and may rely on very long time series. As the CPR survey time series extends it will be important to continually assess the occurrence of tintinnids as well as resolve if there are climate drivers underlying the changes in abundance and distribution.

In Chapter IV, we continued to focus on the zooplankton community, by examining the spatial distribution and relationships with climate drivers for *Calanus*. Previous studies have shown declines in *Calanus finmarchicus* abundance in the NE Atlantic and have linked these changes to the NAOI and westerly wind stress (Planque and Fromentin, 1996). However, in recent years the relationship between the NAOI and *C. finmarchicus* has broken down (Planque and Reid, 1998), and the continued declines were linked to SST. While *C. finmarchicus* has declined in abundance, the congeneric species *C. helgolandicus*, which has a generally warmer water and more southerly distribution has increased (Beaugrand et al., 2002; Fromentin and Planque, 1996). Using the extended time series of the CPR survey (1960-2010), we have shown that the decrease in *C. finmarchicus* and the increase in *C. helgolandicus* in the NE Atlantic region has been maintained in recent years. We have also demonstrated that as time series lengthen, relationships between abundance and climate variables change. The NAOI and westerly wind speed relationship between *Calanus* abundance was reduced, with a clear stepwise decrease in the correlation in 1996. This reduction was also reported by Planque and Reid, (1998). Conversely, over the 50 year time period, SST has shown a significant strengthening correlation with *Calanus* abundance. These results of changing relationships are important, as they highlight the necessity to continually monitor and update plankton abundance and distribution along with climate variables. Given that the CPR series is one of the longest ecological monitoring systems, the instability of the links with climate variables over time suggest that great care must be taken with the interpretation of links found in time series that are much shorter. It is possible that considerable publication bias effects are present in the ecological literature.

While testing for Granger causality, we also noted that the abundance of both *Calanus* species each year is driven by considerable carry over effects. With strong autocorrelation functions over the time step of 1 year. This suggests that the overwintering population has a big impact on the size of the population in the subsequent year and it is the survival of eggs to adulthood that is the principle determinant of adult abundance. This is important as *C. finmarchicus* plays a key trophic role, providing food for the commercially important fish such as cod (Dickson and Brander, 1993). Therefore, changes in *Calanus* abundance can have impacts on subsequent trophic levels e.g. the gadoid outburst in the 1970s (Cushing, 1984).

Lastly, we examined if *Calanus* taxa had adapted to the changing conditions in the NE Atlantic and North Sea. For each decade since the 1960s we developed a 'temperature response curve' by plotting the relationship between abundance and SST across several regions. We found some suggestion that, over time, the temperature response curve for both species was becoming progressively less severe. This implies that the largest effect of temperature changes occurred in the 1960s, and that the per degree response in abundance to temperature was smaller in the 2000s. We note, however, that these trends were statistically significant for *C. finmarchicus* only, and that the data relied on a relatively sparse number of observations in each decade. These changing relationships between abundance and temperature imply that some level of adaptation may be occurring in plankton populations. Adaptation is one possible means by which the impacts of climate change will be reduced in biological systems. However, evidence of the ability of species to adapt to changing conditions is limited. Our results suggest that limited thermal adaptation may be occurring within the *Calanus* complex in the NE Atlantic, but has been insufficient to prevent major range changes occurring over the last 50 years.

The last chapter of the thesis described how a new method was developed to identify the timing of formation and the strength of the seasonal thermocline and thereby examine the spatial and temporal changes in the stratification of the NE Atlantic and North Sea. Many previous papers have linked the northward shifts in plankton abundance in the NE Atlantic to the warming of the ocean (Beaugrand, 2009; Beaugrand et al., 2002), but there is not a direct relationship between changes in stratification and plankton range changes. A possible reason for this is that the effect of SST is indirect, via an intermediate mechanism, and this mechanism is often held to be the degree or timing of ocean stratification which would be favoured (and brought forward in the year) by higher SST. However, in Chapter II we showed how there has been an increase in summer wind speed since the 1970s, which could work to decrease summer stratification. Clearly we cannot assume a simple relationship between individual climate variables and such a complex system as summer stratification. Although there are already some methods available to measure the summer thermocline directly, these do not allow for comparisons on the large spatial and temporal scale we require to compare against species abundance. Therefore we are unable to identify if the dynamics of seasonal thermocline formation has changed in the NE Atlantic over the period of the CPR survey, and assess the possible impacts of these changes to plankton abundance and distributions.

The new technique to estimate the level of stratification was achieved by modelling the whole available temperature profile, and was designed to reduce the problems associated with instrument noise, and to be robust against large depth intervals between measurements within profiles and variable temperature differences between points. Given the way stratification is often invoked as a driver of plankton abundance changes, it may be surprising that we found relatively stable seasonal patterns throughout the NE Atlantic, with no significant shifts in the onset of the thermocline over the four decades. Significant changes in the intensity of the thermocline were found, however these changes were not uniformly shifting northwards, as might have been expected from SST alone, but show a more variable pattern. The southwest region of the NE Atlantic has shown a considerable decrease in the stratification index, while the waters around the French and English coast have shown an increase. These results highlight that simple assumptions that the thermocline intensity is the cause of species shifting northwards no longer apply. A more complex behaviour is occurring and more synergistic approaches need to be used to understand these changes. We propose that our stratification index has wide applicability in ecological studies of change in the North Atlantic.

Within this thesis there are some other aspects which were examined but not included in the results sections. Within Chapter II we only focused on annual SST and summer westerly wind speed. It should be noted that we examined summer SST and annual westerly wind speed and found very similar results. We also looked for Chelton regression and Granger causality relationships for the taxa against the NAOI. However, we found no significant results and therefore this was not included in the results section. It should also be noted that when we first started researching for techniques to examine long-term plankton changes we looked at cross spectral analysis, with the aim of detecting correlations between different cyclical components of the time series. However no strong frequencies were detected in the periodogram (spectral density) calculated for any taxa. This suggests that, over the time scale of the available data, there were no strong cyclical elements to the plankton abundance (other than the expected intra-annual seasonal variability). A reason we found no significant results could be down to the short time scale of the plankton data and although this technique was not included, there remains a considerable potential of this method in the future when the time series lengthens even further. Within Chapter IV, our results focus on the NAOI, and annual SST and westerly wind speed. It should also be noted that the same analysis (modified chelton method, Pearson correlation, Granger causality, and cointergration) was also preformed on summer and winter SST and westerly wind speed. As both seasons (summer and winter) SST and westerly wind speed gave very similar results to the annual data we felt it was unnecessary to include both sets of results.

Throughout this thesis we have attempted to show the importance of long-term ecological surveys and the need to continuously assess plankton and multiple climate variables together. Changes in plankton abundance and distribution have occurred over short time periods, a few years, to several decades. We have also shown that the relationships with plankton and climate variables change over time, with some developing stronger relationships, while others weakening. Conclusions based on a 'single look' at the data, or on time series less extensive than the CPR must be treated with caution. It is important to move to a mechanistic understanding of how plankton are shifting as these changes could have a large impact on subsequent trophic levels. Previous studies have expressed how changing plankton abundances are altering the abundance and distribution of fish taxa in the North Sea (Beaugrand and Kirby, 2010; Beaugrand and Reid, 2003; Reid et al., 2001) e.g. the relationship between *Calanus* and gadoids. Here, we also showed how aquaculture and fisheries can be affected by toxic plankton, and a key defence against these toxins is the continuous monitoring of UK waters.

Throughout the thesis we have also tried to focus on statistical methods that reveal levels of interactions between plankton distributions and climate beyond simple correlations. We have been careful to account for autocorrelation and have introduced (for the first time to our knowledge) criteria of statistical causality to this field. There are several ways in which these methods could be expanded upon in future studies. The most obvious is to develop a full spatio-temporal model for the plankton / climate data sets. Currently we address the spatio-temporal component by partitioning the NE Atlantic into several regions and then examining the average of each over time. There are some advantages to this approach. It is not reliant on a specific statistical model, i.e. we do not have to make mathematical assumptions on exactly how observations made closely in space are related. Such a description of the correlation structure over space would be required because these observations are not independent. It is also likely to be fairly robust, as we are using aggregated data, hence large numbers of observations. However, using this method we are taking averages over large areas, and discarding the finer detail of the data. What could be accomplished is the creation of a matrix for the whole of the NE Atlantic for each time point and estimate the relationship between abundance (interpolated from the CPR with a kriging approach) and the already detailed climate data, formally over space and time. We believe this would be a natural way to take the work here forward, although the application of such a model would not be straightforward. The statistical techniques are available in the methodological literature (Cressie and Wikle, 2011), but the assumptions of the model must be clearly understood before application, particularly for use in regions in which the spatial data are sparse, but if correctly applied this would supply a comprehensive understanding of the movements and relationships of plankton. Combined with Granger criteria it could be used to build 'causality maps' identifying key areas where the links with climate are particularly strong.

A step in a different statistical direction is the development of predictive models. For future studies, we envisage a model with a fairly simple structure: using our existing regression coefficients from the relationship between climate parameters and abundance (along with additional climate variables) we have a set of rules that form the basis for how a particular plankton taxa *should* change over time. These rules could be used directly, or combined with a threshold model to determine how the distribution of plankton contracts or expands over space and time according to the set of environmental rules. We would then split the CPR data into two time periods. Using the earliest CPR dataset we could estimate our rules and use them to simulate the future patterns corresponding to the later time period. Using the later CPR data set we could then validate our predicted results. This type of technique has potential as a valuable tool for understanding the relationships between plankton abundance and climate change.

Looking to the future, one of the key messages from our work has been to highlight the ever increasing power of time series as they lengthen. We look forward to the new discoveries that will be made from the CPR data as the time series extend into their sixth decade.

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