Furthering the understanding of the role of phytoplankton within UK eutrophication monitoring

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Abstract

Estuarine and coastal eutrophication is a worldwide issue, where the elevated input of nutrients and changes in water quality conditions in waterbodies can result in undesirable ecological disturbances. The current metrics used with UK eutrophication monitoring are likely to miss fine scale disturbances, including those in the phytoplankton community. Many researchers have called for the inclusion of a wider range of metrics to monitor and assess the undesirable disturbances of eutrophication and have identified developing the understanding of the relationship between phytoplankton and water quality as essential to progress the effectiveness of monitoring and management. This research asks what additional insight can be gained from utilizing long term monitoring data in unique combinations, and by applying additional metrics, including the Plankton Index tool and long-term trend analysis. Through fieldwork campaigns, the relationship between phytoplankton communities and water quality conditions are investigated along a salinity gradient, to determine the factors which may govern the response of estuarine and coastal waterbodies to eutrophication. Nutrient addition bioassays were used to assess the response of different phytoplankton lifeforms to changes in turbidity to establish if assumptions on which assessment practices are based are suitable. Additional and important insights were obtained from utilising existing data in different ways; however, data availability is identified as a consistent limitation. There is a shift identified in the governing factors along a salinity gradient, which has implications for eutrophication assessment in estuaries and nearshore coastal waters, but importantly also identifies the inclusion of phytoplankton lifeform data in monitoring as important to advance the understanding of phytoplankton community response to changing water quality conditions. The results of the nutrient addition bioassay raise questions about the assumptions within eutrophication monitoring and further highlight the importance of the inclusion of phytoplankton data in order to fully understand the extent of ecological disturbances.

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“The central problem presents an extraordinarily difficult scientific challenge (much harder than rocket science)”

Cloern (2001), on coastal eutrophication

For my husband Ben,

Who held me together when I fell apart

To Naomi and Michelle, thank you for always seeing the bigger picture of this project when I couldn't, having endless patience for my questions and problems, and supporting me with so much kindness throughout this process. Thank you to Mike for insight and guidance, with thanks to Dorothee for being so willing to step in and give support and supervision, and with thanks to Gill for support and guidance from the beginning. With thanks to Carolyn for always answering my calls for help, and with thanks to Eileen for advice and input.

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To Harriet, I will try forever to make it up to you for missing tea parties, story time, and hide and seek in order to write this.

1

Introduction

1.1 Abstract

This chapter will outline the concept, causes, and impacts, of eutrophication, along with describing the UK environmental directives which aim to monitor, assess, and manage the environmental problem in UK waters. The importance of phytoplankton communities and how they respond to elevated anthropogenic nutrient concentrations will be discussed. Different approaches to monitoring and assessing phytoplankton communities will be presented, including methods that form part of the regulatory monitoring in UK coastal and marine waters, alongside further methods that could be part of future monitoring approaches to improve our understanding and assessment of the impact of eutrophication on pelagic community structure. The importance of understanding how phytoplankton are considered within effective eutrophication monitoring and assessment will be presented, and the knowledge gaps and research questions addressed within this thesis will be introduced.

1.2 Nutrient pollution and Eutrophication

Nutrient pollution is the process of inshore, coastal, and marine waters becoming over-enriched with nutrients. The undesirable impacts which result from this are known as eutrophication. Eutrophication can be defined as:

'The enrichment of water by nutrients causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned' (OSPAR, 2005)

Increased nutrient inputs can enter the water column from sources such as agricultural run-off, wastewater inputs, and aquaculture (Neal and Jarvie, 2005; Ulen et al., 2007; Maier et al., 2009; Withers et al., 2014; Wood et al., 2017; Preisner et al., 2020).

Eutrophication is not a new problem, industrial activities in the 19th century increased riverine inputs of nutrients and caused the 'first wave of coastal eutrophication' (Billen et al., 1999). However, global increases in nutrient inputs into coastal and estuarine waters and subsequent eutrophication are currently occurring in many parts of the world (Savchuk, 2018; Devlin et al., 2020; Malone and Newton, 2020; Wang et al., 2020), primarily as a result of anthropogenic nutrient inputs (Vitousek et al., 1997; Jickells et al., 2014; Kelly et al., 2021; Paredes et al., 2021).

This worldwide increase in nutrient pollution and eutrophication comes as a result of polluted terrestrial run off from fertiliser application and anthropogenic waste, as well as atmospheric deposition, and aquaculture which enriches coastal waters with nutrients, primarily nitrogen and phosphorus. This has serious adverse effects on coastal ecosystems. Nutrients can originate from point (direct) and/or diffuse sources. Point sources of nutrients, such as sewage and industrial waste, discharge directly into the waterbody.

1.2.1 Nutrients of concern

The key nutrients that are considered to cause eutrophication are nitrogen (N) and phosphorus (P). N and P occur in a variety of total and dissolved forms (Table 1.1). For the dissolved forms, dissolved inorganic nitrogen occurs primarily as nitrate, nitrite, and ammonium. Dissolved organic nitrogen can be found in amino acids, and urea, for example. Phosphorous can occur organically or inorganically as part of a phosphate molecule. The nutrient parameters primarily considered within this thesis are dissolved inorganic nitrogen (DIN, consisting of nitrate, nitrite, and ammonium), total oxidised nitrogen (TOxN, nitrate plus nitrite), and dissolved inorganic phosphorus (DIP). Dissolved nutrients provide a more accessible food source for phytoplankton, and so whilst other nutrient forms are able to support their growth, dissolved

N and P are considered the key nutrients driving eutrophic conditions and enhanced phytoplankton biomass. Silicate concentrations can also be important when considering nutrient enrichment. Silicate is essential to the growth of diatoms but not dinoflagellates, and so silicate may still limit growth despite enrichment of nitrogen and phosphorus, and silicate concentrations are therefore important to consider within this work (Tye et al., 2024).

Table 1.1 - Nutrients to be considered within the monitoring of eutrophication.

Parameter	**Short description**	**Components**
Dissolved Inorganic nitrogen	DIN	Nitrate, nitrite, ammonium
Dissolved total oxidised nitrogen	TOxN	TOxN = (Nitrate + nitrite)
Ammonium	NH_4^+	Ammonium
Nitrate	NO_3^-	Nitrate
Nitrite	NO_2^-	Nitrite
Dissolved Inorganic phosphorus	DIP	Phosphate
Dissolved Silicate	Si	Silicate

1.2.2 Nutrient imbalances

Environmental management has often focused on reducing the inputs of individual nutrients (Boesch, 2019), either N or P, with many of the improvements focused on reducing P through elimination of P in detergents and fertiliser. However it is now becoming clear that successful management of both nitrogen and phosphorous is required to reduce eutrophic conditions and to ensure healthy coastal marine ecosystems (Howarth and Paerl, 2008; Grizzetti et al., 2012; Burson et al., 2016; Paerl et al., 2016; Paerl et al., 2018; Grizzetti et al., 2021). There has been variation in the success of management practices which aimed to limit the amounts of N and P entering waterways leading to imbalances in the reductions of N vs P. The Urban Wastewater Treatment Directive (UWWTD), a European environmental directive first implemented in 1991, aimed to reduce the adverse effects of urban wastewater by setting out EU-wide rules for collecting, treating, and discharging wastewater. The success of the UWWTD and other environmental directives resulted in considerable reductions of DIP concentrations from sewage treatment plants (Kinniburgh and Barnett, 2010; Neal et al., 2010), however reductions in N inputs to waterways have been less notable. This is largely due to the fact that nitrogen predominantly originates from diffuse sources, agricultural and atmospheric, and these types of sources are much more difficult to reduce. As a result, increases in the ratio of N : P in

riverine inputs have been seen (Burson et al., 2016; Greenwood et al., 2019; Grizzetti et al., 2021) and there is growing evidence that this has major implications for the species composition of phytoplankton communitites, and the prevelance of harmful algal blooms (Davidson et al., 2012; Gowen et al., 2015; Burson et al., 2016). The management of nitrogen is a complex and global issue, evidenced by the concerns of such groups as the United Nations Environment Programme, who have developed a working group on nitrogen, which aims to work towards an international nitrogen management system for policy development (Sutton et al., 2019).

1.3 Impacts of eutrophication

1.3.1 Implications of nutrient imbalances

It is generally accepted that marine phytoplankton take up nutrients in a ratio similar to that of 106 : 16 : 1 for C : N : P, known as the Redfield ratio (Redfield, 1958), however there is known to be variability in this. Uptake ratios have been seen to differ across species, and to vary with the availability of nutrients (Rios et al., 1998; Geider and La Roche, 2002; Hessen et al., 2002; Quigg et al., 2011; Martiny et al., 2013; Branco et al., 2018; Poulton et al., 2019). As the balance of nutrients changes as a result of variations in the success of management of the individual nutrient sources, and the ratio of DIN : DIP increases, phosphate limitation in estuaries becomes more likely. This increased ratio has been observed in coastal waters within Europe (Trommer et al., 2013; Earl et al., 2014; Burson et al., 2016), and worldwide (Zhang et al., 2020; Lu et al., 2021; Beusen et al., 2022; Zhou et al., 2024). A changing nutrient ratio may therefore contribute to a shift in phytoplankton communities, as species which are more tolerant of ratios which deviate from the Redfield ratio of 16 : 1 for N : P may be given a competitive edge. Diatoms have also been shown to be poor competitors relative to flagellates for dissolved inorganic phosphorus, at phosphate concentrations < 0.1 µM (Egge, 1998). Reductions in phosphorus without concurrent nitrogen decreases may therefore support increased (dino)flagellate dominance. Despite the high nutrient ratios observed by Burson et al. (2016), dinoflagellates were limited by both N and P and dominated by mixotrophic species in the samples collected in the North Sea in 2016. Mixotrophic species are able to switch their feeding mode between phototrophy and phagotrophy (Stoecker et al., 2017; Mitra et al., 2023) and may therefore be offered an advantage in nutrient limited environments. Burson et al (2016) highlight that mixotrophic species may have a competitive edge in an environment with a high DIN : DIP ratio, and they may therefore make up an increasing proportion of the community in this environment.

This shift in the balance of nutrient availability and associated P limitation may also increase the importance of dissolved organic phosphorous (DOP). Fitzsimons et al. (2020) documented the preferential uptake of DOP in some marine phytoplankton, these species may be at an advantage in an environment where DIP resources are limited, and this could contribute to changes in community composition. Differing phytoplankton species can have a differing nutritional value for their grazers (Spilling et al., 2018) and further to this, changes in nutrient ratios in coastal waters have been seen to impact the biomolecular composition, stoichiometry, and the nutritional quality of phytoplankton (Burson et al., 2016; Grosse et al., 2017). Shifts in the community composition are therefore likely to have implications at higher trophic levels (Boersma, 2000; Elser et al., 2001).

Attributes of certain phytoplankton may make them better suited and able to adapt more effectively to a changing nutrient balance. For example, the cell shape impacts the potential nutrient uptake, and the ability to form chains with gaps could allow for increased uptake under (phosphate) limited conditions (Pahlow et al., 1997). Under nitrogen-limited conditions, larger phytoplankton are likely to dominate (Stolte and Riegman, 1995; Philippart et al., 2000). Under P limited conditions, Philippart et al. (2007) observed decreases in diatoms, whilst smaller flagellates were able to grow well. Ultimately, an unbalanced system can have wide ranging impacts on the plankton community and needs to be considered in our management of both direct and diffuse sources.

1.3.2 Impacts of nutrient pollution on plankton

Phytoplankton are vital to marine ecosystems and the earth system as a whole, as they provide the basis of marine food webs, take up carbon dioxide and photosynthesise, and are estimated to produce upwards of 50% of the world's oxygen (Field et al., 1998; Falkowski, 2002; Barney, 2022). Enhanced nutrient input into coastal waters can facilitate the enhanced phytoplankton growth that leads to eutrophication, and whilst phytoplankton growth in itself is not unfavourable, highly elevated phytoplankton biomass can result in undesirable consequences. For example, high phytoplankton abundance can reduce the amount of light that is able to penetrate the water column, limiting the growth potential of other life below the surface (Rhodes et al., 2017). Additionally, the bacterial decomposition of phytoplankton can result in a depletion of oxygen, preventing the growth of other organisms and, in extreme cases, create dead zones (Conley et al., 2002; Diaz and Rosenberg, 2011; Breitburg et al., 2018).

Nutrient pollution and subsequent eutrophication can have implications for the wider ecological community. Secondary impacts from eutrophication can include a reduction in submerged aquatic vegetation as a result of reduced light availability to the water column, a

reduction in species that depend on this vegetation, and decreases in water clarity (Ansari et al., 2010; Dorgham, 2014; Rhodes et al., 2017; Malone and Newton, 2020). Ensuring healthy phytoplankton communities is therefore important to reduce undesirable impacts on marine ecosystems.

1.3.3 Harmful algal blooms

The presence of toxic phytoplankton species can result in problems for fisheries, animals, and humans (Turner et al., 2018). Understanding the interactions between nutrient inputs and harmful algal blooms, and being able to effectively monitor them, is therefore important in order to effectively manage their occurrences and reduce their negative impacts. There is debate about the impact that changing nutrient conditions will have on the frequency, intensity, and toxicity of harmful algal blooms (Anderson et al., 2002; Anderson et al., 2008; Davidson et al., 2012). Burson et al (2016) observed a large proportion of mixotrophic species in areas with a high DIN : DIP ratio, of which harmful species were included, but direct relationships between toxic species and a changing ratio are yet to be established.

1.3.4 Climate change interactions

Temperature is an important controlling factor for both the timing and composition of phytoplankton communities. An increase in temperature affects the timings of blooms of different groups and can have implications for the interactions between trophic levels (Edwards and Richardson, 2004; Diehl et al., 2022; Zhu et al., 2023).

Milder winters may have implications for phytoplankton communities, as they typically result in less convective mixing and therefore fewer nutrients mixed up into the higher part of the water column (Wasmund et al., 2017), and increased stratification is expected to intensify the undesirable consequences of eutrophication (Laurent et al., 2018; Sharples et al., 2020). As phytoplankton are the basis of marine ecosystems, it is important to know not only how they respond to nutrients, but how those responses interact with climate impacts. Dinoflagellates grow slower than diatoms, but are able to utilise nutrients from further down in the water column as a result of their ability to move. Therefore, they are able to bloom even if there is limited convective mixing (Wasmund et al., 2017). Changes to typical storm patterns as a result of a changing climate could impact the growth and biomass of phytoplankton and potentially impact the community composition (Rumyantseva et al., 2019; Edwards et al., 2020). These factors may exacerbate the undesirable impacts of eutrophication.

1.4 Management directives

1.4.1 Management and mitigation of nutrient pollution

There are many barriers to the monitoring and management of eutrophication, such as identifying the sources of nutrient inputs, the scientific understanding of interactions between nutrient enrichment and the ecosystem, and the successful implementation of monitoring and management initiatives in complex marine environments (Boesch, 2019). Identifying and effectively managing sources and impacts of nutrient pollution and eutrophication is essential for the protection and health of the coastal marine environment (Friedland et al., 2021; Piroddi et al., 2021).

Nutrients can originate from point (direct) and/or diffuse sources. Point sources of nutrients, such as sewage and industrial waste, discharge directly into the waterbody. Direct sources of nutrients can be easier to manage through direct regulation of the sewage or industrial activity, driving legislative reduction of nutrients. Examples of this in UK include the EU Urban Wastewater Treatment Directive (91/271/EEC) (European Commission, 1991) which required improvements to sewage outfalls across Europe.

In contrast, diffuse sources, which occur mainly from nutrients discharging from agricultural land and atmospheric deposition can be more difficult to manage and regulate (Jickells et al., 2017; Boesch, 2019) . The regulation of diffuse sources requires cohesive management and cooperation from the multitude of users who contribute to the inputs. Identifying sources of diffuse pollution is challenging, and consequently difficult to regulate. Point sources, where there is a clear identifiable source and responsible party, are much easier to impose regulations upon.

In order to address eutrophication, the associated undesirable impacts, and to maintain healthy ecosystems, there are multiple environmental directives aimed at assessing and managing the issues surrounding the causes and the direct and indirect impacts of eutrophication. These directives will be discussed below.

1.4.2 Water Framework Directive

The Water Framework Directive (WFD) was established in 2000 by the European Commission and implemented in 2003 (European Commission, 2000). The WFD required member states to develop river basin management plans in order to monitor and manage their waterbodies with the aim of raising the quality of transitional (estuaries) and coastal waterbodies to good ecological status, in line with the definitions laid out by the directive (European Commission, 2000).

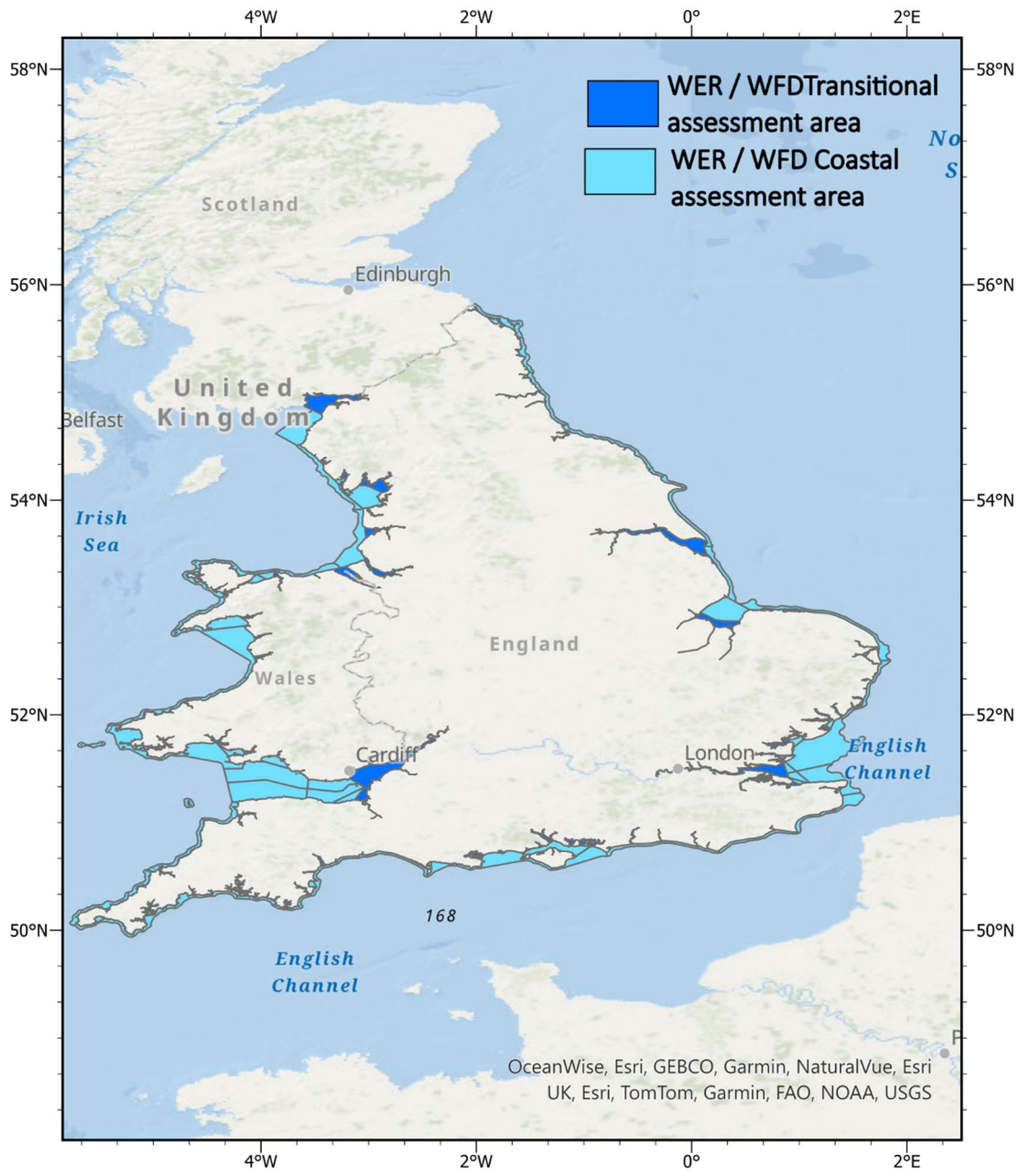

Figure 1.1 - Transitional assessment areas (dark blue) and Coastal assessment areas (teal) for England and Wales as defined in The Water Environment (Water Framework Directive) (England and Wales) Regulations (UK Parliament, 2017) (WFD/WER).

Transitional and coastal waterbodies in the UK are considered within this thesis (Figure 1.1). Transitional water bodies are defined as: *'bodies of surface water in the vicinity of river mouths which are partly saline in character as a consequence of their proximity to coastal waters, but which are substantially influenced by freshwater flows'* (European Commission, 2000). Coastal water bodies are defined as: *'mean surface water on the landward side of a line, every point of which is at a distance of one nautical mile on the seaward side from the nearest point of the baseline'* (European Commission, 2000).

Since its departure from the European Union, the UK continues to engage with the WFD under three separate directives for England and Wales, Scotland, and Northern Ireland. These are known as:

- The Water Environment (Water Framework Directive) (England and Wales) Regulations,
- The Water Environment and Water Services (Scotland) Act 2003 (WEWS Act 2017),
- The Water Environment (Water Framework Directive) Regulations (Northern Ireland) 2017.

These regulations, along with the WFD, will be referred to collectively as WFD/WER hereafter. The WFD/WER requires the ecological status of areas be assessed in 6-year cycles, and their status can be classified from bad to high, with high status indicating the best ecological conditions. The current aim of the WFD/WER is to ensure good ecological status in all waterbodies by 2027 (Poikane et al., 2019).

WFD/WER assessments and classifications are made on a variety of parameters. The metrics relevant to eutrophication which are considered within this thesis are 'phytoplankton' and 'general physicochemical conditions.' General physicochemical conditions are considered to be supporting to the biological indicators, within this thesis the focus is phytoplankton, and they include transparency, thermal conditions, oxygen concentrations, salinity, and nutrient concentrations (Best et al., 2007).

Table 1.2 - Definitions of high, good, and moderate status for phytoplankton and general physicochemical conditions in transitional and coastal waterbodies from The Water Framework Directive (European Commission, 2000).

Parameter	**High status**	**Good status**	**Moderate status**
Phytoplankton	*"The composition and abundance of phytoplanktonic taxa are consistent with undisturbed conditions. The average phytoplankton biomass is consistent with the type-specific physicochemical conditions and is not such as to significantly alter the type-specific transparency conditions. Planktonic blooms occur at a frequency and intensity which is consistent with the type-specific physicochemical conditions."*	*"The composition and abundance of phytoplanktonic taxa show slight signs of disturbance. There are slight changes in biomass compared to type-specific conditions. Such changes do not indicate any accelerated growth of algae resulting in undesirable disturbance to the balance of organisms present in the water body or to the quality of the water. A slight increase in the frequency and intensity of the type-specific planktonic blooms may occur."*	*"The composition and abundance of planktonic taxa show signs of moderate disturbance. Algal biomass is substantially outside the range associated with type-specific conditions and is such as to impact upon other biological quality elements. A moderate increase in the frequency and intensity of planktonic blooms may occur. Persistent blooms may occur during summer months."*
General physico-chemical conditions	*"Physicochemical elements correspond totally or nearly totally to undisturbed conditions. Nutrient concentrations remain within the range normally associated with undisturbed conditions. Temperature, oxygen balance and transparency do not show signs of anthropogenic disturbance and remain within the range normally associated with undisturbed conditions."*	*"Temperature, oxygenation conditions and transparency do not reach levels outside the ranges established so as to ensure the functioning of the ecosystem and the achievement of the values specified above for the biological quality elements. Nutrient concentrations do not exceed the levels established so as to ensure the functioning of the ecosystem and the achievement of the values specified above for the biological quality elements."*	*"Conditions consistent with the achievement of the values specified above for the biological quality elements."*

Table 1.3 – Assessment tools which are used in transitional and coastal waterbodies within the WFD/WER eutrophication assessment (Best et al., 2007; Devlin et al., 2007a; Devlin et al., 2007b).

Type of water body	**Chlorophyll tools**	**Phytoplankton tools**	**Nutrient tools**	**Dissolved oxygen**
Transitional	Average chlorophyll *a* year-round (low and high salinity) in the assessment period Median chlorophyll *a* concentration year-round (low and high salinity) in the assessment period Proportion of samples where mean chlorophyll exceeds predetermined thresholds (low and high salinity) in the assessment period	Proportion of samples in which the count of any single taxa exceeds a threshold value in the assessment period. Proportion of samples in which the count of total taxa exceeds a threshold value in the assessment period.	Winter DIN concentration in the assessment period	5th percentile of year-round surface dissolved oxygen concentration in the assessment period
Coastal	The 90th percentile of all chlorophyll concentrations in the assessment period during the growing season (March to September inclusive)	Average of proportion of months in which diatoms and dinoflagellates fall within the reference envelope. Proportion of samples where counts of a single taxa exceed a threshold value in the assessment period. Proportion of samples in which the count total taxa exceed a threshold value in the assessment period. Proportion of samples in which the number of Phaeocystis cells exceeds a threshold value in the assessment period.	Winter DIN concentration in the assessment period	5th percentile of year-round surface dissolved oxygen concentration in the assessment period

The classification of assessment areas works on the basis of 'one out all out', meaning that the overall classification of an area cannot exceed the lowest classification awarded on any individual metric. The WFD/WER outlines the definitions associated with the classifications of high, good, and moderate status of phytoplankton and general physico-chemical conditions (Table 1.2), and the UK uses a variety of tool to assess these parameters (Table 1.3).

1.4.3 OSPAR Comprehensive Procedure

In addition to the WFD/WER, monitoring and assessment of eutrophication within the UK occurs under the UK Marine Strategy (UKMS), and OSPAR comprehensive procedure. OSPAR assesses the impacts of eutrophication through the Common Procedure (OSPAR, 2005). The first application of the OSPAR Common Procedure (COMP 1) was applied nationally in 2002 with a joint report published in 2003 (OSPAR, 2003). Subsequent applications resulted in joint reports in 2008 and 2017 which contributed to the OSPAR Quality Status Report 2010 and the Intermediate Assessment 2017 respectively (OSPAR, 2005; Heslenfeld and Enserink, 2008; OSPAR, 2008; Foden et al., 2011; OSPAR, 2017). The most recent iteration is the fourth application (COMP 4) with improvements in harmonisation of thresholds and assessment areas across the North-East Atlantic (Devlin et al., 2023). The OSPAR objective under the clean seas theme is to *"Tackle eutrophication, through limiting inputs of nutrients and organic matter to levels that do not give rise to adverse effects on the marine environment" (OSPAR, 2005)*. The results of the COMP 4 application show that this has not been achieved, with 58% of the river plume areas being defined as problem areas (Devlin et al., 2023).

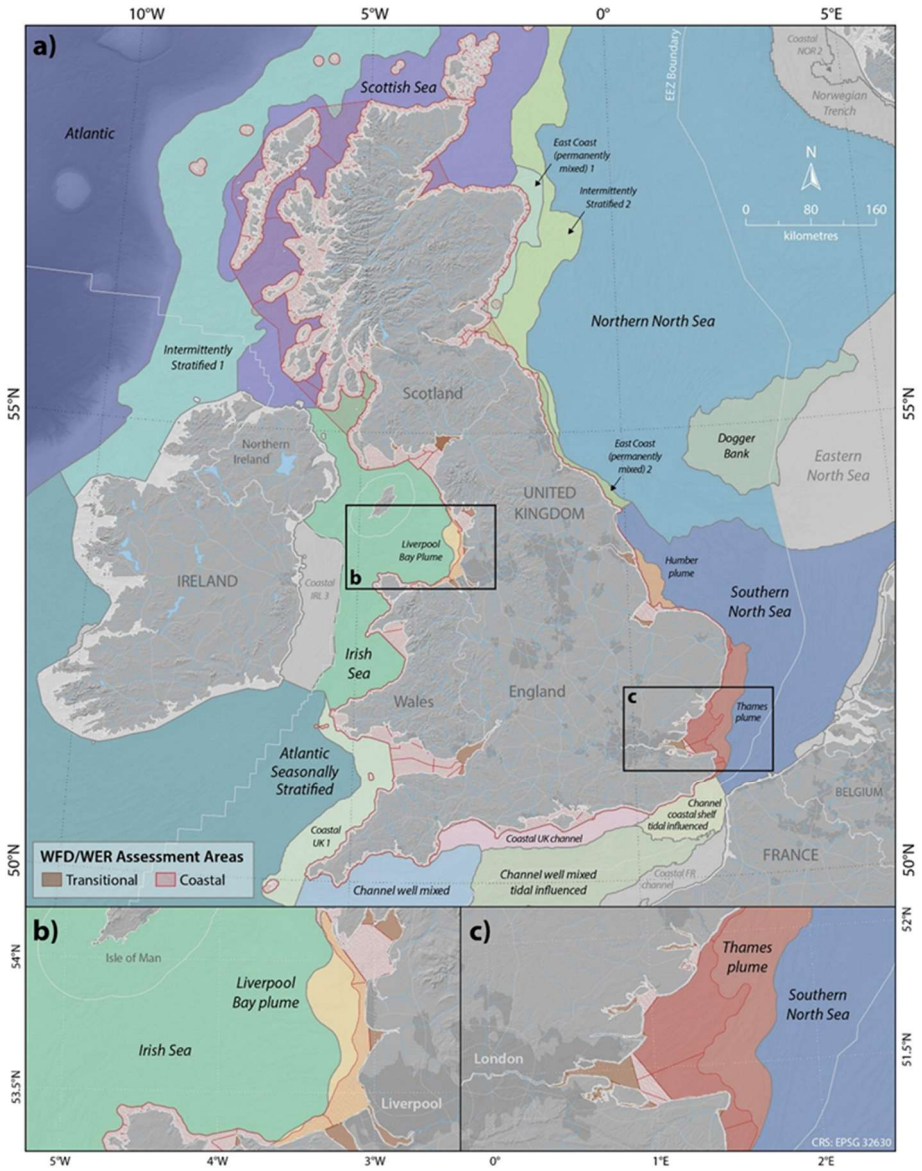

Figure 1.2 – OSPAR assessment areas from COMP 4 relevant to the UK, taken from (Devlin et al., 2025).

OSPAR assesses marine areas from 1 nm offshore, areas inshore of this 1 nm are assessed under WFD/WER (Devlin et al., 2023). In previous applications of the OSPAR assessments, areas were based on geographical boundaries, however in COMP 4, additional and redefined assessment areas were introduced with more ecological relevance (Figure 1.2) (Greenwood et al., 2019; Devlin et al., 2023).

Table 1.4 - Assessment parameters from the fourth application of the OSPAR Common Procedure for assessing eutrophication (OSPAR, 2005; Devlin et al., 2023).

Category I – Degree of nutrient enrichment	**Category II – Direct of effects of nutrient enrichment**	**Category III – Indirect effects of nutrient enrichment**
Area specific levels of winter nutrient concentrations (DIN and or DIP)	Area specific growing season chlorophyll maximum, mean, and / or 90th percentile	Decreased levels and % of oxygen
Area specific winter DIN : DIP ratio	Area specific levels and duration of phytoplankton indicator species	Oxygen or algal toxin related fish kills. Area specific changes in zoobenthos biomass and composition.
Total nitrogen and total phosphorus	Area specific levels and duration of macrophytes	Area specific levels of organic carbon / organic matter
		Transparency of the water column

Eutrophication is assessed by OSPAR using parameters across three categories: nutrient enrichment, direct effects of nutrient enrichment, and indirect effects of nutrient enrichment (Table 1.4). Since COMP 4, the assessment thresholds for the parameters within each of these categories have been area specific (Devlin et al., 2023).

Table 1.5– Common indicators which contribute to the OSPAR waterbody classification (OSPAR, 2005; Devlin et al., 2023).

Category	**Common indicator**
I – nutrient enrichment	Winter mean concentration of DIN and / or DIP
II – direct effects of nutrient enrichment	Growing season mean concentrations of chlorophyll
III – indirect effects of nutrient enrichment	Near seafloor dissolved oxygen concentration

From these assessment parameters, a subset is defined which are the common indicators (Table 1.5), and these contribute to the overall classification of the waterbody. The remainder of the assessment parameters can be applied in areas where they are relevant, to supplement the understanding of the eutrophication problem.

Table 1.6 - Definition of the eutrophication status of water bodies as problem and non-problem areas under the OSPAR assessment (OSPAR, 2005).

Problem areas	**Non-problem areas**
There is evidence of an undesirable disturbance to the marine ecosystem due to anthropogenic enrichment by nutrients.	There are no grounds for concern that anthropogenic enrichment by nutrients has disturbed the marine ecosystem.

OSPAR defines the eutrophication status of waterbodies as problem and non-problem areas (Table 1.6), with this delineation being equivalent to the boundary between good and moderate status in the WFD/WER.

In order to determine these classifications, the individual parameters of the common indicators are assessed. Similar to the WFD/WER, OSPAR classifications use the one out all out where the overall classification cannot be higher than lowest individual parameter assessment outcome. All parameters must achieve a classification of 'good' or higher in order to be considered a non-problem area. There is a caveat to this, however, where it can be demonstrated that nutrient concentrations do not achieve a good status but there is no ecological disturbance as a result of this. In which case the areas will be designated as a 'non-problem area but failing nutrients' (Devlin et al., 2023)

1.4.4 UK Marine Strategy Part One

The UK Marine Strategy Part One (UKMS) (HM Government, 2012) is a further initiative which aims to monitor, manage, and mitigate the issues associated with eutrophication

The UKMS aims to achieve good environmental status in marine waters, and for eutrophication this is defined under descriptor 5 of the directive as:

'Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms and oxygen deficiency in bottom waters ' (European Parliament, 2008).

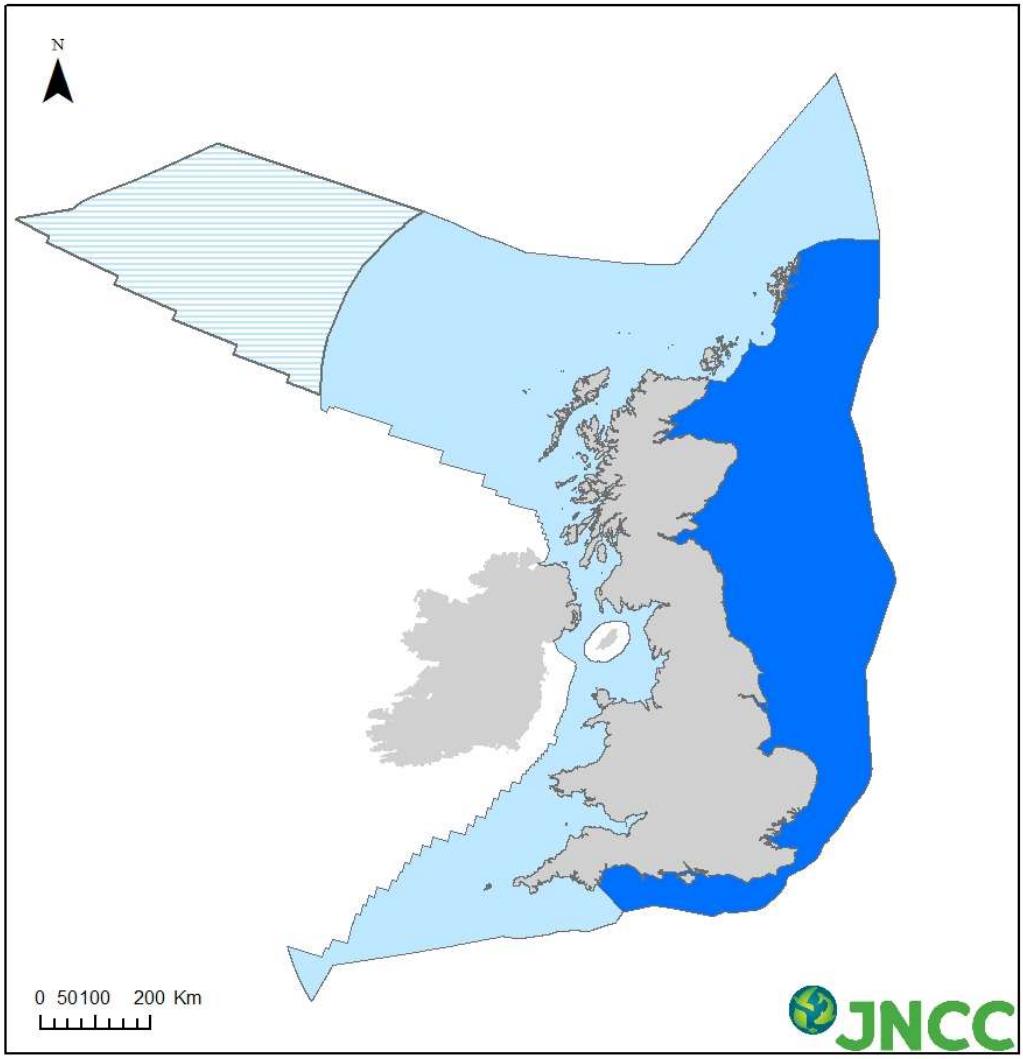

Figure 1.3 - UK Marine Strategy regions (Defra, 2019).

The UKMS covers the areas from the landward boundary of coastal waters to the outer limit of the UK Exclusive Economic Zone, as well as the seabed area where the UK has jurisdiction

(Figure 1.3). Each of these areas falls within a sub-region identified within the overarching Marine Strategy Framework Directive (MSFD) (European Parliament, 2008) which is used in Europe. The indicators used in the UKMS are based on the OSPAR common indicators (Table 1.5) and the thresholds applied in coastal waters are based on those used in the WFD/WER, whilst the thresholds in water further offshore are based on those in the latest OSPAR assessment (Devlin et al., 2023). In the UKMS, the characteristics of good environmental status for eutrophication were defined for each of the common indicators (Table *1.7*) (HM Government, 2012).

Table 1.7 – Characteristics of good environmental status for eutrophication as set out in the UK Marine Strategy Part One (HM Government, 2012).

Category	**Characteristics of 'good environmental status'**
Category I – nutrient enrichment	Nutrient concentrations do not lead to an undesirable disturbance to the balance of organisms present in the water or to the quality of the water concerned resulting from accelerated growth of algae
Category II – direct effects of nutrient enrichment	The direct effects of nutrient enrichment associated with algal growth do not constitute or contribute to an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned
Category II – indirect effects of nutrient enrichment	Indirect effects of nutrient enrichment associated with growth of macroalgae, sea grasses, and reductions of oxygen concentrations do not constitute an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned.

1.5 Assessing phytoplankton communities

A wide range of impacts can occur within phytoplankton communities as a result of eutrophication and management directives are in place which aim to combat these undesirable consequences. The common indicators used within the WFD/WER, OSPAR, and the UKMS which assess the eutrophic condition of waterbodies and health of ecosystems may not, however, be sufficient to assess the full extent of changes within the phytoplankton community (Figure 1.4). The current metrics may also miss changes in the water quality conditions which are responsible for these shifts.

Good environmental status has been achieved for the eutrophication descriptor for the UKMS since 2012, with only 0.41% of estuarine and coastal waters identified as problem areas (Defra, 2019). However, if the metrics used within the management directives were able to identify the full extent of disturbances, or shifts within phytoplankton communities and water quality conditions, this might highlight areas which need further intervention or at risk of undesirable changes imminently. Recently, there has been a focus on developing and implementing methods which aim to assess phytoplankton and eutrophication more holistically (Tett et al., 2007; Tett et al., 2008; Greenwood et al., 2019; Ostle et al., 2021; Devlin et al., 2023; Graves et al., 2023; Devlin et al., 2025; Holland et al., 2025).

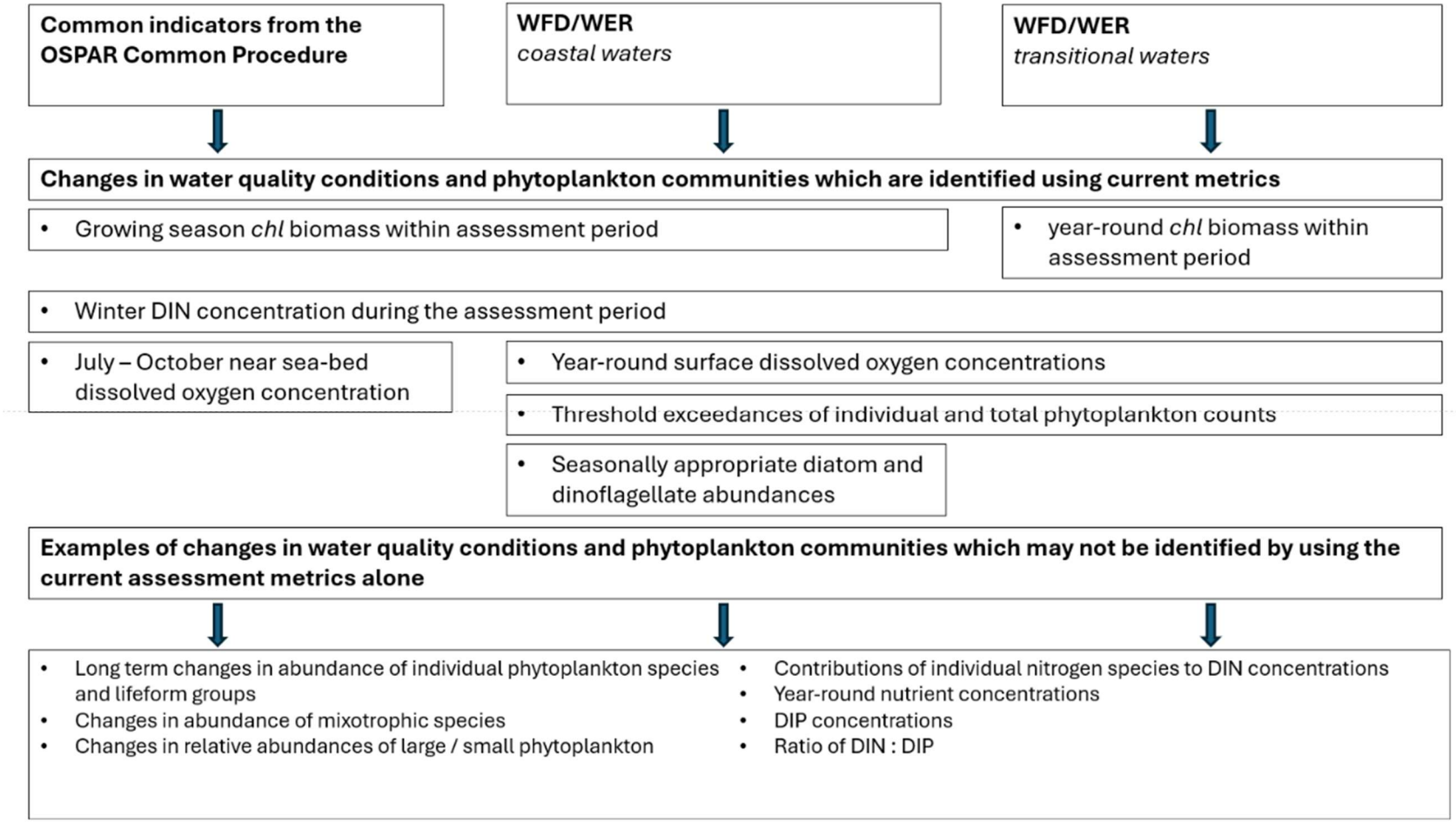

Figure 1.4 - Metrics used by OSPAR and WFD/WER to assess eutrophication and associated undesirable disturbances, alongside examples of changes that would not be identified using the current metrics.

1.5.1 Lifeforms and Lifeform pairs

One of the improvements that is being considered in future eutrophication assessments is a more in depth understanding of how elevated nutrients impact on the plankton community and functioning, as is included in the biodiversity assessments (McQuatters-Gollop et al., 2019). Shifts in water quality conditions are often reflected within phytoplankton community change (Beaugrand, 2005; Tett et al., 2008; McQuatters-Gollop et al., 2019; Bedford et al., 2020; Ostle et al., 2021; Devlin et al., 2023; Graves et al., 2023). Grouping phytoplankton taxa based on their functional traits, and assessing changes within and between these groups, can give information about the drivers contributing to these changes. These lifeforms can be paired together. Lifeform pairs are two lifeforms which have opposing traits but are 'ecologically relevant' (McQuatters-Gollop et al., 2019; Bedford et al., 2020) and are paired together. The rationale behind the pairings is discussed in McQuatters-Gollop et al. (2019) and Bedford et al. (2020). The relative abundance of one to the other can give an indication of ecosystem health (Tett et al., 2008; Wasmund, 2017; Wasmund et al., 2017).

1.5.2 The diatom to dinoflagellate index

Diatoms and dinoflagellates are frequently paired together and often compared when discussing ecosystem health (Wasmund, 2017; Wasmund et al., 2017; Bedford et al., 2020). The relative abundance of one to the other can give indications of the state of the ecosystem (McQuatters-Gollop et al., 2007a). As diatoms take up silicate in order to grow, an increase in the concentration of N and P in the system can mean that Si (silicon) becomes the limiting nutrient, and that diatom growth becomes limited while the relative abundance of dinoflagellates increases (Wasmund, 2017; Wasmund et al., 2017; Spilling et al., 2018)

1.5.3 Plankton index tool

Measuring the change in the relative abundance of lifeform pairs (such as diatoms and dinoflagellates) can be done using the Plankton Index tool (PI tool). The PI tool, developed by Tett et al. (2008) assigns a numeric value to the degree of change in the relative abundances of the lifeforms within the pairs between two time periods. Abundances from the 'assessed' period are plotted, and a 'donut shaped' envelope defining reference conditions is created using 90% of this data (Figure 1.5).

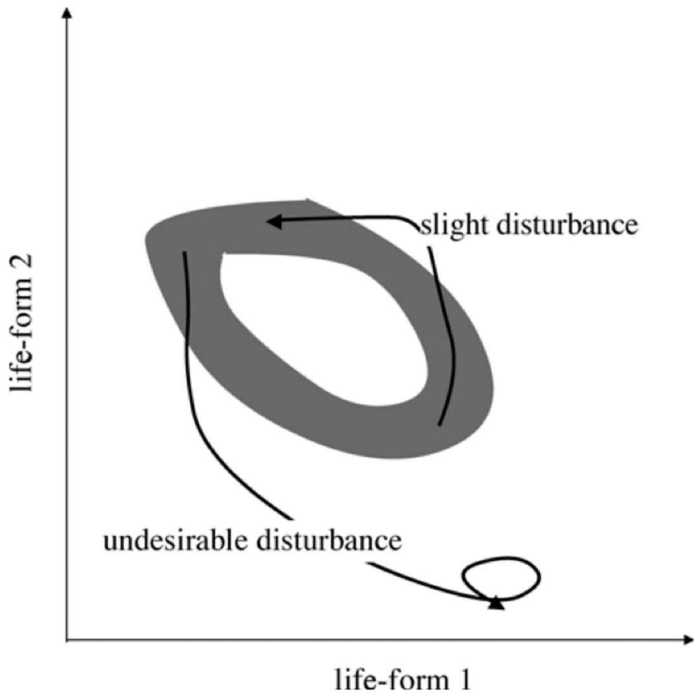

Figure 1.5 – Schematic of the plankton index tool plot, taken from Tett et al. (2008).

Abundances of the lifeforms in the lifeform pair from a separate 'comparison period' are also plotted on the same axes. The plankton index generated is a measure of the proportion of data points from the comparison period which fall within the reference envelope created by the data from the assessed period. A higher proportion of data points being within the reference area shows a smaller change within the relative abundances of the lifeforms within the pair. Therefore, a high PI value indicates little change has occurred between the two time periods, and a low number indicates significant changes have occurred. A PI value of 0.7 or lower is considered to represent a statistically significant change within a lifeform pair (Tett et al., 2008; Greenwood et al., 2019; Graves et al., 2023).

1.6 Study areas

The study areas in this thesis are the Liverpool Bay and the Thames Estuary. There are differences in the predominant land uses in the catchments of these study areas, which provides an opportunity to investigate in contrasting systems. There is literature studying trends in the water quality and phytoplankton communities in these areas (Sanders et al., 2001; Nedwell et al., 2002; Weston et al., 2008; Kinniburgh and Barnett, 2010; Neal et al., 2010; Greenwood et al., 2011; Bowes et al., 2012; Greenwood et al., 2012; Lazar et al., 2012; Greenwood et al., 2019; Fronkova et al., 2022). The availability of this earlier work offers

important knowledge and makes these good study areas to develop the understanding of the role of phytoplankton within eutrophication monitoring.

1.6.1 Liverpool Bay Catchment area

Liverpool Bay sits within the Irish Sea in the northwest of England (Figure 1.6). It is typically less than 50m in depth and can experience stratification for periods of up to several days (Palmer, 2010; Palmer and Polton, 2011; Polton et al., 2011). The Bay is mainly fed by the rivers Dee, Mersey, and Ribble and there is a consistent region of freshwater influence. The northwest catchment area which drains to Liverpool Bay covers an area of 13,200 km^2, with a population of nearly 7 million people, and the major urban centres of Liverpool and Manchester (Environment Agency, 2023a). 80% of the northwest river basin is rural (Environment Agency, 2023a) and the majority of farmed land is permanent pasture (Defra, 2024).

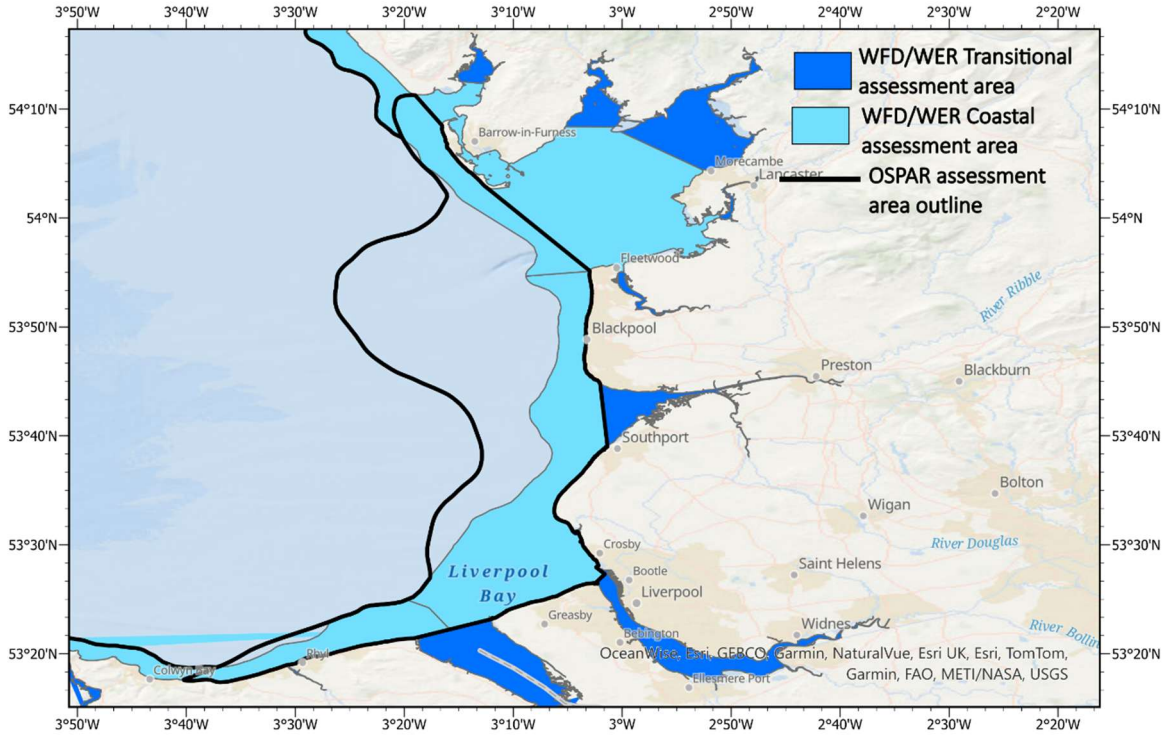

Figure 1.6 – Liverpool Bay study area. The dark blue areas are transitional assessment areas in the WFD/WER. The teal shows the coastal assessment areas in the WFD/WER. The black line outlines the Liverpool Bay plume OSPAR assessment area.

1.6.2 Nutrient inputs and concentrations in Liverpool Bay

Between 1994 and 2016, annual loads of DIN into Liverpool Bay were between 35,800 and 58,500 tonnes of N per year, with nitrate being the dominant form of nitrogen (Greenwood et al., 2019). Annual DIP loads were reported to be between 3,000 and 6,200 tonnes of P per year

in this timeframe (Greenwood et al., 2019). Ammonium, nitrite, and DIP inputs into the bay have significantly decreased over the same time period (1994 – 2016), but no changes were identified in DIN inputs (Greenwood et al., 2019), resulting in a significant increase in the ratio of DIN : DIP inputs (Greenwood et al., 2019). Mean winter nutrient concentrations in Liverpool Bay of 16 µmol/L TOxN, 1 µmol/L DIP, and 10 µmol /L Si have been reported (Greenwood et al., 2011).

1.6.3 Phytoplankton community in Liverpool Bay

Phytoplankton dynamics between 2003 and 2009 in Liverpool Bay show an annual spring bloom, which is dominated by diatoms (Greenwood et al., 2012). Dinoflagellates were identified for short intervals between the months of July and October (Greenwood et al. 2012). Significant changes in the phytoplankton community occurred across transitional and coastal WFD/WER areas in Liverpool Bay between 2006 and 2015, in the form of increasing dinoflagellate abundance (Greenwood et al. 2019).

1.6.4 Liverpool Bay assessment outcomes

In the 2019 results of the WFD/WER, none of the surface waters in the northwest achieved good chemical status (Environment Agency, 2023a). Of the surface waterbodies within the area, 131 of 600 achieved good or high ecological status, whilst the remainder achieved moderate or below (Environment Agency, 2023a). The most common reasons for waters not achieving good ecological status in this river basin district were agricultural pollution from rural areas, and wastewater pollution from the water industry (Environment Agency, 2023a).

The Liverpool Bay plume assessment area (black line, Figure 1.6) was introduced into the OSPAR assessment in COMP 4, and it was awarded a 'high' status in the eutrophication assessment using data from 2015 – 2020 (Devlin et al., 2023). The plume area is based on the contour lines of an SPM concentration of 10 mg/L, has an area of 1661 km^2 and a mean depth of 15 m, and a mean salinity of 30.6 (Greenwood et al., 2019; Devlin et al., 2023).

1.6.5 Thames Estuary catchment area

The Thames Estuary is a well-mixed tidal estuary in the southeast of England where the Thames River flows into the North Sea, with a tidal range of 3-6 m (Middelburg and Nieuwenhuize, 2001). The Thames River basin has a catchment area of 16,200 km^2, and approximately 17% of the basin is urban, whilst the remainder is rural (Environment Agency, 2023b). There is a population of around 15 million people, including urban centres of London, Reading, and Luton (Environment Agency, 2023b). The majority of the farmed land in the Thames Estuary catchment area is arable land (Defra, 2024).

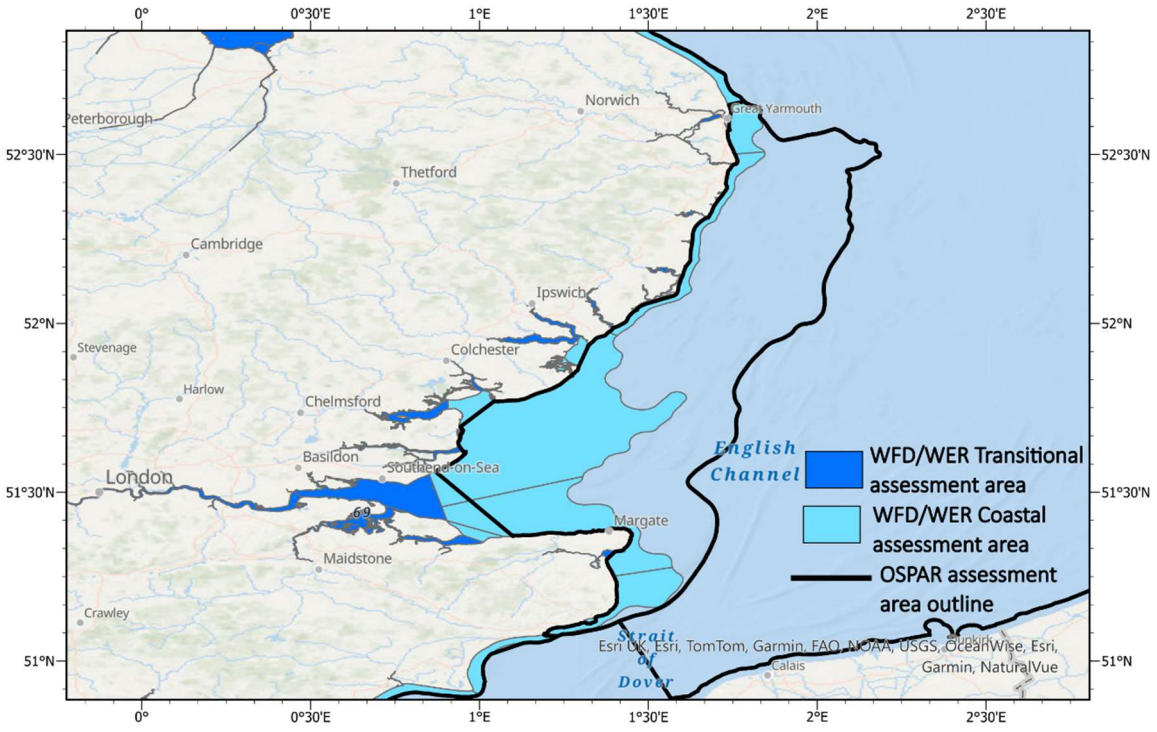

Figure 1.7 - Thames Estuary assessment area. The dark blue areas are transitional assessment areas in the WFD/WER. The teal shows the coastal assessment areas in the WFD/WER. The black line outlines the Thames plume OSPAR assessment area.

1.6.6 Nutrient inputs and concentrations into the Thames Estuary

Nitrogen is the most abundant nutrient entering the Thames Estuary (Middelburg and Nieuwenhuize 2001), with annual loads of DIN varying between 23,700 and 60,500 tonnes of N per year between 1994 and 2016, dominated by nitrate (Greenwood et al., 2019). Ammonium and DIP inputs have decreased in the Thames Estuary (Greenwood et al., 2019), but no statistically significant changes have been identified in the DIN concentrations entering the Estuary between 1994 and 2016. The ratio of DIN : DIP in the nutrient inputs into the Thames has however been seen to increase significantly between 1994 and 2016 (Greenwood et al., 2019). Typical winter concentrations of 45 µmol/L of nitrate, 17 µmol/L of silicate, and 2 µmol/L of phosphate were measured within the Thames plume (Weston et al., 2008).

1.6.7 Phytoplankton community in the Thames Estuary

The phytoplankton community, sampled at the Warp SmartBuoy, showed a spring bloom initially diatom dominated, before a switch to *Phaeocystis* (Weston et al., 2008). The remainder of the year was seen to be diatom dominated (Weston et al., 2008). Significant changes in the phytoplankton community composition were identified across transitional and coastal areas

in the Thames Estuary between the years 2006 and 2015, and this was attributed to increasing dinoflagellate abundances (Greenwood et al., 2019).

1.6.8 Thames Estuary assessment outcomes

In the 2019 WFD/WER, none of the surface waters in the Thames River basin district received good chemical status (Environment Agency, 2023b). No surface waters were awarded high ecological status, only 31 out of 501 surface water bodies were awarded good ecological status and the remaining 470 were rated as moderate or below (Environment Agency, 2023b). The most common reasons for areas not receiving a good status in the Thames River basin were agricultural pollution from rural areas and wastewater pollution from the water industry (Environment Agency, 2023b).

The Thames plume assessment area (black line, Figure 1.7) was introduced into the OSPAR assessment in COMP 4, and it was awarded a 'high' status in the eutrophication assessment using data from 2015 to 2020 (Devlin et al., 2023). The plume has an area of 5523 km^2 is based on the contour lines of an SPM concentration of 25 mg/L It has a mean depth of 22 m, and a mean salinity of 34.4 (Greenwood et al., 2019; Devlin et al., 2023).

1.7 Research questions

Phytoplankton are an essential part of healthy coastal ecosystems, and the implications of eutrophication can be substantial and seen throughout trophic levels. It is therefore important to ensure that the relationships between phytoplankton communities and changing water quality conditions are well understood and that the full extent of changes are sufficiently identified and monitored. This thesis aims to contribute to furthering the understanding of how phytoplankton fit into eutrophication monitoring in the UK marine waters. This will be in terms of how changes can be effectively assessed and monitored, the relationship between water quality parameters and phytoplankton communities, and whether the current policies are sufficient to effectively safeguard ecological communities from the undesirable impacts of eutrophication.

1.7.1 Chapter Three - Implementing new methods into assessments, for a more holistic view of eutrophication in UK marine waters

In Chapter three , the results from the current eutrophication assessment metrics will be discussed alongside those from additional methods, with the aim of addressing the following research questions:

1. *Does long term trend analysis provide more informed assessments of estuarine and coastal waters, and would inclusion of trend information improve current metrics that assess ecological state over 6-year cycles?*
2. *Can the Plankton Index tool offer further insight into the extent of ecological impacts of eutrophication in addition to the current phytoplankton metrics alone?*
3. *What further understanding could be gained about the eutrophic state of coastal and estuarine areas by applying integrated coastal and offshore assessment using both WFD/WER and OSPAR in terms of metrics and time periods?*

1.7.2 Chapter Four - Environmental controls on phytoplankton biomass and community composition in the Thames Estuary and Liverpool Bay

Using data collected under the marine Natural Capital and Ecosystem Assessment programme (mNCEA) (Defra, 2022), relationships will be established between water quality and the phytoplankton community in Liverpool Bay and the Thames Estuary. Chapter four will aim to address:

4. *How do the light and nutrient conditions vary with salinity in Liverpool Bay and the Thames Estuary?*
5. *How does the phytoplankton abundance and community composition vary with salinity in the two study areas of Liverpool Bay and the Thames Estuary?*
6. *Is phytoplankton biomass nutrient limited at an offshore sampling site in the Thames Estuary?*

1.7.3 Chapter Five - Turbidity impacts on the abundance and composition of diatoms and dinoflagellates in coastal waters, and the associated implications for management

Using a natural community from the Thames Estuary area, an assessment will be made on whether current concessions on nutrient concentrations are appropriate to limit the potential undesirable consequences of nutrient enrichment. This research question will be addressed:

7. *How does a natural phytoplankton community respond to sediment additions in a laboratory incubation experiment and what are the implications for current UK assessment criteria?*

1.7.4 Chapter Six – Outlook and synthesis

The findings from each of the research chapters will be brought together and discussed in terms of the additional knowledge that they can offer about the role of phytoplankton within eutrophication monitoring, as well as outlining suggestions for further work in this area.

2

Common methodologies

2.1 Mapping and visualisation of spatial data

ArcGIS Pro was used to visualise assessment areas which are used under OSPAR and WFD/WER monitoring initiatives, and to show sample collection sites. Shapefiles for the WFD/WER transitional and coastal assessment areas were downloaded from the Environment agency at (https://environment.data.gov.uk/dataset/78c2df61-d465-11e4-b839-f0def148f590). OSPAR assessment areas were downloaded from ICES (https://ices-library.figshare.com/articles/dataset/Input_data_files_for_the_OSPAR_COMP_4_eutrophication_assessment_using_COMPEAT/22189111) .

2.2 Analysis

Data exploration and analyses were carried out in R (R Core Team, 2024).

2.3 Discrete sample collection

This section provides the methods of data collection, processing, and analysis of samples collected for this thesis in the Thames Estuary and Liverpool Bay study areas. Core methods common to multiple chapters are outlined here. Further details including vessels, sampling locations, and timings are described in each relevant chapter.

2.3.1 Suspended particulate matter (SPM) Preparation

0.7 µm glass fibre filters were pre-ashed by heating at 450 °C for 1 hour, rinsed with MilliQ water, and then dried at 75 °C for 2 hours. The filter papers were weighed and the mass of each was recorded, and they were stored in a desiccator before use. One sample per station was collected unless an incubation was taking place using water collected from that station, in which case triplicate samples were collected where possible.

2.3.2 SPM Collection and Analysis

To determine SPM concentrations, a small volume of MilliQ water was filtered through the prepared filter paper before filtering a known volume of sample water, typically 100-1000 mL dependent on the turbidity of the water. The measuring cylinder, funnel, and filter paper used were rinsed well with MilliQ water to ensure no sediment remained. The filter papers were kept in a sealed plastic bag and taken back to the lab as soon as possible where filter papers were dried at 75 °C for 24 hours, weighed and the mass recorded, in line with the protocol outlined in Neukermans et al. (2012). The initial mass of the filter paper was subtracted from the mass of the dried filter paper and sediment in order to obtain a value for sediment.

This was converted to an SPM concentration in mg / L using Equation 1:

$$\text{SPM (mg/L)} = \frac{\begin{pmatrix}\text{mass of filter paper} \\ \text{and sediment(mg)}\end{pmatrix} - \left(\text{prepared filter paper mass(mg)}\right)}{\text{Volume of sewater filtered (L)}}$$

Equation 1 - Calculation of suspended particulate matter concentration.

2.3.3 Chlorophyll *a* Collection

A known volume of sample water (typically 100-1000 mL dependent on productivity in the water) was filtered through a 0.7 µm glass fibre filter under vacuum no more than 10 kPa, using acid washed filtering equipment. The filter papers were folded in half with the residue inside, wrapped in aluminium foil and stored in the dark at -80 °C until analysis. Samples were analysed with 12 months of collection.

2.3.4 Chlorophyll *a* Analysis

Chlorophyll concentrations were analysed using fluorometry, in line with the method outlined in Tett et al. (1987) and by the manufacturer (Arar and Collins, 1997). The fluorometer was calibrated by Cefas scientists using a solution of 'chlorophyll *a* free of chlorophyll *b*' (Sigma Aldrich) with the concentration and purity being determined spectrophotometrically with a FLUOstar Omega spectrophotometer. Samples were extracted by placing the filter papers in a test tube with acetone (8 mL, 90%). The test tubes were left in the dark at 4 °C. Within 18-72 hours the test tubes were transferred to a centrifuge at 3500 RPM for 10 minutes. The solution was then transferred to a cuvette and analysed using a Turner 10AU field fluorometer. After the first analysis HCL was added (1.2 M, 2 drops) and the solution was analysed again to obtain a value for the fluorescence contributions from phaeophytin. Quality control of results for discrete samples is assured by participation in the Quality Assurance for Marine Environmental Measurements (QUASIMEME) scheme.

Raw fluorescence values were converted to chlorophyll a concentration using Equation 2 and Equation 3.

$$C_{extract} = F_s \left(\frac{r}{r-1}\right) X\ (R_a - R_b)$$

Equation 2 - Chlorophyll concentration in the extract.

$$C_{sample}\ (\mu g\ L^{-1}) = \frac{C_{extract}\ x\ extract\ volume\ (L) X\ DF}{Sample\ volume\ (L)}$$

Equation 3 - Chlorophyll concentration in sample.

r = the before-to-after acidification ratio of a pure chlorophyll a solution, calculated during calibration

R_b = fluorescence of sample extract before acidification

R_a = fluorescence of sample extract after a acidification.

DF= dilution factor

2.3.5 Inorganic Nutrient Collection

Nutrient sampling and analysis were carried out according to Becker et al. (2020). Sample water was filtered through Minisart 0.45 µm filters into 50 mL sample pots which had been acid washed, and samples were then stored at -12 °C until analysis.

2.3.6 Inorganic Nutrient Analysis

Nutrient samples were fully defrosted for at least 24 hours prior to analysis. Sample tubes were rinsed with sample water and then filled with sample no more than 5 mm from the top. Concentrations of nitrate, nitrite, silicate and ammonium were analysed using a SEAL continuous segmented flow autoanalyzer AA3 by scientists at Cefas. Samples which were beyond the upper limit of quantification were diluted with artificial seawater and re analysed. The upper detection limit and range are outlined in Table 2.1. The % error for all nutrient analyses was ±1% relative to Ocean Scientific International (U.K.) standards. Quality control of results for samples is assured by participation in the Quality Assurance for Marine Environmental Measurements (QUASIMEME) scheme.

Table 2.1 – Detection limits and ranges of each nutrient parameter analysed using the continuous segmented flow autoanalyzer.

Parameter	**Detection Limit (µmol / L)**	**Range (µmol / L)**
Nitrite	0.01	0.01-5
Dissolved total oxidised nitrogen	0.10	0.10-29
Dissolved Silicate	0.10	0.10-20
Dissolved Inorganic Phosphate	0.10	0.10-5
Ammonium	0.10	0.10-10

2.3.7 Salinity

Sample water was collected into 200 mL glass sample bottles. The bottles were rinsed three times with sample water before being filled to the shoulder. The neck and thread were dried with tissue to ensure no salt crystallisation before being stoppered, and the bottles were stored in wooden crates until analysis. One salinity sample was collected at each station, and duplicate samples were collected at the start and end of the crate of bottles for quality control purposes. Samples were analysed by Cefas scientists using a Guildline 8400B salinometer which had been standardised with IAPSO standard seawater.

2.3.8 Phytoplankton Collection

250 mL opaque amber HDPE bottles were filled to the shoulder with sample water and fixed with a pipette (2 mL) of acidified Lugol's iodine. The bottles were kept in the dark until analysis, and where possible were analysed within a year of the collection date.

2.3.9 Phytoplankton Analysis

Samples were analysed by taxonomists at Cefas using the Uttermöhl microscopy method (Uttermöhl, 1958).

Samples were homogenised by inverting ten times and then transferred into settling chambers, the size of chamber determined by the sediment and phytoplankton densities. High sediment samples were pipetted into 1ml Sedgewick-Rafter slide. 5-, 10-, or 25-mL

chambers were used for other samples. Samples were left to stand before being counted under inverted light microscopes.

Taxa were identified to species level where possible and to the lowest taxonomic level above this if not possible. Counts were recorded in cells per litre. 200 cells/L were required per sample and if this was not reached then a second chamber was counted.

The entire baseplate of the chamber was scanned at 200 x magnification for low cell density samples. Transects across the widest part of the chamber were used for moderate cell density samples and a zig-zag pattern across the baseplate of ten random field of views were used for high cell density samples.

3

Implementing new methods into assessments, for a more holistic view of eutrophication in UK marine waters

3.1 Abstract

The monitoring of eutrophication within UK coastal and transitional waters is conducted under WFD/WER. In addition, coastal, plume, and offshore waters are monitored under the UK Marine Strategy Part 1 (UKMS) and OSPAR. The WFD/WER assesses ecological state within an assessment period of 6 years. Eutrophication is assessed using a range of indicators that focus on plankton biomass, nutrients, and dissolved oxygen. Whilst these current indicators are well accepted as being important in the assessment of eutrophication, they may not be fully capturing the extent of ecological disturbances and changes in nutrient concentrations. In addition to this, eutrophication assessments are not integrated along a spatial gradient through WFD/WER and OSPAR assessment areas, with assessment occurring on different time periods. This chapter presents analysis of long-term monitoring data to establish if trend analysis offers additional insight in the assessment of coastal and marine waters. The results suggest that this longer-term view can provide information regarding success of management interventions and identify the likely trajectory of future conditions. In addition, methods for the assessment of plankton community dynamics are used, to assess if extra information regarding the state of phytoplankton communities could be acquired. The findings indicate that there are shifts within phytoplankton communities which are not currently being picked up within eutrophication assessments under WFD/WER and OSPAR, and additional and useful insight could be gained from expanding the assessment parameters.

3.2 Introduction

Monitoring and assessment of eutrophication is conducted under separate pieces of legislation established from The Water Framework Directive (European Commission, 2000), for England and Wales, Scotland, and Northern Ireland. Water Environment Regulations (England and Wales), the Water Environment and Water Services (Scotland) Act 2003 (WEWS Act 2017), and The Water Environment (Water Framework Directive) Regulations (Northern Ireland) 2017, collectively referred to as the WFD/WER. Assessments also take place under the UK Marine Strategy Part One (UKMS) (HM Government, 2012), and OSPAR (OSPAR, 2005) Quality Status Reporting (QSR) in UK waters. Data which feeds into these assessments for Thames and Liverpool Bay are collected by the Environment Agency (EA) and Natural Resources Wales in transitional and coastal assessment areas, and by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) and Agri-Food and Biosciences Institute (Northern Ireland) (AFBI) in the more offshore areas defined by OSPAR.

Table 3.1 Indicators used in the WFD/WER and OSPAR eutrophication assessments.

Directive	**Waterbody type**	**Nutrient parameters assessed**	**Dissolved oxygen parameters assessed**	**Phytoplankton parameters assessed**
WFD/WER	Transitional assessment areas	Winter DIN concentration	Whole year near surface concentration	Chlorophyll a, elevated phytoplankton counts,
WFD/WER	Coastal assessment areas	Winter DIN concentration	Whole year near surface concentration	Chlorophyll a, elevated phytoplankton counts, seasonal succession of diatoms and dinoflagellates
OSPAR fourth Common Procedure for the assessment of eutrophication	River plume areas and larger offshore areas	Winter DIN & DIP concentration	Near-seabed concentration during July – October assessment period	Chlorophyll a, phytoplankton indicator species

The current assessments for eutrophication use a targeted but limited range of indicators (Table 3.1), that provide an overview of status for inshore and offshore waters within a fixed period of time. All assessments rely on what are known as the primary indicators for eutrophication which include dissolved winter nutrients, phytoplankton biomass and dissolved oxygen. Whilst these indicators have been and continue to be valuable indicators for the assessment of eutrophication, they do not capture changes in the pelagic community or measure biodiversity shifts that could be related to eutrophication pressures. The assessments could be enhanced by utilising additional metrics for monitoring change in the plankton community (considered here is the plankton index tool), and by extending the temporal coverage and considering trends over time. Such approaches utilise datasets which already exist and would not require alteration to the sampling efforts of the eutrophication assessments. Many authors have made suggestions for the expansion of the metrics used in assessments (Greenwood et al., 2019; Devlin et al., 2023; Graves et al., 2023; Devlin et al., 2025; Holland et al., 2025), and this work will add to the growing evidence pool.

The range of possible indicators used in the eutrophication assessments compare nutrient inputs, concentrations, and ratios, with the impacts on oxygen concentration, chlorophyll concentration, and phytoplankton total and individual species abundances to pre-determined thresholds, as well as a seasonal succession tool, as outlined in Devlin et al. (2007a) and Devlin et al. (2007b). These indicators aim to determine not only if there are elevated concentrations of nutrients, but if they have resulted in undesirable disturbances to ecosystems, as the assessments are conducted with the knowledge that elevated nutrient concentrations are not *necessarily* an undesirable disturbance if they do not result in ecological disturbances (Devlin et al., 2007b; Foden et al., 2011)

Despite the aim to examine ecological disturbances within waterbodies, there are ecosystem perturbations which cannot be assessed through these metrics. Whilst phytoplankton community change is measured under the OSPAR pelagic habitats assessment of biodiversity (Rombouts et al., 2019), eutrophication assessments do not currently include a phytoplankton community composition metric beyond the seasonal succession tool which is applied only in coastal waters under the WFD/WER. Developments in the understanding of interactions between nutrients, eutrophication, and ecology mean that there is increased awareness of the extent to which ecosystems can be impacted. For example, changes in harmful algal blooms in relation to eutrophic conditions have been investigated (Glibert and Burkholder, 2011; Davidson et al., 2012; Gowen et al., 2012; Glibert and Burford, 2017; Glibert, 2020), but occurrences of harmful species would not be identified in all current assessments. Furthermore, there is evidence that a changing nutrient ratio may impact phytoplankton community composition (Lagus et al., 2004; Vrede et al., 2009; Chu et al., 2014; Burson et al.,

2018). Burson et al. (2016) observed differences in the limiting nutrient across different species, resulting in a shift in the community composition. A low N:P ratio resulted in a community dominated by cyanobacteria in mesocosm experiments (Vrede et al., 2009), and species which could access alternative P sources were more successful in the high N:P ratio experiments by Lagus et al. (2004). As the implications of eutrophication and environmental changes are increasingly more understood, assessment metrics should be developed accordingly in order to monitor and, if necessary, to mitigate the full extent of undesirable disturbances. The phytoplankton index tool (PI) (Tett et al., 2008), is a method of determining change within a lifeform pair. It has been recommended for use as part of eutrophication assessments (Tett et al., 2008; Greenwood et al., 2019) , but has not yet been implemented. The PI is used here to investigate the changes in the diatom/dinoflagellate lifeform pair, as their relative abundances have been linked to eutrophic conditions and changes in water quality (McQuatters-Gollop et al., 2007a; Wasmund, 2017; Wasmund et al., 2017). Responses to nutrient concentrations have been seen to differ between phytoplankton of differing size (Charalampous et al., 2021; Dashkova et al., 2022), the relative abundances of which could also be measured using the PI method. Alternative methods for assessing phytoplankton community change have been developed, such as the species reference list (Devlin et al., 2009) , but to date this has not been formally implemented into the eutrophication assessment methods.

Eutrophication assessments are conducted over a fixed time period under current frameworks. Assessments under the WFD/WER are made using data from 6-year assessment periods. This gives a snapshot of the state of waterbodies but gives limited indication of the long-term health of the assessment areas. There is no metric assessing long term changes and trends within the WFD/WER, and it is not included within the official OSPAR common indicators. Data from the Environment Agency and Cefas is utilised here to present long term trends within the assessment areas to determine whether this could provide extra information and insight which may be beneficial to policy makers and managers, for example on the trajectory of parameters.

Developing the understanding of eutrophication impacts and linking changes seen in the marine environment to their respective drivers is known to be a challenging undertaking (Cloern, 2001), as relationships between environmental variables and phytoplankton response are complex and can be location specific. Extending the range of methods used to assess the available data, here through the PI and long-term trend analysis, may provide a deeper understanding of the relationships between eutrophic conditions and ecological shifts within assessment areas and allow for more effective management.

The aim of this chapter is to present additional metrics and increased temporal coverage alongside the established indicators used in WFD/WER and OSPAR assessments to determine whether they can increase understanding about the impacts of nutrient inputs on the marine environment and about eutrophic water bodies. Key datasets which contribute to the statutory monitoring of eutrophication will be used.

Specifically –

Does long term trend analysis provide more informed assessments of estuarine and coastal waters, and would inclusion of trend information improve current metrics that assess ecological state over 6-year cycles?

Can the Plankton Index tool offer further insight into the extent of ecological impacts of eutrophication in addition to the current phytoplankton metrics alone?

What further understanding could be gained about the eutrophic state of coastal and estuarine areas by applying integrated coastal and offshore assessment using both WFD/WER and OSPAR in terms of metrics and time periods?

Table 3.2 – Overview of the knowledge from current metrics and the potential knowledge gain from harmonising and expanding assessment methods.

Current metrics	Harmonising assessments	Additional metrics
Identifies a classification of status relative to a threshold level within a defined assessment period		**Long term trends -** Assessing trends in the indicators over a longer time period gives information about the trajectory of changes and allows for insight into the success of management practices, and whether water quality in areas is improving or declining, prior to an assessment outcome which indicates intervention is necessary. This can allow for preventative rather than remedial action.
Transitional and coastal, and offshore assessment areas assessed separately through the WFD / WER and OSPAR assessments	**Assessing inshore and offshore areas simultaneously –** Identifying the state of the waterbody along the inshore to offshore gradient simultaneously can give insight into potential at risk areas, if they are neighbouring problematic areas. Assessing changes simultaneously can give insight into how eutrophic conditions manifest along the salinity gradient.	
Universal methods are not used across transitional, coastal, and offshore assessment areas. *Outlined in Table 3.1*	**Implementing the same methods across all assessment areas –** Utilising the full suite of assessment methods across the assessment areas allows for more direct comparisons, and a deeper understanding of ecological disturbances along the salinity gradient, which can inform where certain drivers of undesirable disturbance have the biggest impact.	
Current metrics assess DIN without considering the contributions of different species		**Assessing nutrient species individually –** Identifying changes in individual nutrient species, may offer further insight into the causes, and therefore the necessary management action, of some of the ecological perturbations occurring within the assessment areas.
Phytoplankton metrics may miss fine scale changes across all areas. *Phytoplankton metrics in each assessment area outlined in Table 3.1*.		**Plankton Index tool –** Utilising an addition metric, for example the Plankton Index tool, gives information about finer scale changes in the phytoplankton community which would not be identified in all assessment areas with the current metrics. This is crucial, as these fine scale changes may have undesirable impacts on the ecosystem.

3.3 Study areas

The study areas chosen for this investigation are the Liverpool Bay and Thames Estuary and coastal areas. In the fourth Common Procedure OSPAR assessment (OSPAR, 2005; Devlin et al., 2023) additional ecological assessment areas were defined in both of these study areas (Greenwood et al., 2019), allowing for assessment on a finer scale. These areas have large industrial cities and large catchment areas with a range of agricultural activities. Liverpool Bay has been identified as having high diffuse nutrient inputs (Alldred et al., 2024) and the Thames has been reported to be nutrient enriched (Bowes et al., 2018), and to have high chlorophyll river concentrations (Bowes et al., 2012).

Transects were identified in the study areas. These cover transitional, coastal, and offshore assessment areas and include the regions of freshwater influence used in the OSPAR COMP 4 assessment (Devlin et al., 2023). This coverage should capture the gradient in nutrient concentrations and associated ecological impacts from inshore to offshore as a result of anthropogenic activities.

3.3.1 Thames Estuary

The Thames Estuary is covered in detail in chapter one, but in brief, is in the southeast of England where the Thames river flows into the North Sea, with a tidal range of 3-6m (Middelburg and Nieuwenhuize, 2001). The assessment areas in the Thames estuary cover a transect from inshore transitional assessment areas through London and Essex, into the Essex coastal area and the Thames plume, which is representative of the region of freshwater influence (Greenwood et al., 2019) (Figure 3.1).

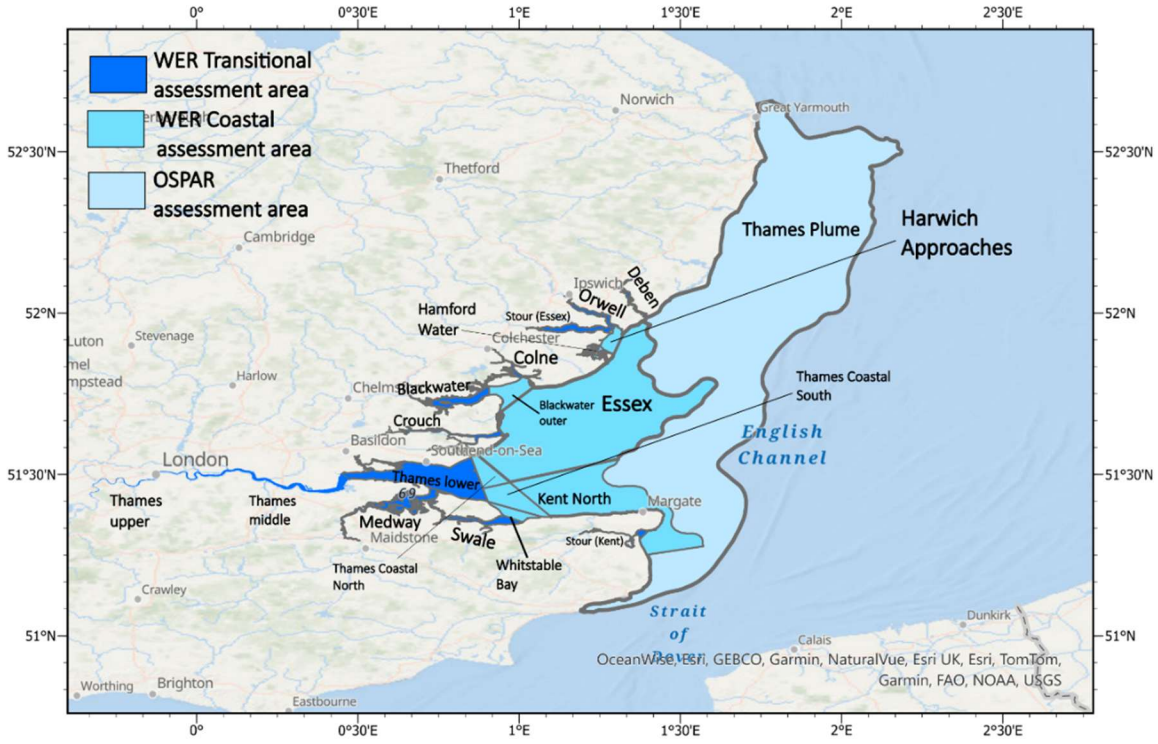

Figure 3.1 - Labelled Assessment areas used in the Thames Estuary study area showing the assessment areas used under the WFD/WER and OSPAR. Dark blue areas are transitional waterbodies (WFD/WER), teal areas are coastal waterbodies (WFD/WER), and the light blue area is the Thames plume assessment area (region of freshwater influence, OSPAR).

3.3.2 Liverpool Bay

The Liverpool Bay area is covered in detail in chapter one, but in brief, sits within the Irish sea in the Northwest of England, and freshwater input dominated by the rivers Dee, Mersey and Ribble creates a consistent region of freshwater influence. The transect identified within the Liverpool Bay study area covers inshore transitional areas including the Mersey and Dee, through coastal areas and into the Liverpool Bay plume, which is representative of the region of freshwater influence (Hopkins and Polton, 2012; Greenwood et al., 2019) (Figure 3.2).

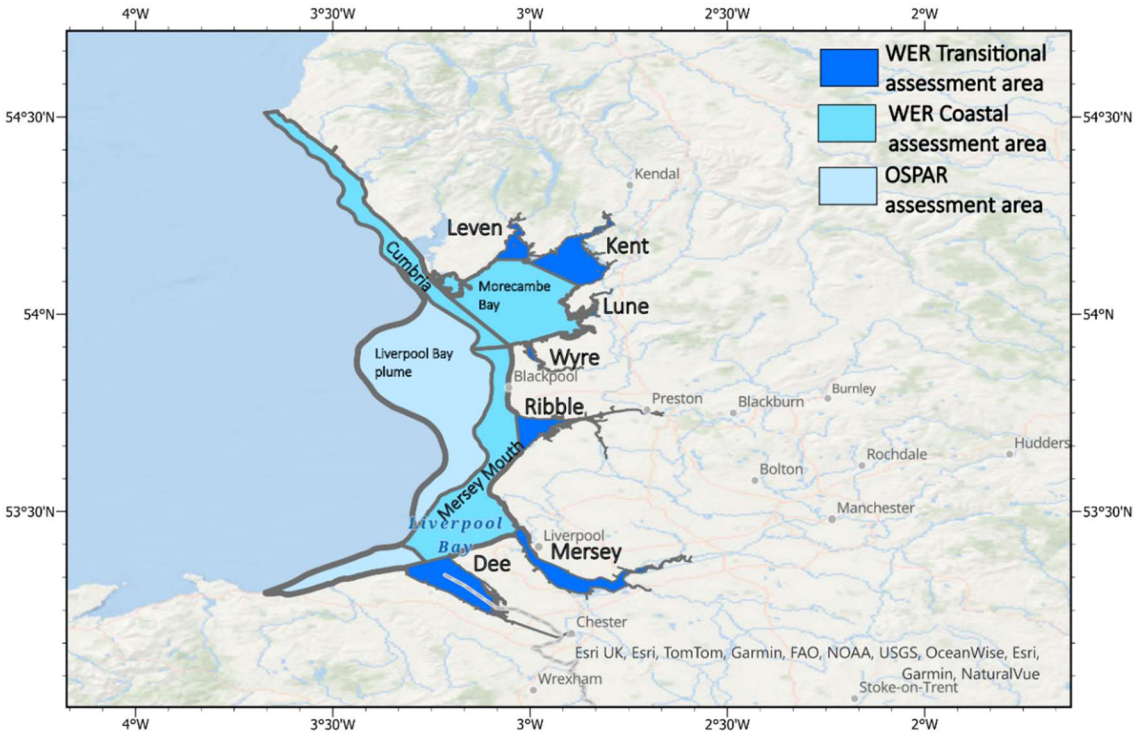

Figure 3.2 – The Liverpool Bay study area showing the eutrophication assessment areas under WFD/WER and OSPAR. Dark blue areas are transitional waterbodies (WFD/WER), teal areas are coastal waterbodies (WFD/WER), and the light blue area is the Liverpool Bay Plume assessment area (OSPAR) which shows the extent of the region of freshwater influence.

3.4 Methodology

The WFD / WER and the OSPAR assessment are not typically considered harmoniously. The WFD / WER and the OSPAR assessment are carried out over different time periods. The most recent WFD / WER assessment was 2014 – 2019 whereas the most recent OSPAR assessment was 2015 – 2020. The data from all assessment areas are considered over the same timeframe within this work. The metrics applied to the different types of assessment areas, and under the different assessments, are not usually applied universally. Data collected for the WER / WFD is not typically combined with that collected for the OSPAR assessments. Combining the data here increases the spatial and temporal coverage in some assessment areas, and applying metrics consistently allows for a better understanding of impacts and changes across different area types at the same time.

Here, data which covers a gradient including transitional and coastal (WFD / WER) and offshore (OSPAR) assessment areas are assessed simultaneously using a common set of metrics, including additional methods. Whilst the benefit of additional metrics and long-term trends has been highlighted in literature (Greenwood et al., 2019; Devlin et al., 2023; Graves

et al., 2023; Devlin et al., 2025), the application of universal, including additional, metrics applied across this spatial extent on the combined dataset is novel, and demonstrates the additional knowledge and insight which could be gained through this this approach.

An assessment of long-term trends in the common indicators were included in the COMP 4 assessment of eutrophication under OSPAR (Devlin et al., 2023), however an analysis of changes over time have not previously been included the WFD / WER assessments. The additional knowledge gained by applying the plankton Index tool to assessment areas is presented in Graves et al. (2023). The results of the plankton index tool assessments from Graves et al. (2023) are utilised within this work. The focus of the work by Graves et al. (2023) is an assessment of the plankton index tool results at varying spatial scales, whilst the results within this work are a comparison to the most recent assessment outcomes of the WFD / WER phytoplankton sub metric classifications.

Data from the Environment Agency was selected if it had been identified as being collected for monitoring purposes, excluding pollution incidences, between 2006 and 2020 was accessed through the EA portal (https://environment.data.gov.uk/water-quality/view/doc/reference) . Data collected between 2006 and 2020 by Cefas was extracted from their online data portal (https://data.cefas.co.uk). A mean monthly value for each parameter was derived from all available data from the identified sources within each assessment area in the long-term analysis which covers 2006 – 2020 and is hereafter referred to as the study period. This time period was chosen as regulatory monitoring associated with the WFD/WER was initiated in 2006, with monthly sampling for nutrients and phytoplankton. Prior to 2006, monitoring was more sporadic with limited phytoplankton data. There is no data available later than 2020 in the Phytoplankton Lifeform Extraction Tool (PLET) (Ostle et al., 2021), which is used for the PI analysis. Data was constrained to the 2006 – 2020 study period to limit analysis being influenced by changes in temporal or spatial data frequency. In an attempt to further address this, areas were only included in the analysis for a parameter if there was data covering at least 10 years of the assessment period. Observations with an unknown associated salinity, a salinity of less than five in line with the WFD/WER assessment protocol, or a salinity of greater than 40 were removed for the analysis.

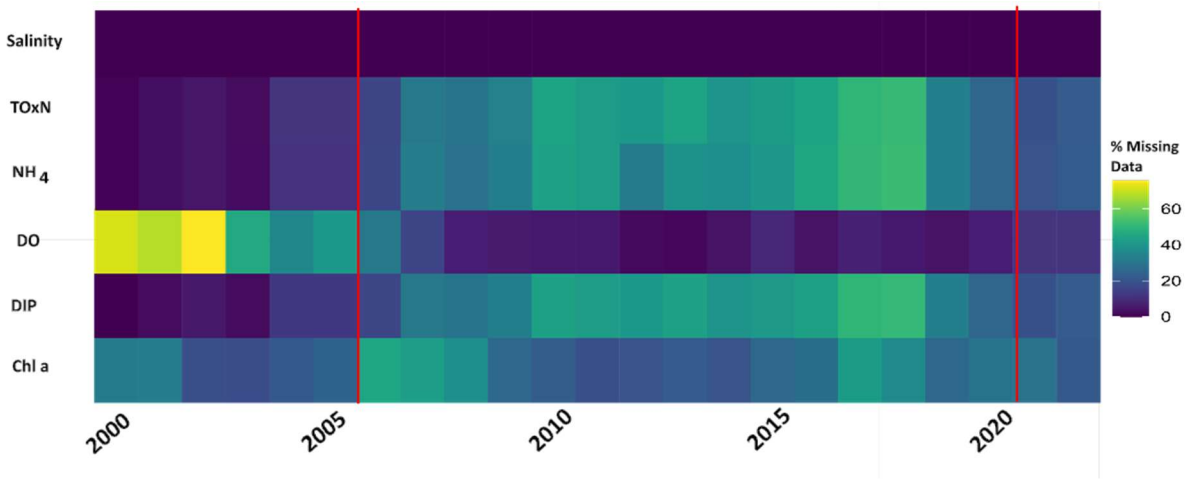

Figure 3.3 - Percentage of mean monthly data missing for each parameter in each year across the assessment areas used in the transect in the Thames Estuary catchment area. Data are compiled from Environment Agency and Cefas eutrophication monitoring programmes. The red vertical lines denote the start and end of the study period.

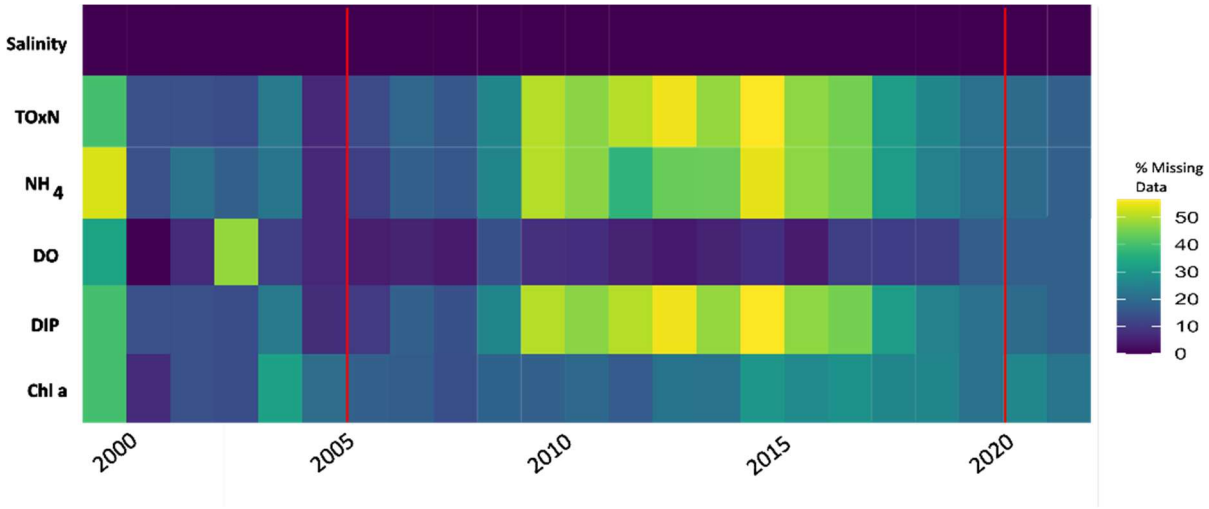

Figure 3.4 - Percentage of monthly mean data missing in each parameter each year across the assessment areas used in this analysis in the Liverpool Bay area. Data are compiled from Environment Agency and Cefas eutrophication monitoring programmes. The red vertical lines denote the start and end of the chosen study period.

Within the chosen time period, there are variations in the availability of mean monthly data in the Thames Estuary area (Figure *3.3*). In the first three years of the assessment period, and 2017 and 2018, there is an increase in the percentage of missing chlorophyll data. In the TOxN, $NH4^+$, and DIP data there is an increase in the percentage of missing data after the first year of the assessment period, and the largest data gaps are observed in 2018 for these variables (Figure 3.3). Dissolved oxygen has a decrease in missing data after the first year of the assessment period and then remains quite consistently available (Figure 3.3). In the Liverpool Bay area, for the nutrient parameters TOxN, $NH4^+$, and DIP, the first four years of the

assessment period show a low percentage of missing data with an increase in the amount of missing data between the years of 2010 and 2017 (Figure 3.4). Chlorophyll and dissolved oxygen have a low percentage of missing data throughout the assessment period (Figure 3.4).

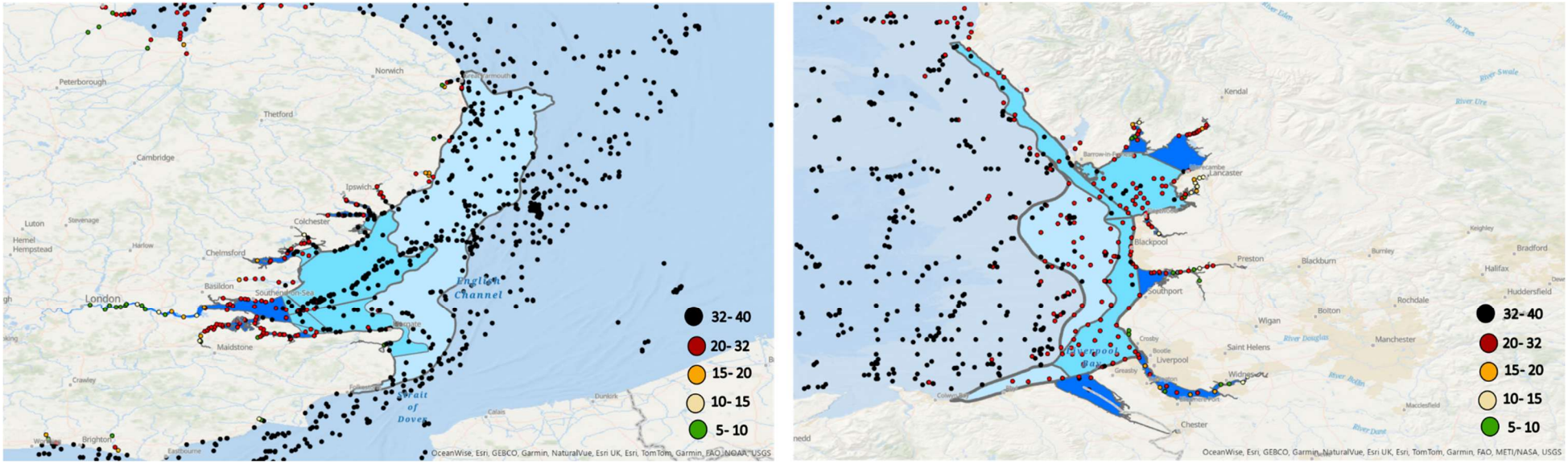

Figure 3.5 – Salinity data availability in the Thames Estuary (left) and in Liverpool Bay (right). Dark blue areas are transitional waterbodies (WFD/WER), teal areas are coastal waterbodies (WFD/WER), and the light blue areas are the Thames plume and Liverpool Bay Plume assessment areas (OSPAR) which show the extent of the region of freshwater influence. Each dot represents an individual sampling occasion, and the shading of the dot represents the salinity associated with that sampling occasion.

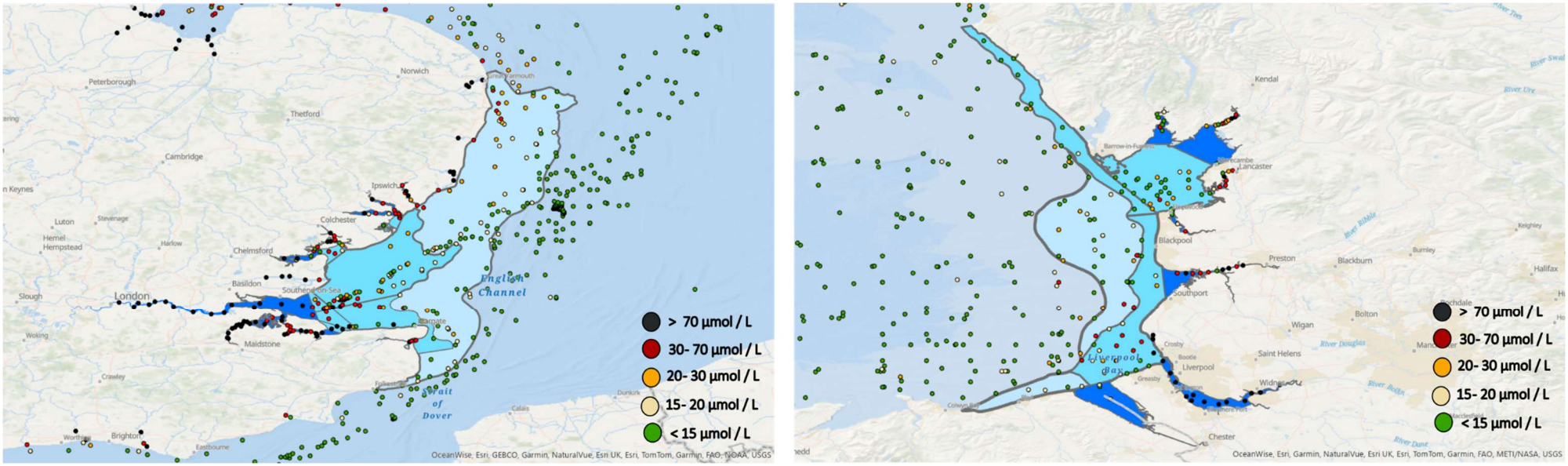

Figure 3.6 – TOxN data availability in the Thames Estuary (left) and in Liverpool Bay (right). Dark blue areas are transitional waterbodies (WFD/WER) teal areas are coastal waterbodies (WFD/WER), and the light blue areas are the Thames plume and Liverpool Bay Plume assessment areas (OSPAR) which show the extent of the region of freshwater influence. Each dot represents an individual sampling occasion, and the shading of the dot represents the TOxN concentration associated with that sampling occasion.

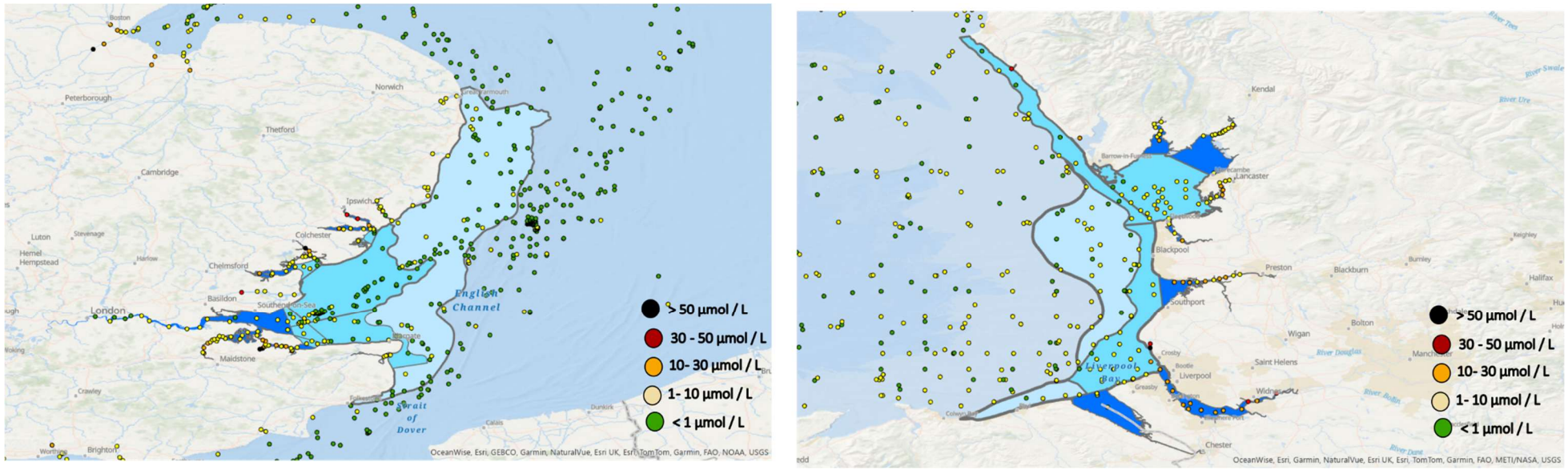

Figure 3.7 – Ammonium data availability in the Thames Estuary (left) and in Liverpool Bay (right). Dark blue areas are transitional waterbodies (WFD/WER), teal areas are coastal waterbodies (WFD/WER), and the light blue areas are the Thames plume and Liverpool Bay Plume assessment areas (OSPAR) which show the extent of the region of freshwater influence. Each dot represents an individual sampling occasion, and the shading of the dot represents the ammonium concentration associated with that sampling occasion.

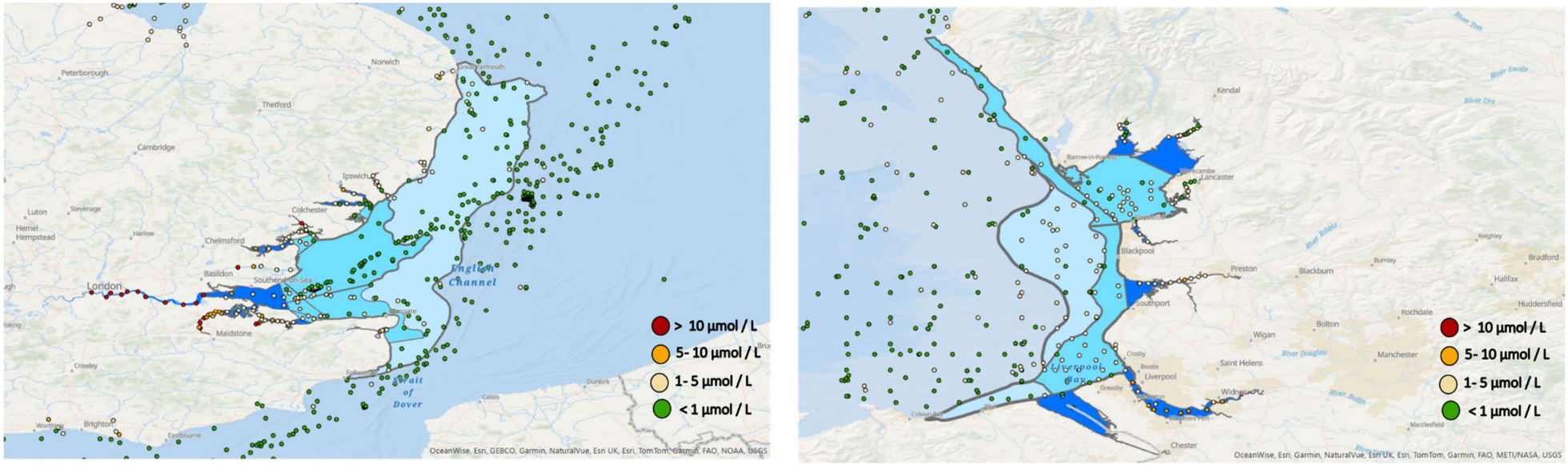

Figure 3.8 – DIP data availability in the Thames Estuary (left) and in Liverpool Bay (right). Dark blue areas are transitional waterbodies (WFD/WER), teal areas are coastal waterbodies (WFD/WER), and the light blue areas are the Thames plume and Liverpool Bay Plume assessment areas (OSPAR) which show the extent of the region of freshwater influence. Each dot represents an individual sampling occasion, and the shading of the dot represents the DIP concentration associated with that sampling occasion.

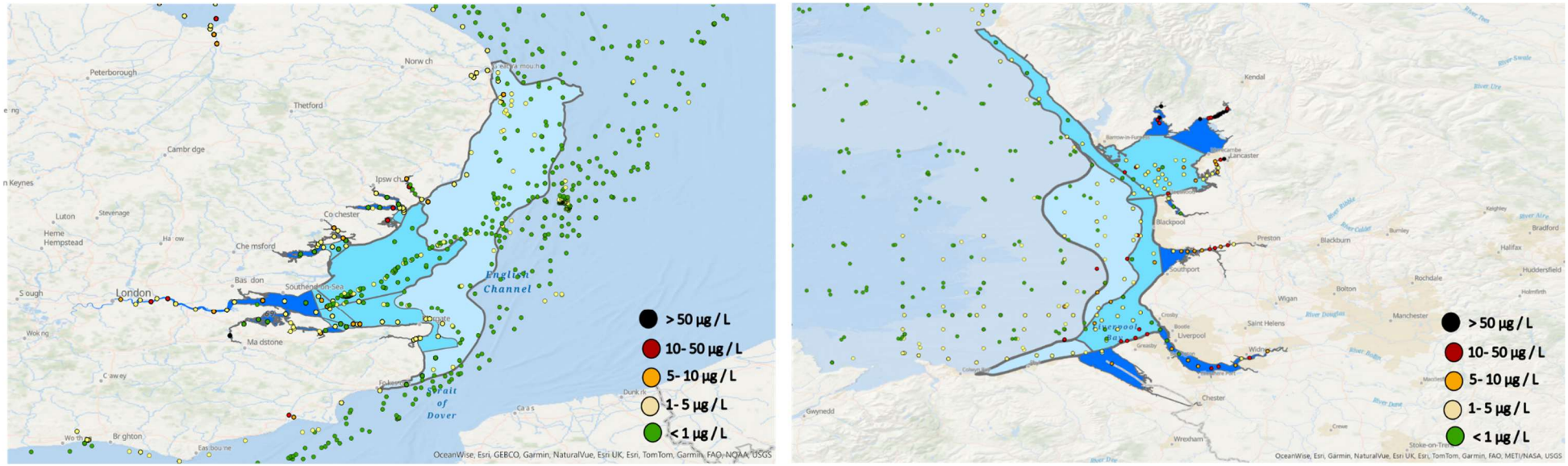

Figure 3.9 – Chlorophyll data availability in the Thames Estuary (left) and in Liverpool Bay (right). Dark blue areas are transitional waterbodies (WFD/WER), teal areas are coastal waterbodies (WFD/WER), and the light blue areas are the Thames plume and Liverpool Bay Plume assessment areas (OSPAR) which show the extent of the region of freshwater influence. Each dot represents an individual sampling occasion, and the shading of the dot represents the chlorophyll concentration associated with that sampling occasion.

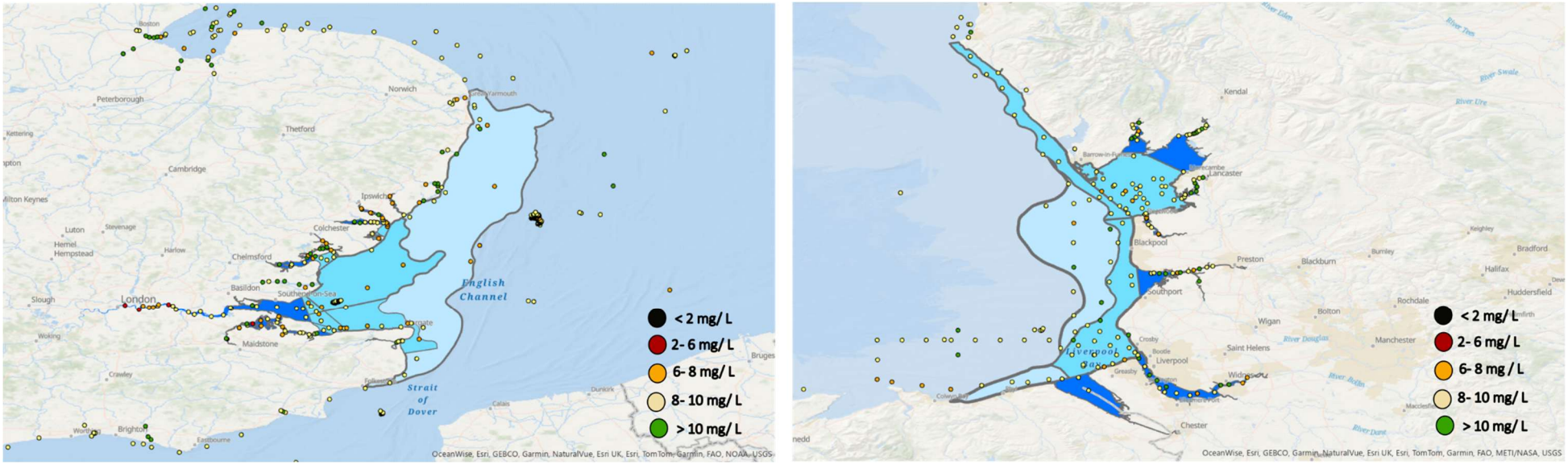

Figure 3.10 – Dissolved oxygen data availability in the Thames Estuary (left) and in Liverpool Bay (right). Dark blue areas are transitional waterbodies (WFD/WER), teal areas are coastal waterbodies (WFD/WER), and the light blue areas are the Thames plume and Liverpool Bay Plume assessment areas (OSPAR) which show the extent of the region of freshwater influence. Each dot represents an individual sampling occasion, and the shading of the dot represents the dissolved oxygen concentration associated with that sampling occasion.

The Thames estuary has salinity values covering the entire range, with transitional assessment areas typically having lower salinity values, whilst the coastal and plume area has a typical salinity of 32 – 36 (Figure 3.5). In the Liverpool Bay area, there are lower salinity values seen in the coastal and plume area, with observations between 20 – 32 typically seen in these areas (Figure 3.5).

TOxN values range between the limit of detection and 700 µmol/L, with over 70% of the values being between the limit of detection and 70 µmol/L (Figure 3.6). In both of the study areas, the highest concentrations are seen inshore in transitional assessment areas, and concentrations decline with distance offshore (Figure 3.6). In coastal and plume areas, the majority of TOxN concentrations are below 30 µmol/L, with the occasional value in the 30 – 70 µmol/L category (Figure 3.6).

$NH4^+$ concentrations range between the limit of detection and 170 µmol/L, however over 95% the of values are below 30 µmol/L (Figure 3.7). Values decrease with distance offshore, with values tending to be higher in the transitional assessment areas, and lower in coastal and plume areas (Figure 3.7). Values are predominantly < 1 µmol/L in the Thames estuary through the coastal and plume areas, whilst values are predominantly between 1 and 10 µmol/L in the coastal and plume areas in Liverpool Bay (Figure 3.7).

DIP values range between the limit of detection and 55 µmol/L (Figure 3.8). Values are typically highest inshore. In the Thames estuary, values above 10 µmol/L are seen in some of the transitional assessment areas, however values in Liverpool Bay do not frequently exceed 10 µmol/L even in transitional areas (Figure 3.8). Values between the limit of detection and 5 µmol/L are seen in coastal and plume areas in both study areas, however a larger proportion of observations are between 1 and 5 µmol/L in Liverpool Bay, compared to the majority of coastal and plume samples being < 1 µmol/L in the Thames estuary (Figure 3.8).

Chlorophyll concentrations range between 0.1µg/L and 167 µg/L, with higher concentrations being seen inshore in both study areas (Figure 3.9). Values of over 50 µg/L are observed in Liverpool Bay, however this is uncommon in the Thames estuary, where are only one observation above 50 µg/L occurs (Figure 3.9). Values in the transitional assessment areas in the Thames are typically below 50 µg/L (Figure 3.9). In the coastal and plume areas, the values are typically below 10 µg/L in the Thames estuary, and below 50 µg/L in the Liverpool Bay area (Figure 3.9).

Dissolved Oxygen concentrations range between 0.6 mg/L and 15 mg/L (Figure 3.10) . The lowest values are typically seen inshore in the transitional assessment areas. Transitional

assessment areas in the Thames estuary often have observations of values of 6 mg/L or below, whilst the coastal and plume areas have observations with values typically between 6 and > 10 mg/L (Figure 3.10). In the Liverpool Bay area, the transitional assessment areas have a few observations of between 6- 8 mg/L, but values typically range between 8 - >10 mg/L throughout areas (Figure 3.10).

Sampling locations are similar across parameters, with the exception of dissolved oxygen, where there is much less spatial coverage of assessment areas. DIN is calculated by summing TOxN and NH_4^+, and DIN : DIP is the ratio of the concentrations of DIN and DIP.

3.4.1 Trend analysis

A linear model of *date ~ concentratio*n was run using the *stats* package in R (R Core Team, 2024), using mean monthly values across the entire study period for TOxN, NH_4^+, DIN, DIP, DIN : DIP, salinity, dissolved oxygen and chlorophyll over the study period (2006-2020) in each individual assessment area in order to determine if a significant trend in each of these monitoring parameters was occurring over time. If the output of the linear model gave a *p*-value below 0.05, the trend was considered to be significant.

3.4.2 Threshold determination

Winter (Data from November – February inclusive) DIN values across the entire study period (2006 – 2020) were plotted against their associated salinity values. A linear regression was fitted and the value of the line at a salinity of 25 for transitional assessment areas, 32 for coastal assessment areas and 34 and 34.5 for Liverpool Bay plume and Thames plume respectively were calculated. Suspended particulate matter (SPM) concessions, where increased nutrient concentrations are permitted in turbid waters under WFD/WER, are not considered here when determining the threshold values. The boundary for good / moderate status in each water body type is 30 µmol/L for transitional waters and 18 µmol/L for coastal waters in line with the WFD/WER threshold values and the method outlined in Devlin, Painting and Best (2007). The winter DIN thresholds for the COMP 4 OSPAR assessment are 22.2 µmol/L for the Liverpool Bay plume, and 16.9 µmol/L in the Thames plume (Devlin et al., 2023).

3.4.3 Plankton Index Tool

Plankton Index values were taken from analysis completed in Graves et al. (2023). There are varying levels of confidence in the PI values for individual assessment areas, and these are identified in Graves et al. (2023). All areas shown here have data which covers at least 10

months across the assessed period. The 'assessed period' is 2016-2020, and this is compared to the 'comparison period' of 2006-2015. A plankton index value below 0.7 is considered to indicate that a significant change has occurred within a lifeform pair between the assessed period and the comparison period.

For the full Plankton Index results, monthly lifeform abundance data (counts as individuals/L) were extracted from the Plankton Lifeform Extraction Tool (PLET) (Ostle et al., 2021) using the Environment Agency 2000-2020 phytoplankton dataset (https://doi.mba.ac.uk/data/1535). These data were analysed using the Phytoplankton Index (PI) method using MATLAB (Tett et al., 2008; Tett, 2021). To allow $\log_{10}$ transformation of zero-values, an estimate of the limit of detection (*z*) was first added to all abundances, taken as half of the lowest observed value for each lifeform. The PI is part of the OSPAR PH1/FW5 indicator 'Changes in phytoplankton and zooplankton communities', and the approach is detailed in previous studies (McQuatters-Gollop et al., 2019; Graves et al., 2023). The PI method is used to identify changes in abundances for the diatom/dinoflagellate lifeform pair by comparing data from the reference period and the comparison period. The PI method involves plotting lifeform pair abundances against one another and creating an envelope containing 90% 'assessed period' data, using an inverted convex hull method. The PI value is then calculated as the ratio between the number of observations in the comparison period which fall within this envelope and to those which fall outside. A PI value of 0.9 indicates that there has been no change, and in the context of the PI being used as an ecological indicator, a value below 0.7 suggests a change has occurred which should be considered statistically significant (generally associated with a binomial p value <0.05) (Tett, 2021).

3.5 Results

3.5.1 Long term trend analysis

For maps showing the nutrient, salinity, and chlorophyll trends (Figure 3.11, Figure 3.12), assessment areas are shaded based on the *p* value obtained from a linear model fitted to the data. *P* values and time series figures are presented in the appendix (7.1 – 7.2).

The concentrations of DIN, DIP and TOxN significantly decline in the Thames plume , while there are no significant changes in DIN : DIP and the concentrations of $NH4^+$ and chlorophyll (Figure 3.11). Salinity increases from 2006 to 2020 in the Thames plume (Figure 3.11). The smaller Essex assessment area, which sits within the Thames plume, has no significant trends in DIN, TOxN, DIP, DIN : DIP, or chlorophyll, whilst there is significant increase in the $NH4^+$concentration and in salinity (Figure *3.11*). There are significant increases in DIN and TOxN concentrations in the Thames middle and Stour (Essex) assessment areas, and $NH4^+$

increases in Thames Lower, Stour (Essex) and Blackwater (Figure 3.11). These changes are not mirrored in the DIP concentrations, for which limited significant changes are observed. There are declines in DIP concentrations a few transitional areas (Figure 3.11), and a singular increasing trend in the Stour (Kent) (Figure 3.11). The DIN : DIP ratio increases in the Orwell and Stour (Kent) and decreases in the Deben, with no significant changes elsewhere (Figure 3.11). Thames Lower, Stour (Essex), Blackwater and Colne show significant declines in chlorophyll concentrations, Swale shows an increasing trend, and the remaining areas have no significant trends (Figure 3.11). Salinity increases from 2006 to 2020 in the majority of assessment areas in the Thames estuary area (Figure 3.11), with a singular decreasing trend in the Stour (Kent). Dissolved oxygen concentrations decrease in the Thames plume area and increase in the Thames middle but otherwise no significant trends are identified (Figure 3.11).

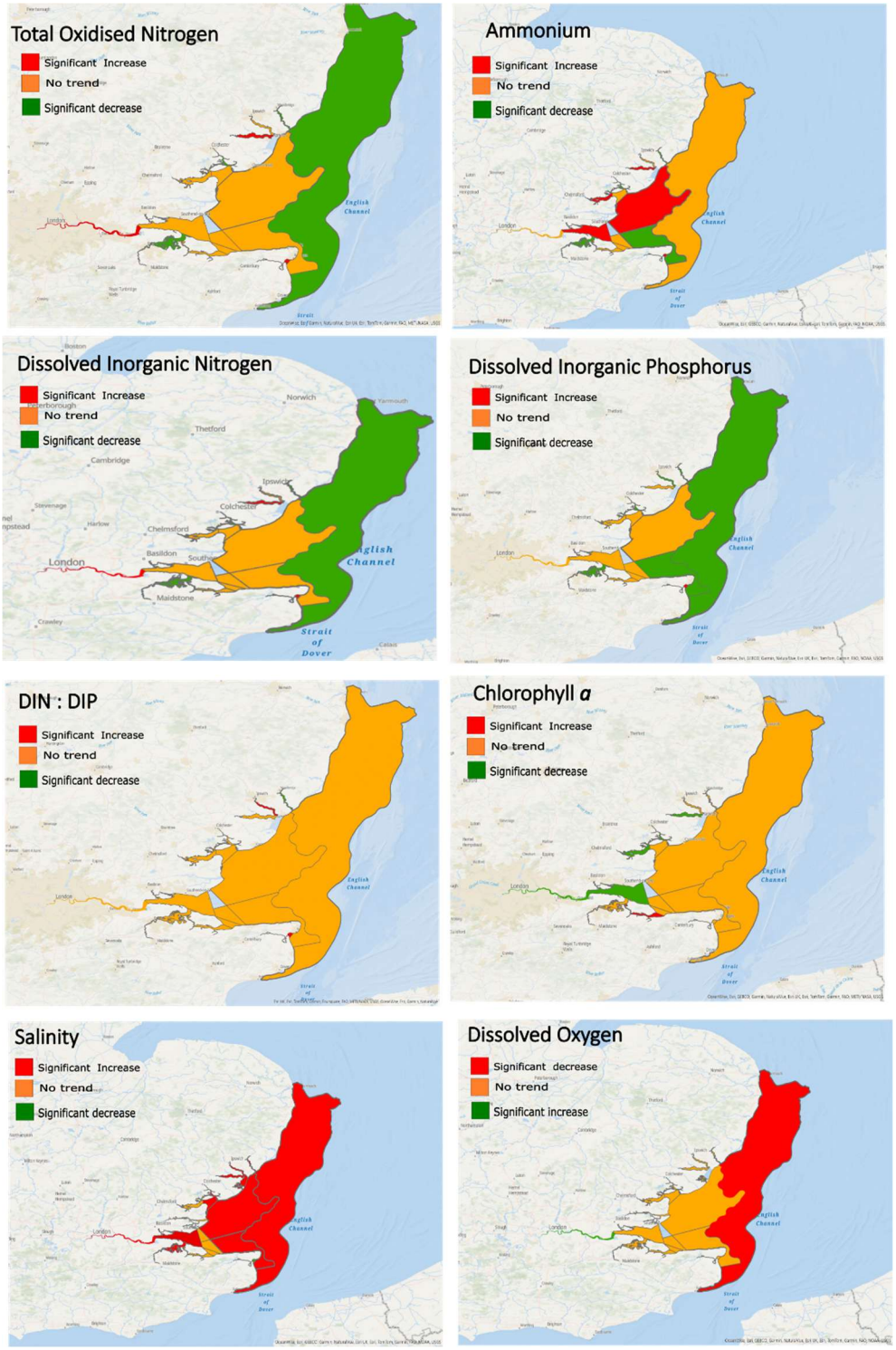

Figure 3.11 - Trends in nutrient concentrations and ratios, chlorophyll, salinity, and dissolved oxygen concentration between 2006 and 2020 in the Thames Estuary area, as determined by a linear model. A significant trend is indicated where $p < 0.05$. Red indicates a significant increasing trend, orange represents no trend, and green represents a significant declining trend. The colour scale identifying the direction of the change is reversed for dissolved oxygen, based on the ecological impacts, and therefore red indicates declining oxygen concentration whilst green indicates an increasing oxygen concentration.

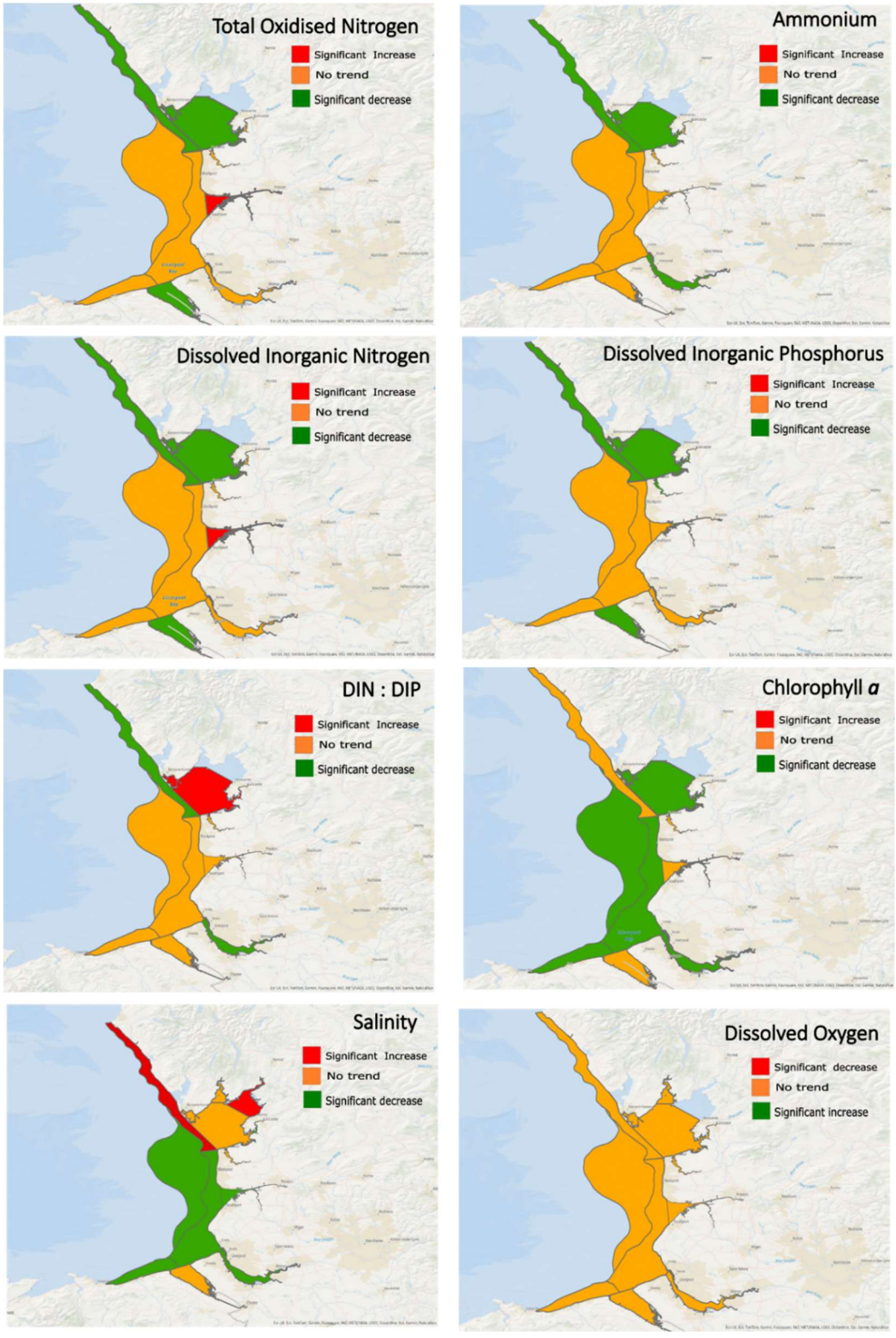

Figure 3.12 - Trends in nutrient concentrations and ratios, chlorophyll, salinity, and dissolved oxygen concentration between 2006 and 2020 in Liverpool Bay, as determined by a linear model. A significant trend is indicated where $p < 0.05$. Red indicates a significant increasing trend, orange represents no trend, and green represents a significant declining trend. The colour scale identifying the direction of the change is reversed for dissolved oxygen, based on the ecological impacts, and therefore red indicates declining oxygen concentration whilst green indicates an increasing oxygen concentration.

A significant decline in chlorophyll and salinity is observed in both the Liverpool Bay plume and the Mersey Mouth, but no significant trend is seen in any other parameter in these assessment areas (Figure 3.12). Morecambe Bay has declining concentrations in nutrients, chlorophyll, dissolved oxygen and salinity and an increase in the DIN : DIP ratio (Figure 3.12). The Cumbria assessment area shows a decline in nutrients and in the DIN : DIP ratio, an increase in salinity, and no trend in chlorophyll or dissolved oxygen concentrations (Figure 3.12). The Ribble assessment area exhibits increasing concentrations of DIN, and TOxN alongside a declining salinity and no trend in the chlorophyll concentration.

3.5.2 Threshold determination

The winter DIN threshold tool was run using data collected in the study period of 2006 – 2020.

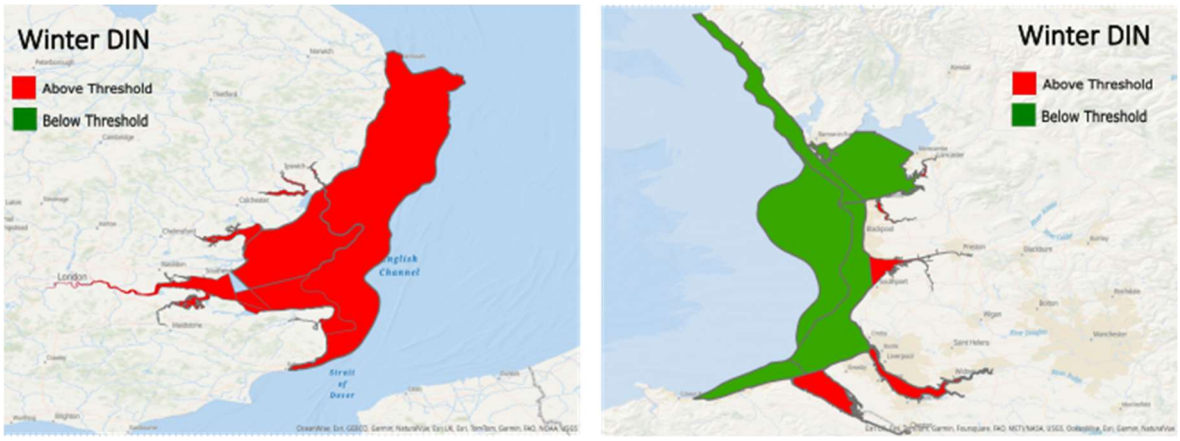

Figure 3.13 – Outcomes of the WFD/WER and OSPAR winter DIN assessment metric for the assessed period of 2006-2020 in the Thames Estuary (left) and Liverpool Bay (right) areas. Areas coloured red exceeds the assessment threshold, those coloured green are below the assessment threshold.

The results of the winter DIN threshold tool show that, using the study period of 2006 -2020 four assessment areas do not exceed the winter DIN threshold (Figure 3.13), specifically the Liverpool Bay plume, Mersey mouth, Morecambe Bay, and Cumbria. No assessment areas in the Thames Estuary area are below the winter DIN thresholds within this assessment period (Figure 3.13).

3.5.3 Phytoplankton WFD/WER assessment tools

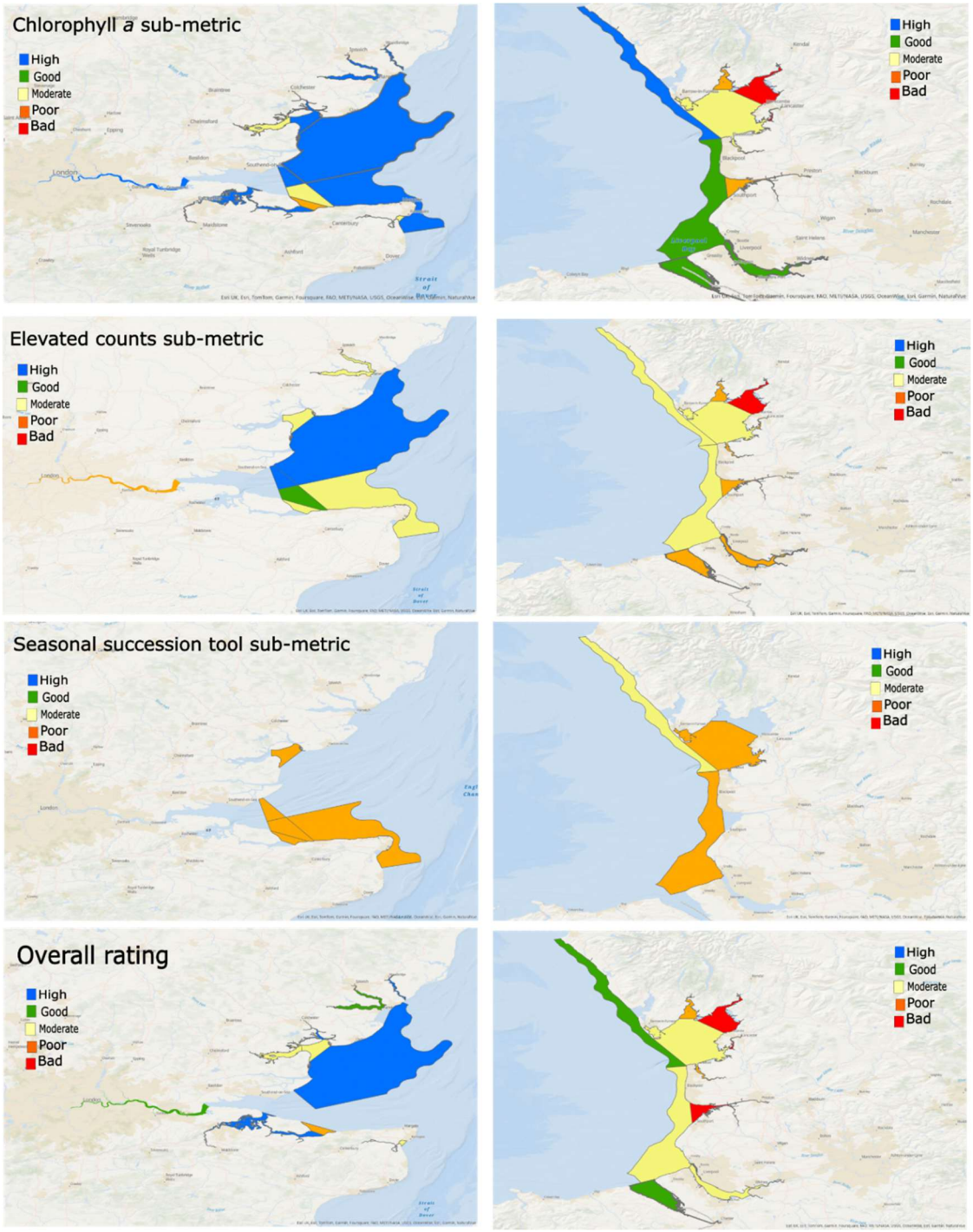

Figure 3.14 – Results of the WFD/WER phytoplankton tool sub metrics and overall rating for the Thames Estuary (left) and Liverpool Bay (right). Areas from the most recent WFD/WER assessment outcomes (2019) which fall within the period assessed here (2006-2020). Seasonal succession is not applied in transitional waters. If minimum data availability requirements for the sub metrics in coastal water bodies are not met, they are calculated but they do not contribute to an overall rating. Therefore, some coastal assessment areas have results for an individual sub metric but no overall classification. If there is no data available for a sub metric, the overall classification is calculated but flagged within the assessment.

The chlorophyll sub-metric shows that only one area is classified as bad status in these areas, which is the transitional area, Kent, in the Liverpool Bay catchment (Figure 3.14). In the Thames Estuary area, there is only one area classified as poor, which is Whitstable Bay. Ribble and Leven have been classified as poor in the Liverpool Bay area. The remainder of the assessment areas in both study areas are classified as at least moderate for the chlorophyll sub metric. The outcomes of elevated phytoplankton counts shows no areas classified as good or high in the Liverpool Bay area (Figure 3.14), and only two (Essex and Whitstable Bay) in the Thames plume area (Figure 3.14). The Kent assessment area is classified as bad in the elevated phytoplankton counts sub-metric (Figure 3.14). The seasonal succession tool is only used in coastal assessment areas, and with the exception of the Cumbria assessment area in the Liverpool Bay, all areas are classified as poor (Figure 3.14). The overall classification of the WFD/WER assessment areas show that Kent and the Ribble in the Liverpool Bay area are classified as bad, whilst only Cumbria and Dee are classified as good. There are no areas in the Liverpool Bay area classified above moderate (i.e. achieving good or high status). In the Thames Estuary area, Whitstable Bay is classified as poor, with the remaining areas classified as moderate or above (Figure 3.14).

3.5.4 Plankton Index tool

The results of the Plankton Index analysis from Graves et al. (2023) are mapped below. Additionally, the full results of the Plankton Index Tool are presented for the Cumbria and Mersey Mouth assessment areas. These areas were chosen as they had data available for all sub-metrics in the WFD/WER assessment, and have PI values calculated with high confidence, as noted in Graves et al. (2023).

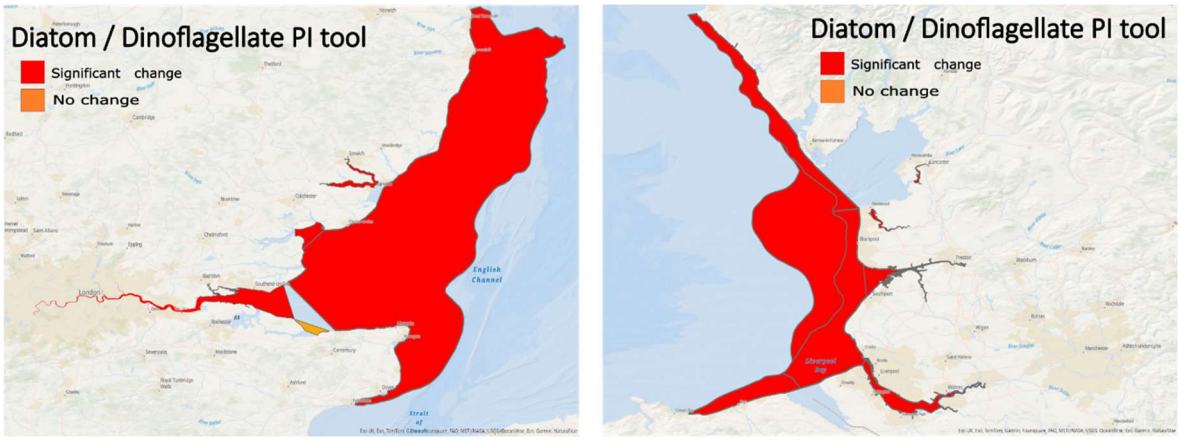

Figure 3.15 – The results of the diatom / dinoflagellate plankton index tool assessment in the Thames Estuary (left) and Liverpool Bay (right) study areas for 2006 to 2020. Changes are considered to be statistically significant if the calculated PI value is below 0.7.

All assessment areas in the Liverpool Bay catchment and in the Thames Estuary area, except Whitstable Bay show a significant change in the diatom/dinoflagellate community between 2006 and 2020 (Figure 3.15).

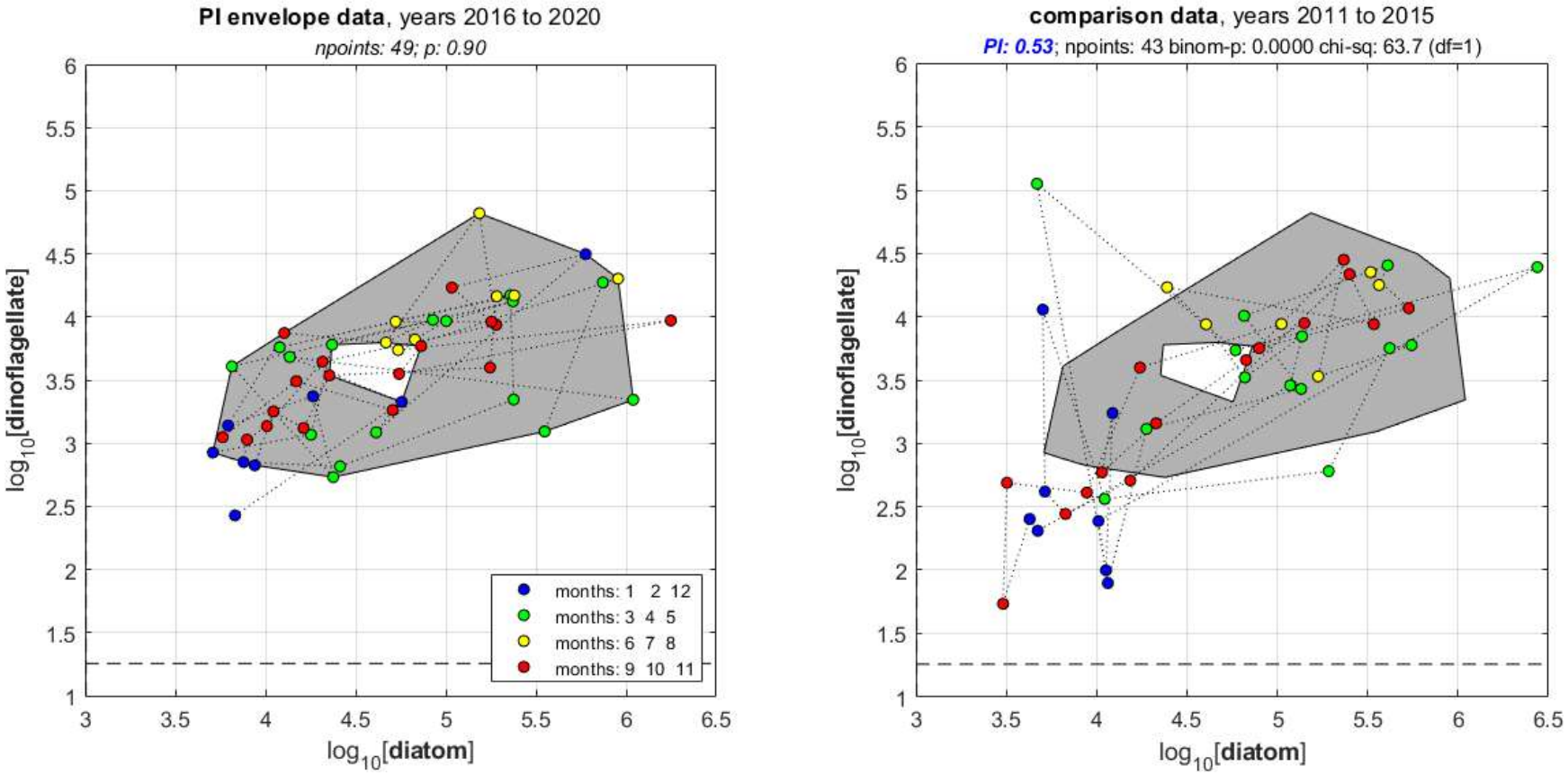

Figure 3.16 – The Plankton Index 'donut' plot for the Cumbria assessment area using all available data from 2006 – 2020. The 'assessed' period of 2016 – 2020 (left). The 'comparison' period for 2011 to 2015 (right). There is no phytoplankton data for the diatom / dinoflagellate lifeform pair in this area before 2011. The Dashed line represents the limit of detection (z). For the analysis of results, spring is defined as months 3, 4, 5, summer is months 6, 7, 8, autumn is months 9, 10, 11 and winter is months 12, 1, 2.

There is a significant change in the diatom/ dinoflagellate lifeform pair in between the two periods (Figure 3.16), PI = 0.53. In the later assessed period there is a smaller range in the abundance of dinoflagellates in winter, with increased abundances of diatoms in the same months. Autumn has higher abundances of both diatoms and dinoflagellates in 2016 – 2020 compared to 2011- 2015. The spring months show a larger range in both dinoflagellates and diatoms abundance in the comparison period compared to the assessed period.

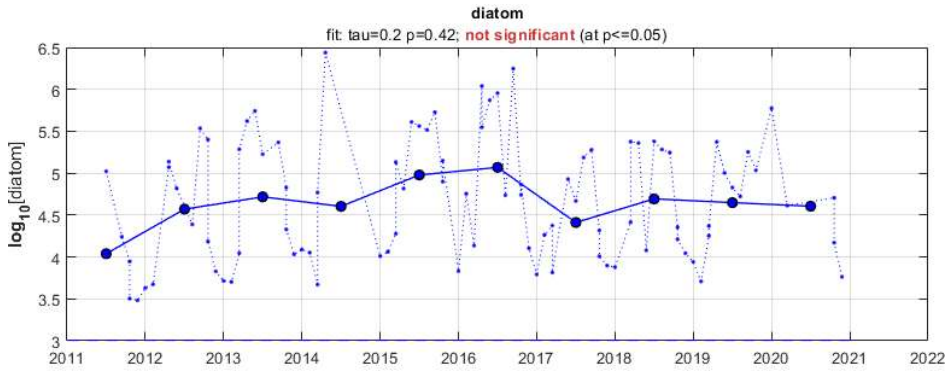

Figure 3.17 – Time series of diatom abundance in the Cumbria assessment area for 2011 to 2020. (No data are available data before 2011.). The small blue dots and dotted line represent the monthly mean abundances, and the larger dots and solid blue line show the mean annual abundance.

There is no significant change in the annual mean abundance of diatoms in the Cumbria assessment area (Figure 3.17). Mean monthly abundances reach maximum values in 2015 and 2016 (Figure 3.17).

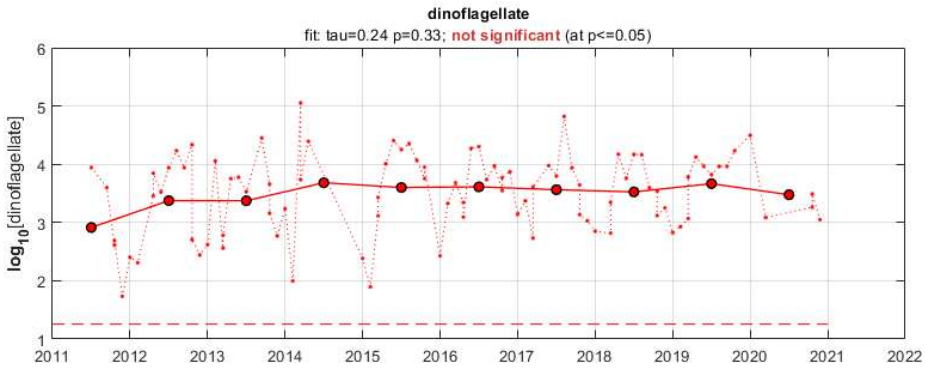

Figure 3.18 – Time series of dinoflagellate abundance in the Cumbria assessment area for 2011 to 2020. No data are available before 2011. The small red dots and dotted line represent the monthly mean abundances, and the larger dots and solid red line show the mean annual abundance. Dashed line represents the limit of detection (z).

There is no significant increase in the annual mean abundance of dinoflagellates in the Cumbria assessment area, with similar mean monthly abundances observed throughout the time series (Figure 3.18).

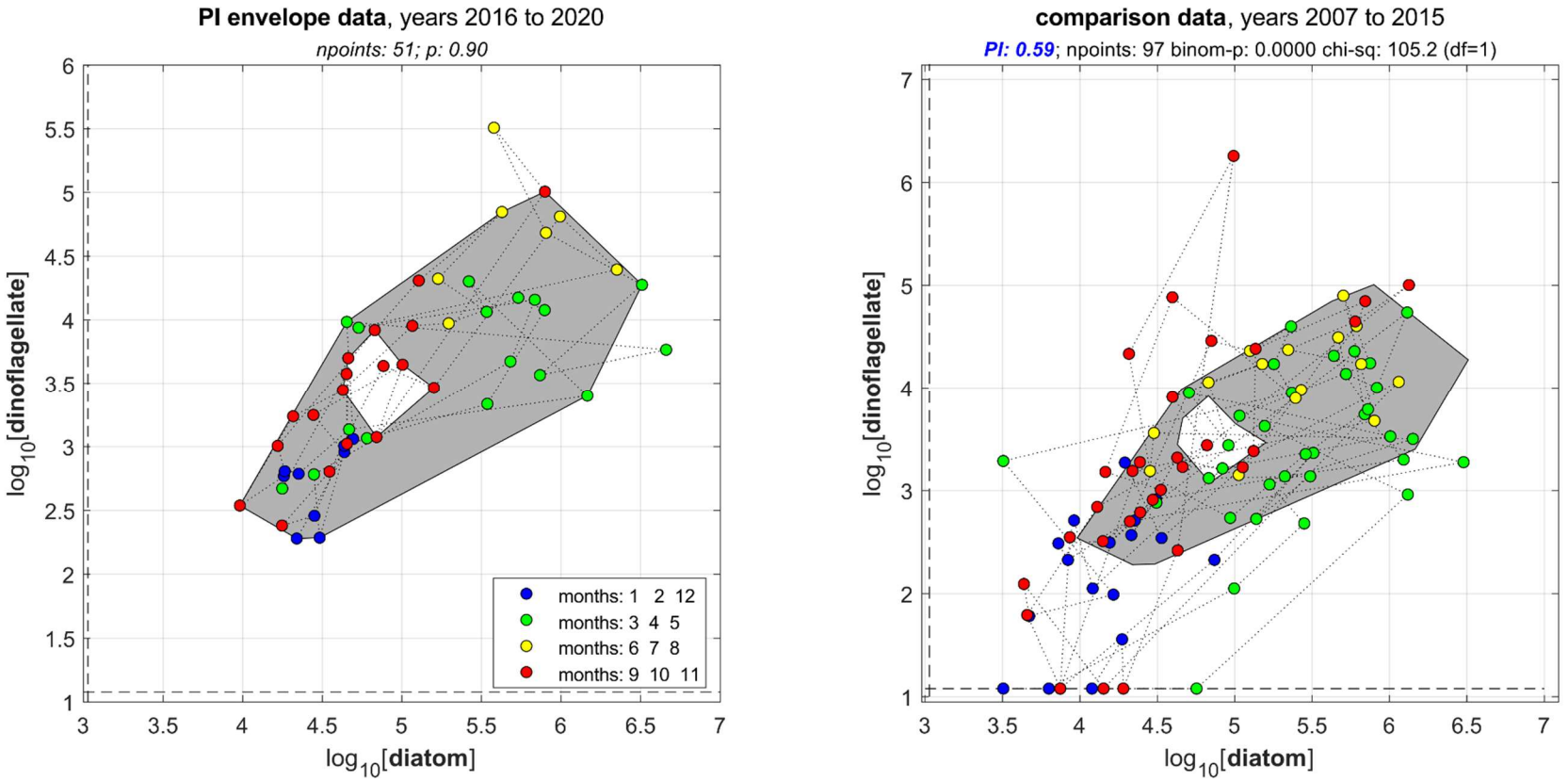

Figure 3.19 - The Plankton Index 'donut' plot for the Mersey Mouth assessment area using all available data from 2006 – 2020. The 'assessed' period of 2016 – 2020 (left). The 'comparison' period for 2011 to 2015 (right). There is no phytoplankton data for the diatom / dinoflagellate lifeform pair in this area before 2007. Dashed line represents the limit of detection (z). For the analysis of results, spring is defined as months 3, 4, 5, summer is months 6, 7, 8, autumn is months 9, 10, 11 and winter is months 12, 1, 2.

There is a significant change in the diatom/ dinoflagellate lifeform pair in between the two periods in the Mersey Mouth (Figure 3.19), PI= 0.59. Diatom and dinoflagellate abundances increase in summer in the later assessed period compared to the earlier comparison period. There are no observations of dinoflagellate abundances being close to the limit of detection in the later assessed period, whereas there are numerous observations of this occurring autumn and winter in earlier comparison period (2007 – 2015), and one observation in the spring. The range of both the diatom and dinoflagellate abundances also decrease in the later assessed period in spring, autumn, and winter.

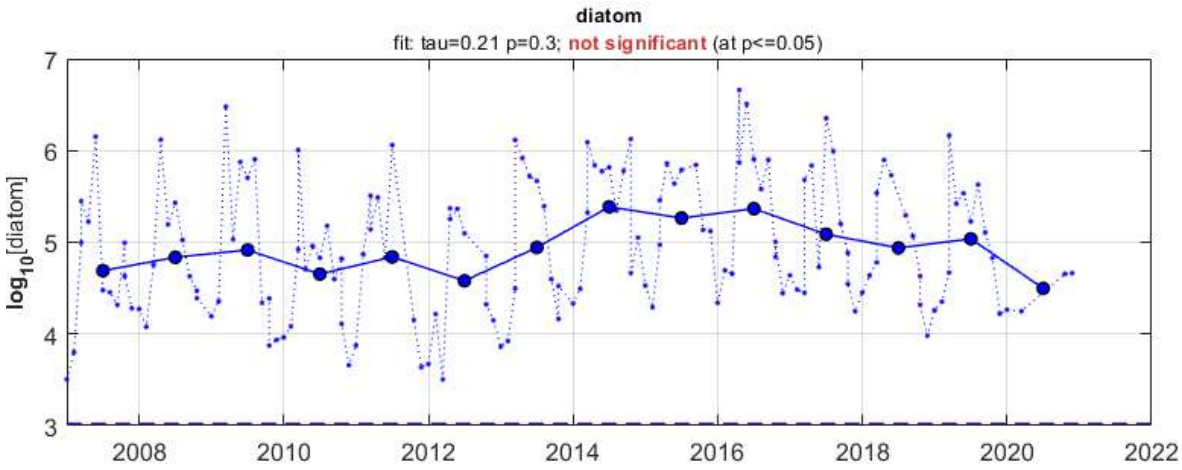

Figure 3.20 - Time series of diatom abundance in the Mersey Mouth assessment area for 2007 to 2020. (No data are available data before 2007. The small blue dots and dotted line represent the monthly mean abundances, and the larger dots and solid blue line show the mean annual abundance.

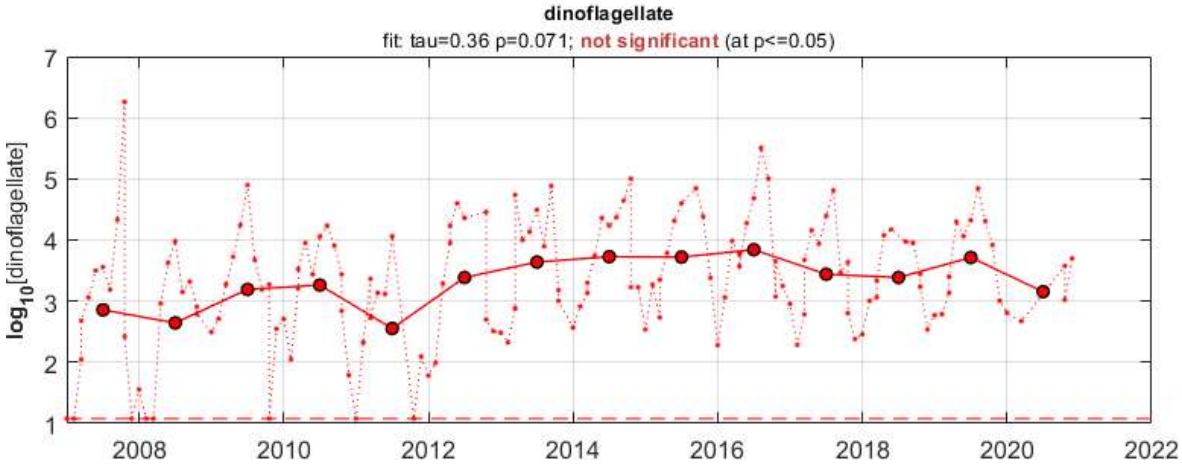

Figure 3.21 - Time series of dinoflagellate abundance in the Mersey Mouth assessment area for 2007 to 2020. No data are available before 2007. The small red dots and dotted line represent the monthly mean abundances, and the larger dots and solid red line show the mean annual abundance. Dashed line represents the limit of detection (z).

There is no significant increase in the annual mean abundance of diatoms in the Mersey Mouth assessment area (Figure 3.20). However, between the year of 2014 and 2018, abundances do not fall to values as low as those seen in the preceding years. There is no significant increase in the annual mean abundance of dinoflagellates in the Mersey Mouth assessment area (Figure 3.21). However, there is a low annual mean recorded in 2012. After 2012, there are no observations of no dinoflagellates being recorded.

3.6 Discussion

3.6.1 Long term trends in nutrient concentrations

Increases in DIN concentrations are seen in transitional waters in the Thames Estuary and Liverpool Bay, but not further offshore (Figure 3.11, Figure 3.12). This suggests that despite increasing concentrations in the transitional areas, the dilution or cycling occurring has not yet resulted in a statistically significant increase in concentrations further offshore. Alongside the increases in DIN, increases in TOxN are observed, but are not always accompanied by increases in ammonium. Nitrate and nitrite are therefore likely responsible for the DIN increases seen in the transitional areas (Figure 3.11, Figure 3.12). Ammonium inputs into the Thames and Liverpool Bay estuaries significantly declined between 1994 – 2016 (Greenwood et al., 2019), however increases in some areas are observed here (Figure 3.11, Figure 3.12).

Estuarine ecohydrology is an important factor when considering the transport of nutrients offshore, specifically the fine sediment that is available for retention and cycling (Jickells et al., 2014). Alterations to the physical characteristics of the estuaries over the study period may have changed the nutrient retention potential and increased the proportion of nitrogen which remains in the water column. The release of legacy nutrients may be contributing to the increased nitrogen concentrations seen in the inshore areas however nitrogen release has been seen to be dominated by $NH4^+$ (Jarvie et al., 2020), and NH_4^+ concentrations do not increase in this study period in the transitional areas where DIN increases are observed.

The increasing DIN concentrations in the transitional areas suggests that coastal and offshore areas risk becoming significantly enriched and at risk of eutrophication in the future and should be monitored, to determine whether there is an increase in nutrient transport offshore. Harmonising the WFD/WER and OSPAR assessments allows for information about the water quality status along the inshore to offshore gradient within the same time period and gives further insight and warning signs about future challenges associated with nutrient enrichment and potential eutrophication. This does, however, rely on the significance limit of $p < 0.05$ used with the linear model, and so changes may still be occurring, but they are not statistically

significant. Using a linear model to assess long-term trends may not be the most suitable way to capture the full extent of any changes, but it demonstrates that additional information can be obtained by considering the data over a longer period compared to only using the current metrics for the assessment of eutrophication in the UK over the six-year assessment cycle.

Multiple transitional and coastal waterbodies areas show no change in DIN concentrations from 2006 to 2020 (Figure 3.11, Figure 3.12) alongside exceedances of the Water Environment Regulation thresholds (Figure 3.13). This suggests that the management practices in these areas and their catchments are not sufficient to reduce the DIN concentrations. However, whilst the Thames plume fails the WFD/WER winter DIN threshold over the period assessed here, the DIN concentration is declining (Figure 3.11), and this suggests that management practices may be having a positive effect. Alternatively, this decline may be a function of sampling locations, as salinity increases in the Thames plume (Figure 3.11). An increased number of further offshore, higher salinity, samples may have been collected later in the assessment period. Samples collected further offshore are likely to have lower nutrient concentrations as a result of dilution.

Nitrogen has been the subject of management initiatives prior to the Water Environment Regulations. In 1991, the Nitrates Directive (ND) was introduced in order to address the problem of diffuse pollution originating from agricultural sources, by implementing management requirements in nitrate vulnerable zones. The approach of the ND and WFD/WER has been described as ineffective for managing diffuse pollution, as the complexity and localised nature of the issues are not sufficiently acknowledged (Sharma, 2020). Nitrogen concentrations in transitional and coastal waters remain above thresholds considered acceptable by the WFD/WER in many areas (Greenwood et al., 2019), and total oxidised nitrogen concentrations increased by 23% between 2015 – 2022 in the Wensum, an eastern English arable catchment (Cooper and Hiscock, 2023). This highlights that management practices concerning nitrogen have not been effective at reducing DIN concentrations in transitional and coastal waters sufficiently. The additional metrics suggested here may offer further insight into barriers to successful management. For example, long term trends can offer insight into the direction of change in concentrations. If the results of trend analysis in area suggest increasing concentrations, but this is alongside a concentration which does not yet exceed the upper threshold, then preventative rather than remedial action may be able to be implemented. This foresight is important, as the accumulation of nutrients increases the difficulty of successfully managing eutrophication (Khan and Mohammad, 2014). Prevention and advance action are therefore preferable. Current WFD/WER metrics offer limited insight into the success, or lack thereof, of management practices implemented within the assessment areas to address eutrophication problems. In the Thames Estuary area (Figure

3.11), and Liverpool Bay area (Figure 3.12) many of the areas exceed the DIN winter threshold values. By considering a singular pass or fail measurement for DIN concentrations relative to a pre-determined threshold rather than also including the longer-term trends presented here, there is limited evidence of the impact management practices are having. Understanding the trend direction of the DIN concentrations gives insight for managers into the success of practices, and whether a continuation of current measures is likely to achieve desired results in the future, or whether alternative actions are necessary. This information is not available under the current assessment metrics of the WFD/WER. An indication as to whether concentrations are above or below a threshold in each WFD/WER cycle, 6 years, is much more limited than also looking at long term trends. This means that there are no metrics available to managers which can offer a 'warning' for areas which could become eutrophic, and this would not be flagged under the current metrics until concentrations fail the thresholds, are already considered problematic, and are having negative impacts on the pelagic ecosystem.

DIP concentrations have significantly declined in the larger offshore Thames plume and in a few inshore transitional areas (Figure 3.11) but otherwise show no significant trend across the period assessed elsewhere in the Thames Estuary (Figure 3.11). A similar pattern is found in Liverpool Bay, with declining concentrations or no change in concentrations (Figure 3.12). DIP concentrations have been in decline in many areas across Europe, often more strongly than nitrogen, as a result of legislation which was enacted (Skarbovik et al., 2014; Burson et al., 2016; Westphal et al., 2020). In 1991 the Urban Waste Water Treatment Directive (UTTWD) required the removal of phosphorus from wastewater discharging into areas considered eutrophic (European Commission, 1991). A requirement of the UTTWD is for phosphorus concentrations of incoming wastewater to be reduced by 80 % at treatment plants, or a total phosphorus limit based on the population size. In the Thames River catchment, implementation of phosphorous removal at wastewater treatment works began in 1996 and was fully in place by 2008. This resulted in an 88% reduction in phosphorus loading into the Thames catchment in 2004-2006 compared to 1991-1993 (Kinniburgh and Barnett, 2010). This timeline of phosphorus removal at wastewater treatment centres suggests that the tail end of the declines in DIP concentrations, as a result of the implementation of the UWWTD, might be captured within this assessment period (2006 – 2020), and multiple areas with declining DIP concentrations are seen in both study areas (Figure 3.11, Figure 3.12).

The trends in the period assessed here show few significant changes in DIN : DIP ratio. Increasing ratios have been seen in the Thames riverine inputs between 1994 – 2016 (Greenwood et al. 2019) but this is not reflected in the analysis of these areas for 2006 to 2020 in this study. The DIN : DIP ratio is monitored under the WFD/WER regulations, as to whether it falls within the acceptable bracket of 8-24, but there is no formal assessment of changes

over time. There is evidence that a changing DIN : DIP ratio can impact the community composition of phytoplankton, and reductions in both nitrogen and phosphorus simultaneously have been called for in order to successfully mitigate the negative impacts of eutrophication (Turner et al., 2003; Philippart et al., 2007; Grizzetti et al., 2012; Paerl et al., 2014; Burson et al., 2016; Paerl et al., 2016).

3.6.2 Considering nutrient speciation to strengthen eutrophication assessments

DIN is a combination of NH_4^+ and TOxN, however under the current eutrophication monitoring methods, winter DIN concentrations are the exclusive nutrient measurement included in the assessment for coastal and transitional waters (Poikane et al., 2019). Concentrations of NH_4^+ and TOxN are not considered separately. This can limit the understanding of trends or changes which may occur within the relative contributions of NH_4^+ and TOxN. In the Thames Estuary, the DIN concentrations are declining in the plume area, along with the TOxN concentrations (Figure 3.11). This is not true for NH_4^+ concentrations, which have no trend in the plume, and increase in the Essex coastal area, which sits within the plume (Figure 3.11). The source of nitrogen has been seen to impact the community composition of phytoplankton (Blomqvist et al., 1994; Domingues et al., 2011; Donald et al., 2013; Glibert et al., 2016; Shilova et al., 2017). Domingues et al. (2011) saw no diatom response to $NH4^+$ inputs, but reliance of $NH4^+$ by cyanobacteria. An increase in the relative proportion of NH_4^+ to DIN concentrations could result in an increased dominance of cyanobacteria, which might have negative implications (Zhang et al., 2022). Donald et al. (2013), saw an increase in phytoplankton biomass after $NH4^+$ addition compared to nitrate, but responses differed by phytoplankton genus. Evidence that the nitrogen source can impact on the phytoplankton abundance and community composition highlights that the contributions of TOxN and NH_4^+ should be a consideration when collecting monitoring data to support investigation into the impacts of nutrient enrichment and eutrophic conditions on phytoplankton. Shifts in the community composition of phytoplankton can have implications for the wider marine ecosystem (Spilling et al., 2018). The relative dominance of diatoms and dinoflagellates within the community has important implications for nutrient cycling within a waterbody (Spilling et al., 2018), and alterations to the community composition can have implications upwards in the marine food web as available food sources change (Taipale et al., 2019).

3.6.3 Enhancing phytoplankton metrics to improve eutrophication assessments

As well as monitoring nutrient concentrations, OSPAR and the WFD/WER have metrics to monitor the health of phytoplankton communities, through measures of chlorophyll concentration, abundances of individual and total taxa, and seasonality of certain species.

These metrics are classified from high to bad, based on the extent to which conditions deviate from reference conditions (Devlin et al., 2007a).

For chlorophyll, this gives a snapshot of a typical growing season value for chlorophyll within the 6-year assessment period, exact metrics are outlined in chapter one (Table 1.3). There are data requirements to ensure that the spatial and temporal variability are represented within the final calculated value. These include sampling across salinity bands, a multitude of statistics including mean, median, and threshold exceedances, but still the variation over time is not able to be understood from this one score. Within the analysis presented here, chlorophyll concentrations have shown declines in some areas and no significant trends in the remainder of the assessment areas, with no significant increases documented (Figure 3.11, Figure 3.12). Comparing trends in the chlorophyll concentration to the trends in nutrients show some decreasing chlorophyll concentrations despite no change seen in the nutrients in the area, or in the case of the Ribble, increasing nutrient concentrations. This suggests that nutrients are not the only driver behind changes in chlorophyll concentrations in these areas, and successful management must take an integrated approach, looking at a wider range of variables over a longer time period, to understand what other factors may govern primary productivity. The method presented here aims to assess chlorophyll concentrations over a longer time scale, which is important to be able to identify the potential drivers of changes and implement a comprehensive approach. Declines in primary productivity have been identified in the North Sea through time series of chlorophyll (Capuzzo et al., 2018), linked to nutrient inputs and sea surface temperature. Additional long term analysis of chlorophyll concentrations in the North Sea has identified climate change and water clarity as key drivers of increases in primary productivity (McQuatters-Gollop et al., 2007b). The findings in these analyses highlight different driving factors, but importantly both take a long-term view of changes in chlorophyll concentrations to identify causes. Understanding drivers behind change is important to ensure best use of resources when implementing management measures. Incorporating an assessment of changes over time in the WFD/WER analysis could help to do this. OSPAR has incorporated longer-term trend assessments as a supplement to the Common Procedure since COMP 3, (OSPAR, 2005; Devlin et al., 2023) extending this into the WFD/WER would be beneficial.

As well as studies documenting trends in chlorophyll concentrations over time, changes in phenology have been identified (Desmit et al., 2020), with blooms occurring earlier in the year due to warming. Chlorophyll a concentrations, as used within the WFD/WER and OSPAR eutrophication assessments, give information about phytoplankton biomass but do not provide information about the extent to which the phytoplankton community has been altered. There are many potential changes within the phytoplankton community which would not be

apparent through the use of chlorophyll concentration alone, including phenology, and shifts in dominant species (Tett et al., 2008; Thackeray et al., 2008; Wasmund et al., 2017; Taipale et al., 2019). The results of the chlorophyll sub-metric (Figure 3.14) show that there are multiple areas which are classified as high, indicating that there is little deviation from reference conditions during the WFD/WER assessment cycle, however, this gives no indication of shifts beyond abundance, and significant changes in phytoplankton communities have been identified in these same areas within other phytoplankton sub metric tools for elevated counts and seasonal succession (Figure 3.14). Furthermore, a changing climate will complicate the management of eutrophication in coastal waters, as a result of the complex interactions between nutrient and climate dynamics, alongside anthropogenic activity (Rabalais et al., 2009; Moss et al., 2011; Sinha et al., 2017; Vigouroux et al., 2021). Now, and especially under future scenarios of a shifting climate, chlorophyll concentration alone will likely be insufficient to capture the extent to which phytoplankton communities are being altered through the eutrophication process. The relationship between chlorophyll concentration and phytoplankton biomass has been seen to vary (Alvarez-Fernandez and Riegman, 2014), and so ensuring there is a suite of indicators to monitor changes in phytoplankton communities is important.

The elevated counts indicator offers information about blooms of specific species or total species exceeding a threshold. This is useful for monitoring the extent of large blooms of nuisance species and short-lived increases in all phytoplankton species. However, this metric may miss increases in groups of species which share a common trait (lifeforms), but of which an individual species would not exceed a threshold. There is evidence linking shifts in the relative abundances of diatoms and dinoflagellates to changes in the eutrophication status of a water body (McQuatters-Gollop et al., 2007a; Wasmund, 2017; Wasmund et al., 2017). The WFD/WER seasonal succession tool delivers information about this in coastal water bodies but is not routinely used as an indicator across transitional and offshore waterbodies. The seasonal succession tool measures the percentage compliance of diatoms and/or dinoflagellates within monthly reference conditions (Devlin et al., 2007a). The results of the seasonal succession tool from the latest WFD/WER assessment (Figure 3.14) indicate a poor classification in the majority of the assessed areas. This is in agreement with the PI (Figure 3.15) where significant changes in diatoms and dinoflagellates are identified in the majority of assessment areas. The results presented here, utilising the plankton index tool, offer insight into changes within phytoplankton community composition by giving information on shifts within seasonality and relative abundance of diatoms and dinoflagellates, which is not assessed under the WFD/WER outside of coastal areas. This method could also be applied to

other lifeform pairs in order to gain more understanding about changes within the wider phytoplankton community. For example, large and small phytoplankton.

The results of the plankton index tool are presented for the Cumbria and Mersey Mouth assessment areas, where the overall classifications based on the WFD/WER phytoplankton metric for the areas are 'good' and 'moderate', respectively, and, by WFD/WER definition,

Good - 'Phytoplankton show slight signs of disturbance and changes do not indicate any accelerated growth of algae resulting an undesirable disturbance to the balance of organisms present' (European Commission, 2000)

*Moderate - 'The composition & abundance of planktonic taxa show signs of moderate disturbance. Algal biomass is substantially outside the range associated with type specific reference conditions and is such as to impact on other biological quality elements.' (*European Commission, 2000)

The changes documented within the plankton index tool show shifts in seasonality rather than significant changes in annual abundance for both Cumbria and the Mersey Mouth (Figure 3.16, 3.19). In the Cumbria assessment area, increased abundances of both diatoms and dinoflagellates are seen in the autumn months in the later assessed period (2016-2020), as well as increased diatom abundances in the winter months. In the Mersey Mouth, dinoflagellate abundances increase in the autumn and winter in the later assessed period. Autumn and winter blooms as a result of river discharge from increased rainfall have been seen in other parts of the world (Ding et al., 2024), and whilst the system studied in their research is likely different from the Thames and Liverpool Bay areas, it gives an indication of the potential interactions between eutrophication and climate change and suggests that shifts in typical seasonal patterns are possible. The seasonal succession tool in the Cumbria area gives a classification of moderate, and the Mersey Mouth is classified as poor. Whilst the seasonal succession tool is important in identifying that a shift has occurred in the seasonal distribution of diatom and dinoflagellate abundances, and has here successfully identified alterations, the use of the Plankton Index tool offers further insight. The PI is able to identify when and which lifeforms, further helping to ensure that management measures are targeted and effective.

Utilising the plankton index tool, alongside the current WFD/WER and OSPAR metrics, can create a body of evidence in order to further understand the slight and moderate disturbances identified here and aid in identifying the most effective actions to ensure minimal deviation from reference conditions, and minimal impact on other biological elements in the marine ecosystem. The data used in the assessment of nutrient conditions under the WFD/WER are from the months of November to February inclusive, as these are the nutrients which are

available to support the spring bloom once light and temperature conditions allow. Under a changing climate, where typical phenology may be altered, extending the time scale for which nutrients are assessed might be important. Increasing the temporal coverage to not only look at longer term trends, but also to take a full year view within the 6-year WFD/WER assessment cycle would allow for the assessment of the impacts of nutrient conditions on abundance and community structure beyond winter concentrations.

Under the WFD/WER regulations, oxygen concentrations are monitored as a 'supporting element' (Best et al., 2007), similarly to nutrients in that they are considered to shape the ecological status. Low concentrations of dissolved oxygen are detrimental to many organisms (Best et al., 2007) and shifts in oxygen concentrations can occur as a result of changes in water quality and the development of eutrophic conditions. When eutrophication causes excess blooms of phytoplankton, low oxygen concentrations can occur as phytoplankton remnants sink and decay (Cabral et al., 2019), and in extreme cases anoxia may develop, where limited life can be supported. Declining oxygen concentrations are being seen worldwide (Breitburg et al., 2018). There are many deviations from reference conditions which could occur as a result of eutrophication, before dissolved oxygen begins to become depleted. Whilst oxygen concentration is an important parameter for ecosystem health, monitoring of subtler, important, impacts of eutrophication, such as shifts within diatoms and dinoflagellates abundances, using methods such as the Plankton Index tool is important to detect undesirable disturbances before such drastic changes as oxygen depletion develop. Changes to oxygen concentrations are also likely to occur in some areas under a shifting climate, and eutrophication is not the only driver of change (Mahaffey et al., 2023). Therefore, it is important to have a range of metrics to monitor the undesirable effects of eutrophication, as the impacts of other drivers will vary across areas, and without a variety of methods it may be difficult to identify the cause of phytoplankton community shifts.

By increasing the range of metrics and applying them across WFD/WER and OSPAR areas, a holistic view of the changes occurring along an inshore to offshore gradient can be obtained. Currently, WFD/WER and OSPAR assessments occur independently to one another. Harmonising the assessments to utilise the same range of metrics will allow for a more coordinated assessment of systems.

3.7 Conclusion

Outcomes presented in this chapter have shown that by increasing and harmonising the temporal and spatial coverage of the WFD/WER and OSPAR assessments to look at longer term changes in water quality parameters, an increased knowledge can be gained about the

eutrophic state of water bodies and the effectiveness of the management measures. Determining the trajectory of changes gives insight into whether a waterbody is heading towards problematic conditions, before it fails the assessment threshold, and intervention can happen before there are severe undesirable consequences. Having simultaneous results across assessment area types, understanding the pressures which occur and the ecological impacts these have throughout a waterbody system, could help to guide appropriate and successful management. Adding the Plankton Index to the suite of assessment methods offers an understanding about critical changes within the phytoplankton community which would otherwise be missed across the entire inshore to offshore gradient using the current metrics. The Plankton index tool has been shown to be an effective way of capturing changes in abundance and seasonality of a lifeform pair. Understanding these finer scale changes is likely to become more important under a changing climate and so the addition of the PI tool, or a similar method, would be beneficial to ensure effective monitoring of the full range of potential undesirable disturbances.

So What? – The use of the data in this way, in combination and with additional metrics, has shown that there are spatial and temporal trends and patterns which would not be captured currently. The use of plankton Index, or similar metric, gives further insight into the details of shifts within the phytoplankton community in all assessment areas, beyond those currently included in the assessments. This can help environmental managers decide where to focus efforts and resources. Having this additional understanding means that preventative rather than remedial action could be taken, and waterbodies may not have to reach 'less desirable' states before the need for intervention is recognised.

4

Environmental controls on phytoplankton biomass and community composition in the Thames Estuary and Liverpool Bay

4.1 Abstract

Eutrophication in UK transitional and coastal waters can be difficult to monitor and manage due to the complexities of connecting eutrophication impacts with the occurrence of high nutrients, and the extent and nature of the undesirable disturbance associated with nutrient pollution is dependent on the specific environment. Elevated phytoplankton biomass and shifts in the phytoplankton community composition can be an undesirable disturbance associated with nutrient pollution and eutrophication. In order to support effective management of the relative risks associated with nutrient pollution in different estuarine and coastal environments, it is important to identify the main drivers that influence the severity of the eutrophication impacts. This chapter utilises data from the marine Natural Capital and Ecosystem Assessment (mNCEA) programme in the Thames Estuary and Liverpool Bay, as well as an additional data collection effort in Liverpool Bay, in order to identify the factors that govern the abundance and community composition of phytoplankton along a salinity gradient in these two study areas. The results of the analysis indicate that despite high inshore nutrient concentrations, which decline with increasing salinity, chlorophyll concentrations are similar along the salinity gradient in the Thames estuary and decline or remain similar with distance offshore in Liverpool Bay. The results presented in this chapter indicate that the inshore environment is limited by the light environment, and this moves towards a nutrient limited system further along the salinity gradient.

4.2 Introduction

The undesirable effects of eutrophication can have significant implications throughout coastal and marine environments. Disturbances can include shifts in phytoplankton community composition (Gowen et al., 2015; Wasmund, 2017; Wasmund et al., 2017; Van Meerssche and Pinckney, 2019), abundance (Gowen et al., 1992; Allen et al., 1998; Ptacnik et al., 2008), and phenology (Desmit et al., 2020; Nohe et al., 2020). It is therefore important to ensure that phytoplankton communities are appropriately assessed and managed, to ensure healthy coastal and estuarine ecosystems. An increased understanding of the specific drivers behind changes in phytoplankton communities is needed in order to be able to identify the causes and allow effective mitigation strategies, that are appropriate to the specific stressors.

The tools used in the UK for assessing the eutrophic state of estuarine and coastal waters are outlined in Chapter One. There are variations in the methods used in different water body types, and by different monitoring and assessment directives (Figure 1.5). However, in general, eutrophication is assessed by a measure of elevated nutrients and plankton biomass, alongside secondary indicators such as dissolved oxygen (Best et al., 2007). Assessment of eutrophication requires the identification of an undesirable disturbance, such as elevated algal growth leading to oxygen depletion. Therefore, it is not sufficient to assume that high nutrient concentrations will universally result in negative disturbances, as the responses between environments will differ (Painting et al., 2007; Foden et al., 2011). Upper thresholds to assess nutrient concentrations in transitional and coastal waters under WFD/WER and OSPAR are in place, but the final classification of a waterbody is decided by nutrient concentrations in conjunction with any measured negative ecological impacts they have (Devlin et al., 2007b). Therefore, it is important to understand the factors which govern the response of ecological systems to nutrient pollution, and to establish the interactions which may result in undesirable disturbances that contribute to eutrophication. Specifically investigated here are the responses of phytoplankton communities, in terms of their abundance and composition of the plankton community in transitional and coastal waters.

In order to successfully assess and mitigate the threats of nutrient enrichment to estuarine and coastal waterbodies and phytoplankton communities, drivers behind the variations in the nature of responses need to be well understood. The interactions between nutrient pollution (by nitrogen and phosphorus) and additional water quality parameters should be considered within the evaluation of overall health and function of the phytoplankton community and wider

ecosystem. For instance, silicate limitation is important in relation to diatom growth but is not relevant for dinoflagellate abundances.

Phytoplankton, typically, require nutrients and light in order to grow, although there are increasing observations of mixotrophic phytoplankton (Mitra et al., 2016), for which light conditions exert less control.

The extent of growth and community composition of phytoplankton is also governed by the physical characteristics of a waterbody, and it is known that there can be considerable variability in the susceptibility of coastal and estuarine waters to eutrophication (McQuatters-Gollop et al., 2009; Cloern and Jassby, 2010; Foden et al., 2011; Plew et al., 2020). For example, the light environment and water residence time are important controlling mechanisms for the extent and nature to which enhanced nutrient concentrations cause undesirable impacts (Cloern, 1987; Fichez et al., 1992; Cloern, 1999; Ferreira et al., 2005; Painting et al., 2007; Lueangthuwapranit et al., 2011; Shen et al., 2011; He et al., 2017; Burson et al., 2018). Consequently, the WFD/WER allows higher nutrient concentrations in turbid environments on the assumption that light limitation will prevent excessive growth (Painting et al., 2007; Devlin et al., 2007b). The suitability of this concession is discussed in detail in chapter Five.

The ratio of nitrogen to phosphorus has increased within riverine inputs over time as a result of differences in management practices (Turner et al., 2003; Grizzetti et al., 2012; Burson et al., 2016; Longphuirt et al., 2016; Greenwood et al., 2019; Shi et al., 2022), as discussed in chapter one. An increasing DIN : DIP ratio may push the environment to become phosphorus limited, and the DIN : DIP ratio is known to impact the community composition of phytoplankton (Li et al., 2011; Gowen et al., 2015; Shangguan et al., 2017; Nohe et al., 2020).

Changes in phytoplankton community composition and abundance are unlikely to show consistent responses across different areas and at different distances offshore. Understanding how changes in water quality parameters contribute to shaping the phytoplankton abundance and community composition is useful to identify the key drivers of change, and where policy decisions may be best focussed for effective management. The use of (phyto)plankton lifeforms offer a way to assess change within the community at a functional trait level (McQuatters-Gollop et al., 2019; Bedford et al., 2020).

The aim of this chapter is to outline the variations in nutrient concentrations and light environment at varying distances offshore in the two selected study areas of Liverpool Bay and the Thames Estuary, in order to consider how this variation may impact upon phytoplankton biomass and lifeform abundance. Variables which exert significant control in the two areas will be identified, and these results will be considered within the context of current eutrophication

monitoring in the UK, alongside the additional insight they can offer into the complex mechanisms which govern phytoplankton response to nutrient enrichment.

The marine Natural Capital and Ecosystem Assessment programme (mNCEA) (Defra, 2022; Devlin et al., 2023) was a 3-year Defra initiative (2022-2025). It aimed to provide the evidence needed for a natural capital approach to be integrated into marine and coastal management (Devlin et al., 2023). The natural capital approach is one that recognises the economic and societal value of the marine ecosystem. Data collected under this programme, alongside further sampling effort, will be utilised here to address the following research questions.

Specifically:

- *How do the light and nutrient conditions vary with salinity in Liverpool Bay and the Thames Estuary?*
- *How does the phytoplankton abundance and community composition vary with salinity in the two study areas of Liverpool Bay and the Thames Estuary?*
- *Is phytoplankton biomass nutrient limited at an offshore sampling site in the Thames Estuary?*

Outcomes of these questions will enhance the understanding of how the undesirable impacts of nutrient pollution on phytoplankton communities manifest along a salinity gradient in different environments.

4.3 Methods

4.3.1 Water sampling

As part of the mNCEA programme, data was collected in the Thames Estuary (Figure 4.1) and Liverpool Bay (Figure 4.2) onboard the *Thames Guardian* and the *Mersey Guardian* respectively from July 2022 until January 2025.

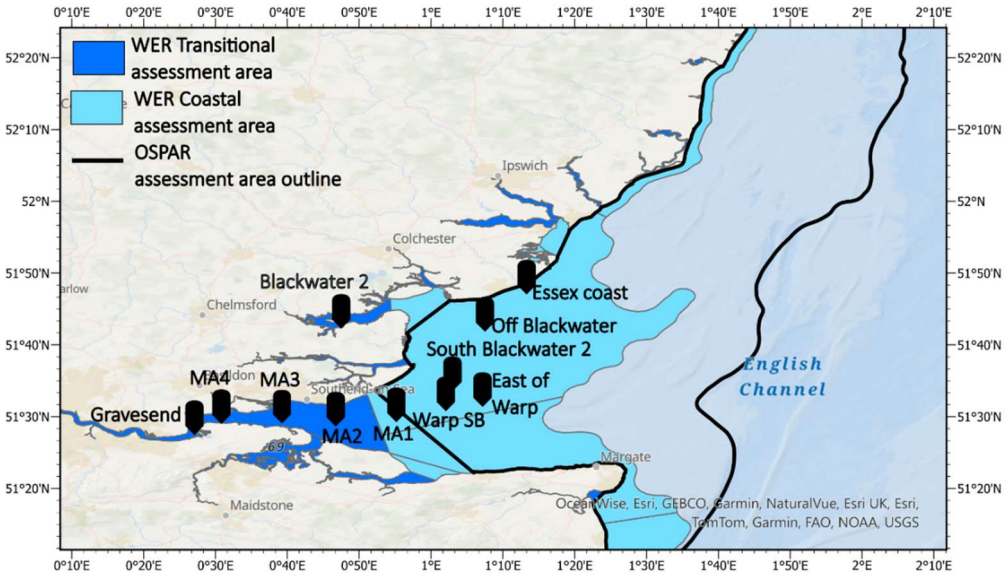

Figure 4.1- Map showing the sites of data collection under the mNCEA programme between July 2022 and June 2024 in the Thames Estuary (black pins). The sampling sites cover an inshore to offshore transect through transitional and coastal WFD/WER assessment areas. Discrete nutrient, salinity, and phytoplankton samples were collected at stations, alongside water column profiles using a CTD.

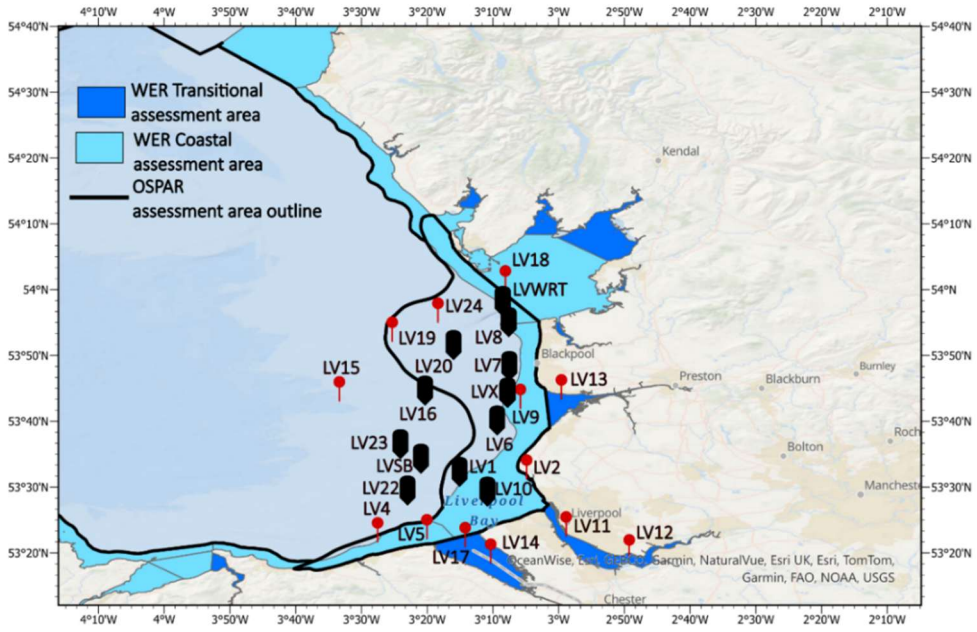

Figure 4.2 - Map showing the sites of data collection under the mNCEA programme between July 2022 and June 2024 in Liverpool Bay (black pins) and sites where samples were only collected as part of the April 2022 sampling effort (red pins). The sampling sites cover an inshore to offshore transect through transitional and coastal WFD/WER assessment areas. Discrete nutrient, salinity, and phytoplankton samples were collected at each station, alongside water column profiles using a CTD.

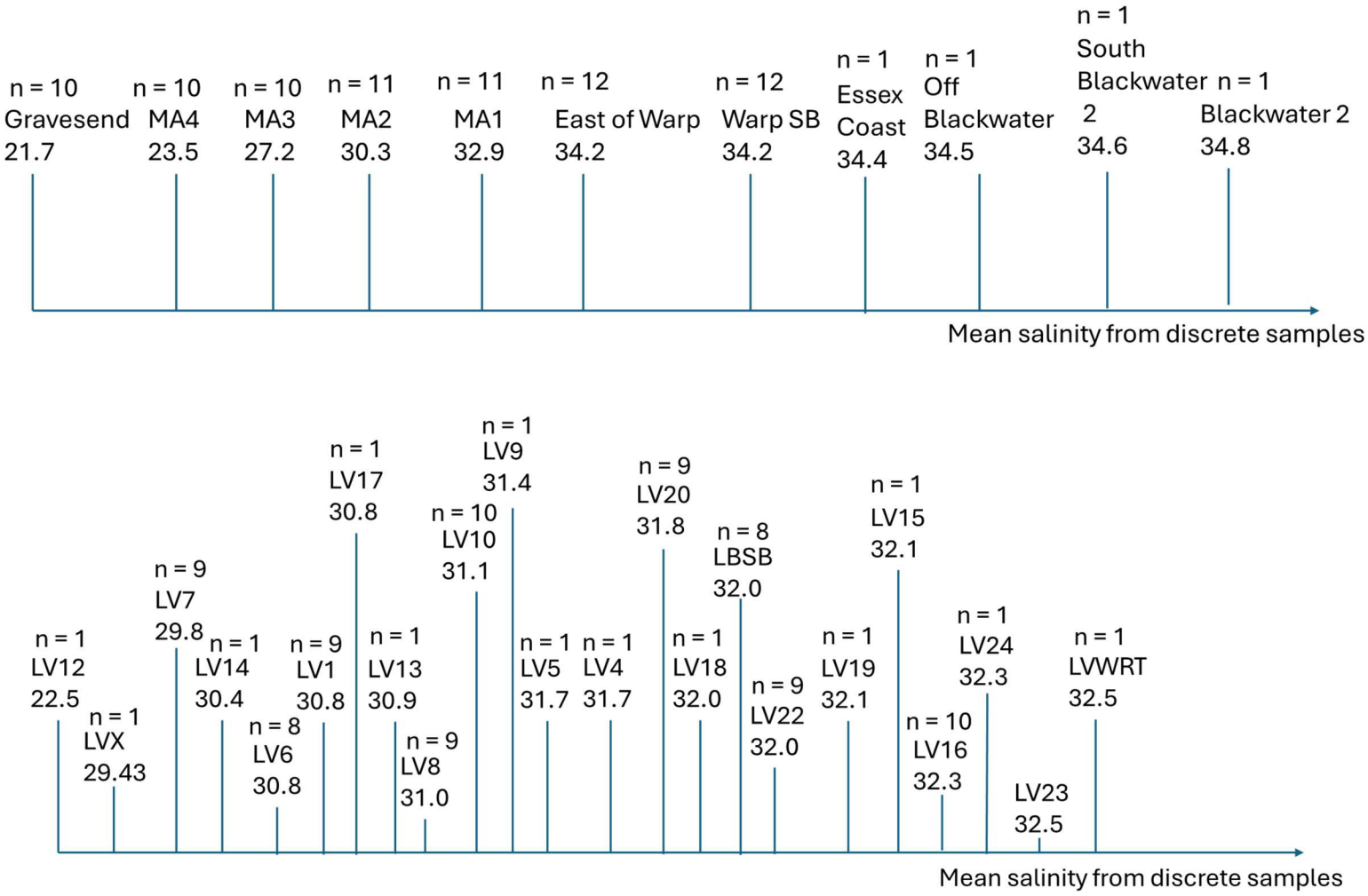

Figure 4.3 – Sampling station names against mean salinity in the Thames estuary (Top) and Liverpool Bay (bottom)

Sampling occasions are outlined in the appendix (7.4). Samples for the determination of inorganic nutrients, salinity, chlorophyll, and phytoplankton were collected in surface water using a bucket and analysed in line with the methods outlined in the methods chapter (Chapter Two). Nutrient concentrations recorded below the limit of detection, at < 0.01 µg/L for nitrite, and at, < 0.1 µg/L, for TOxN, $NH4^+$, DIP, and silicate, were assigned the value of the limit of the respective value of detection for inclusion in figures and models. An RBR Maestro CTD (https://rbr-global.com/products/standard-loggers/#large-multi-channel) was used to collect oceanographic profiles of the water column at each sample site. Salinity is measured on the Practical Salinity Scale of 1978 (UNESCO, 1981) and is dimensionless. Turbidity data was collected using a RBR*tridente* turbidity sensor which has a range of 0-500 Formazin Turbidity Units (FTU) and a detection limit of 0.001 FTU. Underwater light intensity was collected using an RBR*coda*^{3} PAR sensor (https://rbr-global.com/products/sensors/rbrcoda3-par-rad/). Data from these sensors was collected at a frequency of 16Hz and a surface mean value for each parameter was calculated using readings from the upper 2m of the water column. An additional data collection effort, not part of the mNCEA programme, occurred in Liverpool Bay in April 2022 onboard the *Mersey Guardian* (Figure 4.2). Samples for the determination of inorganic nutrients, salinity, chlorophyll and phytoplankton species composition and abundance were collected from the surface using a bucket and processed using the methods outlined in the methods chapter (Chapter Two). Oceanographic data at a surface, middle, and bottom depth at each sample site, including turbidity data, were collected using an Idronaut Ocean Seven 305/89 CTD (https://www.idronaut.it/multiparameter-ctds/oceanographic-ctds/. The surface values were used in this work.

4.3.2 Data Analysis

For each sample, TOxN and $NH4^+$ were added together to obtain a value for DIN. Concentrations of nutrients, SPM, and chlorophyll were plotted against salinity determined on individual water samples. Values for turbidity and photosynthetically active radiation were plotted against the salinity values obtained from the corresponding CTD deployment. Values with no associated salinity were not included in the analysis. All turbidity observations were removed from Liverpool Bay data for June 2023 due to equipment malfunction being identified. Turbidity observations from August 2022 and January 2023 were removed from LV10 and MA2 due to an abnormally high values being identified due to suspected equipment malfunction. Observations are grouped into seasons for the calculations of linear models (Table 4.1).

Table 4.1 Months assigned to each season for the analysis of biogeochemical and phytoplankton variables.

Season	**Month**
Autumn	September, October, November
Winter	December, January, February
Spring	March, April, May
Summer	June, July, August

4.3.3 Nutrient addition Bioassay in the Thames estuary

Seawater was collected by Cefas scientists from the Warp SmartBuoy site (Figure 4.1) at a depth of 1 m from the survey vessel on 14th July 2022 at 10:00 GMT. The location of the site was 51.5332°N, 1.0498°E. The salinity at the site at the time of collection was 34.755.

Water was collected into 2 x 25 L carboys which were washed with distilled water and pre rinsed with sample water. This was collected by repeat deployments of a 10 L niskin bottles. The carboys were covered with black plastic in order to reduce light exposure upon collection and were transported back to the UEA laboratory within 12 hours of sampling. Once back at the laboratory, water was filtered through a 200 µm net to filter out zooplankton and remove grazing pressure (Weston et al., 2008). Water from the two carboys was mixed together and gently shaken to ensure even distribution. 1.5 L of this filtered bulk water was transferred into each of the acid washed 2 L polycarbonate Nalgene bottles.

Table 4.2 – Final nutrient concentrations in each bottle in the Thames Estuary bioassay

Bottles	**Final concentration of nutrients in each 1.5 L of sample**
1, 2, 3	+ 60 µmol/L $NaNO_3$
4, 5, 6	+ 3 µmol/L KH_2PO_4
7, 8, 9	+ 25 µmol/ L Na_2SiO_4
10,11,12	+ 60 µmol/ L $NaNO_3$, + 3 µmol/ L KH_2PO_4
13,14,15	+ 60 µmol /L $NaNO_3$, + 3 µmol/ L KH_2PO_4, + 25 µmol/ L Na_2SiO_4
16,17,18	Control

Nutrient additions were made to reach concentrations (Table 4.2) which would ensure concentrations of at least the mean 90th percentile of winter nutrient concentrations at this site using historic data from the Cefas SmartBuoy (https://www.cefas.co.uk/data-and-publications/smartbuoys/). The bottles were randomly distributed within the growth cabinet

in a temperature-controlled room set to 13.5 ˚C on a light : dark cycle set to the same hours as at the collection site on 14 July 2022, resulting in a 16.5 light to 7.5-hour dark cycle. The bottles were incubated for 48 hours.

Every 24 hours, 100 mL from each bottle was decanted into a measuring cylinder and filtered onto a 25 mm 0.7 µm glass fibre filter, with a maximum vacuum of 10 kPa. The filters were wrapped in aluminium foil and frozen at -80 °C for chlorophyll analysis (see Chapter Two).

4.4 Results

4.4.1 Thames Estuary Results

Results are grouped and presented in three parameter groups of nutrient variables, physical environmental variables, and biological variables.

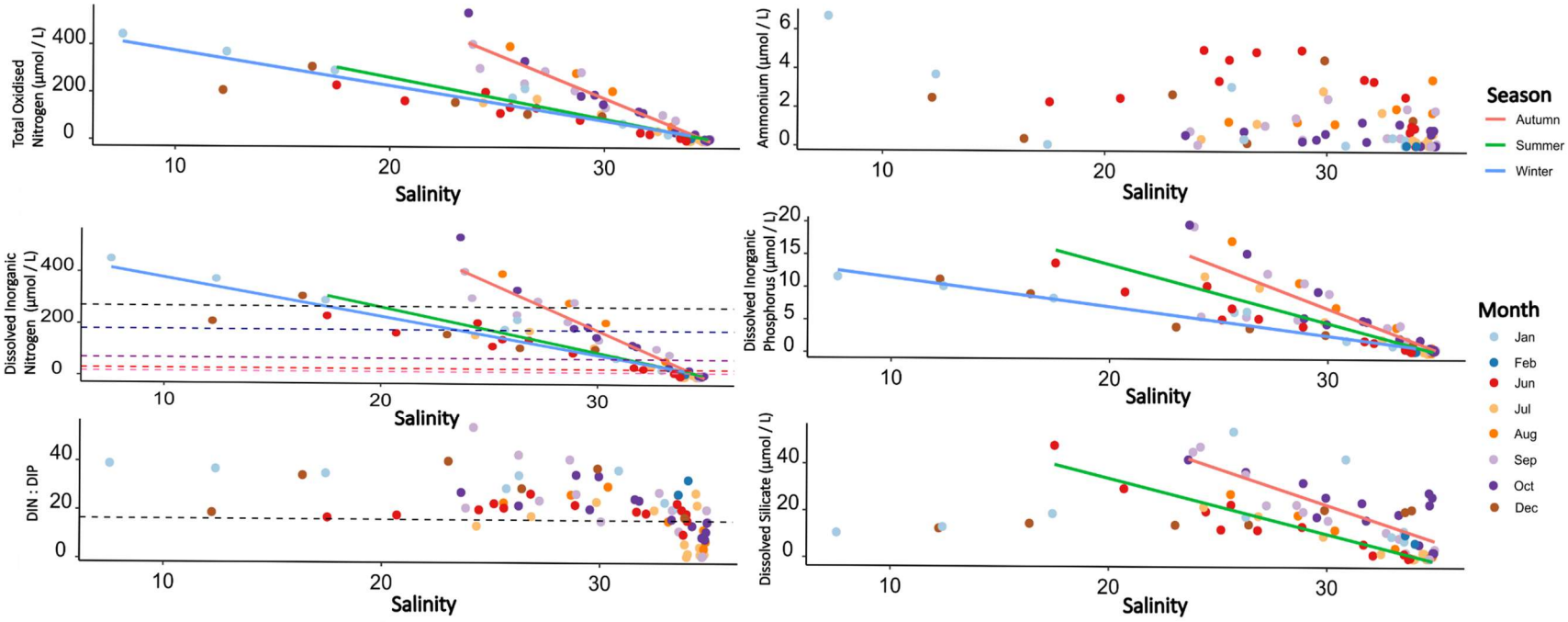

Figure 4.4 –Nutrient results from the Thames estuary. Top row: Total oxidised Nitrogen (left), Ammonium (right); Middle row: Dissolved Inorganic Nitrogen (left), Dissolved Inorganic Phosphorus (right); Bottom row: the ratio of DIN : DIP (left), dissolved Silicate (right) as a function of salinity. Observations are coloured by month. Observations are grouped by seasons for linear models and those linear models with an R^2 of 0.5 or greater are displayed on the figures. On the DIN figure, the dashed lines show the WFD/WER assessment thresholds for **pink:** non-turbid coastal waters (18 µmol/L); **red:** non-turbid transitional waters (30 µmol/L); **purple:** Intermediate turbidity waters (70 µmol/L), **blue:** Turbid waters (180 µmol/L), and **black:** very Turbid waters (270 µmol/L). On the DIN : DIP figure, **black:** the 16: 1 Redfield N : P ratio.

Table 4.3 - Summary of R^2 and *p* values for linear models in each season for each nutrient parameter in the Thames Estuary. Significant *p* values ($p < 0.05$) are shaded green.

	Summer R^2	Summer *p* value	Autumn R^2	Autumn *p* value	Winter R^2	Winter *p* value
TOxN	0.61	4.64×10^{-8}	0.92	2.86×10^{-16}	0.89	2.09×10^{-9}
$NH4^+$	0.19	0.01	-0.04	0.92	0.32	0.01
DIN	0.62	3.64×10^{-8}	0.92	2.74×10^{-16}	0.89	1.97×10^{-9}
DIP	0.75	3.81×10^{-11}	0.73	3.85×10^{-9}	0.95	5.56×10^{-12}
DIN : DIP	0.14	0.02	0.49	2.10×10^{-5}	0.12	0.09
Silicate	0.91	2.2×10^{-16}	0.67	5.22×10^{-8}	-0.06	0.76

TOxN and DIN in the Thames Estuary values range between ~ 538 µmol/L at Gravesend in winter, and the limit of detection (0.1 µmol/L) at South Blackwater 2 and the Warp SmartBuoy in summer. The DIN threshold for very turbid waters is occasionally exceeded at salinities below 30, whilst the threshold for intermediate turbidity waters is consistently exceeded at salinities below 30 (Figure 4.4). Values from samples above a salinity value of 30 do not consistently exceed the DIN thresholds (Figure 4.4). 48% of samples with a salinity value of over 30, have a DIN : DIP ratio which exceeds the Redfield ratio of 16 : 1 (Figure 4.4)Values for $NH4^+$ concentrations range between the limit of detection (0.1 µmol/L), and a peak value of 6.7 µmol/ L (Figure 4.4) observed at MA4 in winter. Low DIP values are at the limit of detection (0.1 µmol/L), with a peak value of 19.7 µmol/L (Figure 4.4) at Gravesend in winter. Dissolved silicate concentrations have low values at the limit of detection (0.1 µmol/L), with the highest value being 54 µmol/L (Figure 4.4) at MA2 in winter. Significant relationships ($p < 0.05$) are identified in the summer, autumn, and winter seasons for TOxN, DIN, and DIP, and in summer and autumn for dissolved silicate (Table 4.3). In the winter season the concentration of dissolved silicate shows a peak around a salinity of 25 before the concentrations of dissolved silicate decline again as salinity increases (Figure 4.4). The highest silicate concentrations are observed in the months of January and June (Figure 4.4). The concentration of TOxN, DIN, and

DIP show a significant linear decline with increasing salinity in the autumn, summer, and winter seasons (Table 4.3). The highest concentrations of DIN and TOxN are observed in October, whilst the highest concentrations of DIP are observed in September and October ()The highest concentrations of $NH4^+$ are observed in June, January, and December (Figure 4.4). and no strongly linear relationship with salinity has been identified, however concentrations do decline significantly with salinity in summer and winter (Figure 4.4, Table 4.3). The highest values of DIN : DIP ratios are observed in September, December, and January (Figure 4.4). The values of the DIN : DIP ratio remain consistent with salinity in the winter months, whilst there is a significant decline in the ratio of DIN : DIP in the summer and autumn months (Figure 4.4 , Table 4.3).

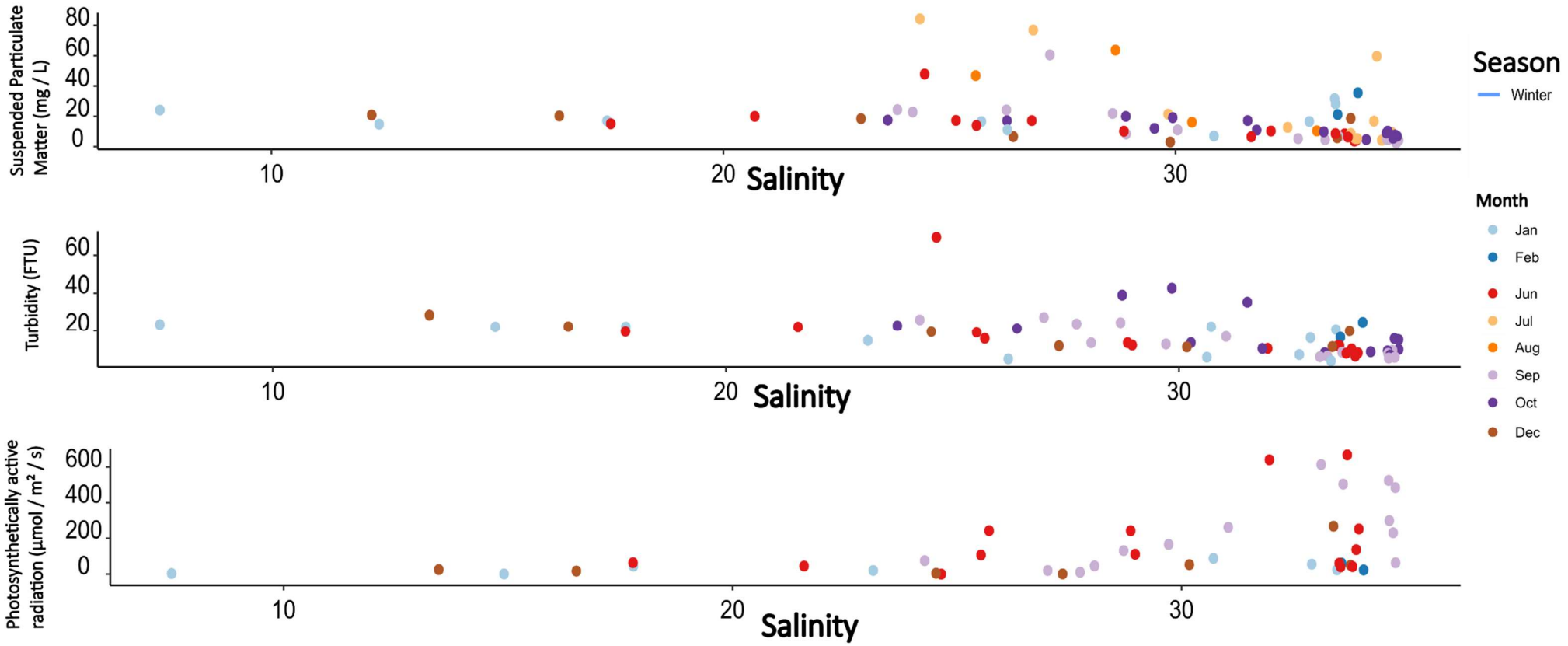

Figure 4.5 - Suspended particulate matter (top), Turbidity (middle), and photosynthetically active radiation (bottom) averaged over the upper 2m of the water column, as a function of salinity in the Thames Estuary. Observations are coloured by month.

Table 4.4 - Summary of R^2 and *p* values for linear models calculated in each season for each physical variable in the Thames Estuary. Significant *p* values ($p < 0.05$) are shaded in green.

	Summer R^2	**Summer *p* value**	**Autumn R^2**	**Autumn *p* value**	**Winter R^2**	**Winter *p* value**
Suspended Particulate Matter	0.18	0.01	0.41	1.30×10^{-4}	-0.06	0.99
Turbidity	0.18	0.08	0.39	1.70×10^{-4}	-0.05	0.03
Photosynthetically active radiation	0.05	0.23	0.41	7.87×10^{-3}	0.13	0.10

SPM values peak at 84 mg/L at Gravesend in summer, and the lowest value at ~2 mg/L (Figure 4.5) at East of Warp in autumn. Turbidity values range between approximately 3 and 40 FTU, with one exception at 69 FTU at Gravesend in summer L (Figure 4.5). PAR values range between lows of 0.005 µmol/m^2/s at Gravesend in summer, and peak values of over 667 µmol/m^2/s L (Figure 4.5) at MA1 in summer. Suspended particulate matter concentrations show a significant decline with increasing salinity in the summer and autumn seasons, but the relationships are not strongly linear (Table 4.4). The highest concentrations of SPM are observed in July L (Figure 4.5). No strongly linear relationship with salinity is observed for turbidity, but a significant decline with increasing salinity is observed in the autumn and winter seasons (Table 4.4). Photosynthetically active radiation in the upper 2 m of the water column shows a significant increase with increasing salinity in the autumn season (Table 4.4). The highest values of PAR are observed in June and September L (Figure 4.5).

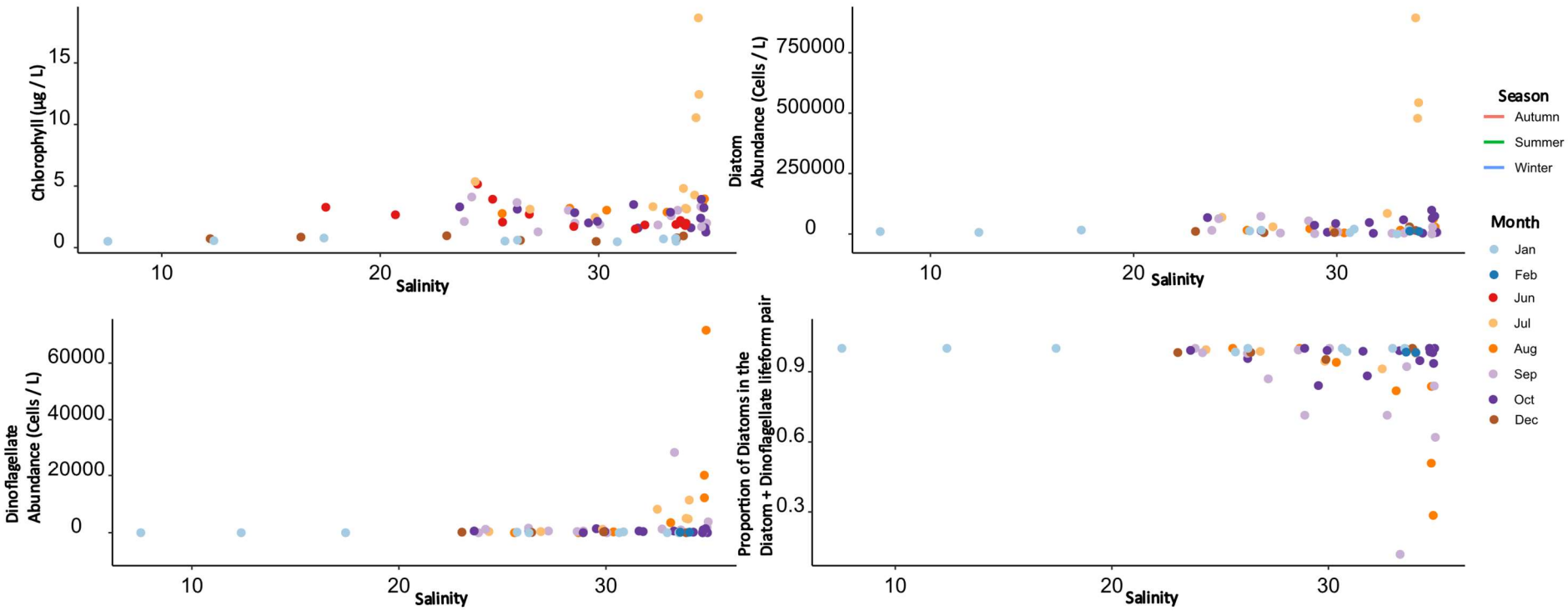

Figure 4.6 - Top row: Chlorophyll concentration (left), Diatom abundance (right); Bottom row: Dinoflagellate abundance (left), the diatom proportion of the diatom and dinoflagellate pair (right) as a function of salinity in the Thames Estuary. Observations are coloured by month.

Table 4.5 - Summary of R^2 and *p* values for linear models calculated in each season for each biological parameter in the Thames Estuary.

	Summer R^2	**Summer *p* value**	**Autumn R^2**	**Autumn *p* value**	**Winter R^2**	**Winter *p* value**
Chlorophyll concentration	0.01	0.26	0.05	0.13	-0.07	0.81
Diatom abundance	0.07	0.18	-0.01	0.40	-0.01	0.37
Dinoflagellate abundance	0.18	0.07	-0.02	0.50	0.05	0.19
Proportion of diatoms in the diatom + dinoflagellate lifeform pair	0.20	0.06	-4.21×10^{-3}	0.36	-0.02	0.40

Chlorophyll values are typically 5 µg/L or below, with three occasions of peak values higher than this, at 18.6, 12.4, and 10.5 µg/L (Figure 4.6), seen at South Blackwater 2, Warp SmartBuoy, and Off Blackwater in summer. Diatom abundances are typically between 200 and 10 0000 cells/L, with three observations of higher values, at 894400, 543740, and 478920 cells/L (Figure 4.6) observed at East of Warp, MA1, and Warp SmartBuoy in summer. Dinoflagellate abundances range between 0 cells/L and a peak of approximately 71620 cells/L at MA1 in summer, with 89% of samples having less than or equal to 5000 cells/L (Figure 4.6). No significant linear relationships with salinity are observed for any of the biological parameters in the Thames Estuary (Table 4.5). High values of chlorophyll and diatom abundance are observed in July, at high salinities (Figure 4.6). Peak values in dinoflagellate abundance are seen at the highest salinities, in July, August, and September (Figure 4.6). The proportion of diatoms within the diatom and dinoflagellate lifeform pair is above 0.6 for all observations below salinity values of 30. Above a salinity of 30, there are observations from July and September where diatoms make up a lower proportion of the lifeform pair (Figure 4.6).

4.4.2 Liverpool Bay Results

Results are grouped and presented in three parameter groups of nutrient variables, physical environmental variables, and biological variables.

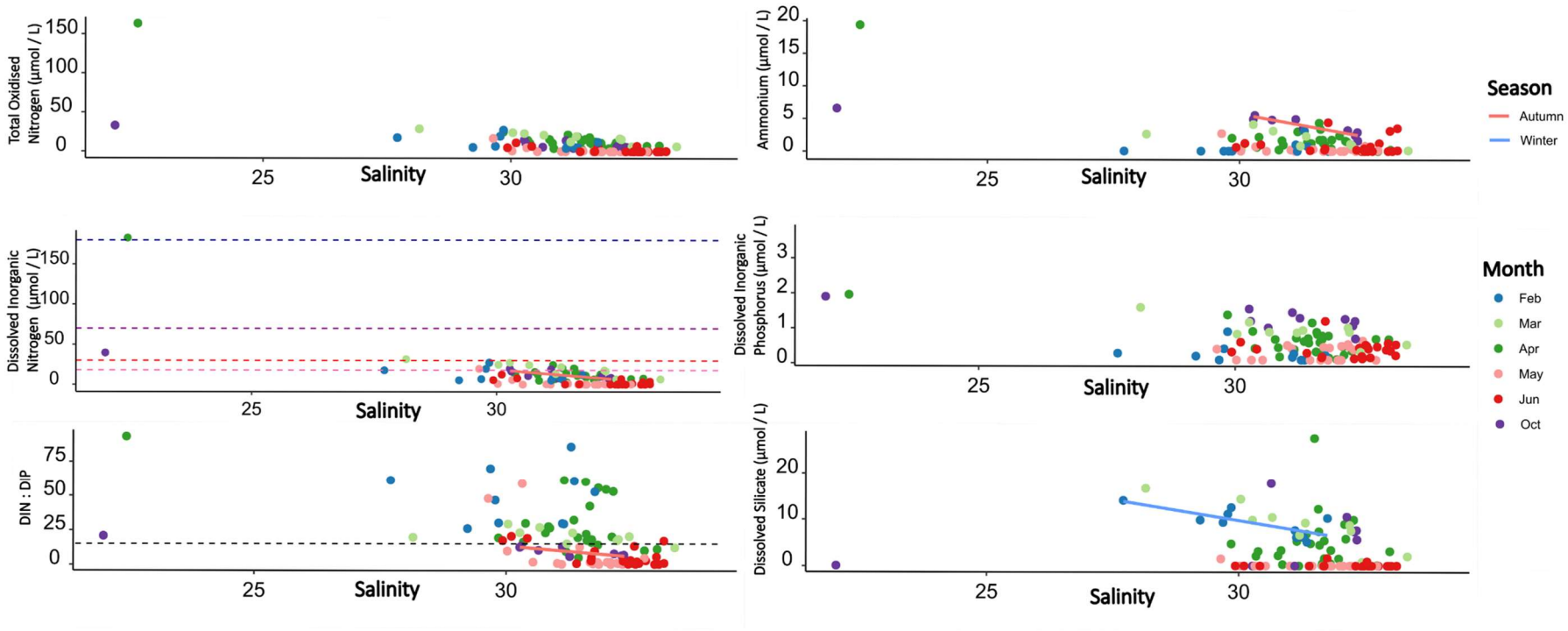

Figure 4.7 - Top row: Total oxidised Nitrogen (left), Ammonium (right); Middle row: Dissolved Inorganic Nitrogen (left), Dissolved Inorganic Phosphorus (right); Bottom row: the ratio of DIN : DIP (left), Dissolved Silicate (right) as a function of salinity in Liverpool Bay. Observations are coloured by month. Observations are grouped by seasons for linear models and those linear models with an R^2 of 0.5 or greater are displayed on the figures. On the DIN figure, the dashed lines show the WFD/WER assessment thresholds for **pink:** non-turbid coastal waters (18 µmol/L); **red:** non-turbid transitional waters (30 µmol/L); **purple:** Intermediate turbidity waters (70 µmol/L), **blue:** Turbid waters (180 µmol/L).

TOxN and DIN values range between a high of ~163 µmol/L at LV12, and the limit of detection (Figure 4.7). The WFD/WER DIN threshold for Intermediate and Turbid waters are only exceeded once, and 86% of observations are below the DIN threshold for non-turbid coastal waters (Figure 4.7). $NH4^+$ concentrations peak at 19.4 µmol/L at LV12, whilst the remainder of the observations are 6 µmol/L or below (Figure 4.7). DIP concentrations have low values at the limit of detection (0.1 µmol/L), and the highest DIP concentrations observed in this data set are at approximately 2 µmol/L (Figure 4.7) at LV12. The DIN : DIP ratios observed in Liverpool Bay range between 93 at LV12 and 0.4 at LV23 and the Liverpool Bay SmartBuoy, with all observations at a salinity below 30 having a DIN : DIP ratio which exceeds the 16 : 1 Redfield ratio (Figure 4.7). Dissolved silicate concentrations have a range between the limit of detection (0.1 µmol/L), and 27 µmol/L seen at LV23, with 98% of observations falling below 20 µmol/L. Significant ($p < 0.05$) non-linear decreases in concentration with increasing salinity are identified for all parameters in the spring season, with the exception of DIN : DIP where $p = 0.05$ (Table 4.6). TOxN, NH_4^+, DIN, and DIN : DIP show significant negative relationships with salinity in autumn. For NH_4^+, DIN, and DIN : DIP, this relationship has an R^2 above 0.5, but this relationship is non-linear for TOxN. In the summer, TOxN, DIN, and DIN : DIP show a significant ($p < 0.05$) non-linear negative relationship with salinity. Silicate concentrations show a significant decline with increasing salinity in the winter season (Table 4.6). Silicate concentrations show a moderately linear ($R^2 > 0.5$) relationship with salinity in the winter (Table 4.6). There are samples obtained at lower salinity sites in April and October, and the April inshore value for all variables is typically highest, with the exception of DIP, where the values are comparable (Figure 4.7). Concentrations of nutrients at the highest salinity values are generally highest in the months of March and April for TOxN and DIN, whilst DIP and $NH4^+$ concentrations are highest in October (Figure 4.7). The highest ratios of DIN : DIP at highest salinity values are observed in February, and silicate concentrations are highest in April (Figure 4.7). Across all parameters, the lowest values are observed in May and June ((Figure 4.7), however high concentrations of $NH4^+$ are also observed in June (Figure 4.7). In these months, silicate concentrations are consistently depleted, with values being at the limit of detection (Figure 4.7).

Table 4.6 – Summary of R^2 and p values for linear models calculated in each season for each nutrient parameter in the Liverpool Bay study area. Significant p values ($p < 0.05$) are shaded in green.

	Spring R^2	Spring p value	Summer R^2	Summer p value	Autumn R^2	Autumn p value	Winter R^2	Winter p value
TOxN	0.25	4.16×10^{-5}	0.44	5.86×10^{-4}	0.39	0.04	0.08	0.21
NH_4^+	0.14	3.27×10^{-3}	-0.05	0.98	0.83	3.94×10^{-4}	0.07	0.23
DIN	0.26	4.54×10^{-5}	0.31	4.98×10^{-3}	0.53	0.02	0.05	0.26
DIP	0.10	0.01	-0.05	0.72	0.14	0.17	-0.05	0.47
DIN : DIP	0.05	0.05	0.38	1.83×10^{-3}	0.63	6.28×10^{-3}	-0.12	0.89
Si	0.06	0.04	-0.05	0.88	-0.05	0.45	0.53	0.01

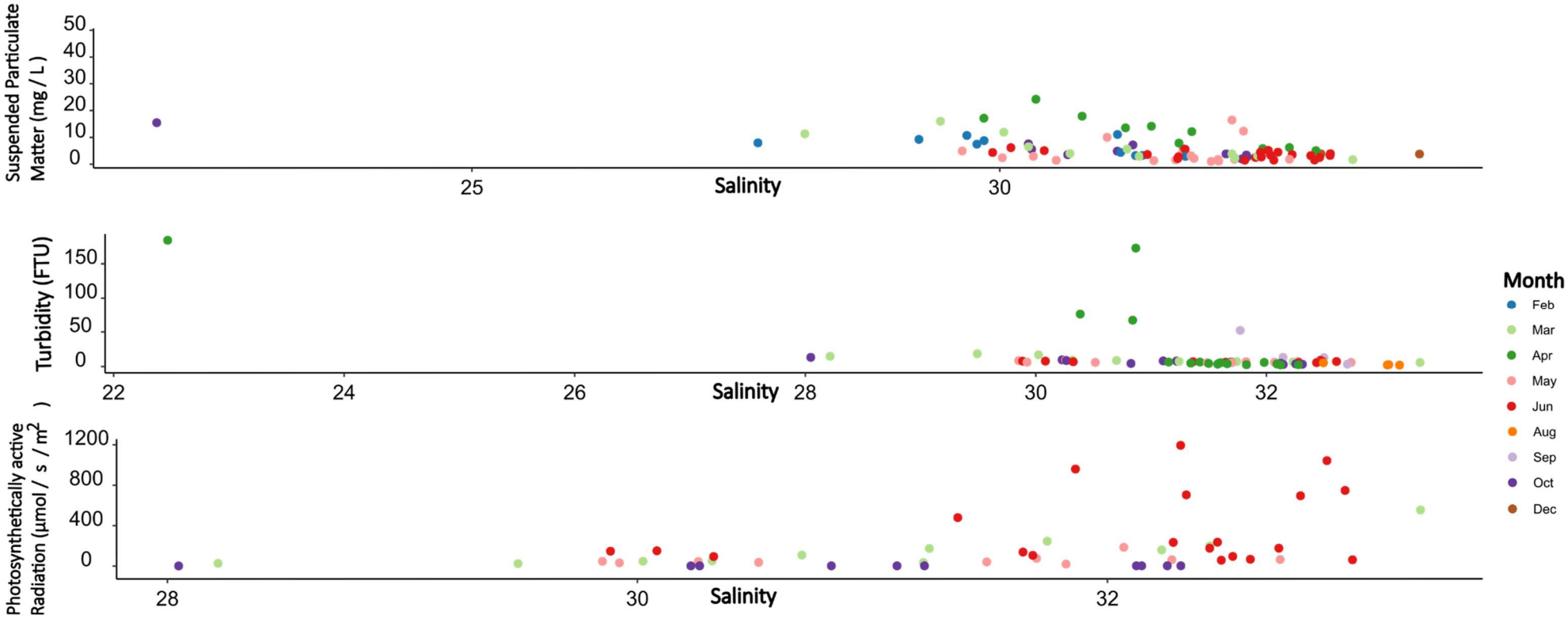

Figure 4.8 - Suspended particulate matter (top), Turbidity (middle), and photosynthetically active radiation (bottom) averaged over the upper 2m of the water column, as a function of salinity in Liverpool Bay. Observations are coloured by month.

Table 4.7 - Summary of R^2 and *p* values for linear models calculated in each season for each physical parameter in the Liverpool Bay study area. Significant *p* values ($p < 0.05$) are shaded in green.

	Spring R^2	**Spring *p* value**	**Summer R^2**	**Summer *p* value**	**Autumn R^2**	**Autumn *p* value**	**Winter R^2**	**Winter *p* value**
SPM	0.04	0.08	0.21	0.02	0.45	0.03	0.36	0.02
FTU	0.03	0.15	0.31	0.02	-0.08	0.83	*NA*	*NA*
PAR	0.33	4.10×10^{-3}	0.03	0.23	0.19	0.12	*NA*	*NA*

Suspended particulate matter concentrations peak at 24 mg/L at LV8 in winter and reach a low of 0.9 mg/L (Figure 4.8) at LV23 in spring. The highest turbidity values are 184 and 172 FTU at LV12 and LV13 respectively in spring, with all remaining observations at or below 75 FTU, and 80% below 20 FTU. PAR values peak at 1193 µmol/m^2/s at the Liverpool Bay SmartBuoy in summer, with low values of ~2 µmol/m^2/s in autumn. SPM concentrations decrease significantly with salinity in the autumn, summer, and winter seasons. There is a significant increase in PAR with salinity in the Spring season (Table 4.7), although the relationship is not strongly linear (Table 4.7, Figure 4.8). Turbidity significantly declines with increasing salinity in the summer season (Table 4.7). The highest concentrations of SPM are observed in April and March, and observations of high turbidity are also seen in April (Figure 4.8). PAR values are highest in June, whilst the lowest values are observed in October (Figure 4.8).

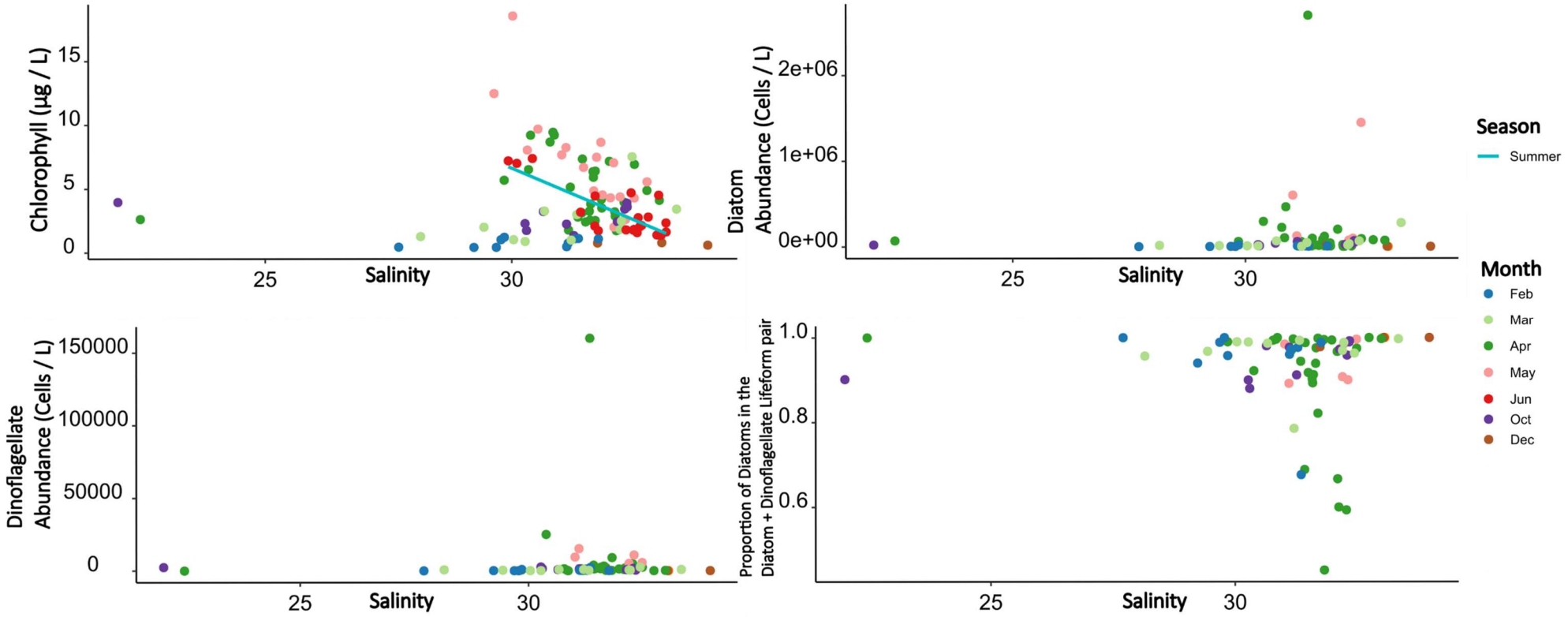

Figure 4.9 -Top row: Chlorophyll (left) , Diatom abundance (right); Bottom row: Dinoflagellate abundance (left), and the diatom proportion of the diatom and dinoflagellate pair (right) as a function of salinity in Liverpool Bay. Observations are coloured by month. Observations are grouped by seasons for linear models and those linear models with an R^2 of 0.5 or greater are displayed on the figures.

Table 4.8 - Summary of R^2 and *p* values for linear models calculated in each season for each physical parameter in the Liverpool Bay study area. Significant *p* values ($p < 0.05$) are shaded in green.

	Spring R^2	**Spring *p* value**	**Summer R^2**	**Autumn R^2**	**Autumn *p* value**	**Summer *p* value**	**Winter R^2**	**Winter *p* value**
Chlorophyll	0.03	0.09	0.6218	0.29	0.08	1.32×10^{-5}	-0.05	0.52
Diatom abundance	-0.02	0.78	*NA*	0.14	0.17	*NA*	-0.07	0.66
Dinoflagellate abundance	-0.02	0.83	*NA*	0.28	0.08	*NA*	-0.09	0.91
Proportion of Diatoms in the Diatom / Dinoflagellate lifeform pair	-7.27×10^{-4}	0.33	*NA*	0.41	0.04	*NA*	-0.09	0.94

Chlorophyll concentrations have peak values of 18.6 µg/L, and 12.5 µg/L at LV6 and LV1 respectively in spring, with the remainder of samples having chlorophyll concentrations between 10 and 0.4 µg/L. Diatom Abundances have peak values at 2700000 cells/L at LV1 in spring, and at 1448320 cells/L at LV20 in spring, with all other samples having abundances below 6000000 cells/L. There is a high dinoflagellate abundance of 160000 cells/L at LV1 in spring, and all other samples have dinoflagellate abundances below 25000 cells/L. Chlorophyll concentrations significantly decline with increasing salinity in the summer months (Figure 4.9, Table 4.8). There is a significant increase in the proportion of diatoms within the diatom and dinoflagellate lifeform pair in the autumn season (Figure 4.9, Table 4.8). The highest concentrations of chlorophyll are recorded in the months of May and April, with peak values being measured in May (Figure 4.9). The highest abundances of diatoms and dinoflagellates are also observed in April and May, with peak values being observed at high salinities in April for both lifeforms (Figure 4.9). The proportion of diatoms within the diatom and dinoflagellate lifeform pair remains above 0.8 for the majority of samples, however between salinity values of 31 and 32, there are observations below 0.8 in the months of March, April, and February (Figure 4.9).

4.4.3 Nutrient Addition Bioassay Results in the Thames Estuary

Nutrient additions were made to the samples (Table 4.9) in order to reach final concentrations (Table 4.2) which would ensure concentrations of at least the mean 90th percentile of winter TOxN, DIP, and dissolved silicate concentrations at this site using historic data from the Cefas SmartBuoy (https://www.cefas.co.uk/data-and-publications/smartbuoys/).

Table 4.9- Nutrient concentrations at t = 0 at the Warp SmartBuoy site.

TOxN (µmol/L)	**Nitrite (µmol/L)**	**Phosphate (µmol/L)**	**Silicate (µmol/L)**	**$NH4^+$ (µmol/L)**
<0.10	<0.01	0.13 ± 0.06	0.73 ± 0.29	0.70 ± 0.12

The nutrient concentrations at the time of sampling of the water used in the bioassays show values below the limit of detection for TOxN and nitrite (Table 4.9). The concentrations of the other nutrients were all below 1 µmol/ L.

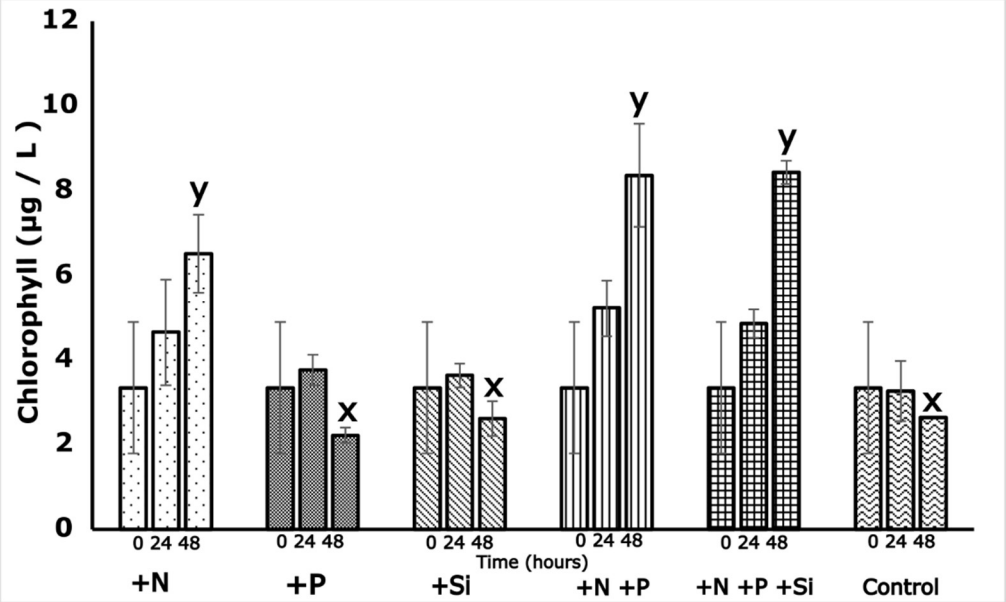

Figure 4.10 - The chlorophyll concentration (µg/L) in the nutrient addition bioassays using a natural community collected from the Warp SmartBuoy site in July 2022. Error bars represent the standard deviation (n = 3), except the control treatment at t = 48 hours where n = 2 and no standard deviation could be calculated. The results of the one-way ANOVA are displayed using letter groupings. Treatments which share letter groupings have no statistically significant difference at a confidence level of $p < 0.05$. Treatments with different letter groupings are statistically significant at a confidence level of $p < 0.05$.

The chlorophyll concentration increases over time in the +N, +N+P, and +N+P+Si treatments (Figure 4.10). The +P and +Si treatments show slight initial increases in chlorophyll concentration compared to the control treatment, but the chlorophyll concentration has declined after 48 hours. At t = 48 hours the treatments of +N, +N+P, and +N+P+Si are statistically significantly different from the control and the other treatments. The additions of P and Si individually show no statistically significant difference from the control.

4.5 Discussion

4.5.1 Nutrient trends

In the Thames Estuary, $NH4^+$ concentrations are low compared with TOxN concentrations, with TOxN reaching 400 µmol/L in some cases (Figure 4.4), whilst $NH4^+$ peaks at 6 µmol/L but does not often exceed 4 µmol/L (Figure 4.4). An $NH4^+$concentration of 4 µmol/L, coupled with a limited residence time, has previously been presented as an upper threshold for which NH_4^+ concentrations reduce NO_3^- uptake and therefore limit algal biomass (Dugdale et al., 2012; Wilkerson and Dugdale, 2016). In contrast, added $NH4^+$ has been documented as supporting increased phytoplankton biomass compared to added nitrate, during in situ mesocosm experiments (Donald et al., 2013). $NH4^+$ concentrations are higher Liverpool Bay (Figure 4.7) than in the Thames Estuary (Figure 4.4). However, chlorophyll concentrations are also higher in Liverpool Bay, with 67% of spring and summer values below 5 µg/L (Figure 4.9), whereas 85% of concentrations are below 5 µg/L in the spring and summer in the Thames Estuary (Figure 4.6). The inhibiting role of NH_4^+ is not observed in this PhD research, potentially due to differences in physical characteristics in the Thames Estuary and Liverpool Bay study areas compared to those investigated by Dugdale et al. (2012) and Wilkerson and Dugdale (2016). Diatoms have been reported to have a preference for nitrate uptake relative to $NH4^+$ uptake and therefore may not always be inhibited by elevated concentrations of $NH4^+$ (Glibert et al., 2016; Andersen et al., 2020), nonetheless diatoms dominated the community observed by Dugdale et al. (2012) and Wilkerson and Dugdale (2016). Diatoms dominate the community in the Thames Estuary and Liverpool Bay areas studied here (Figure 4.6, Figure 4.9). The observations made within this study do not identify increased $NH4^+$ concentrations as an inhibiting factor to phytoplankton growth, and higher chlorophyll concentrations are seen in Liverpool Bay where $NH4^+$ concentrations are higher, than in the Thames estuary. However, the available data here captures the spring bloom in Liverpool Bay but not in the Thames Estuary, and this will impact the chlorophyll concentrations observed. This suggests that increased $NH4^+$ concentrations are not inhibiting growth, as similar chlorophyll concentrations are observed where comparable data is available in both areas (e.g. October).

DIP significantly declines with increasing salinity in both study areas in spring, and also in autumn and winter in the Thames Estuary (Figure 4.4, Figure 4.7). Phosphorus concentrations in Liverpool Bay do not exceed 2 µmol/L and 79% of values are below 1 µmol/L (Figure 4.7). Higher DIP concentrations are observed in the Thames Estuary (Figure 4.4), but observations below 1 µmol/L are made at high salinities. At DIP concentrations below 1 µmol/L, diatoms have been observed to be poor competitors compared to dinoflagellates (Egge, 1998). Despite the lower DIP values seen in Liverpool Bay compared to the Thames estuary, diatoms are dominant across the salinity gradient in both study areas (Figure 4.6, Figure 4.9). Higher proportions of dinoflagellates are seen only at higher salinities in both areas (Figure 4.6, Figure 4.9), and this may in part be a result of the increasing competitiveness of dinoflagellates compared to diatoms with low phosphorus concentrations. Coastal waters have previously been considered nitrogen-limited (Howarth and Marino, 2006), however, the differences in the success of management practices aiming to reduce nutrient pollution have resulted in a more significant decline in phosphorus concentrations that in nitrogen concentrations (Paerl, 2009; Lewis et al., 2011; Grizzetti et al., 2012; Burson et al., 2016; Greenwood et al., 2019). This has consequently led to an increase in the DIN : DIP ratio within riverine inputs and coastal waters (Burson et al., 2016; Greenwood et al., 2019).

In the Thames Estuary there is a significant decline in DIN:DIP with increasing salinity in the autumn and summer seasons (Table 4.3). Rather than a gradual decline with increasing salinity there is a sharp decline in the DIN : DIP ratio at salinities above 33 (Figure 4.4), with values reducing below the Redfield ratio (Redfield, 1958). This suggests a switch from phosphorus-limited conditions at lower salinities, to nitrogen-limited conditions at higher salinities. The majority of observations along the salinity gradient in Liverpool Bay from the months of May, June and October are below the Redfield ratio, whilst winter observations are generally above the 16 : 1 Redfield ratio (Figure 4.7). Observations in April and March span the 16:1 ratio (Figure 4.7), suggesting that there is a switch from phosphorus-limited conditions to nitrogen-controlled conditions within these months, based on the Redfield ratio. A change in the controlling nutrient from phosphorus-controlled to nitrogen-controlled conditions along a salinity gradient was previously observed within the North Sea by Burson et al. (2016) during multiple bioassay addition experiments. They observed the switch 250 km offshore from the Dutch coast, north of the island of Terschelling. This is considerably further offshore than the sampled stations within this study, where a switch in conditions has been observed here much closer to the coast.

The use of the Redfield ratio as a proxy for identifying the limiting nutrients is only indicative, as many deviations have been identified in the ratios in which phytoplankton take up nutrients (Rios et al., 1998; Geider and La Roche, 2002; Ptacnik et al., 2010; Glibert and Burkholder,

2011). Nutrient addition bioassays can there offer further insight into nutrient limitation patterns. The nutrient addition bioassay conducted within this work at the Warp SmartBuoy site in the Thames Estuary indicates nitrogen limitation and nitrogen and phosphorus co-limitation (Figure 4.10). A nutrient bioassay at the Warp SmartBuoy site by Weston et al. (2008) showed a moderate response to enrichment for treatments of +N+P, and a strong response to the addition of +N+P+Si. The nutrient addition bioassays conducted by Weston et al. (2008) took place on days 172 and 201, June 21st and July 20th, respectively. The nutrient addition experiment conducted within this PhD research took place on 14th July 2022 (day 195) and is therefore likely representative of similar seasonal conditions. Silicate enrichment alone (+Si) resulted in no response in phytoplankton biomass within this 2022 experiment, indicating that silicate was not limiting growth. The initial silicate concentration in the 2022 experiment was 0.72 µmol/L, which is in line with the concentrations observed by Weston et al. (2008) for June – August. Silicate is only a relevant limiting nutrient for diatoms, whilst other phytoplankton are not reliant on it. No response to silicate addition, despite concentrations which have previously been identified as limiting, could be an indication that the community is not diatom-dominated, and the added nutrients are instead being utilised by an alternative lifeform. Weston et al. (2008) observed a switch in dominating taxa of the spring bloom from initially diatom-dominated to *Phaeocystis*. They also noted a switch back to diatom-dominance in the summer, post bloom, when their bioassay was conducted. There is no phytoplankton data for the 2022 nutrient addition bioassay, but phytoplankton analysis from July 2023 indicates that diatom abundance was approximately 25 times higher than dinoflagellate abundance at salinities similar to those at the Warp SmartBuoy site (Figure 4.6). It therefore seems likely that the community was diatom-dominated, and the lack of response to silicate addition is driven by a different factor. The incubation experiments conducted by Weston et al. (2008) lasted 5 days, whereas the experimental results presented here are from a 48-hour incubation. It is also possible that had growth been monitored for longer, the treatments with silicate addition may have shown a response, as diatoms continued to grow and deplete silicate concentrations.

4.5.2 Phytoplankton composition and abundance

Silicate concentrations along the salinity gradient in the Thames Estuary show a significant decline with increasing salinity in the autumn and summer seasons, but no significant change over the salinity gradient in the winter season (Figure 4.4). The fraction of diatoms within the diatom / dinoflagellate pair declines with increasing salinity in the summer season in the Thames Estuary. This decline is close to a significant p value (p = 0.06), but is statistically, significant at a significance level of $p < 0.05$ (Figure 4.6, Table 4.5). The increase in the dinoflagellate proportion with salinity is predominantly seen in the months of July and September, where the silicate concentration is lowest at high salinities. Concentrations

measured in June also shows low silicate concentrations but there is no phytoplankton data available in June. Dinoflagellate growth upon silicate limitation of diatoms is an established pathway for the succession of diatoms into dinoflagellates and has been observed in the Thames Estuary before (Weston et al., 2008). In Liverpool Bay, silicate concentrations are observed to be highest in the months preceding the spring bloom of April and May identified by (Greenwood et al., 2011) (Figure 4.7). Silicate concentrations are depleted in the May and June samples across the salinity gradient in Liverpool Bay (Figure 4.7), and there are observations of an increased dinoflagellate proportion within the diatom - dinoflagellate lifeform pair within May and June (Figure 4.9). However, there are no observations for the months of June to September in Liverpool Bay. Diatoms have previously been seen to dominate the majority of the duration of the spring bloom in Liverpool Bay at the SmartBuoy site, with dinoflagellates briefly dominating in July (Greenwood et al., 2012). This pattern is not observed here, with the diatom proportion of the diatom and dinoflagellate lifeform pair dropping below 0.5 only once, in April (Figure 4.9).

Chlorophyll concentrations in the Thames Estuary show no significant change with salinity (Table 4.5). The higher nutrient concentrations observed at lower salinities in the Thames Estuary do not result in increased chlorophyll at these salinities. The premise with which transitional and coastal waters are managed, i.e. that nutrient concentrations alone are not necessarily problematic (Devlin et al., 2007b; Foden et al., 2011), is supported here. However, peak chlorophyll concentrations have previously been observed in May (Weston et al., 2008), and this month is not sampled in the Thames Estuary within the dataset used in this PhD research. Data from the spring bloom are unlikely to have been captured within the sampling occasions in the Thames Estuary, as no samples were taken in March, April and May in this dataset. This makes it difficult to assess the nutrient controls on phytoplankton, when other environmental conditions are likely to be optimal for supporting increased growth. In Liverpool Bay, there is a significant decline in the concentration of chlorophyll with increasing salinity in the summer, but not in other seasons (Figure 4.9). The spring bloom has previously been observed in April / May in Liverpool Bay (Greenwood et al., 2011), and no significant change in chlorophyll concentrations with increasing salinity are observed in the spring season (Figure 4.9), despite a significant decline in the concentration of nutrients (Figure 4.7).

4.5.3 Nutrient concentrations relative to assessment thresholds

DIN concentrations consistently exceed the upper thresholds imposed by the WFD/WER (Devlin et al., 2007b) in the Thames Estuary at salinities below 30 (Figure 4.4). At salinities below 30, the SPM concentrations typically fall within the intermediate category of 10-70 mg/L (Figure 4.5), resulting in an upper DIN threshold of 70 µmol/L. This is exceeded by all of the DIN

observations at salinities below 30. The majority of observations at higher salinities, where waters are non-turbid, have DIN concentrations below the non-turbid threshold (Figure 4.4). The SPM concentrations observed in Liverpool Bay in waters above 32 are in the non-turbid category, and those at a salinity below 32 in the intermediate category (Figure 4.8). All bar three samples from Liverpool Bay are below the DIN non-turbid threshold for coastal waters across the entire salinity gradient (Figure 4.7). Despite the elevated nutrient concentrations in the Thames Estuary at low salinities, which exceed the acceptable associated threshold, no significant change in chlorophyll concentration with salinity is identified (Figure 4.6). Chlorophyll concentrations are higher in Liverpool Bay, despite much lower concentrations of DIN compared to the Thames Estuary. It is important to note that the spring bloom is not thought to have been captured within the Thames Estuary data collection efforts. Nevertheless, results are available for months around the spring bloom in both areas. The results discussed above further support the suggestion that elevated concentrations of nutrients alone do not support an increased chlorophyll concentration, along a salinity gradient or in different study areas.

4.5.4 Light environment

The turbidity of a waterbody and associated light environment is consistently recognised within the literature as a factor which governs the response of a waterbody to nutrient pollution (Cloern, 1987; Painting et al., 2007; Burson et al., 2018). Suspended particulate matter concentrations of 50 mg/L and above have been described as limiting to phytoplankton growth (Cloern, 1987; Shaw et al., 1998; Weston et al., 2008). SPM has been observed to be a governing factor for the spatial distribution of phytoplankton blooms (Domingues et al., 2011; Gameiro et al., 2011; Liu et al., 2018). In the Thames Estuary, suspended particulate matter concentrations decline with salinity in the summer relative to autumn and winter (Table 4.4). Observations of an SPM concentration above 50 mg/L are not common at salinities above 30 (Figure 4.5). Despite this declining concentration of SPM with salinity, PAR does not have a significant relationship with salinity in the Thames Estuary (Table 4.5), however peak PAR values are observed at the highest end of the salinity gradient (Figure 4.5). Chlorophyll concentrations do not significantly change with salinity in the Thames Estuary (Figure 4.6). Although again it should be noted that the spring bloom is not represented within these results, in the absence of samples for March, April and May for the Thames Estuary. The peak chlorophyll concentrations and phytoplankton abundances are observed at the highest salinities (Figure 4.6), where PAR has peak values, and suspended particulate matter concentrations are at their lowest. This may suggest that on occasion the lower SPM/higher PAR allows for high abundance blooms to occur at these high salinities; however, PAR data is not available when chlorophyll and phytoplankton peaks are observed, and chlorophyll does

not show high values in the months where PAR is highest. Nonetheless, these high chlorophyll concentrations are only observed on for one sampling occasion and increasing chlorophyll concentration with increasing salinity is not the typically observed pattern within this dataset. In Liverpool Bay, chlorophyll concentrations significantly decline with increasing salinity in the summer season (Table 4.8). PAR concentrations in the summer have no significant relationship with salinity, but peak PAR values are observed at high salinities in June (Figure 4.8). In the spring in Liverpool Bay, PAR concentrations increase significantly with salinity (Table 4.7).

4.5.5 Limiting factors along the salinity gradient

At locations along the salinity gradient, typically high salinity, where environmental (SPM and/or PAR) conditions are suitable for elevated growth, the nutrient concentrations may not be sufficient to support increased phytoplankton biomass. In the Thames estuary this results in no change in chlorophyll concentration along the salinity gradient (Figure 4.6), and in Liverpool Bay there is a decrease in chlorophyll concentration with increasing salinity in the summer, and no change observed in other seasons (Figure 4.9). Nutrient inputs assessed here are usually sufficiently reduced by the time they reach the high salinity coastal waters to be below WFD/WER acceptable thresholds (Figure 4.4, Figure 4.7). The patterns observed within this study suggest that there is no, or limited, overlap in environmental conditions which allow for high algal biomass, i.e. high nutrient concentrations and high amounts of PAR or low amounts of SPM. In Liverpool Bay, but not the Thames Estuary, this includes the months containing the spring bloom. Peak chlorophyll concentrations within these months (April and May) are observed around a salinity of 30 (Figure 4.9). This salinity window of increased phytoplankton biomass may be indicative of a sweet spot of a combination of nutrient concentrations and light environment which support elevated phytoplankton growth. The results of the nutrient addition bioassay (Figure 4.10), indicate nutrient limitation in July 2022 in the Thames Estuary. This experiment was conducted with a natural community collected at the Warp SmartBuoy site which lies at the higher end of the salinity gradient assessed here. The results of the bioassay support the suggestion that phytoplankton growth at high salinities is limited by nutrient concentrations. Light limitation may govern phytoplankton growth inshore, resulting in a uniform concentration of chlorophyll along the salinity gradient in the Thames Estuary (Figure 4.6). If nutrient inputs were to increase, then this nutrient limitation may be alleviated, and higher chlorophyll concentrations may be seen at high salinity sites, and with them the undesirable impact of high algal biomass, such as oxygen depletion (Best et al., 2007; Diaz and Rosenberg, 2008). Similarly, if the light environment inshore were to improve for phytoplankton, increased inshore chlorophyll concentrations may be observed.

4.6 Conclusion

Studies (Dugdale et al., 2012; Wilkerson and Dugdale, 2016) have identified the potential for the relative contribution of $NH4^+$ to DIN to have an impact on the phytoplankton community as a result of inhibition. This is not identified within this data, and chlorophyll concentrations are higher in Liverpool Bay, despite an increase in $NH4^+$ compared to values seen in the Thames Estuary. There is a switch from phosphate limited conditions at low salinity to nitrogen limited conditions at high salinity around a salinity of 33 in the Thames Estuary, and around 30 in Liverpool Bay. This is supported by the results of the nutrient addition bioassay at the Warp SmartBuoy which indicated nitrogen limitation, and nitrogen and phosphorus co-limitation in July 2022 in the Thames Estuary. In the Thames Estuary, SPM significantly decreased with increasing salinity in the summer and autumn, but despite the changing environmental conditions, no significant changes in chlorophyll concentrations were observed along the salinity gradient in any season. Concentrations of chlorophyll declined with increasing salinity in the summer in Liverpool Bay despite a decline in SPM concentrations and peak PAR values at high salinities. The inshore light limitation may prevent excessive phytoplankton growth at low salinities, and once the light environment has become conducive for enhanced phytoplankton growth at higher salinities, nutrient concentrations are sufficiently depleted to not be able to support sustained high algal biomass. This consecutive limitation currently results in a constant chlorophyll concentration along the salinity gradient in the Thames Estuary, and constant or declining concentration with increasing salinity in Liverpool Bay. However, under increased nutrient inputs scenarios, higher nutrient concentrations further along the gradient may results in elevated phytoplankton biomass being supported at the higher end of the salinity gradient. There are data availability issues within this dataset, notably that the spring bloom is not captured within the available data in the Thames Estuary, and the data distribution in Liverpool Bay means low salinities are represented by only two samples. However, despite data limitations, the outcomes of this work show that there are shifts along the salinity gradient in the environmental drivers.

So what? - Understanding how the effects of nutrient inputs manifest along a salinity gradient can help to identify where and when undesirable consequences are most likely to occur, and where management decisions and resources might be best focused. Monitoring and developing this understanding may become increasingly important under a changing climate, as interactions between parameters become increasing complicated. It is likely that there are more complicated non-linear relationships which could be identified. This work has also highlighted the importance of consistent and well-resourced data collection efforts in order to make sound and robust conclusions.

5

Turbidity impacts on the abundance and composition of diatoms and dinoflagellates in coastal waters, and the associated implications for management

5.1 Abstract

Water quality condition can be monitored through the assessment of nutrient, chlorophyll, and suspended particulate matter concentrations as well as other indicators of plankton community and composition. However, concessions for UK transitional waters can be made on pre-established nutrient concentrations in areas which have high suspended particulate matter (SPM) and resulting high turbidity. These allowances are made as phytoplankton are assumed to be light limited in such environments, and these waters would therefore not be able to support anthropogenic increased primary production from high nutrient levels. This was tested through two experiments , where abundance and community composition were analysed at varying concentrations of suspended particulate matter over two different time periods using a natural community from the Thames estuary. The first time period represented the early summer post spring bloom peak (June) with the second time period representing the start of the mid-autumn period (October). Both diatom abundances and chlorophyll concentrations showed increases over time with increasing turbidity. The very turbid treatment showed diatom abundances increase by a factor of 2.3 compared those seen in the control treatment in early summer, and 1.9 times in mid-autumn. This increase was not seen in dinoflagellate abundances. Dinoflagellate communities tended towards a community of *armoured dinoflagellates* and/or *Scrippsiella* in both experiments. Point of sampling results showed severe silicate limitation in early summer with an N : Si ratio of 20.23 :1. Silicate limitation was not severe in mid-autumn, with an N : Si ratio of 2.01 :1 recorded. The results seen here suggest that the increased SPM has not limited diatom growth and may have provided a source of nutrients which had previously been limiting. Further investigation is required to establish if the WFD/WER concession allowing higher nutrient concentrations in turbid water is appropriate for ensuring coastal and estuarine water bodies do not suffer the negative impacts of eutrophication.

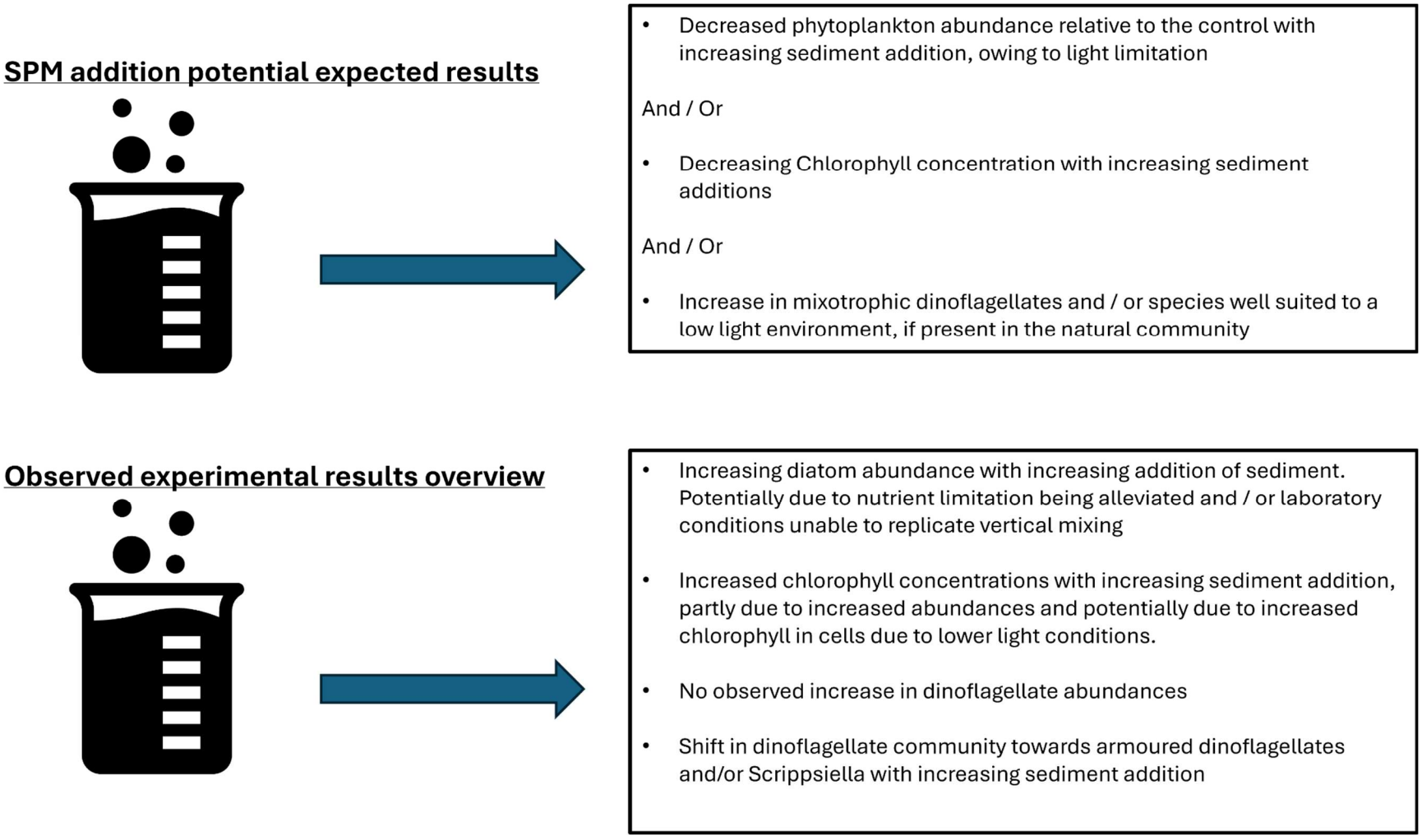

Figure 5.1 – Infographic of expected versus observed experimental outcomes

5.2 Introduction

Nutrient pollution in coastal and estuarine waters can result in increased phytoplankton biomass, shifts in dominant species and lifeforms, and the presence of harmful algal blooms. Good coastal and estuarine water quality is important to ensure healthy and resilient ecosystems and supporting balanced phytoplankton communities (Shao et al., 2019; Barçante et al., 2020). Shifts or declines in water quality can have impacts on the composition of phytoplankton communities, change the species diversity and alter the dominance of key species (Garmendia et al., 2013; Song et al., 2022).

Changes in the relative abundance of phytoplankton lifeforms within the community can be used as indicators of changing or deteriorating water quality (Tett et al., 2008; McQuatters-Gollop et al., 2019). This has been investigated in areas including in the Baltic (Wasmund, 2017; Wasmund et al., 2017; Spilling et al., 2018), Chesapeake Bay (Marshall et al., 2006; Li et al., 2015) and many others (Willén, 2000; Webber et al., 2005; Devlin et al., 2019; Bi et al., 2021; Chen et al., 2022).

Water quality conditions are intrinsically linked with phytoplankton; changes in the water quality and environmental conditions can impact the stoichiometry of phytoplankton cells (Grosse et al., 2017) as well as their feeding mode (da Costa et al., 2024) and cell size (Marshall et al., 2006) which can alter palatability of phytoplankton to their predators (Atkinson et al., 2021). The relative abundances of diatoms and dinoflagellates have been established as key indicators of changes in water quality (McQuatters-Gollop et al., 2007a; Wasmund, 2017; Wasmund et al., 2017; Bedford et al., 2020). Devlin et al. (2009) present multiple methods of assessing changes in phytoplankton communities, including the use of a reference species list. Effective assessment and management are essential to ensure that these communities remain healthy and functional. In order to do this, is it imperative to understand the specific effects of changes in different water quality parameters on phytoplankton communities.

There are variations in the susceptibility of a marine environment to eutrophication (Painting et al., 2007), and to what extent nutrient enrichments will results in undesirable disturbances. The response of phytoplankton to nutrient enrichment has previously been considered to be a more simplistic, linear, relationship, however developments in knowledge have resulted in an understanding that the differences in coastal and estuarine systems act as a 'filter' which will vary the response of these environments to nutrient loading (Cloern, 2001), of which light and water clarity is one.

Turbidity is considered to exert a spatial and temporal control over phytoplankton biomass within estuaries (Cloern, 1987; May et al., 2003; Painting et al., 2007) as light availability becomes the limiting factor as opposed to nutrient concentrations. Estuaries with low and moderate light environments, including the Thames considered here, are thought to be less likely to exhibit the undesirable impacts of eutrophication (Painting et al., 2007).

Turbidity, and the associated change in light, is an important variable when considering the impacts of environmental variables on phytoplankton e.g. (May et al., 2003; Lueangthuwapranit et al., 2011)Increased turbidity increases light attenuation (Devlin et al., 2008), thus reducing light availability in the water column for phytoplankton photosynthesis. The potential of reduced light availability in a higher turbidity water column leads to a concession in the WFD/WER assessment for dissolved inorganic nitrogen (DIN) concentrations in transitional and coastal waters. Nutrient concentrations are assessed by the WFD/WER, compared to predetermined thresholds, and subsequently classified ranging from high to bad (Devlin et al., 2007b). However, the upper limit of DIN is altered, dependent on the turbidity category assigned to the area, based primarily on calculations and values which result in eutrophic conditions, as defined in Nixon (1995). The turbidity category of an area is based on the concentration of suspended particulate matter present in the water column (Table 5.1). In increasingly turbid water, increased amounts of DIN are allowed to be present before the area would be considered not in good status for this particular variable (Table 5.1).

Table 5.1 - Upper dissolved inorganic nitrogen (DIN) thresholds for the boundary between good and moderate in Transitional and Coastal assessment areas for the WFD/WER (Devlin et al., 2007b). The threshold is a mean winter DIN value for Not Turbid waters, and is an annual 99th percentile for Intermediate, Turbid, and Very Turbid waters.

SPM concentration (mg / L)	**Water category**	**Transitional DIN upper limit (µmol / L)**	**Coastal DIN upper limit (µmol / L)**
0 – 10	Not Turbid	30	18
10 - 70	Intermediate	70	70
70 - 300	Turbid	180	180
300 +	Very Turbid	270	270

Whilst turbidity reduces light availability for phytoplankton communities, in turn reducing the available resources for photosynthesis, there is emerging evidence that the reduced light availability may not be inhibiting for all lifeforms. Mixoplankton are lifeforms which are able to obtain nutrition through both phototrophic and phaogtrophic modes, and are able to adapt

their feeding mode of based on the available resources, including light (Mitra et al., 2016). If light limitation does not inhibit growth of these lifeforms, and they can supplement their photosynthesis through phagotrophy, then the increased nutrients which are allowed in turbid conditions may be fuelling the growth of mixotrophic species and contributing to the undesirable effects of eutrophication they aim to prevent. Growth through mixotrophic methods has been reported to allow for increased biomass compared to phototrophy alone (Adolf et al., 2006) and potentially favours harmful species (Burkholder et al., 2008).

Reductions in water clarity over time, which would be seen with increasing turbidity, have been reported. Capuzzo et al. (2015) present findings of a Secchi depth decrease of 25-75% in the North Sea, including coastal waterbody areas, post-1950 compared with pre-1950, and attribute this to increases in suspended sediment. Opdal et al. (2019) report a reduction in clarity in the North Sea, and with it a 3-week delay in spring bloom timing in both shallow and deep waters. Reductions in Secchi depth in inshore waters have also been seen in the North and Baltic Seas (Dupont and Aksnes, 2013). The effects of increased turbidity are already being seen in ecological communities, from impacts on seagrass distribution (Davis et al., 2016) to microbenthic communities (Liess et al., 2015). As water clarity may be seen to decrease, contributing to the understanding of how phytoplankton respond to this becomes increasingly important if marine environments are going to be managed effectively and kept in good ecological status. Given the complexity of phytoplankton responses to water quality conditions in marine environments, turbidity may not be universally preventative of the undesirable consequences of eutrophication and so investigating the possible variations in responses is valuable.

Appropriate monitoring is the first step to the successful management of these marine environments. Transitional and coastal water bodies in the United Kingdom are managed under WFD/WER (Best et al., 2007; Devlin et al., 2007a; Devlin et al., 2007b; UK Parliament, 2017). Transitional and coastal areas in the UK are outlined in Chapter One.

As part of the monitoring under the WFD/WER, phytoplankton metrics are measured through chlorophyll concentration and phytoplankton counts. Both chlorophyll concentrations, and phytoplankton counts which exceed a predetermined threshold are measured across both water body types. For coastal waters the seasonal succession tool is also used to assess the amount of time during which diatom and dinoflagellate abundances fall above or below a monthly reference score, but this is not used in transitional assessment areas. A 12-month periodicity, with a spring phytoplankton bloom, is a common phytoplankton seasonality (Winder and Cloern, 2010). This increased phytoplankton abundance is usually initially diatom dominated and then followed up by increasing concentrations of dinoflagellates (Figure 5.2)

(Zhou et al., 2017b; Zhang et al., 2019; Wang et al., 2022). However, this pattern is not universal and phytoplankton seasonality in coastal and inshore ecosystems can exhibit high variability (Winder and Cloern, 2010), as well as being heavily influenced by small scale processes (Cloern and Jassby, 2008).

Here, a natural community from the Thames estuary is used in laboratory incubations with increasing amounts of sediment added, with the aim of answering the question

7. How does a natural phytoplankton community respond to sediment additions in a laboratory incubation experiment and what are the implications for current UK assessment criteria?

The impact of increased turbidity on the abundance and community composition of diatoms and dinoflagellates is investigated. The implications of these results for eutrophication assessments going forward will be considered, specifically whether a concession on the allowed concentrations of certain nutrients is appropriate in higher turbidity waters.

5.3 Methods

5.3.1 Sample collection

Seawater was collected from site MA2, in the year round well-mixed Thames estuary (Figure 5.3) at a depth of 1 m from onboard the survey vessel *Thames Guardian* on 16th June 2023, representing early summer conditions, and on 14th October 2023, representing mid-autumn conditions. The location of the site was 51.490 ˚N, 0.778 ˚E and the water column depth is approximately 11 m. The water temperature and salinity at 1 m depth were 18 ˚C and 32.11 in June and 17 ˚C and 33.28 in October, respectively. Water was collected using a 10 L Niskin bottle and transferred into a 25 L carboy which was pre rinsed with sample water. The carboy was kept chilled, in the dark, and was taken back to the lab within 24 hours. Water was not filtered, so as not to remove any sediment. SPM concentration at the point of sampling was determined using an RBR*tridente* turbidity sensor, which has a range of 0-500 Formazin turbidity unit (FTU) and a detection limit of 0.001 FTU. The relationship between SPM and turbidity was assumed to be SPM = 1.46 X Turbidity in the month June and considered to be SPM = 1.01 x Turbidity in October (Jafar-Sidik et al., 2017). Discrete SPM samples were also collected and processed in order for the SPM concentrations to also be calculated gravimetrically (see Chapter Two).

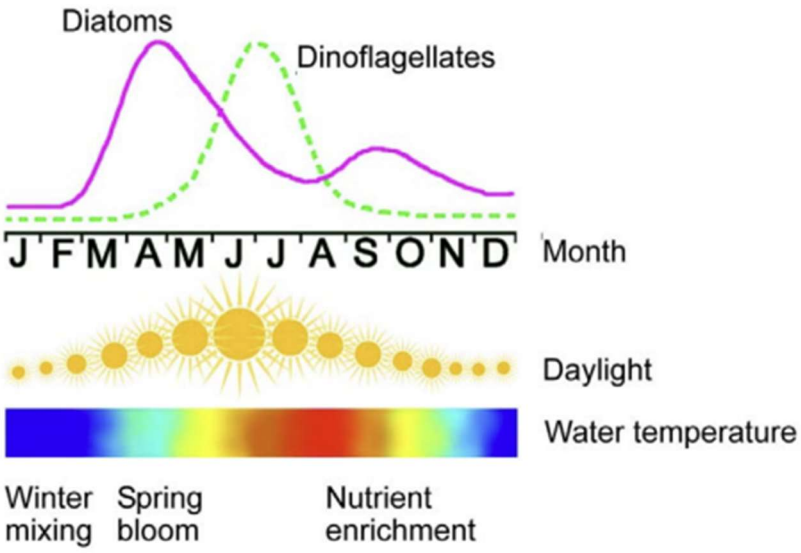

Figure 5.2 – Seasonal succession of diatoms and dinoflagellates and associated environmental conditions (Swan and Davidson, 2007)

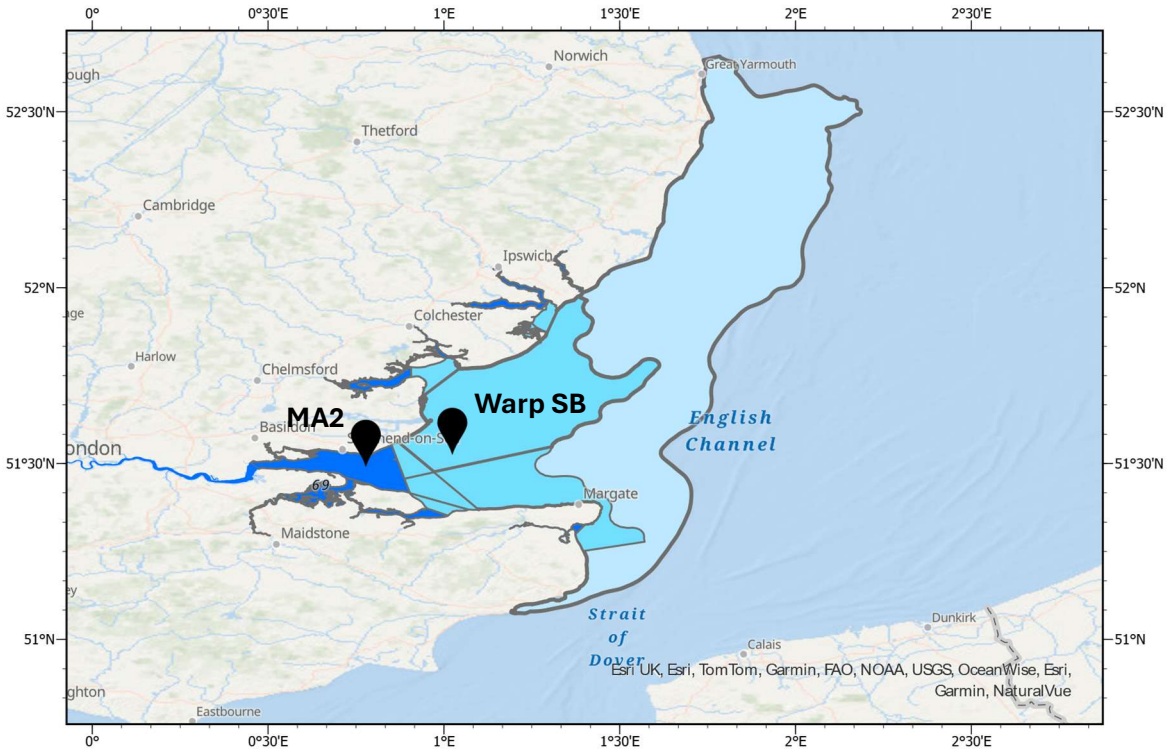

Figure 5.3 – Sampling sites within the Thames estuary area. Water for the incubations was collected from MA2, suspended sediment for the incubations was collected in a sediment trap at the Warp SmartBuoy (SB) site. Dark blue areas are transitional waterbodies (WFD/WER teal areas are coastal waterbodies (WFD/WER), and the light blue area is the Thames plume assessment area (region of freshwater influence, OSPAR).

5.3.2 Laboratory incubations

The experimental conditions were setup in order to consider the impacts of suspended particulate matter concentrations holistically, i.e. not aiming to separate the impacts of light limitation and nutrient additions. SPM from a location close to the sample collection was used, in order for this to be representative of the type of sediment which may be present in high SPM conditions in this location. Suspended sediment is a key driver of increased light attenuation at this site (Devlin et al., 2008) and so the addition of sediment was used to induce a changing light environment.

All water used in the experiment came from the same carboy. 1.5 L of the bulk water was decanted into each of the acid washed 2 L polycarbonate Nalgene bottles. The sediment used in the experiment was collected from the Warp SmartBuoy sediment trap which was deployed from 01/05/2022 – 30/09/2022. The sediment was kept at -20°C after collection. The Warp SmartBuoy lies close to the collection site of MA2 at 51.524 ˚N, 1.024 ˚E (Figure 5.3) and suspended sediment collected at this site can be considered representative of the suspended sediment which would typically be found at MA2. Nutrient and SPM samples were taken from site MA2 at the point of sampling. Day Zero was considered to be when the experiment was set up in the lab, approximately 24 hours after the point of sampling, and phytoplankton samples were taken at day zero.

Samples were collected every 24 hours for chlorophyll and phytoplankton (Figure 5.4) in line with the methods outlined in Chapter Two, and were collected on day four for nutrients (Figure 5.4). Water samples for nutrient analyses were filtered through Minisart 0.45 µm filters into 50 mL sample pots and stored at -12 °C until analysis using a SEAL Analytical continuous segmented flow autoanalyzer AA3 (See Chapter Two). Sediment was added in amounts (Table 5.3, Table 5.4) which would result in SPM concentrations that equated to each turbidity classification under the WFD/WER assessment (Table 5.1). The bottles were randomly distributed and redistributed daily within the growth cabinet under lights which aimed to match an in-situ measurement taken in at 1m depth in June as closely as possible, however there was a range either side of the measured value. The PAR value was based on a single measurement and therefore may not actually be representative of the natural environment. There was no PAR reading taken in situ in October and the same light set up was used in both experiments. Lights were set to the same daylight hours as the collection site, resulting in a 16.5:7.5 light : dark cycle in June and a 10.5:13.5 light : dark cycle in October. The temperature remained at 14 °C throughout the experiments. This is lower than the temperature recorded in June of 18°C and 17°C in October and is a limitation of the available facilities. The bottles were placed on orbital shakers set to 86 revolutions per minute to keep the sediment in suspension. The bottles were

incubated for four days as this time was thought to allow for acclimation to a change in the light regime, in line with similar experiments (Carter et al., 2005; Ok et al., 2019; Tomkins et al., 2020).

5.3.3 Determining nutrient concentrations in sediment

Known masses of wet sediment were dried in a 75 °C oven for 24 hours to determine the dry sediment content. The mean (*n* = *3*) dry sediment content was 72.3 % of the wet mass. The equivalent mass of wet sediment to 1 g of dry sediment was added to 1 L of distilled water and stored at ambient temperature for 4 days and was inverted once every 24 hours. Leaving the water sample with added sediment for 4 days was considered sufficient to establish the maximum concentration of dissolved nutrients which could have been released during the laboratory experiment. The distilled water with sediment added was filtered through a 0.7 µm GF/F filter and the filtrate was analysed for nutrient concentrations using the SEAL analytical continuous segmented flow autoanalyzer AA3 (see Chapter Two).

Table 5.2 - Dissolved Nutrient concentrations released from sediment in distilled water over 4 days

DIN	Phosphate	Silicate
3.79 ± 1.44 µmol/g dry sediment	1.13 ± 0.23 µmol/g dry sediment	13.0 ± 2.89 µmol/g dry sediment

Table 5.3 - Sediment additions to the 1.5 L of bulk water and SPM concentrations in each bottle in early summer 2023.

Bottle	Treatment	Starting [SPM] from turbidity meter (mg/L)	Wet Sediment added to 1.5 L bulk water (g)	Dry equivalent of sediment added to 1.5L bulk water (g)	[SPM] calculated from turbidity meter starting SPM + sediment addition (mg/L)	WFD/WER Turbidity Category	[SPM] measured using Gravimetric method (mg/L)
1	Control	23.2	0	0	23.2	Intermediate	46
2	Control	23.2	0	0	23.2	Intermediate	15
3	Control	23.2	0	0	23.2	Intermediate	47
4	1	23.2	0.2667	0.1928	196.9	Turbid	96
5	1	23.2	0.2606	0.1884	192.8	Turbid	120
6	1	23.2	0.2570	0.1858	190.5	Turbid	117
7	2	23.2	0.5192	0.3754	380.2	Very turbid	191
8	2	23.2	0.5045	0.3648	355.5	Very turbid	388
9	2	23.2	0.5176	0.3742	364.2	Very turbid	238

Table 5.4 - Sediment additions to the 1.5L of bulk water and SPM concentrations in each bottle in mid-autumn 2023.

Bottle	Treatment	Starting [SPM] from turbidity meter (mg/L)	Wet Sediment added to 1.5 L bulk water (g)	Dry equivalent of sediment added to 1.5L bulk water (g)	[SPM] calculated from turbidity meter starting SPM + sediment addition (mg/L)	WFD/WER Turbidity Category	[SPM] measured using Gravimetric method (mg/L)
1	Control	9.5	0	0	9.5	Not turbid	15
2	Control	9.5	0	0	9.5	Not turbid	41
3	Control	9.5	0	0	9.5	Not turbid	13
4	1	9.5	0.0616	0.0444	39.07	Intermediate	39
5	1	9.5	0.0614	0.0442	38.97	Intermediate	40
6	1	9.5	0.0621	0.0447	39.31	Intermediate	38
7	2	9.5	0.3600	0.2590	182.3	Turbid	126
8	2	9.5	0.3623	0.2609	183.4	Turbid	126
9	2	9.5	0.3623	0.2609	183.4	Turbid	124
10	3	9.5	0.7052	0.5077	348.0	Very Turbid	208
11	3	9.5	0.7061	0.5084	348.4	Very Turbid	259
12	3	9.5	0.7038	0.5067	347.3	Very Turbid	240

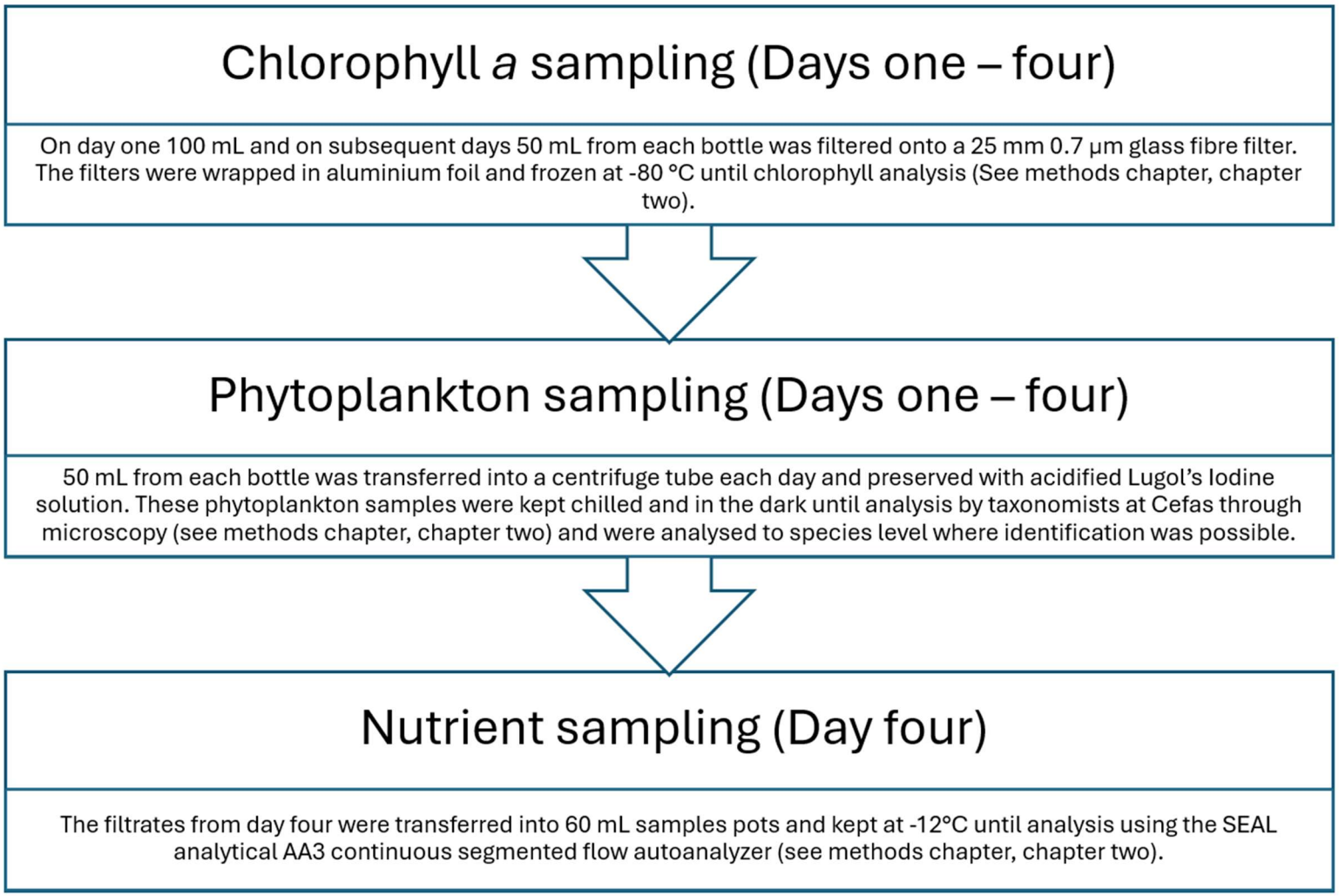

Figure 5.4 - Methods flow chart of sampling process during incubation experiments.

5.4 Results

Nutrient concentrations were analysed at the point of sampling in both early summer and mid-autumn (Table 5.5), and at end of the experiment in mid-autumn 2023 (Table 5.6). Concentrations of total oxidised nitrogen (TOxN = nitrite + nitrate), nitrite, phosphate, and silicate are greater at the time of sampling in mid-autumn than in early summer, whilst ammonium is lower. The N:Si ratio is lower in mid-autumn than in early summer, at 1.9:1 compared to 18:1. At day 4 in mid-autumn 2023 concentrations of TOxN and phosphate decrease with increasing turbidity. The silicate concentration is low in the intermediate and turbid waters but is slightly higher in the very turbid water.

Table 5.5 - Nutrient (n=1), Turbidity (n=1), and SPM (n=3) concentrations at the point of sampling from MA2 in early summer and mid-autumn.

Parameter	**Early summer MA2 at point of sampling**	**Mid-Autumn MA2 at point of sampling**
TOxN (µmol /L)	28.80	37.0
Nitrite (µmol /L)	0.52	1.78
DIP (µmol /L)	1.70	2.32
Silicate (µmol /L)	1.60	19.00
$NH4^+$ (µmol /L)	3.40	1.20
DIN (TOxN + $NH4^+$) (µmol / L)	32.20	38.20
Turbidity (FTU)	29.80	9.50
Mean SPM gravimetric (mg / L)	41.9 ± 29.6	32.2± 5.6
DIN : P ratio	18.90	16.40
DIN : Si	20.13	2.01

Table 5.6 - Mean nutrient concentrations and ratios ± standard deviation at day Four in mid-autumn 2023 (n=3).

Parameter	Not turbid	Inter-mediate	Turbid mean	Very Turbid
TOxN (µmol /L)	27.36 ±3.67	20.33 ±2.30	15.06 ±2.99	11.30±2.71
Nitrite (µmol /L)	1.71 ±0.01	1.44 ±0.05	1.13 ±0.26	1.21±0.08
DIP (µmol /L)	1.74 ±0.23	1.31 ±0.04	0.977 ±0.13	0.80±0.06
Silicate (µmol /L)	0.50 ±0.69	<0.1	<0.1	0.40±0.10
$NH4^+$ (µmol /L)	0.20 ±0.10	<0.1	0.13±0.06	0.17±0.12
DIN (µmol /L)	27.57 ±3.62	20.43 ± 2.30	15.20 ±2.98	11.43 ± 2.79
DIN : DIP ratio	15.88 ± 0.50	15.68 ±1.28	15.36 ±1.26	15.57 ± 2.67
DIN : Si	179.05 ± 134.93	204.33 ± 23.03	152.00 ± 29.31	30.52 ± 12.20

5.4.1 Diatom and Dinoflagellate abundance and community composition

The results below (Figures 5.4 – 5.13) show the changes in diatom and dinoflagellate abundance and community composition during the 4-day incubation period. Turbidity categories were derived using the SPM concentrations calculated from the amount of sediment added to the initial SPM concentration of the sample, measured using the turbidity meter at the point of sampling (Table 5.7).

Table 5.7 – Shading key for the WFD/WER turbidity categories in Figures 5.3 – 5.12.

Calculated Turbidity Category	Not Turbid	Intermediate	Turbid	Very Turbid
Key				

There is an increasing abundance of diatoms with turbidity in both the early summer and mid-autumn experiments (Figure 5.5). The abundance of diatoms in the samples for mid-autumn were approximately one order of magnitude higher than in those for early summer (Figure 5.5).

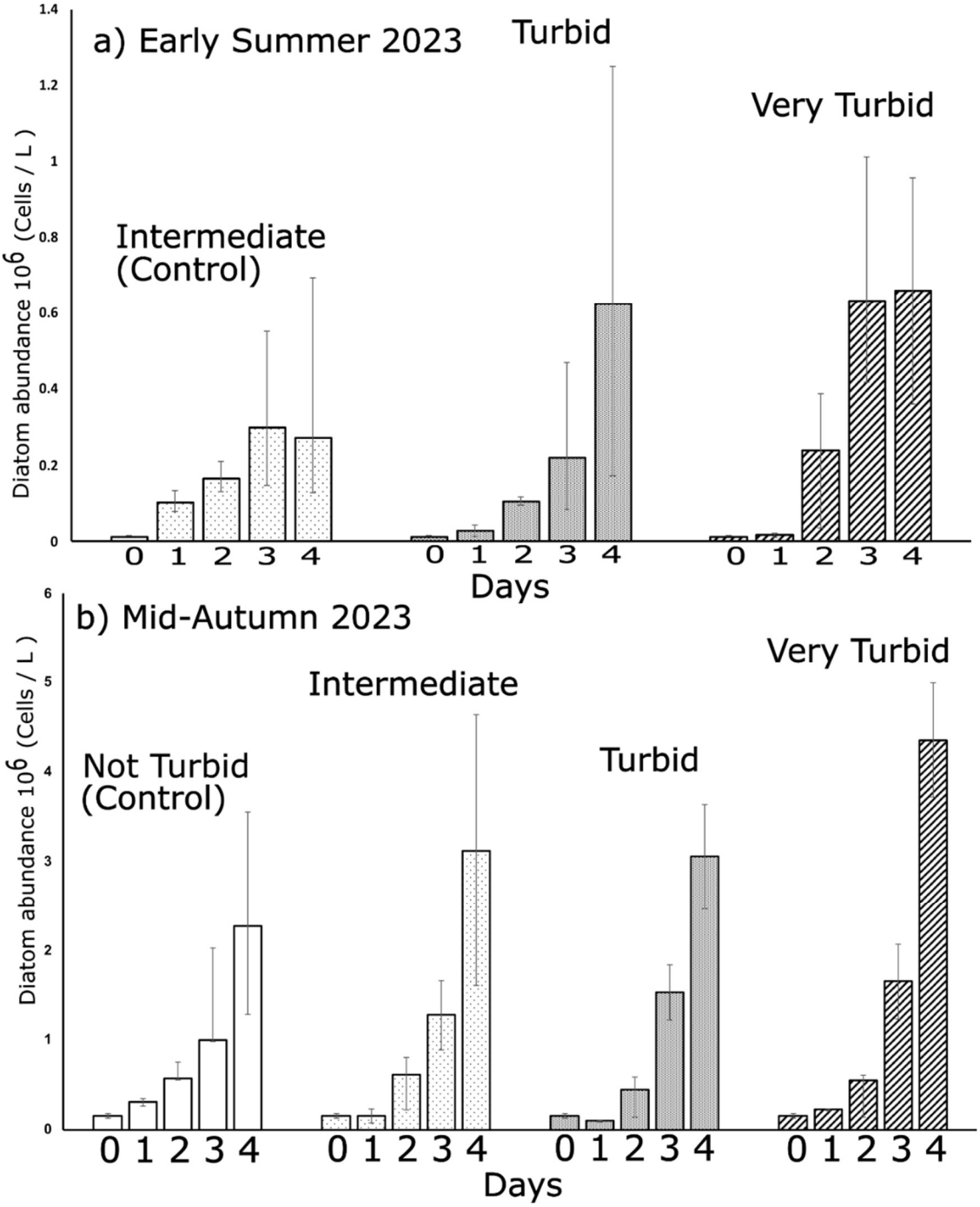

Figure 5.5 - Mean daily diatom abundances for each treatment in a) early summer (n = 3) and b) mid-autumn (n = 2) 2023. Error bars represent the range across repeat samples. Figures are shaded based on the WFD/WER turbidity category of the water determined from SPM concentration using the concentration calculated from the amount of sediment added to the initial SPM concentration measured with the turbidity meter. Analysis of variance showed no significant differences in the day four abundances between treatments.

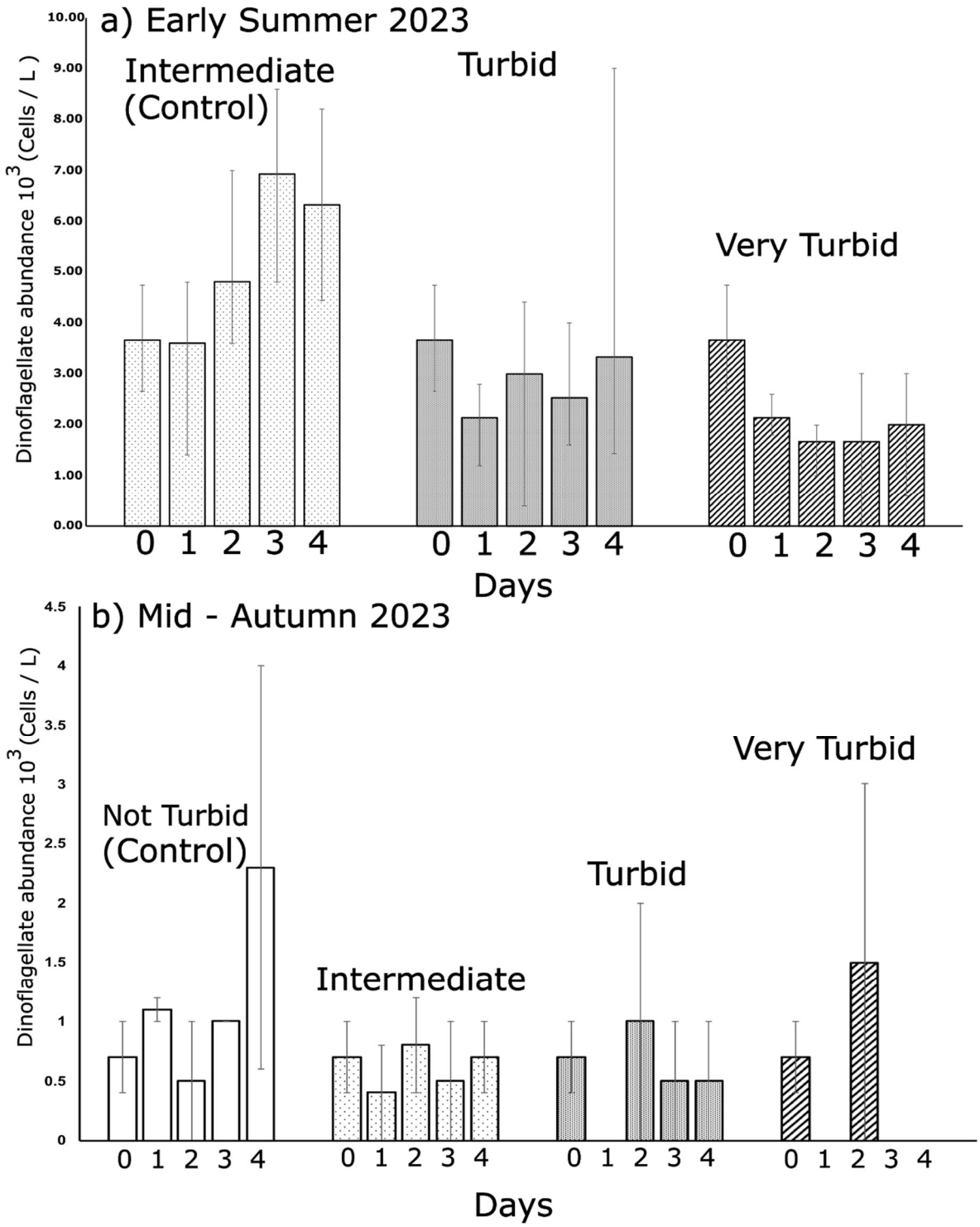

Figure 5.6 - Mean daily dinoflagellate abundances for each treatment in a) early summer (n = 3) and b) Mid - autumn (n = 2) 2023. Error bars represent the range across repeat samples. Absent bars indicate that no dinoflagellates were identified. Figures are shaded based on the WFD/WER turbidity category of the water determined from the SPM concentration using the concentration calculated from the amount of sediment added to the initial SPM concentration measured with the turbidity meter. Analysis of variance showed no significant differences in the day four abundances of dinoflagellates between treatments.

Whilst dinoflagellate abundance increases over time in the control (intermediate) treatment in early summer, the community does not change significantly in the turbid and very turbid treatments respectively (Figure 5.6). Dinoflagellate abundance at the start of the incubations is ~ five times lower in mid-autumn than in early summer (Figure 5.6). Dinoflagellate abundance does not change much over the first three days in the control and treatments in mid-autumn, larger values are seen on day four in the control and very turbid treatment. The number of days on which no dinoflagellates were identified increases with turbidity in mid-autumn 2023 (Figure 5.6).

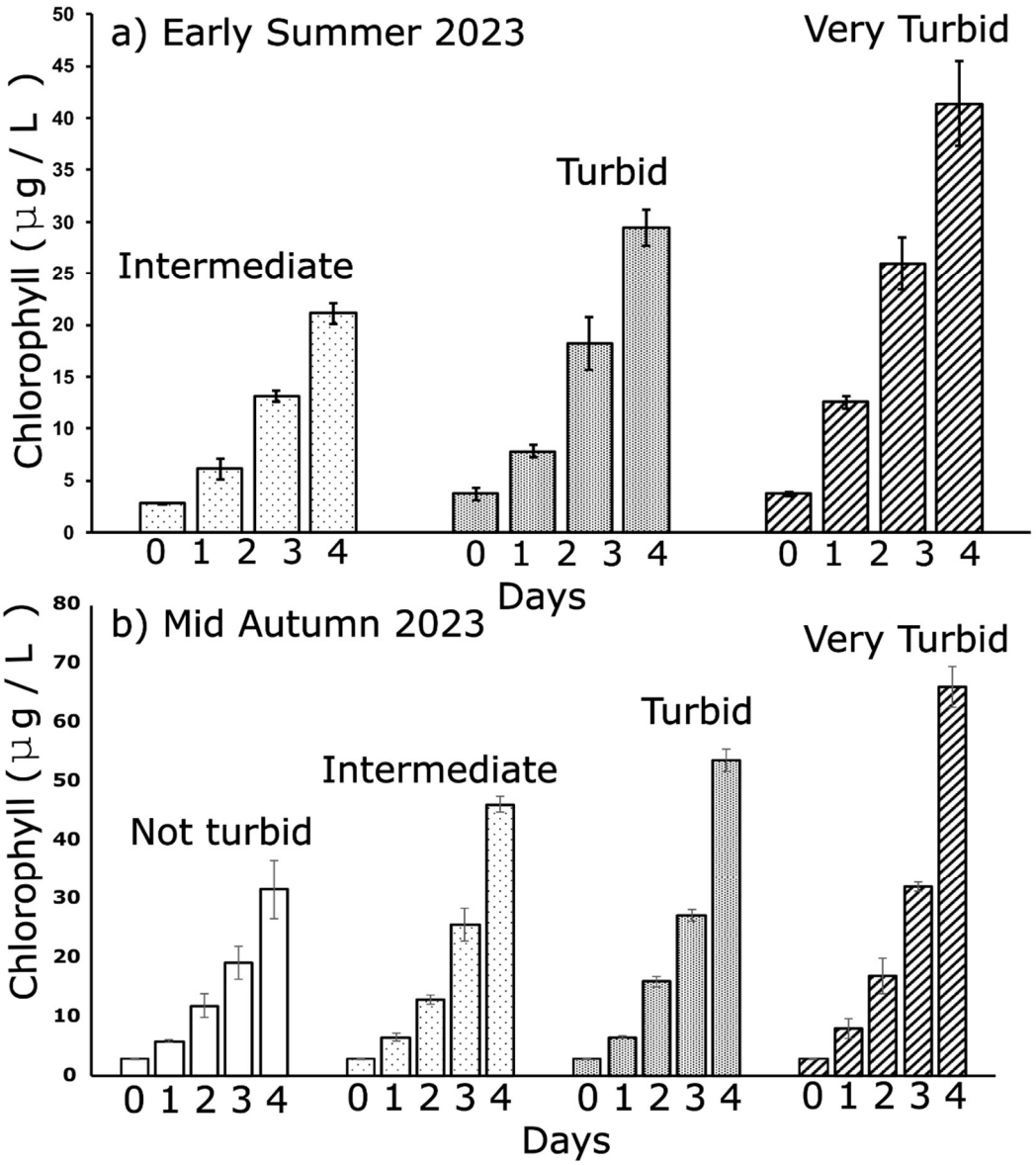

Figure 5.7 - Mean (n = 3) Chlorophyll concentration from each treatment in a) early summer and b) mid-autumn 2023. Error bars represent the standard deviation. Figures are shaded based on the WFD/WER turbidity category of the water determined from the SPM concentration using the concentration calculated from the amount of sediment added to the initial SPM concentration measured with the turbidity meter.

The chlorophyll concentration increases during the experiments in all the treatments in both early summer and mid-autumn (Figure 5.7). The peak chlorophyll concentration increases when more sediment is added in both experiments. Chlorophyll concentrations are elevated in all treatments in the mid-autumn experiment compared to the early summer experiment.

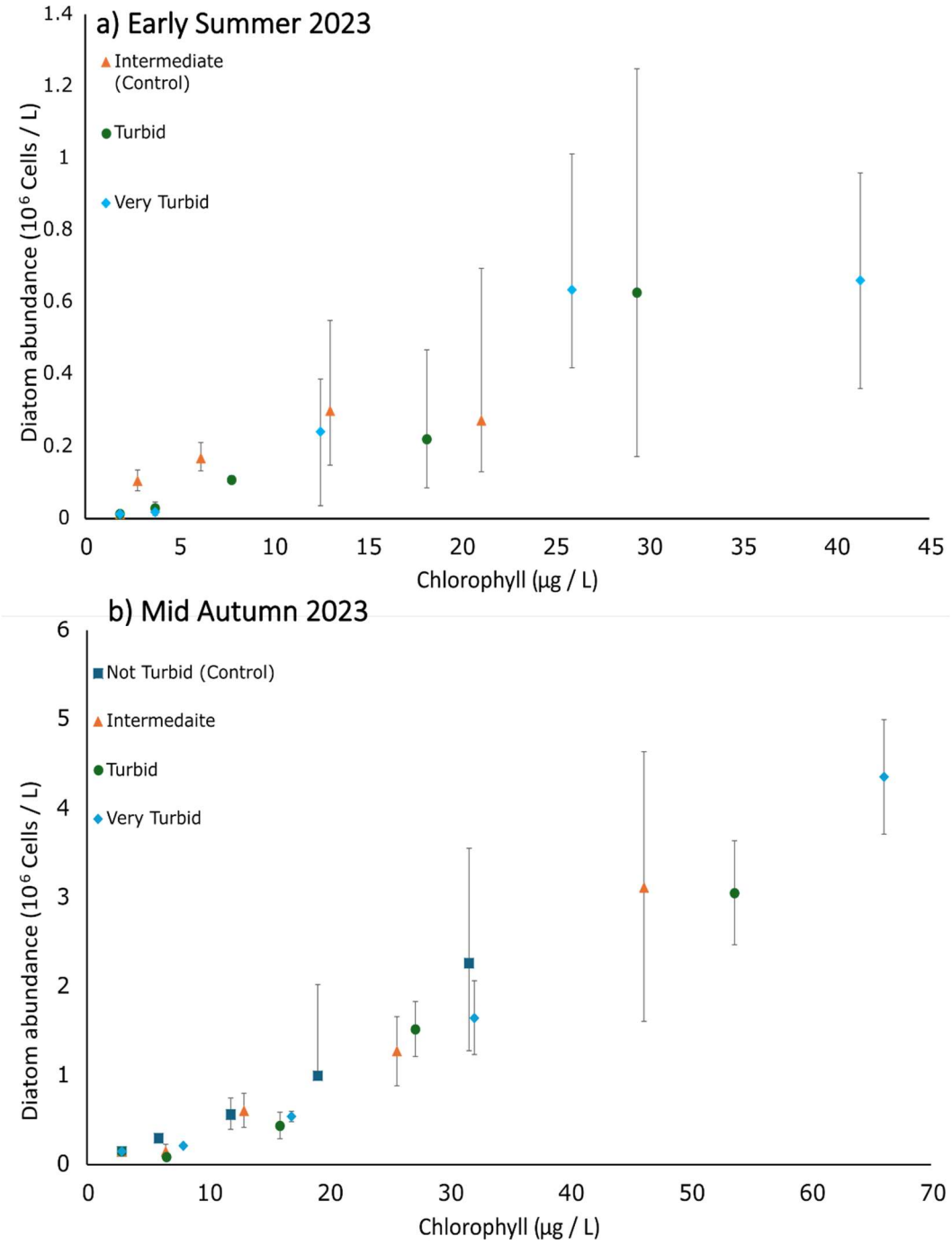

Figure 5.8 - Mean diatom abundance (n = 3 in early summer, n = 2 in mid-autumn) plotted against mean chlorophyll concentration in a) early summer and b) mid- autumn. Dark blue squares represent the not turbid data points. Orange triangles represent the intermediate treatment, green circles represent the turbid data points, and the light blue diamond represents the very turbid data points. Error bars represent the range of repeat samples.

In early summer 2023 there are increasing trends in both chlorophyll and diatom abundance in all of the turbidity treatments (Figure 5.8). The very turbid treatment shows a levelling off of the gradient at higher abundances. The turbid and very turbid treatment show values on the right-hand side of the intermediate treatment on days 1 and 2. The mid-autumn 2023 experiment shows the intermediate, turbid, and very turbid treatments consistently to the right-hand side of the not turbid treatment, representing higher chlorophyll concentrations for a comparable diatom abundance.

The majority of the early summer community is comprised of *chain diatoms (ribbons), Skeletonema*, and *centric diatoms* at 32 %, 14 % and 9 %, respectively (Figure 5.9). The mid-autumn community is dominated by *Chaetoceros (Hyalochaete), Brockmanniella brockmannii, Leptocylindrus minimus*, and *Chaetoceros (Phaeoceros)* at 24 %, 16 %, 9 %, and 6 %, respectively (Figure 5.10).

The dinoflagellate community in early summer 2023 is dominated by *armoured dinoflagellates* at 60 %, *Scrippsiella* is the second most abundant taxa recorded (Figure 5.11). The mid-autumn community from MA2 at day zero also contains a high percentage of *armoured dinoflagellates*, but they make up a smaller proportion at 43 % (Figure 5.12). The other taxa represent equal percentages of the community at 14 % in mid-autumn. There are no common dinoflagellates recorded in the initial community composition in early summer and mid-autumn except from *armoured dinoflagellates* (Figure 5.11, Figure 5.12).

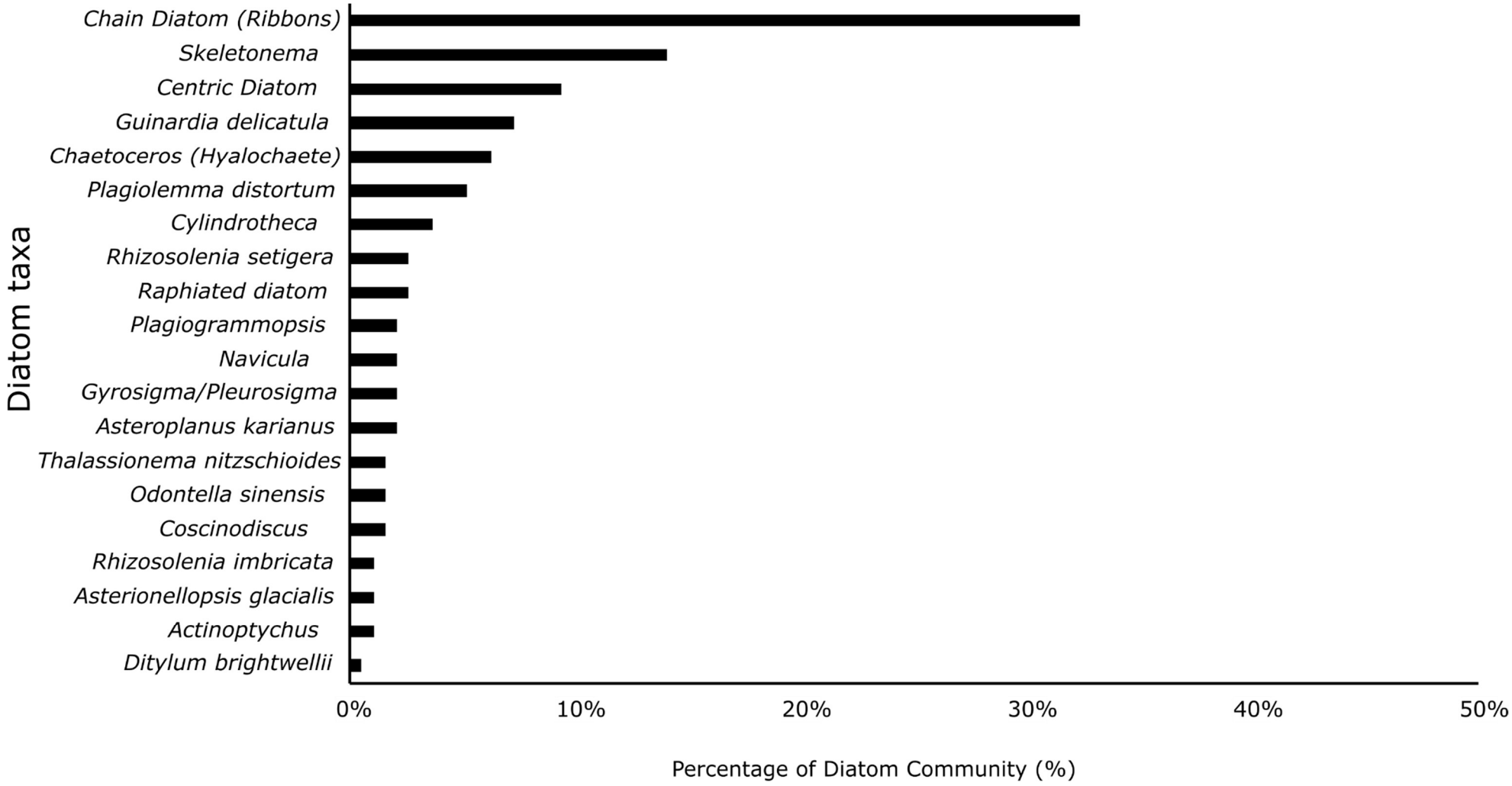

Figure 5.9 - Mean composition of diatoms at day zero in the bulk water in early summer (*n = 3*) 2023 as a percentage of the total diatom community.

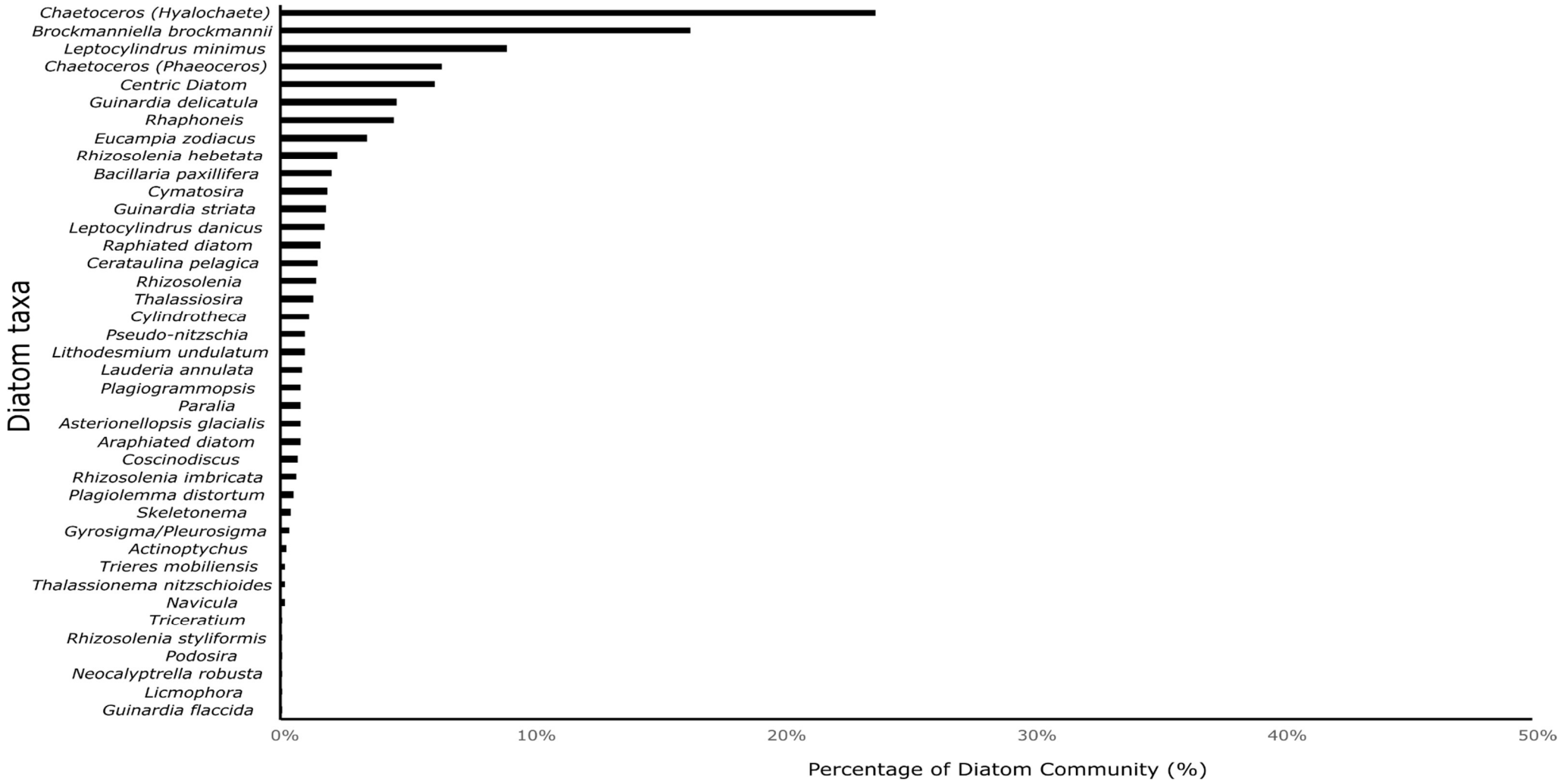

Figure 5.10 - Mean composition of diatoms at day zero in the bulk water in mid-autumn (*n* = *2*) 2023 as a percentage of the total diatom community.

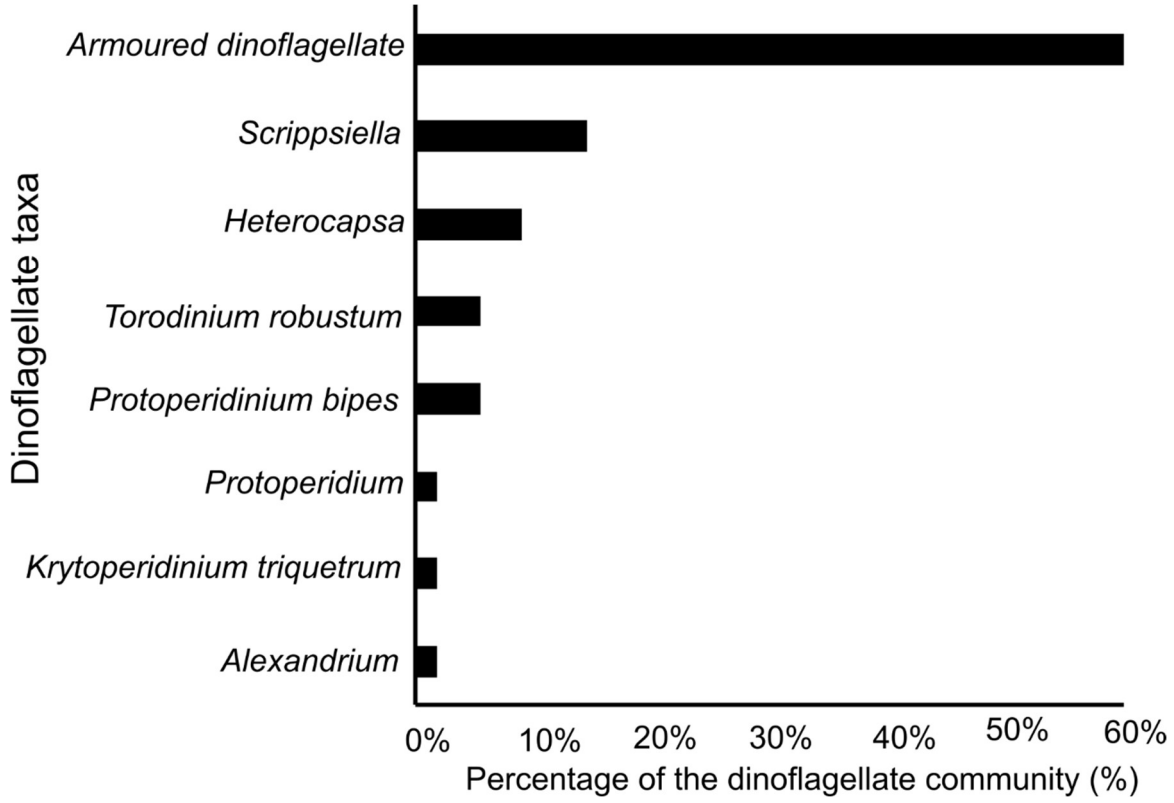

Figure 5.11 – Mean abundance of dinoflagellates at day zero in the bulk water in early summer (*n = 3*) 2023 as a percentage of the total dinoflagellate community.

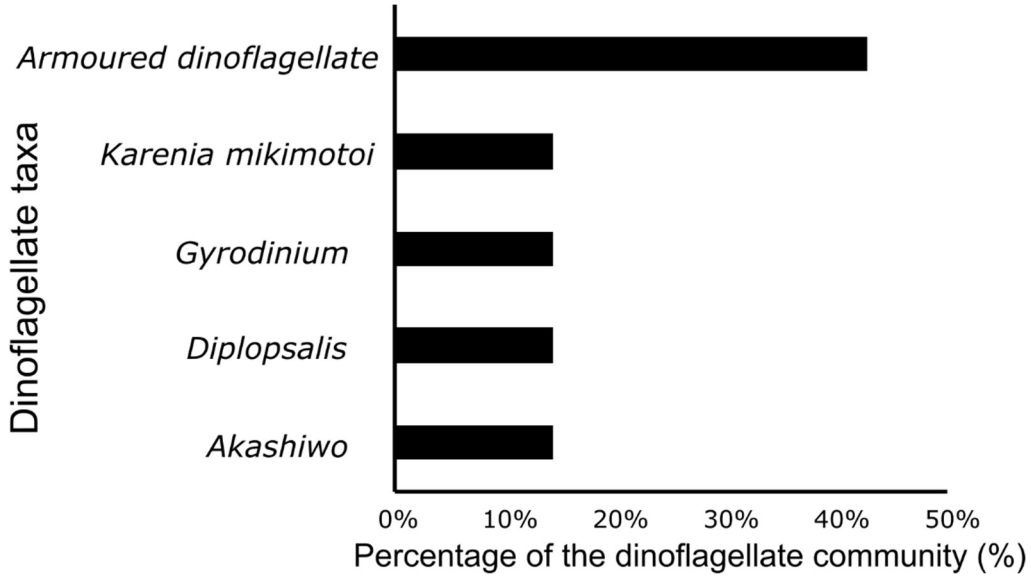

Figure 5.12 - Mean abundance of dinoflagellates at day zero in the bulk water in mid-autumn (*n = 2*) 2023 as a percentage of the total dinoflagellate community.

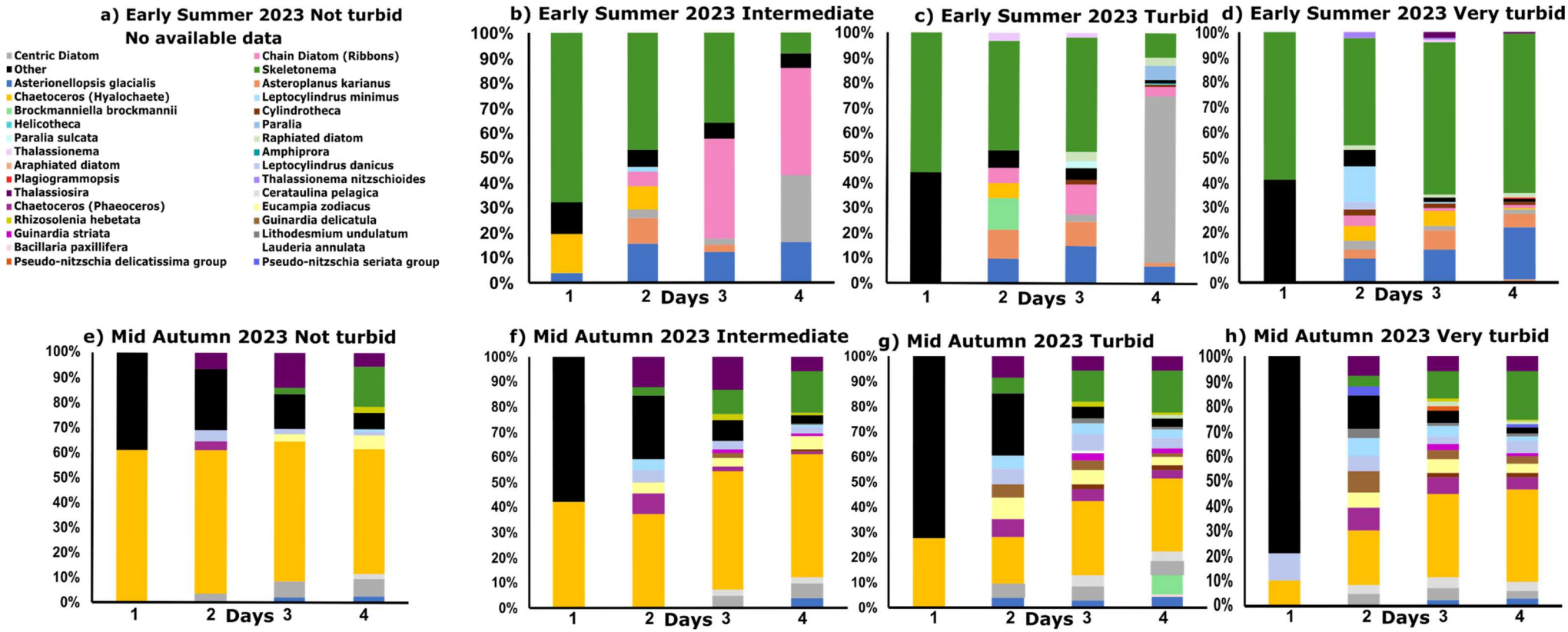

Figure 5.13 - Mean daily diatom community composition for the incubations in early summer (top row, n = 3) and mid-autumn 2023 (bottom row, n = 2). Taxa were assigned an individual colour if the abundance is >= 3,000 cells /L in early summer and 19,000 cells /L in mid-autumn otherwise they were amalgamated into an 'Other' Category. These thresholds were chosen as they gave the top 20 most abundant taxa across each experiment. There are no results for not turbid water in early summer 2023 as the bulk water was already in the intermediate category at the point of sampling. Turbidity increases between the subplots from left to right.

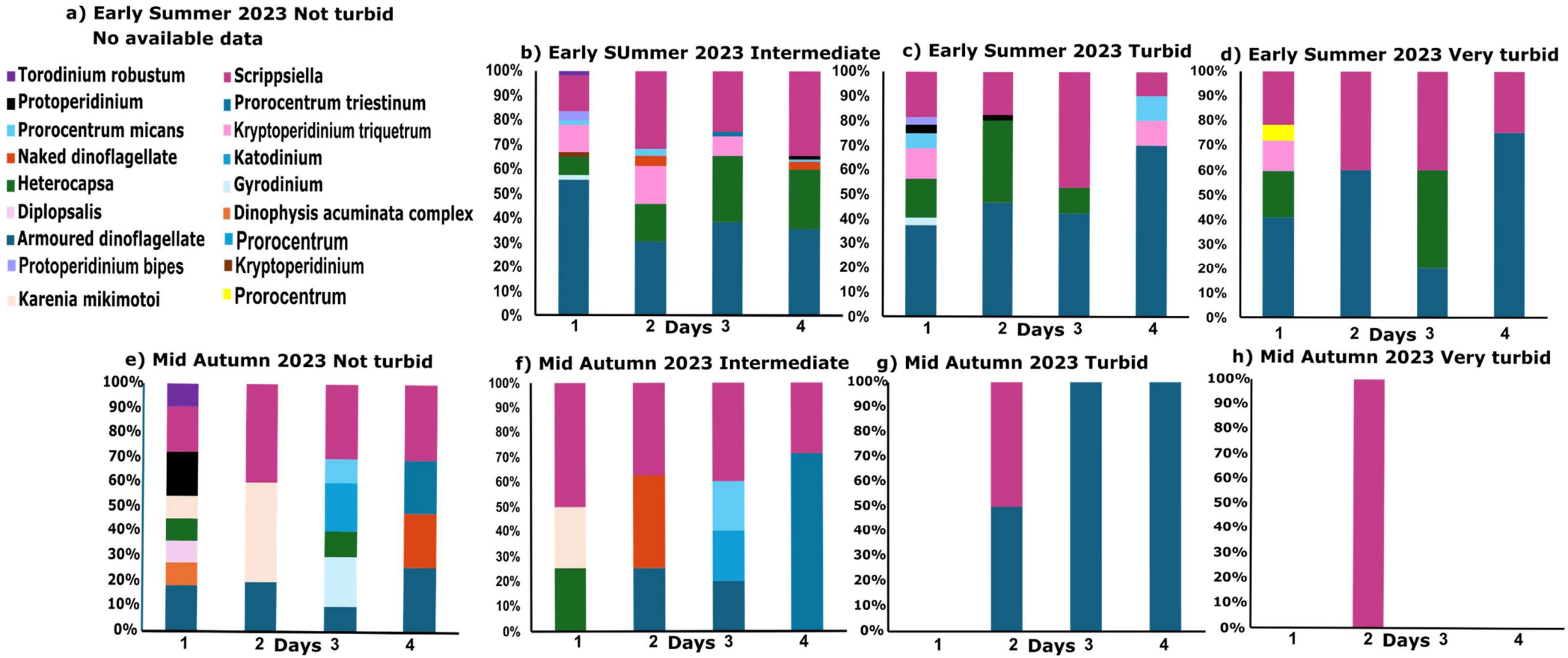

Figure 5.14 - Mean daily dinoflagellate community composition for the incubations in early summer top row, n = 3) and mid-autumn 2023 (bottom row, n = 2). Turbidity increases between the subplots from left to right.

As well as the changes in the diatom community composition throughout the experiments, there is variation between the early summer and mid-autumn communities, where the early summer community has a higher percentage abundance of *Skeletonema* while *Chaetoceros (Hyalochaete)* is more dominant in the community in mid-autumn. There is a decline in the relative abundance of *Skeletonema* over the course of the early summer experiment in the control and intermediate treatment, but this is not seen in the very turbid treatment (Figure 5.13). *Chain diatoms* increase their proportion of the community in the control over time but not in more turbid treatments in early summer (Figure 5.13). By the end of the experiment *centric diatoms* dominate in the early summer turbid incubation. *Chaetoceros (Hyalochaete)* dominates the non -turbid water incubation (the control) in mid-autumn throughout the incubation. The relative abundance of *Chaetoceros (Hyalochaete)* is lower in treatments where more sediment is added (Figure 5.13). There is an increase in the number of diatoms identified at an abundance of over 3,000 cells/L in early summer and 19,000 cells/L in mid-autumn in treatments with more sediment added.

The dinoflagellate *Scrippsiella* is abundant in both experiments in early summer and mid-autumn (Figure 5.14). With increasing turbidity, a higher proportion of the community consists of a combination of *armoured dinoflagellates* and *Scrippsiella* species (Figure 5.14). There is a decrease in the identified species present in the dinoflagellate community with increasing turbidity. *Heterocapsa* is present in all treatments in early summer 2023. In mid-autumn 2023 the community is exclusively comprised of *Scrippsiella* species and *armoured dinoflagellates* on day 2, and exclusively of *armoured dinoflagellates* on day 3 and 4 in turbid water. *Scrippsiella* is the only taxa recorded in the very turbid treatment in mid-autumn (Figure 5.14).

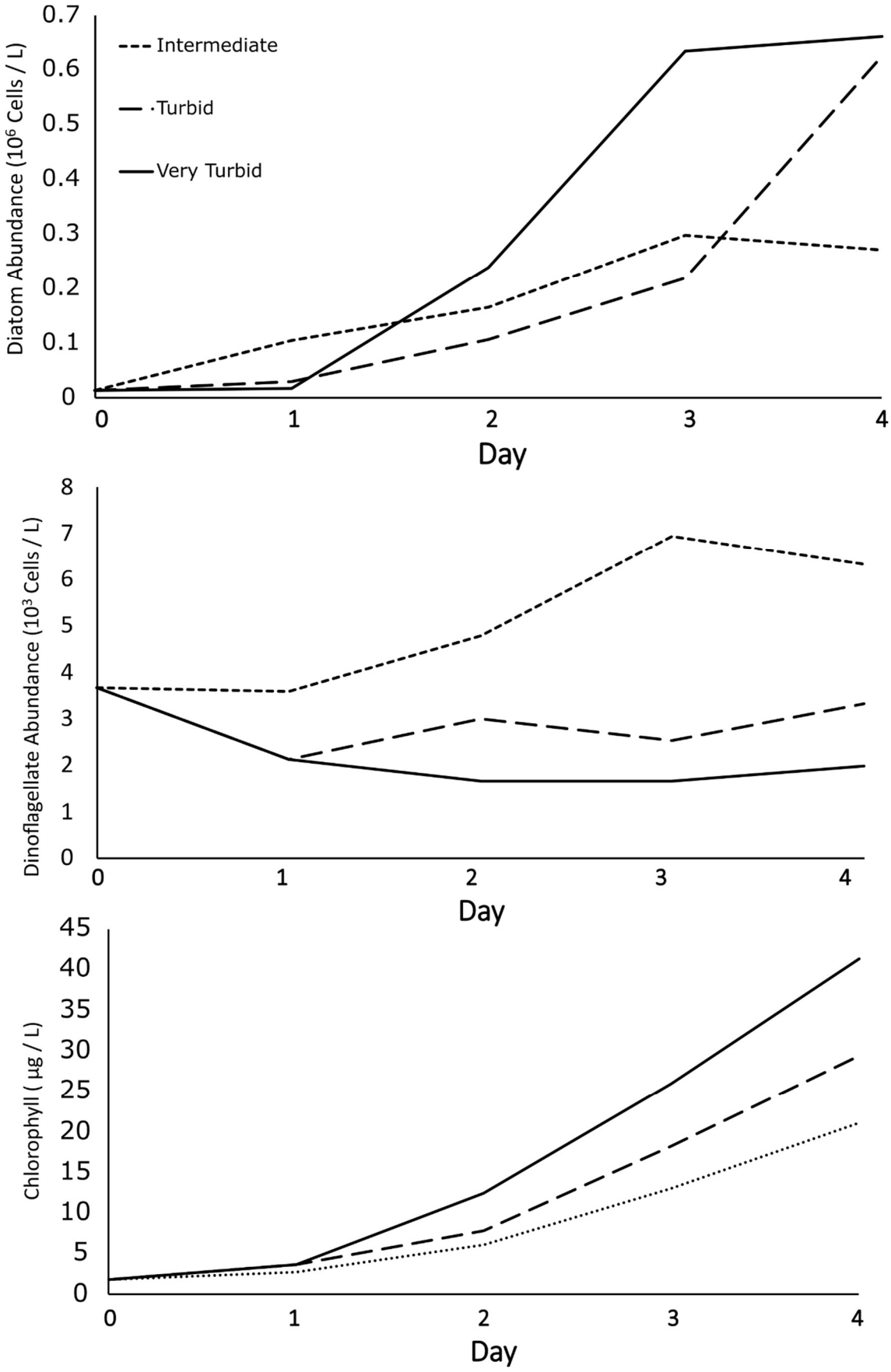

Figure 5.15 - Line graph of mean daily diatom abundance (n=3), mean daily dinoflagellate abundance (n=3), and mean daily chlorophyll abundance (n=3) in samples from the early summer experiment.

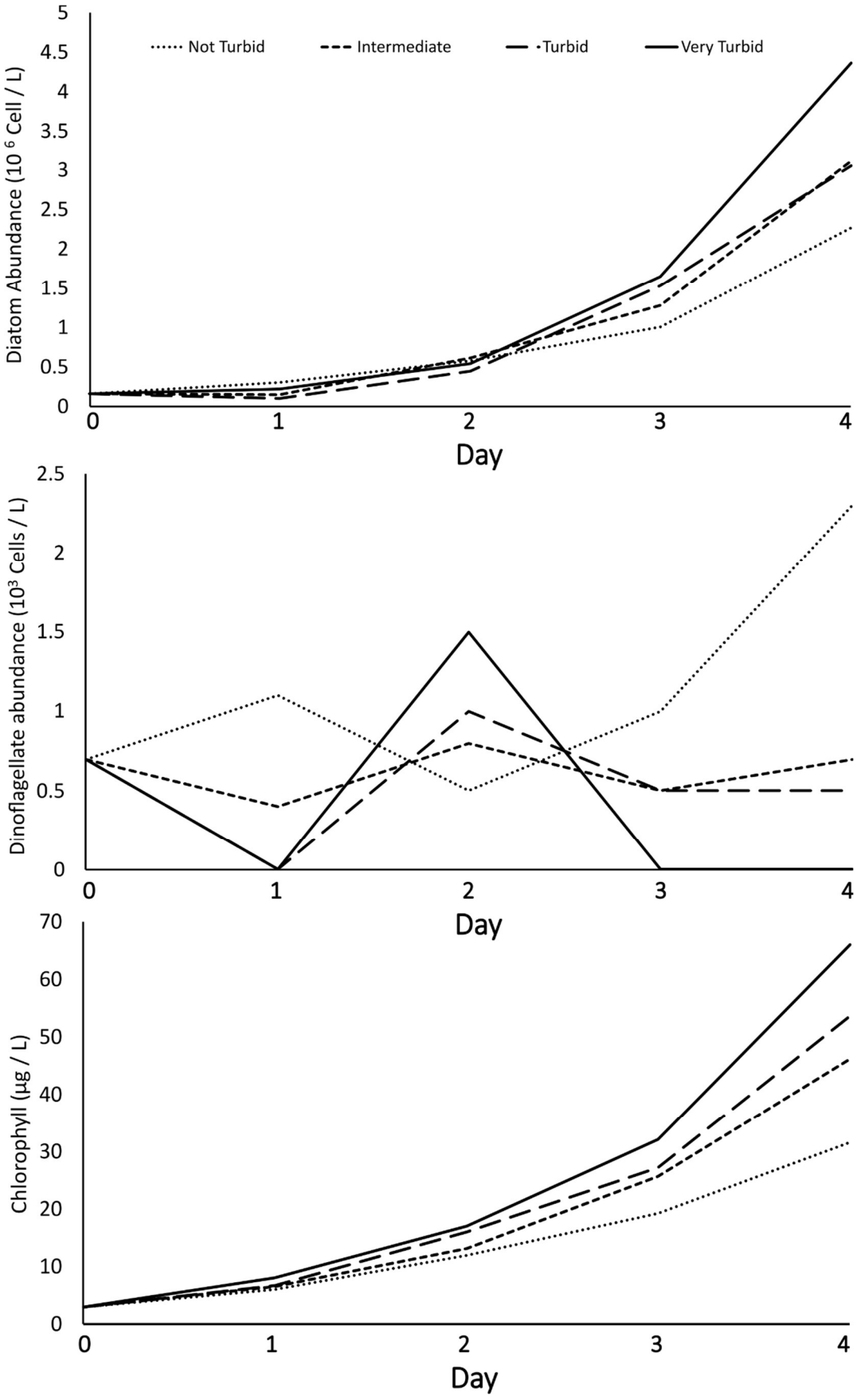

Figure 5.16 - Line graph of mean daily diatom abundance (n=2), mean daily dinoflagellate abundance (n=2), and mean daily chlorophyll abundance (n=3) in samples from the mid-autumn experiment.

There is an increase in the abundance of diatoms and chlorophyll in all turbidity treatments in early summer 2023 (Figure 5.15). This is not the case for dinoflagellates however, where declines over time are observed in turbid and very turbid treatments (Figure 5.15). In mid-autumn, diatom and chlorophyll concentrations increase with time in all treatments (Figure 5.16). Dinoflagellate abundances do not show similar patterns in all treatments. There are increased dinoflagellate abundances over time in the control treatment. The dinoflagellate abundance in the intermediate treatment initially increases, but by day four has an equal abundance to the starting community, whilst the turbid and very turbid treatments decline to values lower than the abundances seen in the starting community (Figure 5.16).

5.5 Discussion

5.5.1 Phytoplankton response to increasing turbidity in early summer and mid-autumn

In the incubations conducted in early summer, sediment additions were made to bring the SPM concentrations in line with the turbidity categories outlined by the WFD/WER (Table 5.1). Increasing sediment additions resulted in an increase in the abundance of diatoms (Figure 5.5, Figure 5.8), and of diatom species above 3,000 cells/L (Figure 5.13). In early summer 2023, the abundance of diatoms on day four increased by a factor of 2.3 and 2.4 between the control (intermediate turbidity) and the turbid and very turbid treatments respectively (Figure 5.5). Increased dinoflagellate abundances over time were observed in the control treatment in early summer (Figure 5.6), whilst this was not seen in the incubations in both the turbid and very turbid treatments in early summer for dinoflagellates (Figure 5.6), with abundances ranging from ~ 1.5 – 3.5 x 10^3 cells/L in the two treatments with sediment added. The number of dinoflagellate species declined with increasing sediment additions (Figure 5.14), from 6 on the final day of the control treatment to 2 on the final day of the very turbid treatment. There was a 1.4 factor increase in diatom abundance between the intermediate treatment to the very turbid treatment in mid-autumn, whilst the abundance in the very turbid treatment was 1.9 times higher than the control (Not Turbid). Dinoflagellate abundances in mid-autumn ranged between 0 - ~ 1.5 x 10^3 cells/L in the treatments with sediment added, whilst abundances in the control treatment increased to ~2.5 x 10^3 cells /L at the end of the incubation. There were more observations of no occurrence of dinoflagellates with increasing sediment addition in mid-autumn.

5.5.2 Sediment addition and nutrient availability

In both early summer and mid-autumn, higher turbidity treatments support an increasing abundance of diatoms, whilst dinoflagellate abundances declined relative to the control in early summer, and there are increasing observations of no dinoflagellates observed in the mid-autumn incubation. Grazing pressures in the higher turbidity treatments may reduce, given that

zooplankton are visual predators. A higher turbidity would make it more difficult for them to identify their prey (Hart, 1988; Eiane et al., 1999; Kiørboe, 2011). This may be a contributing factor in the increased abundances of the diatom community seen in the higher turbidity treatments in early summer and mid-autumn (Figure 5.8, Figure 5.15, Figure 5.16).

The nutrient data collected at MA2 at the point of sampling in both early summer (DIN = 32.2 µmol/L, DIP = 1.70 µmol/L) and mid-autumn (DIN = 38.2.0 µmol/L, DIP = 2.32 µmol/L) show that TOxN and DIP are not depleted, with an N : P ratio of 19 : 1 in early summer and 16 : 1 in mid-autumn 2023 (Table 5.5). The nutrient concentrations at the end of the incubation for mid-autumn (Table 5.6) show that DIN and DIP have reduced over the different treatments. This suggests that N and P have been taken up by phytoplankton despite the increase in turbidity, as evidenced by the higher diatom abundances and chlorophyll concentrations seen in the higher turbidity treatments (Figure 5.5, Figure 5.7). Silicate concentrations at the point of sampling in early summer 2023 are low and give an N : Si ratio of 20.13 : 1 (Table 5.5). This is high compared to the Redfield-Brzezinski nutrient ratio for diatoms of C : Si : N : P = 106 : 15 : 16 : 1 (Redfield, 1958; Brzezinski, 1985) and suggests silicate limitation. Gilpin et al. (2004) report Si limitation in diatoms at an N : Si ratio of 4 : 1. There is not such an extreme limitation in mid-autumn, where the N : Si ratio is 2.01 : 1 at the point of sampling (Table 5.5), however this is still higher than the Redfield-Brzezinski ratio (Redfield, 1958; Brzezinski, 1985). Despite the large disparity in potential silicate limitation, there is a similar increase in diatom abundances as a result of sediment addition. The addition of the sediment is likely to bring nutrients, and the concentration of nutrients released from the sediment after 4 days has been calculated here (DIN = 3.79 µmol/g dry sediment, DIP = 1.13 µm/g dry sediment, Si = 13.0 µm/g dry sediment, Table 5.2). The silicate released from the sediment over the course of the experiment may be enough to relieve some of the limitation and support growth.

There is a 1–2-day lag in the increase of diatom abundances after the addition of sediment in the higher turbidity treatments (Figure 5.5), but there is no delay in the growth in the control treatment. This lag is not as pronounced in the mid-autumn samples as it is in the early summer samples. The difference in the lag times could be explained by the reduction in silicate limitation, as the DIN : Si is much lower and there is less severe silica limitation in the mid-autumn samples. At the point of sampling the DIN : Si ratio was 20.13 in early summer, compared to 2.01 in mid-autumn (Table 5.5).

Nutrients are released from the sediment to become bioavailable, and a higher dependence on sediments being released after sediment addition, rather than those already in the waterbody, may result in a more pronounced delay to increased growth. The lag time which is seen in the results from the incubations may also be a result of the diatom community adapting

to the changing light environment. Photo-acclimation has been shown to take 3-5 days for diatoms (Tomkins et al., 2020), and may be a further controlling factor for the differences observed between the two time periods. The day length and light intensity is lower in samples collected in mid- autumn compared to samples collected in early summer with some photo-acclimation already.

5.5.3 Chlorophyll concentrations and phytoplankton abundances

Chlorophyll concentrations follow a similar pattern to diatom abundance, as expected given they are a proxy for plankton biomass. In both early summer and mid-autumn, chlorophyll increases with increasing turbidity, but the lag seen in diatom abundances is not seen in the chlorophyll concentrations (Figure 5.15, Figure 5.16). In the results from the early summer incubation, the chlorophyll concentrations increase continuously in the very turbid treatment, whilst diatom abundances remain relatively stable from days zero to one, and then from day three compared to day four (Figure 5.15, Figure 5.16). Chlorophyll concentrations on day four are higher in the very turbid sample than in in the turbid treatment, whilst diatom abundances are similar in both treatments on day four (Figure 5.15, Figure 5.16). The increases in chlorophyll despite this not being matched by abundance could be further representative of the diatoms acclimating and producing increased chlorophyll in order to more effectively photosynthesise in a lower light environment. Adaptations to differing light environments through a change in chlorophyll concentration have been reported in the literature (Anning et al., 2000; Shi et al., 2016). Higher chlorophyll concentrations were seen for similar diatom abundances in both early summer and mid-autumn (Figure 5.8). This supports the idea that the lag, at least in part, is a result of the diatoms increasing photosynthetic pigment before the increases in abundance are seen.

5.5.4 Dinoflagellate Diatom competition

Silicate concentrations are not limiting for dinoflagellate growth as they do not require this nutrient for their shells (Egge and Aksnes, 1992). In the control treatments in both early summer samples and mid-autumn samples, where no sediment is added, dinoflagellates have the most success compared to treatments where sediment is added (Figure 5.6). Dinoflagellates are most competitive when diatoms are experiencing silicate limitation, and this is most likely in the control treatment where no sediment and potential nutrient additions have been made. In early summer, dinoflagellate abundances do not increase with time, but after initial declines, their abundances are maintained throughout the experiments in each sediment addition treatment (Figure 5.6). Dinoflagellates may be less successful in increasing turbidity as grazing may become harder for heterotrophic and mixotrophic species, as encounter rates with prey decrease with increasing SPM concentrations. There is the further

possibility that the turbulence created by the use of orbital shakers within the experiment that dampened the growth of dinoflagellates in all treatments including the control. Turbulence is known to be detrimental to dinoflagellates, (van de Waal et al., 2014) and may have prevented further growth. However, species of *Scrippsiella* have been described as highly sensitive to turbulence (Berdalet et al., 2007; van de Waal et al., 2014), and *Scrippsiella* was still able to persist in this experiment. It is possible that either specific species of *Scrippsiella* present in the natural community used here are not highly sensitive to turbulence, or the turbulence did not have a big impact on the phytoplankton during the experiment.

Dinoflagellates are noticeably less abundant in the mid-autumn experiment compared to early summer, with there being days in both turbid and very turbid treatments where no dinoflagellates have been identified (Figure 5.6). The initial abundance of dinoflagellates is approximately 5 times lower in mid-autumn than in early summer (Figure 5.6). By contrast, the mid-autumn diatom abundance was around one order of magnitude higher than in early summer 2023 (Figure 5.5). Weston et al. (2008) documented a spring bloom in the Thames plume between days 95-150 which is April-May. The Weston et al. (2008) study site is approximately 18 km further offshore than the collection site of MA2 (Figure 5.3), but with a similar salinity recorded of ~32-34, compared to the 32.111 and 33.281 recorded at MA2 in early summer and mid-autumn, respectively. The samples for the first experiment were collected in June. Therefore, it is likely that the natural phytoplankton community collected from MA2 in early summer 2023 was towards the tail end of a spring bloom. There are frequent observations of diatom to dinoflagellate successions during blooms (Zhou et al., 2017a; Zhang et al., 2019; Wang et al., 2022) with various triggers identified for the progression. The Thames estuary has been seen to remain diatom dominated year-round (Sanders et al., 2001), with lower abundances in the summer, where Sanders et al. (2001) suggest silicate limitation. The samples in early summer, with lower diatom abundances and relatively high dinoflagellate numbers, may have gone through this succession, potentially as a result of Silicate limitation as previously suggested for this location. Silicate depletion is supported by the high DIN : Si ratio of 20.13 : 1 recorded at MA2 at the point of sampling in early summer (Table 5.5).

5.5.5 Community composition changes in response to increasing turbidity

The diatom *Skeletonema* is the most abundant taxa on day one in the early summer community in all treatments (Figure 5.13). In the control and in turbidity treatments, *Skeletonema* maintains a high relative abundance for the duration of the experiment (~ 40-60 %) with the notable exception of day four of the turbid treatment (10%). Here, *centric diatoms* become dominant (~70%). In the mid-autumn community, the relative abundance of *Skeletonema*

increases over time in the control and the three turbidity treatments, but *Skeletonema* never dominates to the same extent as in the early summer community (Figure 5.13). These increases in relative abundance of *Skeletonema* over time may mean that it is well suited to a turbid environment but takes time to acclimatise and so is seen later in the time series. Both *Skeletonema* and *Chaetoceros (Hyalochaete)* have been reported in low light environments (Ramakrishnan et al., 2018), however there is likely to be governing factors other than low light adaptation, as *Chaetoceros (Hyalochaete)* declined with increasing turbidity. The stark differences in community composition between the early summer and mid-autumn communities (Figure 5.13), accompanied by similar patterns in abundance, (Figure 5.5), suggest that there are a multitude of species with the ability to adapt to the changing conditions increased turbidity brings, and that a wide variety of diatom communities might be able to demonstrate a similar growth response to increased SPM concentrations.

The number of identified dinoflagellate taxa declines with increasing turbidity during both experiments (Figure 5.14). In both early summer and mid-autumn, the communities tend towards a community dominated by *armoured dinoflagellates*, *Scrippsiella*, or a combination of the two. These were the two most abundant taxa recorded in the initial community collected in early summer, however *Scrippsiella* was not identified in the initial community in mid-autumn (Figure 5.12). *Armoured dinoflagellates*, along with *Scripsiella,* are the only identified taxa on days two and four of the very turbid treatment in early summer, and the entirety of the turbid treatment in mid-autumn (Figure 5.14). The *armoured dinoflagellates* encompass a large variety of possible species, which have not been identifiable. This taxa group may represent single or multiple species. It is unknown whether the *armoured dinoflagellate* taxa group represents the same species throughout the experiment, and therefore the a*rmoured dinoflagellates* recorded in each treatment may not be the same taxa. In the mid-autumn experiment, increasing observations of no dinoflagellates were recorded with increasing turbidity (Figure 5.14), and *Scrippsiella* was the only identified taxa in the very turbid treatment (Figure 5.14). It could be that the unknown *armoured dinoflagellates* which persisted in the early summer experiment, were not present in the mid-autumn community, and those which were present were not as well suited to a turbid environment.

Specific species of *Scrippsiella* have the ability to be mixotrophic (Mitra et al., 2023; You et al., 2023). Mixotrophic ability may allow *Scrippsiella* to continue to grow in a low light environment, or in an environment with strong competition from other photosynthetic organisms. In increasing turbidity, as replicated in these experiments, mixotrophy may be an available mechanism to aid continued successful growth in the lower light environment which comes with increased SPM concentrations. Although, this would rely on the specific species of *Scrippsiella* with mixotrophic ability to have been present in the community during

these experiments. Unfortunately, it was not possible to identify the taxa for *Scrippsiella* to this level. However, *Torodinium Robustum*, another mixotrophic dinoflagellate (Strom et al., 2024), was offered no advantage by its potential for mixotrophy. *Torodinium robustum* is recorded in the initial community collected in both early summer and mid-autumn and is identified on day one in the control treatment in early summer but is not seen elsewhere in the experiments. This suggest that an alternative trait(s), potentially in conjunction with mixotrophy, allows *Scripsiella* the advantage over other dinoflagellate taxa in the experiments.

Literature documents turbidity as a factor limiting the growth of phytoplankton biomass (Cloern, 1987; May et al., 2003; Painting et al., 2007; Devlin et al., 2007b; Gameiro et al., 2011; Pan et al., 2016; Burson et al., 2018). Dijkstra et al. (2019) describe growth as being limited by 'sediment-induced deterioration of the light climate'. In contrast, other studies find growth supported by increased sediment (He et al., 2017). Zhang et al. (2017) document increased phytoplankton biomass with increased suspended solids due to fish presence in shallow lake mesocosms. Deininger et al. (2016) describe a short-lived increase in diatom abundance immediately after soil addition in their coastal lagoon mesocosm, but dinoflagellates increased immediately and then again on day 12 of their experiment. If the incubations in this study had continued longer there might have been a subsequent increase in dinoflagellate abundance.

Small diatoms, *Chaetoceros*, were favoured in Deininger et al. (2016) 's experiment. Whilst *Chaetoceros (Hyalochaete)* represented a large proportion of the mid - autumn community throughout the results of this turbidity experiment (Figure 5.14), the relative abundance of *Chaetoceros (Hyalochaete)* declined with increasing turbidity. This difference with Deininger et al. (2016) 's experiment highlights the fact that there are (many) other specific conditions which govern a dominant taxa. Burson et al. (2018) saw a slight decline in diversity with light limitation, under nitrogen and phosphorous replete conditions. Burson et al. (2018) also describe how, under nutrient limited conditions, co-limitation of nutrients with light can allow for the co-existence of species with different niches. Whilst *Skeletonema* does increase with increasing turbidity, diatoms do not become completely dominated by a single taxa and the co-existence due to differing niches offers an explanation for this lack of exclusion of all other taxa. Burson et al. (2018) present results which show the green alga *Chlorella marina* became the most successful under light limited conditions. Domingues et al. (2011) found that cyanobacteria were the only taxa able to acclimate to low light conditions. The research presented within this chapter focused on diatoms and dinoflagellates as they are a commonly used indicator of water quality. However, the literature presented above presents results of

increased turbidity on the abundance and composition of other lifeforms, and this too should be considered in management and policy decisions.

The results observed within this experiment indicate that dinoflagellate abundances are depleted relative to the control treatment, but are not completely diminished during the experiment and are still able to maintain their abundances despite the increasing turbidity in some cases (Figure 5.6). Mixotrophic ability, in combination with specific physiological factors, may allow for these dinoflagellates to be successful and not completely controlled by turbidity. Dinoflagellates can pose problems when it comes to harmful algal blooms and toxic species (Jeong, 1999; Panton and Purdie, 2022). Increased nutrients being allowed under the WFD/WER in coastal and transitional waters with increasing turbidity may allow for harmful and toxic dinoflagellate growth. *Scrippsiella* has been seen to remain despite the turbid conditions in this experiment (Figure 5.14), and species of *Scrippsiella* are common harmful species (Baek et al., 2003). Increased turbidity may therefore not only allow for increased growth, but also potentially select for taxa which have the potential to cause harmful algal blooms.

This work does not support the basis of the WFD/WER nutrient concession policy. Turbidity supports rather than dampens diatom growth in this experiment and does not uniformly reduce dinoflagellate growth. The increased nutrient concentrations which are allowed to occur before a water body does not receive a good standard in transitional and coastal waters therefore potentially are able to be utilised by diatoms, and dinoflagellates to an extent. The success of any phytoplankton in water with a high concentration of suspended particulate matter is contrary to the assumptions made in the WFD/WER, where the light limitation associated with turbid water has been assumed to reduce a water body's capacity to support primary production.

Table 5.8 – Examples of literature which support / do not support the results in this chapter

Papers with supporting evidence of allowing increased nutrients in waterbodies with higher SPM	**Papers with evidence against allowing increased nutrient concentrations in waterbodies with higher SPM concentrations**
• (Cloern, 1987) – light limitation is a control on phytoplankton as turbidity relates to the ratio of photic depth to mixed depth. • (May et al., 2003) - Model of spatial and temporal mechanisms which have a control on turbidity and phytoplankton growth • (Gameiro et al., 2011) - Nutrient replete estuary where phytoplankton	• (Deininger et al., 2016) - Soil additions made to mesocosms of 2m water column depth in a coastal Lagoon. Diatoms increased immediately after addition and then decreased. Dinoflagellates peaked immediately and decreased and then peaked again on day 12. Short lived blooms

growth was suspected to be light limited. • (Irigoien and Castel, 1997) – Estuary where nutrients are not considered limiting, and light / turbidity governs growth. There are observations of chlorophyll in high turbidity areas, and two mechanisms are discussed, imported chlorophyll and phytoplankton adaptation. • (De Swart et al., 2009) – Idealised model in turbid estuaries. Growth rates are dependent on nutrient and suspended sediment concentrations. • (Grobbelaar, 1985) – Mixed layer depth is important regulating factor in turbid waters • (Wang et al., 2019) – High turbidity in nearshore waters resulted in higher light attenuation, and this was a controlling factor in phytoplankton blooms. • (Liu et al., 2018) – Model of phytoplankton bloom dynamics in turbid estuaries. SPM concentration is a controlling factor for blooms. • (McSweeney et al., 2017) – surface suspended sediment is a key controlling factor in a high nutrient low growth turbid estuary. • (Jiang et al., 2021) – SPM increased light attenuation and reduced growth rate in *A. Carterae*. • (Sobolev et al., 2009) – Turbidity limited phytoplankton abundance despite high nutrients in an artificial reservoir. This may be conducive for floating macrophytes due to decreased competition for nutrients. • (Cloern and Alpine, 1988) – Highest growth rates observed where photic depth was large portion of mixed depth. • (Diehl et al., 2002) – experimental evidence supporting model prediction that high turbidity reduces algal production. • (Colijn and Cadée, 2003) - light limitation more important factor than nutrients in some cases. • (Kromkamp et al., 1995) – light limited phytoplankton growth in	• (He et al., 2017) – Mesocosm study in a shallow lake in which higher turbidity was induced by crucian carp through sediment resuspension, and additional nutrients additions were made. Higher turbidity resulted in increased phytoplankton biomass and chlorophyll concentrations. The results are in contrast to those of similar studies, where light limitation was observed. • (Örnólfsdóttir et al., 2004) – Phytoplankton growth rates increased in response to nutrient inputs despite decreased surface irradiances in a lab-based bioassay. Authors note that the lack of different in growth rates between treatments suggests that phytoplankton had experiences light conditions which included all experimental conditions, and they also note that the decreased irradiance used may not have been low enough. • (Nunes et al., 2022) – Turbidity did not reduce chlorophyll concentrations in some treatments in a sediment addition bioassay, dependent on the initial chlorophyll concentration. • (Pinckney et al., 1999) – sediment additions showed higher productivity and biomass in mesocosm experiments; however, authors note this is probably as a result of phytoplankton within the sediment additions, and growth rate was not impacted. • (Kim et al., 2025) Dinoflagellate compensation to low light environments, including mixotrophy. • (Mena et al., 2025) Phagotrophy may offer an advantage to some mixotrophic dinoflagellates in short low light or low nutrient periods. • (Fichez et al., 1992) – Field observations of phytoplankton growth in high turbidity due to balance of critical depth and mixing depth. • (Hansen, 2011) and references therein – Review of mixotrophic behaviour of dinoflagellates.

certain part of a high turbidity high nutrient estuary • (Painting et al., 2007) – Model demonstrating that estuaries with low / moderate light levels are not very likely to show a biological response to nutrient inputs. • (Jeong et al., 2018) – Light intensity had no impact on the growth rate or ingestion rate of a mixotrophic dinoflagellate. There was no growth in complete darkness. • (Cole et al., 1992) – Light and mixing limited phytoplankton growth. However, there are observations of blooms. Authors suggest these occur in specific shallower parts of the estuary. • (Cloern, 1999) – light availability regulates how nutrient enrichment manifests in coastal estuaries. • (Cole and Cloern, 1984) – growth was highest in the regions of lowest turbidity. Spatial and temporal variation in primary productivity explained by light availability in San Francisco Bay. • (Cloern et al., 2014) – review of phytoplankton growth in estuaries, including sediment and light limitation. • (Monbet, 1992) – review of data from micro and macrotidal estuaries. Higher amounts of suspended solids resulted in chlorophyll decrease. • (Fisher et al., 1988) Chlorophyll maximum is observed seaward of the turbidity maximum, in clearer waters. • (Pennock and Sharp, 1986) – Model of phytoplankton growth limiting factors in the Delaware estuary. Model suggests light limitation throughout the year in upper estuary and in winter in the lower estuary. • (Randall and Day Jr, 1987) – Authors suggest light limitation at low salinities due to turbidity in Louisiana estuary. Decreased production in moving incubations. • (Domingues et al., 2011) – Light limited phytoplankton growth of a natural community in a turbid	• (Kocum et al., 2002) – The highest phytoplankton biomass was found at the head of the estuary where nutrient levels were highest even though there was high light attenuation. Authors cite a shallow well mixed water column. However, overall production was low, and phytoplankton were light limited.

estuary. Light and nutrient co limitation found in summer. • (Stoecker et al., 1997) Mixotrophy does not appear to be a mechanism by which *Prorocentrum minimum* responds to light limitation. • (Joint and Pomroy, 1981) – Higher growth rates were seen in less turbid areas of the Bristol channel. • (Cloern and Alpine, 1988) – Phytoplankton growth rate was highest when the photic depth was the large compared to the mixing depth. • (Gazeau et al., 2005) – Light limited rather than nutrient limited phytoplankton due to high concentrations of SPM. • (Wofsy, 1983) – Suspended sediment is a control on phytoplankton biomass. • (Kocum et al., 2002) – Phytoplankton was light limited, below reported bloom levels for other systems considered similar, despite high nutrient levels. • (O'Donohue and Dennison, 1997) – Productivity limited by light as a result of high SPM. • (Hansen, 2011) and references therein – Review of mixotrophic behaviour of dinoflagellates. • (MacIntyre and Geider, 1997) – Decline in photosynthesis greatest in turbid rapidly mixed waters.	

This list of papers is definitely not exhaustive but does give an indication of the types of observations which support or contradict the work presented here.

Observations which might support the concession on nutrient thresholds, and those which don't, cite vertical mixing and depth as important for (inhibition of) growth in turbid environments, parameters which were not replicated in the laboratory environment. Observations of increased growth through sediment addition are seen in shallow environments, and the mesocosm results presented here are not from estuarine environments for which the concession exists. The results seen in this experiment are unlikely to be representative of a natural response, and in situ experiments which can more accurately replicate the environment would be beneficial. This also suggests that SPM **alone** is not a governing factor, and response to turbidity could be very spatially or temporally dependent.

Further investigation and work are warranted. Mixotrophy has been seen to offer advantage in some species in low light environment in the papers considered here, and to not offer an advantage for others. It appears a species-specific response and may therefore also be very location and community driven. It is unknown whether the light values considered in the papers would correspond to realistic values in turbid estuaries. Furthermore, if high turbidity environments were to induce a mixotrophic response and offer certain species an advantage, establishing if the growth could cause an undesirable disturbance, and if extra nutrients permitted under the concession would actually support or fuel any further growth would help to establish the suitability of the additional threshold. A switch of feeding mode could mean that the inorganic nutrient concentrations become increasingly unimportant.

There are observations within the papers listed here of increased growth as a result of sediment addition through nutrient addition, which supports the results observed within this experiment. However, an important consideration is whether or not this increased growth would result in undesirable consequences, as is the approach taken by the WER / WFD.

5.6 Conclusion

The incubations carried out in this study demonstrate that turbidity may exert an influence on the community composition of phytoplankton. Diatoms responded favourably to the increase in the sediment additions in comparison to dinoflagellates. It is demonstrated here that increased turbidity as a result of sediment additions have not reduced the ability of the waterbody to support increased phytoplankton biomass, and this challenges the effectiveness of the WFD/WER policy which is in place. This response is seen in samples collected in both early summer and mid-autumn, where the communities are notably different and are representative of different ecological and environmental conditions. The results presented here are in contrast to literature, which presents turbidity and light limitation as a key limiting factor for the growth of phytoplankton. These differing results highlight the need for further work investigating and unravelling the complicated relationship between turbidity and phytoplankton biomass and community composition and determining whether the WFD/WER concession should indeed be in place. The response to increasing sediment addition differs between species, implying that the initial composition may be large factor in determining the response of the community, and therefore responses are likely to differ on both spatial and temporal scales.

So what? – The results of this experiment have indicated that there may be scenarios in which the increased nutrient inputs into the water column as a result of increased suspended particulate matter concentrations could be fuelling growth of certain phytoplankton or altering community composition. This could make the higher threshold for acceptable

nutrient concentrations under the WER / WFD in higher SPM waters on the assumption of light limited growth, unsuitable. However, there needs to be more research into whether this is realistic in the natural environment or whether these results are a function of a laboratory setting, and whether the increased growth which is supported is sufficient to result in an undesirable disturbance to the ecosystem, as results were not statistically significant. This work demonstrates that additional research across a variety of different waterbody types to investigate how SPM might support or not support problematic phytoplankton growth is necessary.

6

Outlook and synthesis

6.1 Overview

The aim of this PhD research was to further the understanding of the role of phytoplankton within UK eutrophication monitoring and how they may be considered within associated assessment frameworks. The current metrics fail to identify the true extent of changes happening within water quality conditions and phytoplankton communities over space and time. In addition, factors which may govern the response of a water body to nutrient enrichment are not fully considered within assessments. The research has focused on Liverpool Bay and the Thames estuary and the wider marine area. Specifically, this PhD research has examined the following research questions:

1. *Does long term trend analysis provide more informed assessments of estuarine and coastal waters, and would inclusion of trend information improve current metrics that assess ecological state over 6-year cycles?*

Long term trend analysis has offered increased information about the patterns of nutrient enrichment over time, giving indications of areas where there are increasing, decreasing, or no changes occurring over time. This information could give managers insight into the effectiveness of decisions and management efforts and offer warnings about potentially deteriorating areas prior to the outcome of a pass / fail metric relative to good/moderate status, and areas which may be consistently failing, with no identification of an improving trend, can be prioritised for action. This could mean, for example, altering permits, or altering land management practices as outlined in Environment Agency (2024). Additional mitigation activities and their importance under a changing climate are considered in Duarte and Krause-Jensen (2018), and long term trend analysis could help to consider their effectiveness.

Ecosystem recovery can be complicated, lengthy, and highly variable (McCrackin et al., 2017). This suggests that prevention may be preferable to recovery activities, where possible. McCrackin et al. (2017) also highlight the importance of long-term monitoring to assess ecosystem recovery.

The costs of damage as a result of eutrophication versus addressing problems have been calculated for freshwater (Pretty et al., 2003), and the authors conclude that there would be cost reduction for prevention. Whilst considerations of damage costs would be different in coastal and marine waters compared to freshwaters, the principal of the cost of damage exceeding the cost of response may still stand.

2. *Can the Plankton Index tool offer further insight into the extent of ecological impacts of eutrophication in addition to the current phytoplankton metrics alone?*

The plankton Index tool, as used here, offers more detailed information about the shifts seen within a phytoplankton lifeform pair compared to the currently used phytoplankton sub metrics. Specifically, the phytoplankton index tool gives information about when the shifts in the community composition are occurring, which will become increasingly important under a changing climate when phenology is likely to be impacted e.g. (Mészáros et al., 2021; Fernández-Barba et al., 2025). (Phyto)plankton has societal value beyond being the base of the marine food web (Grigoratou et al., 2025). Grigoratou et al. (2025) present six groups where phytoplankton are considered to have value – Biogeochemistry, ecology, culture recreation and wellbeing, evolution of science, economy, and climate. Ensuring that changes within the community can be properly monitored, assessed, and managed is integral to mitigating undesirable disturbances and safeguarding phytoplankton and associated value.

3. *What further understanding could be gained about the eutrophic state of coastal and estuarine areas by applying integrated coastal and offshore assessment using both WFD/WER and OSPAR in terms of metrics and time periods?*

   Assessing the data collected for the WER / WFD and the OSPAR assessment simultaneously and with universal and additional metrics give an indication of how nutrient enrichment is changing along a spatial gradient of assessment areas. This means that potential problem areas may be able to be identified before an assessment is 'failed'/ does not achieve good status, and preventative rather than remedial actions could be taken. This approach also gives information about the spatial variation of eutrophication impacts in more detail, which can again further assist management as there may be indications regarding drivers of change in different locations, which can inform appropriate actions. Combining the datasets may improve temporal and spatial coverage in some areas, potentially making information about trends more accurate. Additionally, using the metrics presented here across all areas will allow for the identification of changes and shifts in areas which would not previously be identified.

4. *How do the light and nutrient conditions vary with salinity in Liverpool Bay and the Thames Estuary?*
5. *How does the phytoplankton abundance and community composition vary with salinity in the two study areas of Liverpool Bay and the Thames Estuary?*
6. *Is phytoplankton biomass nutrient limited at an offshore sampling site in the Thames Estuary?*

There is a suspected shift from inshore light limited conditions to nutrient limited conditions further offshore. This has, currently, created a consistent chlorophyll concentration in the Thames estuary area along the gradient. Understanding the governing factors along the salinity gradient can help to determine how the ecosystem may react the changing environmental conditions. This could include increases in nutrient inputs, but it will also help environmental managers to be aware of how shifts in the climate may impact locations differently. Understanding the governing factors could help to determine how effective and impactful policy / legislation decisions may be at managing the undesirable consequences of eutrophication, especially under a climate change scenario.

7. *How does a natural phytoplankton community respond to sediment additions in a laboratory incubation experiment and what are the implications for current UK assessment criteria?*

The results of the addition experiments indicate that sediment additions supported increased diatom abundances rather than preventing growth through light limitation, as would be expected under the WER / WFD assessment. An evaluation of the literature indicates that vertical mixing, which could not be replicated in the laboratory setting, is an important mechanism when considering light limitation as a result of turbidity, and so additional experiments are necessary. Unravelling the relationships may become increasingly important under a changing climate, as more extreme weather events may increase the amount of sediment added to waterbodies through run off, and increased mixing may result in more resuspension. This means that understanding how increased concentrations of suspended sediment are impacting the water column could become more important to make informed and effective management decisions. A different response to the sediment addition was seen in diatoms compared to dinoflagellates, which suggests that the response to SPM concentrations may be governed, in part, by the phytoplankton community, and responses may be very location dependent.

In terms of the WER / WFD assessment results and classification outcomes, it is possible that despite high nutrient concentrations, a turbid waterbody could be classified as ‘good’ under the nutrient metric if concentrations are below the concession thresholds. However increased growth and or shifts in the phytoplankton community could still be occurring which the phytoplankton sub metrics might capture and given the ‘one out all out’ policy used for classification in the WER / WFD, a good overall classification might not be achieved.

These research questions and their conclusions all aim to contribute to the knowledge pool of how eutrophication monitoring can be improved by properly considering the impacts on phytoplankton communities. This is achieved through the inclusion of a wider range of metrics,

improved understanding of interactions between water quality and phytoplankton, and the impact of sediment on phytoplankton response. Field sampling of water quality parameters and community composition provided insight into the changes in phytoplankton abundance and community composition occurring along salinity gradients, identifying succession of light and nutrient limitation from inshore to offshore.

Several large, long term, datasets which have been collected under multiple monitoring initiatives were combined and analysed in order to establish if changes within the phytoplankton community in the Thames Estuary and Liverpool Bay could be identified and understood (Chapter Three). The findings from this demonstrate that by utilising additional metrics to those currently included within monitoring directives, including the phytoplankton index tool, finer scale shifts within the phytoplankton community can be identified. Utilising metrics over a longer time frame than the current 6-year assessment periods reveals that the inclusion of trend data or information on the trajectory of change enhance eutrophication assessments, offering insights into the direction of eutrophication impacts and informing preventative rather than remedial management efforts.

The variation in salinity and nutrients were explored (Chapter Four) at sites in the Thames Estuary and Liverpool Bay, both large impacted embayments which experience high nutrient inputs from direct and indirect sources. Sites were located along a salinity gradient ranging from ~ 8 to 35. Assessing the nutrient concentrations and ratios, the light environment, and phytoplankton abundance along the salinity gradient has identified a shift from inshore to offshore in the factors which regulate and govern the phytoplankton community in the Thames Estuary and Liverpool Bay.

The response of phytoplankton communities within changing suspended sediment concentration scenarios was investigated (Chapter Five) through laboratory experiments, namely addition bioassays. Abundances of diatoms were not observed to decrease between treatments, despite higher turbidity, however increased turbidity did result in community composition shifts in both diatoms and dinoflagellates. This chapter highlighted that there may be a more complicated relationship between turbidity and phytoplankton than is considered within current eutrophication monitoring and may not be identified using the current metrics. These laboratory experiments have brought into question the suitability of some of the concessions associated with nutrient thresholds set by the WFD/WER in transitional and coastal waters.

6.2 Current metrics for eutrophication monitoring and assessment do not capture the full extent of the important changes occurring within the phytoplankton community

Some potential scenarios of shifts within the phytoplankton community which may not be identified utilising the current monitoring methods alone were outlined (Figure 1.4). This included changes in the relative abundance within lifeform pairs and long-term changes in abundance. The findings from the research within this thesis have confirmed shifts over time and space within the phytoplankton community, through the use of the Plankton Index tool, which would not otherwise have been observed within eutrophication monitoring, and which could have consequences for the wider ecological community. Results from the Cumbria and Mersey Mouth assessment areas were presented (Figures 3.16 – 3.19). The outcomes of the WFD/WER phytoplankton metrics gave a classification of 'good' and 'moderate' for these areas respectively (Figure 3.14). These WFD/WER metrics identified shifts in the area, however the detail of these, which is important to ensure appropriate management actions, was not. Applying the Plankton Index tool to the available data within the assessment areas resulted in a significant change in the diatom / dinoflagellate lifeform pair being identified (Figure 3.16, Figure 3.19). The inclusion of this method has identified an ecological disturbance which may have undesirable impacts and might previously have been overlooked. The importance of small-scale changes has been discussed throughout the thesis in terms of their implications for the wider ecosystem. Confirming the presence of changes within assessment areas which are not represented within the current assessment outcomes supports the recommendation made in previous studies (McQuatters-Gollop et al., 2009; Greenwood et al., 2019; Devlin et al., 2023; Graves et al., 2023; Devlin et al., 2025; Holland et al., 2025) that metrics for eutrophication monitoring must be expanded, in order for the monitoring and mitigation of eutrophication to be effective. A feasible example, of the Plankton Index tool (Tett et al., 2008), is utilised within this research.

Furthermore, differences in the response of diatoms and dinoflagellates were identified along the salinity gradient in the Thames estuary and in Liverpool Bay (Chapter Four), where increases in the relative abundance of dinoflagellates were only observed at higher salinities (Figure 4.4, Figure 4.9). Laboratory experiments have similarly identified differing responses to changes in water quality conditions, as diatom abundance increased with the addition of suspended particulate matter, but this trend was not observed in dinoflagellates (Figure 5.4, Figure 5.5). The community composition differed with changing concentrations of suspended particulate matter; dinoflagellate communities tended towards a reduced number of specific species. The changes identified within this thesis represent only a few of the community level impacts of eutrophic conditions, but identifying even this small number has confirmed that

there are important and consequential impacts of eutrophication which are currently being missed within WFD/WER and OSPAR monitoring.

6.3 The suitability of the concessions allowing higher nutrient concentrations in turbid waters has been brought into question

In transitional and coastal waters, the permitted nutrient concentrations for a water body are higher in turbid waters than in non-turbid waters. This is based on modelling primary productivity as functions of definitions of eutrophication by Nixon (1995), as light limitation is assumed to accompany increased concentrations of suspended particulate matter and limit phytoplankton growth (Cloern, 1987; Painting et al., 2007; Devlin et al., 2007b). The results presented here of the addition bioassay indicate that the increased suspended particulate matter concentrations did not prevent the continued growth of phytoplankton, despite the assumed associated change in the light environment; diatom abundances were observed to be higher in the treatments where more sediment was added (Figure 5.4). The same was not observed for dinoflagellates however, and there was an increased number of samples where no dinoflagellates were identified in the more turbid treatments relative to non-turbid treatments (Figure 5.5). Alongside the trends observed within the phytoplankton abundances, shifts in the community composition were observed for both of the lifeforms. With increasing turbidity, dinoflagellates tended towards a community comprised of *Scrippsiella* species and *armoured dinoflagellates*. Diatoms showed an increased number of species occurring at increased abundances. Not only do these results indicate that the permitted increase of nutrient concentrations in turbid waters under the WFD/WER warrants further investigation, it also indicates that the response to changing turbidity may differ between lifeforms in the diatom dinoflagellate pair.

6.4 The existing monitoring data has more to offer when combined

This PhD research has shown that the data collected for the WFD/WER and OSPAR assessments could be utilised more effectively in order to provide a more in depth understanding of eutrophication on wider temporal and spatial scales. The data analysed in Chapter Three comes from both the Environment Agency and Cefas, and whilst these datasets would not usually be combined with one another when carrying out eutrophication assessments, doing so here has offered additional insight into the changes occurring over time and along the salinity gradient. Fusing these datasets together makes it possible to assess trends over longer temporal and more cohesive spatial scales, which can provide valuable information on the trajectory of changes, measure the success of management initiatives, or identify areas where further intervention is needed. The WFD/WER assesses in 6-year cycles, and whilst the outcomes of each cycle will be compared, trends in the long-term data are not

assessed. The results presented in Chapter Three confirm that utilising the data in this way and looking at trends over a longer period of time rather than a binary pass or fail within each 6-year assessment period can be beneficial for informing management practices. For example, multiple areas are identified which have winter DIN concentrations exceeding the threshold (Figure 3.13), but no significant long-term change in DIN concentrations has been identified over multiple assessment cycles (Figure 3.11, Figure 3.12). This extra information, which would not be routinely revealed under current assessments, clearly demonstrates an area where concentrations are both elevated and unchanging and suggests that the management initiatives have not been successful in reducing this. In a similar manner, longer term assessments can offer warnings by identifying areas which may become eutrophic. Under the current metrics, initiatives to address elevated nutrient concentrations may not be implemented until the status of a waterbody is identified as problematic. By taking a more holistic view of the data and assessing the trajectory of changes within the marine environment, earlier intervention can occur, supporting prevention rather than remediation.

Combining data collected under different directives also supports assessment of the state of waterbodies along the salinity gradient. Transitional and coastal waterbodies in the WFD/WER would ordinarily be assessed separately to OSPAR areas, however considering the data simultaneously is valuable. A non-linear relationship between nutrient concentration and phytoplankton biomass is demonstrated (Chapter Four), and that a response elicited in one place may not be present in another. The conclusions made in Chapter Four also highlight that the factors which govern waterbody response to eutrophication can change along the salinity gradient, which was identified as inshore light limitation and offshore nutrient limitation. Being able to assess the eutrophic state of transitional, coastal, and offshore areas simultaneously can assist in identifying areas where the specific water quality conditions create an environment which may be more susceptible to eutrophication, and where attention and intervention may therefore be best focused.

6.5 Future directions and recommendations

6.5.1 Considering phytoplankton response to turbidity in eutrophication assessments

The results of this research bring into question the suitability of the higher thresholds for permitted nutrient concentrations in more turbid waters. The increased concentrations of suspended particulate matter added during the bioassay (Chapter Five) did not dampen the growth of diatoms, and in fact increased growth was seen. However, the field observations (Chapter Four) do suggest that inshore growth in the Thames is light limited, and the excess nutrients are not being utilised. The concessions on allowed nutrient concentrations within the WFD/WER were enacted on the understanding that the elevated levels would not result in the

undesirable consequence of increased phytoplankton biomass due to light limitation. The contrasting observations presented in Chapter Four and Chapter Five suggest that there are further governing factors which regulate the phytoplankton response to increased turbidity. It is possible that these differing results are a function of the laboratory-based environment compared to the conditions found *in situ*. Whilst every effort was made to replicate the natural environment, an exact match for conditions is not realistic.

Further work investigating phytoplankton response to changes in turbidity would be advantageous in order to unravel the complicated relationship between phytoplankton, nutrients, and light, and could offer valuable insight into whether the higher allowed nutrient concentrations are resulting in the unintended effects of eutrophication unnecessarily. Repeating the experiments from Chapter Five on a larger scale may be a useful way to investigate this, and using *in situ* mesocosms or a field-based bioassay may be a suitable method for upscaling this work. Nevertheless, the results presented here have raised compelling questions regarding the assumptions which underpin eutrophication guidelines. Recommendations for further work are timely, given the shifts in water clarity being observed (Capuzzo et al., 2015; Opdal et al., 2019). The results also highlight the challenges associated with translating laboratory results into management practices, as well as the importance of long-term field monitoring.

6.5.2 Data availability

Data obtained from long-term monitoring has underpinned this research. However, the often-inconsistent availability of this data has been identified as a limitation throughout the thesis. The methods presented in Chapter Three currently could not be applied universally across all areas due to the sparse nature of some of the data, and in Chapter Four direct comparisons between the study areas were limited by the data coverage. The nature of fieldwork at sea means that gaps are inevitable as a result of the challenges associated with weather dependent activities, but further to this, the availability of financial resources and skilled personnel can play a large part in the data coverage.

Unravelling the complex relationship between phytoplankton and water quality relies on substantial quantities of simultaneously collected variables, which is a considerable workload. This makes the case for the increased use of high frequency monitoring equipment, as is also made by Rozemeijer et al. (2025), to supplement water quality surveys, such as the autonomous Cefas SmartBuoys, and sensors seen in streams and rivers (Halliday et al., 2015; Bieroza et al., 2023). SmartBuoy data was not used within this thesis as the focus was on spatial relationships between phytoplankton and water quality, but the use of autonomous technologies which could provide high frequency data across a spatial range could

undoubtedly deepen the understanding of these complex systems. The salinity range covered in Liverpool Bay is limited, partly due to the physical characteristics of the bay itself but also somewhat as a result of the available access to the more inshore sites. The samples which are available from the most inshore stations in Liverpool Bay were obtained using different survey vessels to those used for the remainder of the stations, and the logistics associated with this mean that data collection along the entire salinity gradient is not viable for all surveys. The use of an autonomous data collection method could widen the spatial coverage of data collection in areas with more difficult access.

Water sampling surveys remain an integral part of the long-term monitoring however, and their consistent funding is key to the successful understanding of eutrophication in coastal and transitional waterbodies. The mNCEA programme, from which data has been used for the analysis in Chapter Four, is no longer running. The programme has offered some insight into the water quality along the salinity gradient, but a continuation in the data collection would allow for more robust conclusions and comparisons across a wider range of temporal and spatial scales. For eutrophication to be successfully monitored and managed, especially under a changing climate where trends may become unpredictable, consistent long-term monitoring which provides comprehensive datasets from the field is key. The discrepancy observed in Chapters Four and Five between laboratory-based results and field monitoring confirms the importance of both data types, and the conclusions drawn in Chapter Three highlight the benefits of data which covers wider temporal and spatial scales.

In order to make monitoring programmes for eutrophication more ‘fit for purpose’ , a few changes could be considered, as similarly discussed in Graves et al. (2023), Devlin et al. (2025), for example,

- Utilising the available methods, such as the plankton index tool or equivalent, to be able to identify more detailed information about phytoplankton community change. This will allow for a deeper understanding of changes occurring in water bodies and help to ensure that waterbodies more accurately reflect the classification awarded to them.
- Data sharing across monitoring programmes. For example, combining data collected under the WER / WFD and the OSPAR assessments. This could increase spatial and temporal resolution in some places and would contribute to the most effective use of already existing data.
- Considering data and assessments holistically by synchronising assessment metrics and time periods, will give information about how nutrient enrichment is manifesting in different areas.

- Including an analysis of long-term trends in the assessments, this can offer increased understanding about potential problem areas, and information about the success of management practices.
- Ensure that monitoring programmes have resources which allow for sufficient temporal and spatial coverage to make robust conclusions. This will mean that monitoring initiatives may be more impactful and worthwhile.

6.6 Concluding remarks

In conclusion, the results from this thesis have highlighted fine scale shifts which are occurring within the phytoplankton community in transitional and coastal waters, are not revealed by the current eutrophication monitoring and assessment methods. The assumptions on which the nutrient thresholds are based have been tested, which creates opportunity for important future work. Separate datasets have been amalgamated to deepen the understanding of eutrophic conditions and their relationship to phytoplankton across temporal and spatial scales. The varying response by different lifeforms observed within this research cast phytoplankton as a governing factor within a waterbody's response to eutrophication, as well as a factor which itself is impacted by the development of eutrophic conditions. Most importantly, this thesis has established the important role that phytoplankton data can play within eutrophication monitoring far beyond its current inclusion, in understanding the health and wellbeing of transitional and coastal ecosystems, if the appropriate data assessment methods are used.

7

Appendix

7.1 Thames estuary long term trend results

7.1.1 Thames plume

Table 7.1 – *p* values in the Thames plume assessment area

Variable	*P* value
Chlorophyll	0.30
DIP	0.00
Ammonium	0.39
TOxN	0.00
Salinity	0.00
DIN : DIP	0.09
DIN	0.00
Dissolved Oxygen	0.04

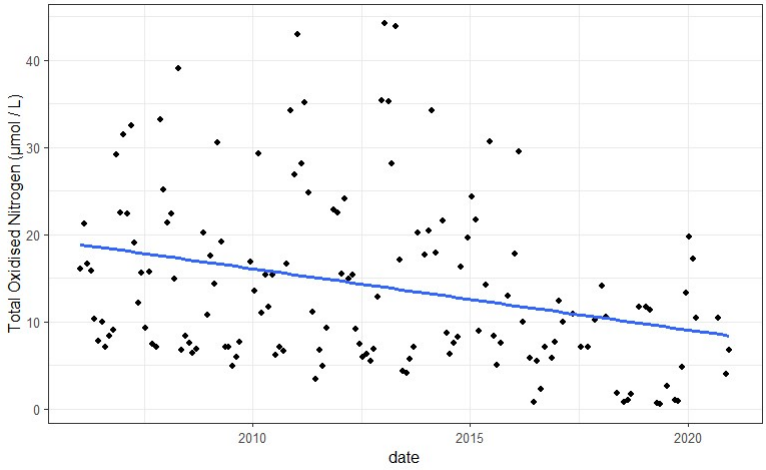

Figure 7.1 - TOxN concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

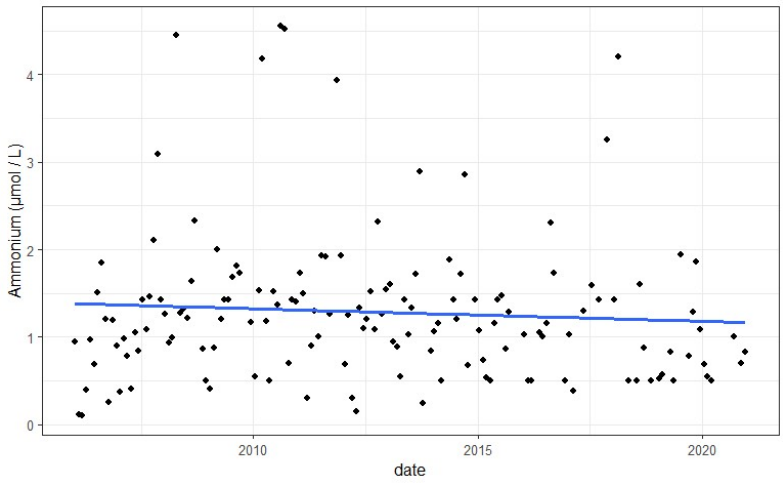

Figure 7.2 - Ammonium concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

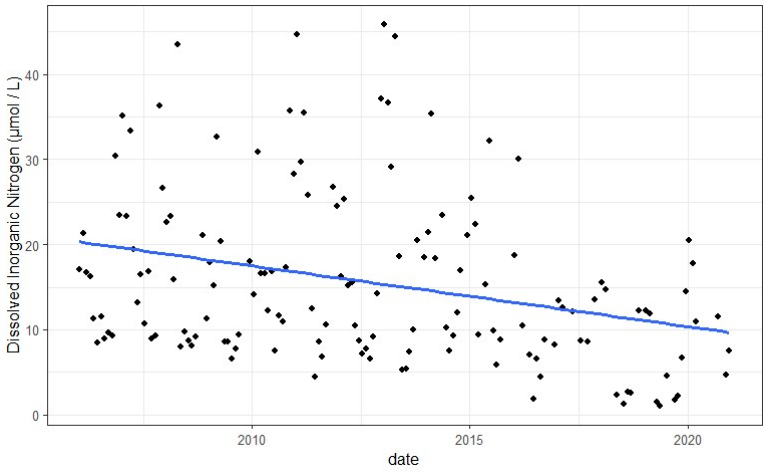

Figure 7.3 - DIN concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

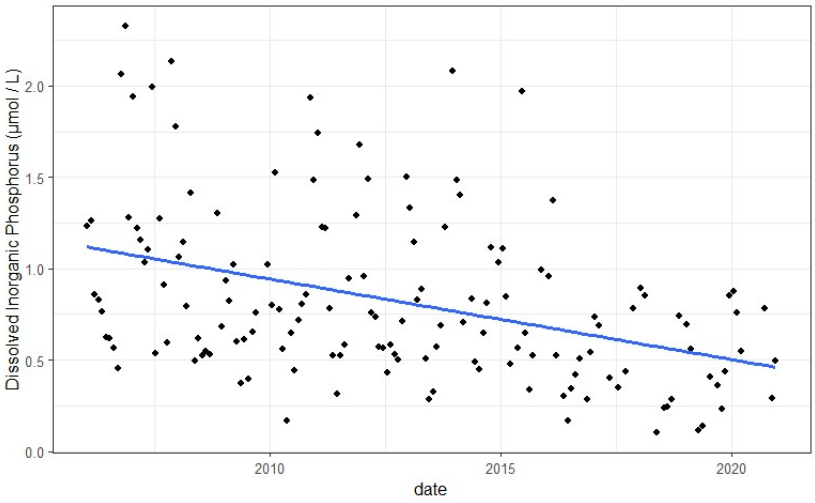

Figure 7.4 - DIP concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

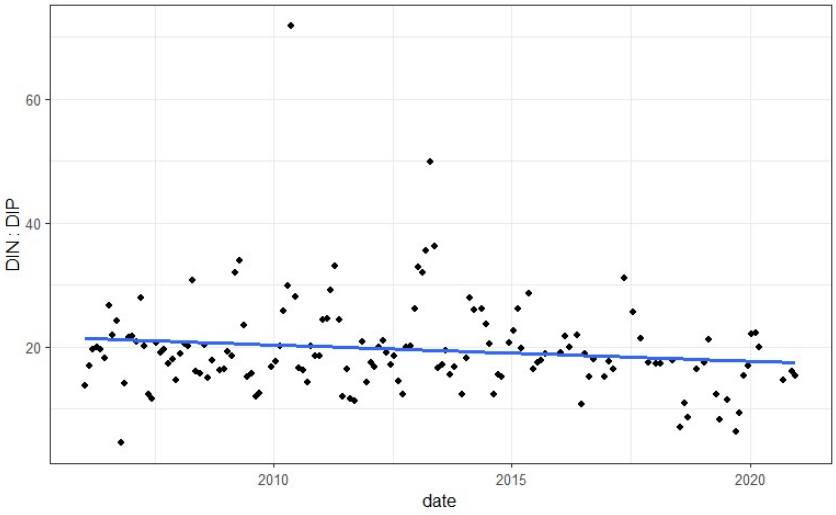

Figure 7.5 – DIN : DIP as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

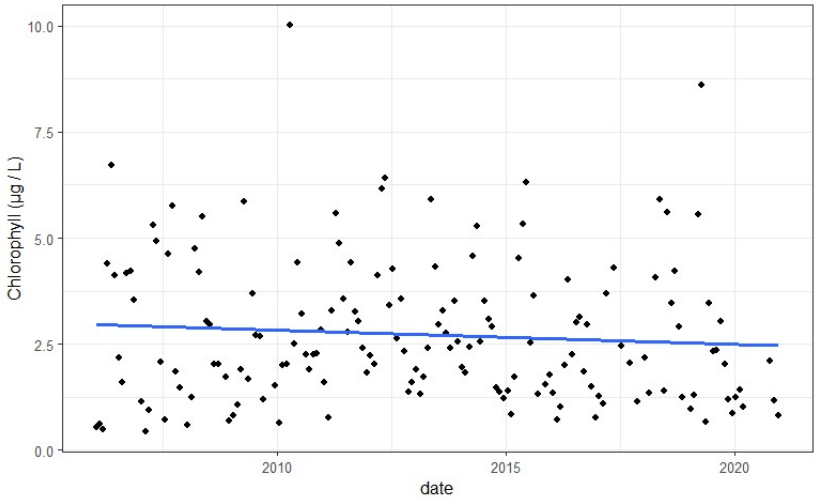

Figure 7.6 – Chlorophyll concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

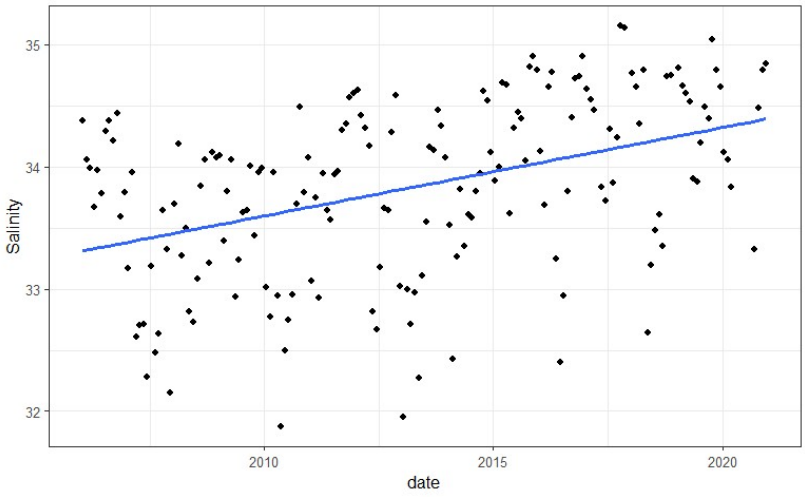

Figure 7.7 - Salinity as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

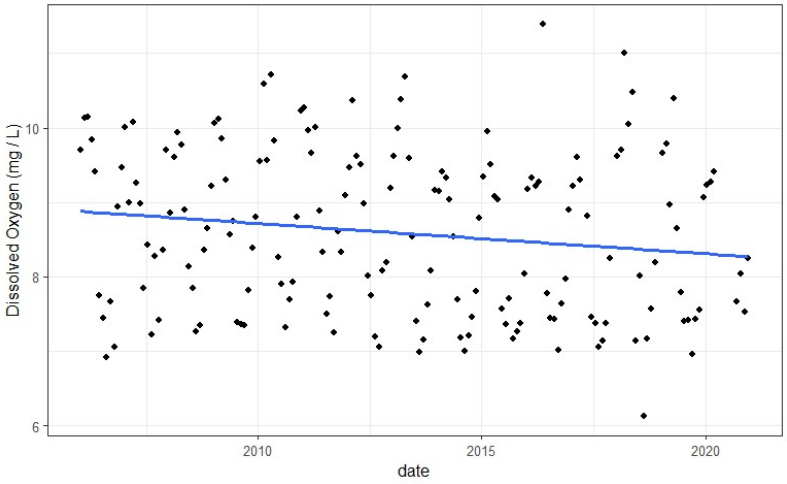

Figure 7.8 – Dissolved oxygen concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.2 Essex

Table 7.2 - *p* values from the results of the linear models in the Essex assessment area.

Variable	***P* value**
Chlorophyll	0.17
DIP	0.24
Ammonium	0.00
TOxN	0.46
Salinity	0.00
DIN : DIP	0.31
DIN	0.83
Dissolved Oxygen	0.06

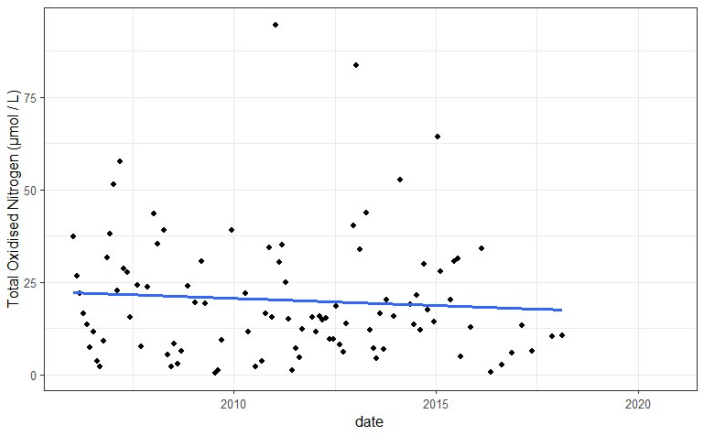

Figure 7.9 – TOxN concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved TOxN.

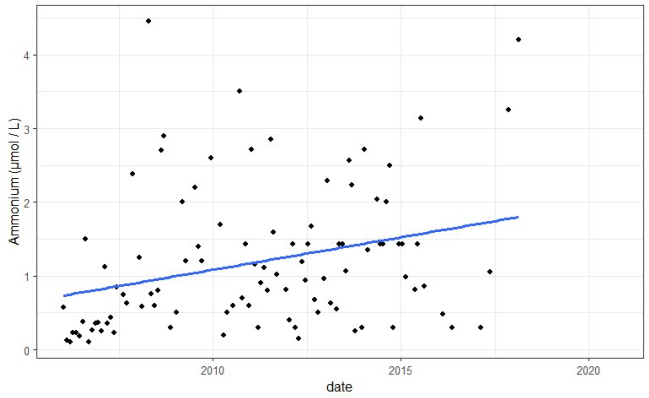

Figure 7.10 - Ammonium concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved ammonium.

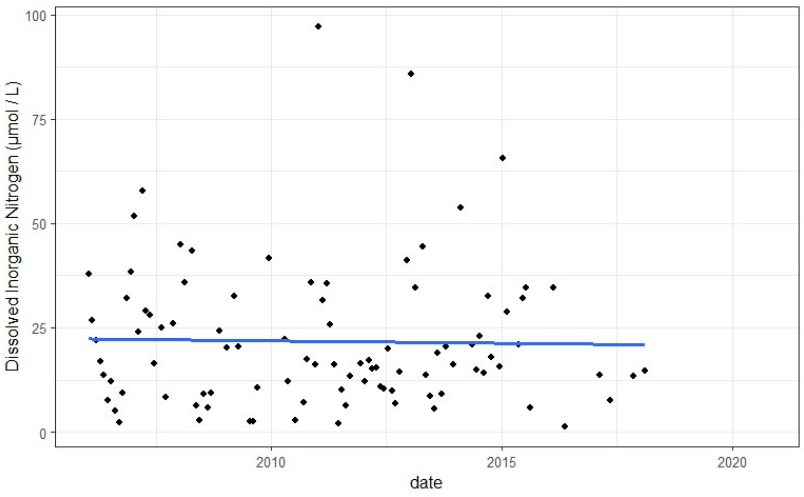

Figure 7.11 - DIN concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved DIN.

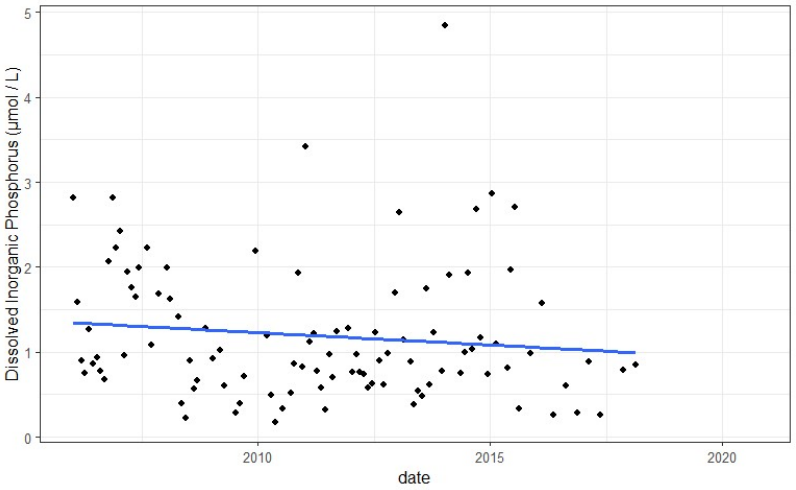

Figure 7.12 – DIP concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved DIP.

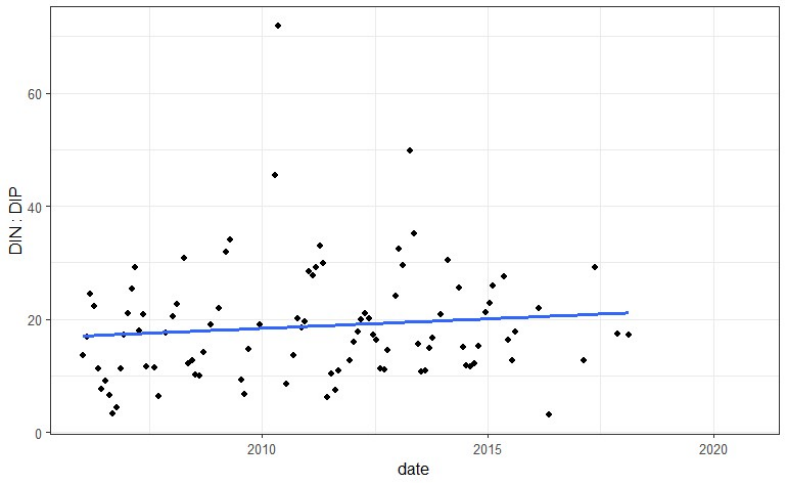

Figure 7.13 – DIN : DIP as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved DIN : DIP.

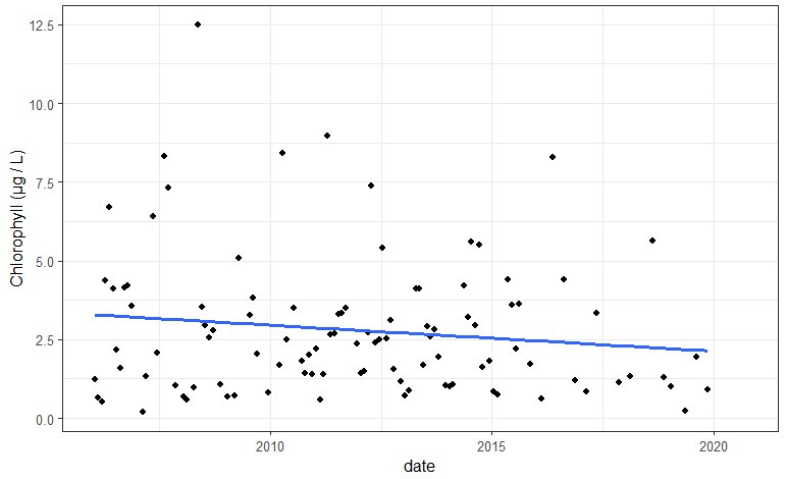

Figure 7.14 - Chlorophyll concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved chlorophyll.

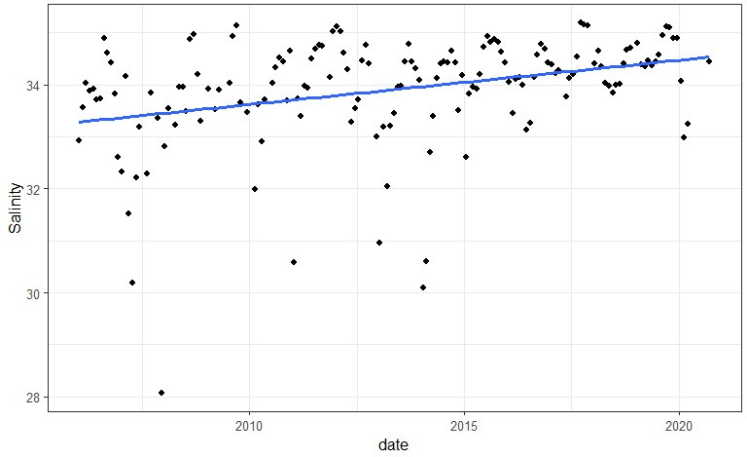

Figure 7.15 - Salinity as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

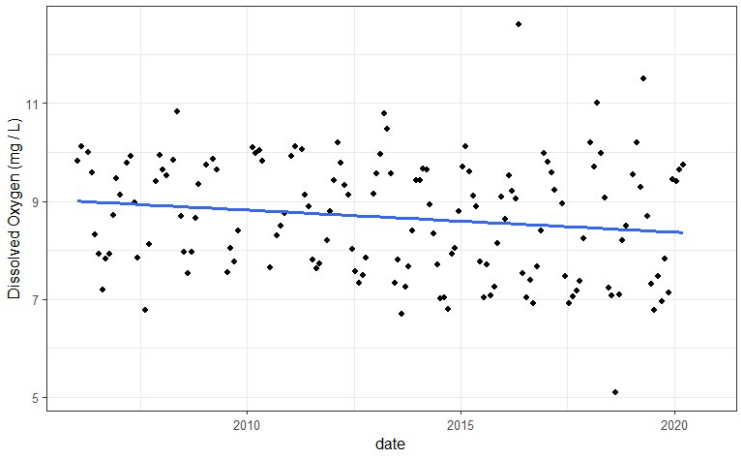

Figure 7.16 – Dissolved oxygen concentrations as a function of time in the Thames plume. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.3 Thames Lower

Table 7.3 - *p* Values from the results of the linear models in the Thames lower assessment area.

Variable	***p* value**
Chlorophyll	0.01
DIP	0.08
Ammonium	0.00
TOxN	0.45
Salinity	0.01
DIN : DIP	0.90
DIN	0.40
Dissolved Oxygen	0.58

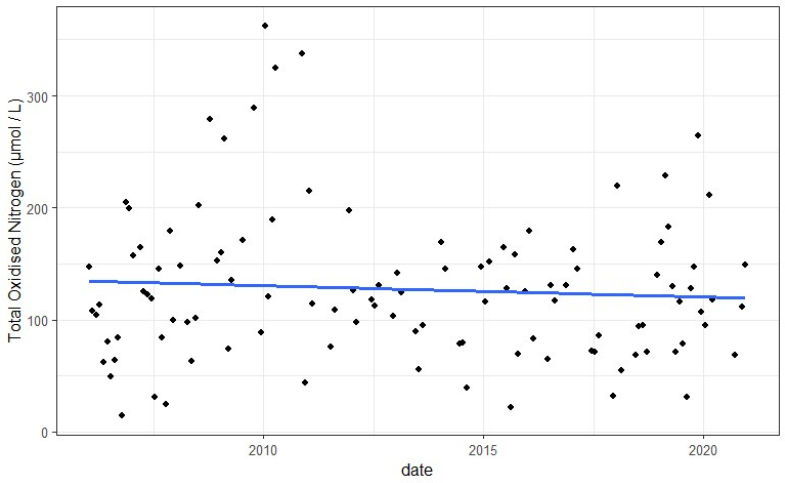

Figure 7.17 – TOxN concentrations as a function of time in the Thames lower assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

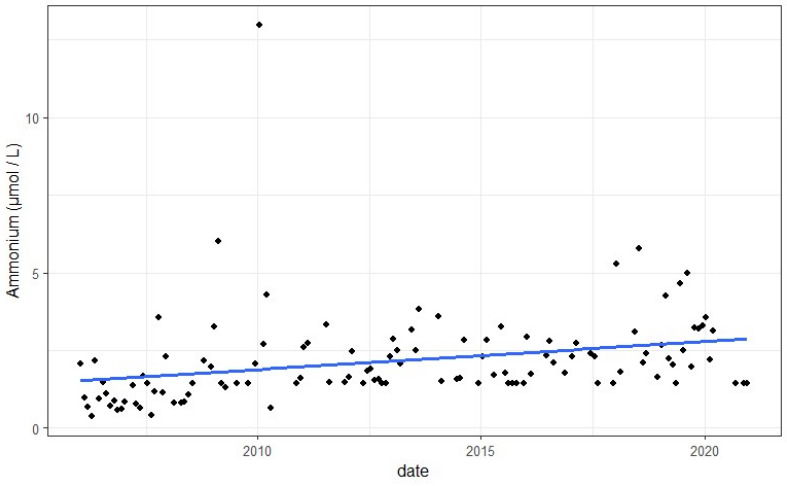

Figure 7.18 - Ammonium concentrations as a function of time in the Thames lower assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

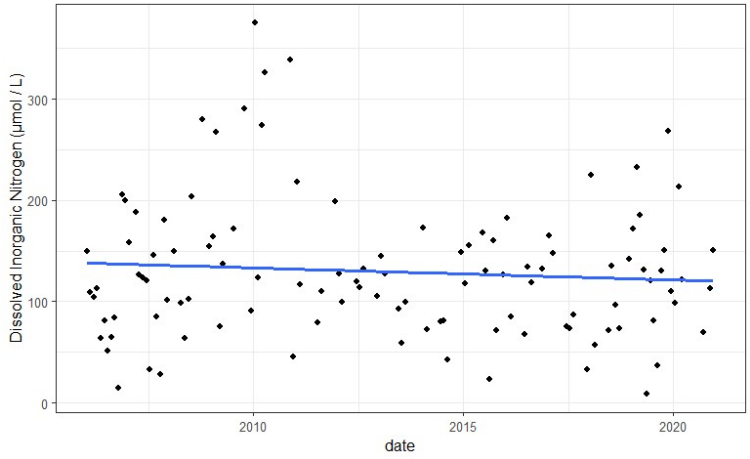

Figure 7.19 - DIN concentrations as a function of time in the Thames lower assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

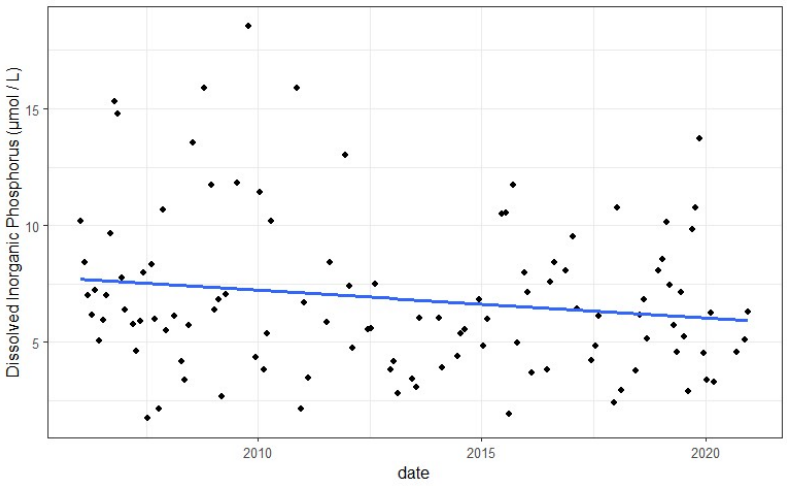

Figure 7.20 - DIP concentrations as a function of time in the Thames lower assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

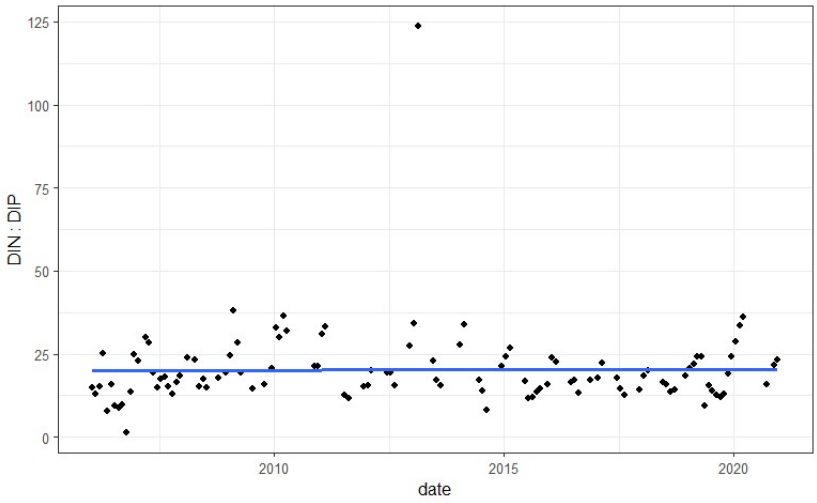

Figure 7.21 – DIN : DIP as a function of time in the Thames lower assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

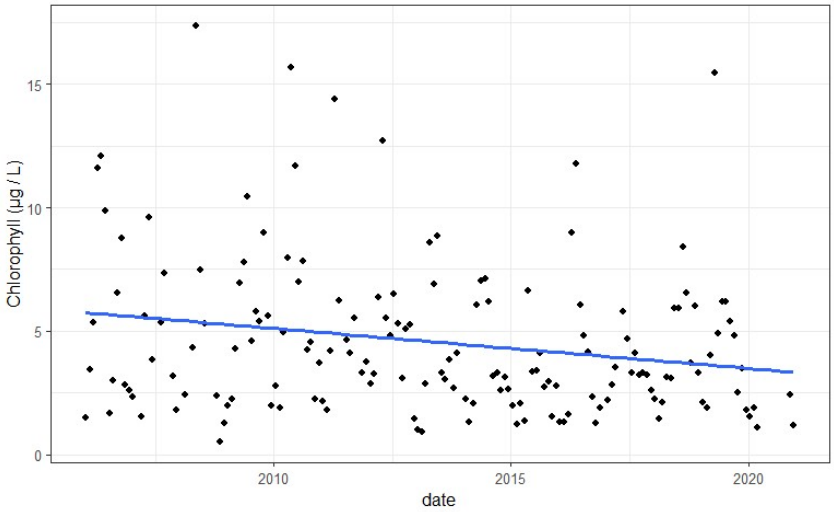

Figure 7.22 - Chlorophyll concentrations as a function of time in the Thames lower assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

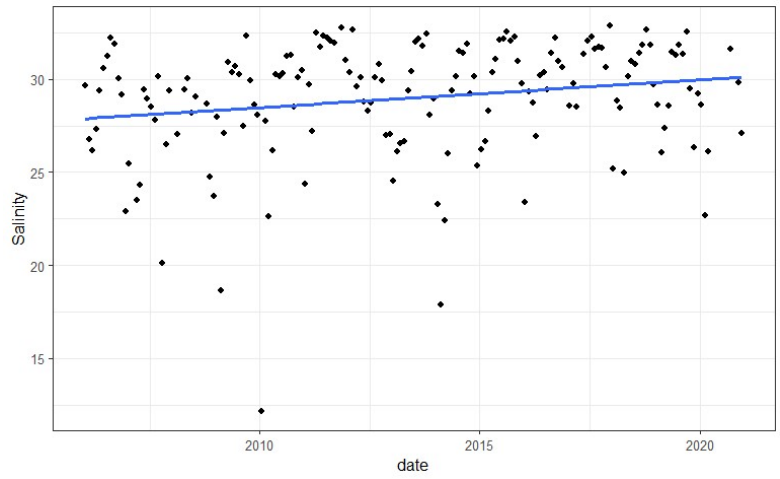

Figure 7.23 - Salinity as a function of time in the Thames lower assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

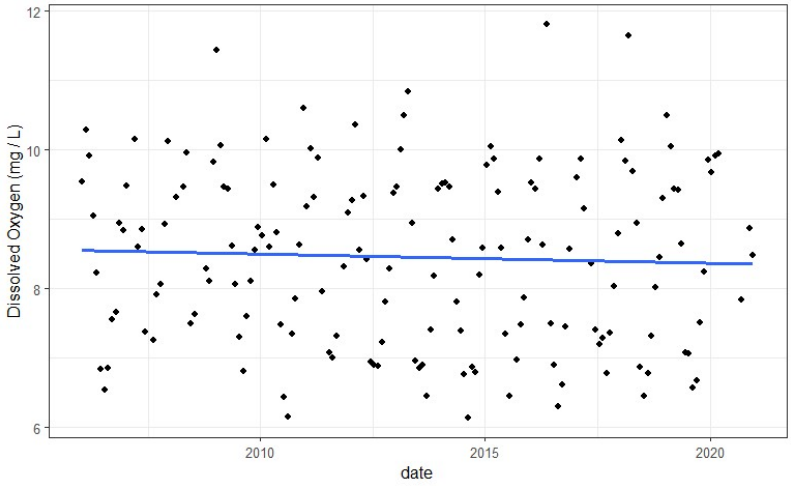

Figure 7.24 – Dissolved oxygen concentrations as a function of time in the Thames lower assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.4 Thames Middle

Table 7.4 - *p* Values from the results of the linear models in the Thames middle assessment area.

Variable	***P* value**
Chlorophyll	0.03
DIP	0.77
Ammonium	0.69
NO2	0.03
TOxN	0.00
Salinity	0.00
DIN : DIP	0.42
DIN	0.00
Dissolved Oxygen	0.00

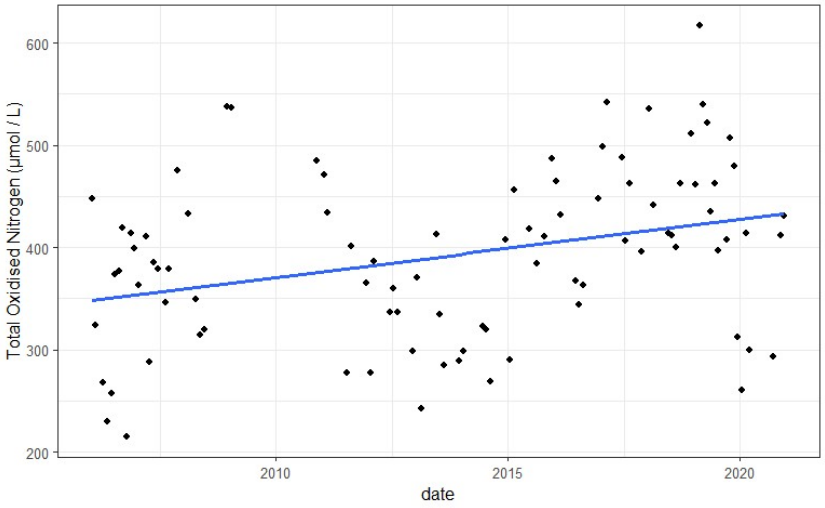

Figure 7.25 - TOxN concentrations as a function of time in the Thames middle assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

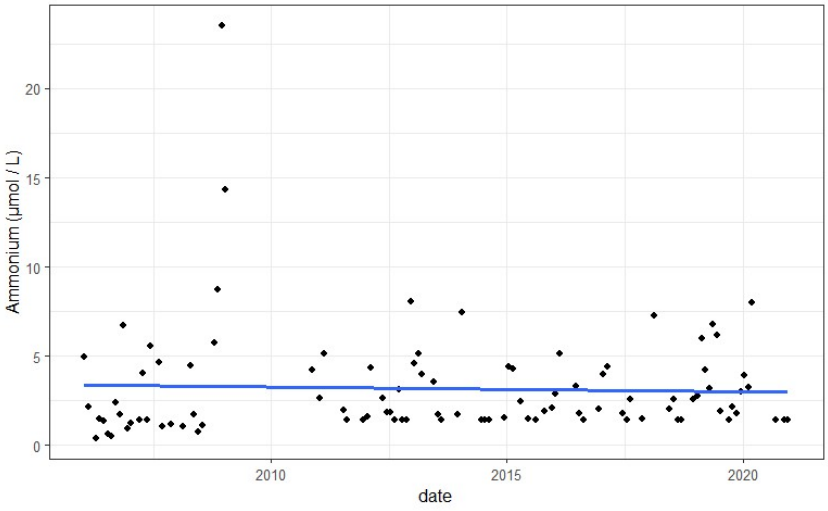

Figure 7.26 – Ammonium concentrations as a function of time in the Thames middle assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

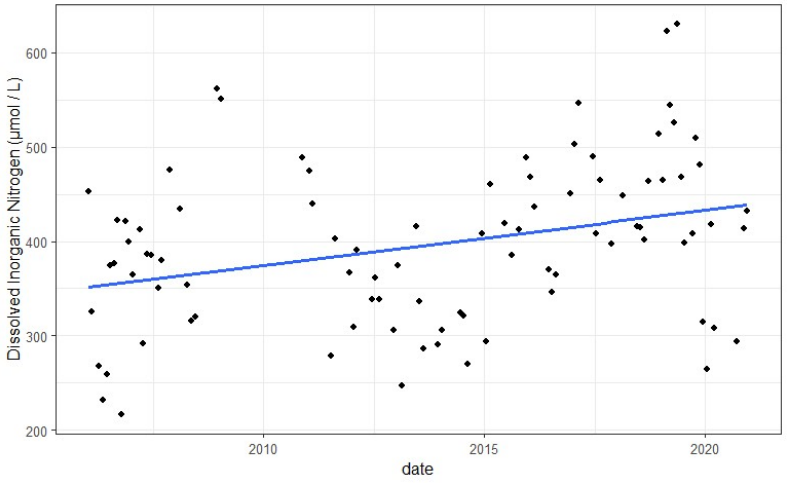

Figure 7.27 - DIN concentrations as a function of time in the Thames middle assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

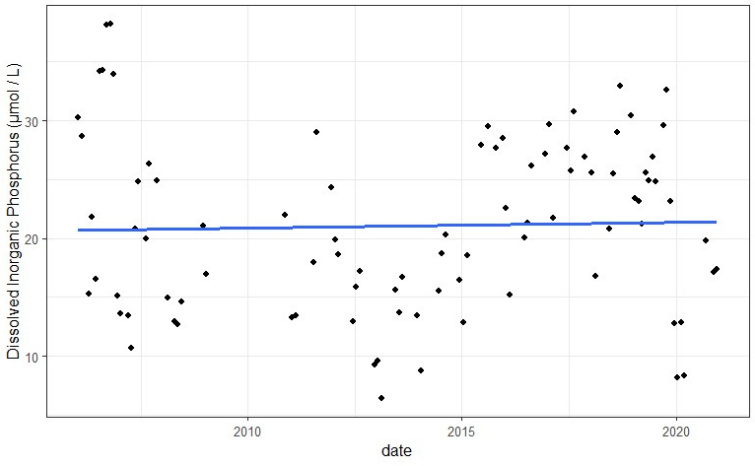

Figure 7.28 - DIP concentrations as a function of time in the Thames middle assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

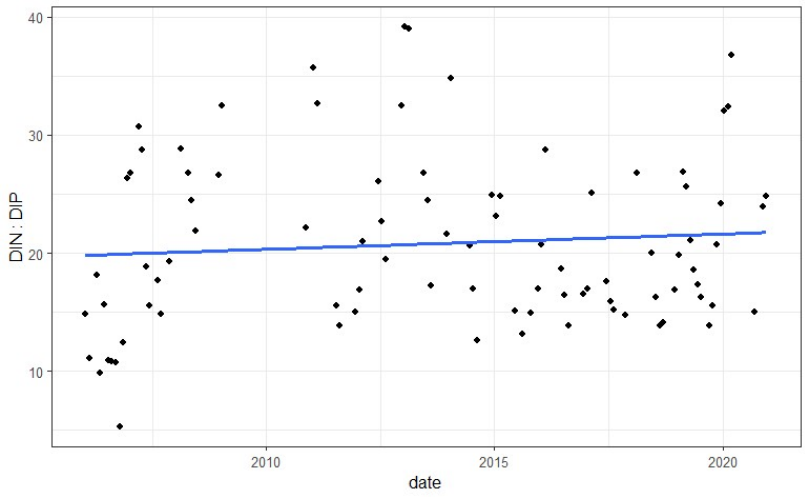

Figure 7.29 – DIN : DIP as a function of time in the Thames middle assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

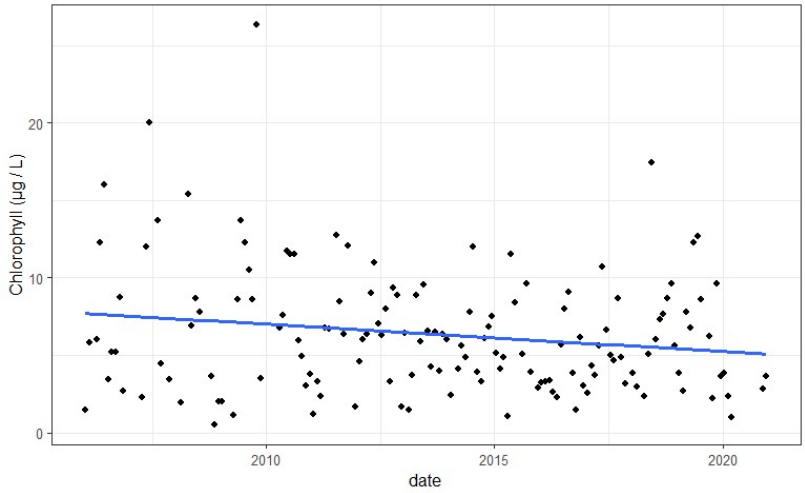

Figure 7.30 - Chlorophyll concentrations as a function of time in the Thames middle assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

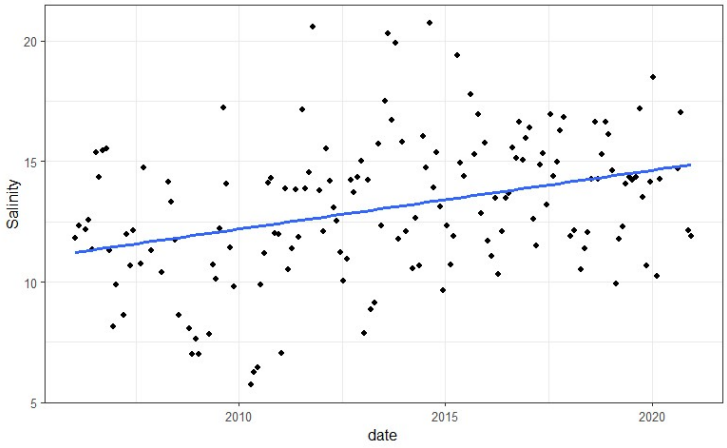

Figure 7.31 - Salinity as a function of time in the Thames middle assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

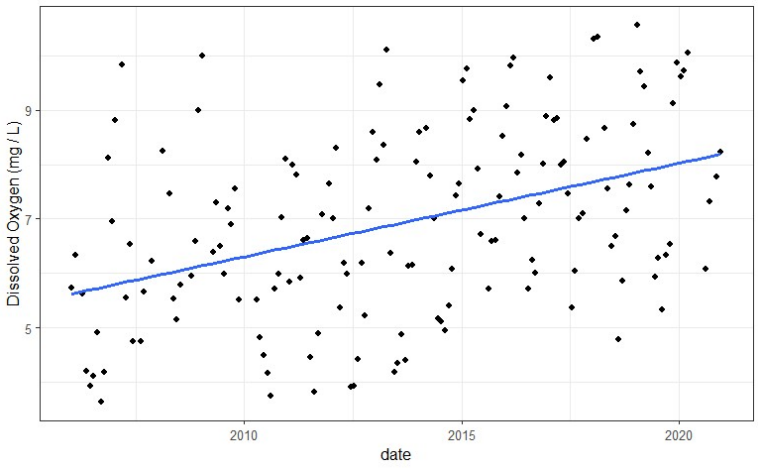

Figure 7.32 – Dissolved oxygen concentrations as a function of time in the Thames middle assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.5 Thames Coastal South

Table 7.5 - *p* Values from the results of the linear models in the Thames Coastal South assessment area.

Variable	***P* value**
Chlorophyll	0.83
DIP	0.28
Ammonium	0.74
TOxN	0.80
Salinity	0.35
DIN : DIP	0.82
DIN	0.92
Dissolved Oxygen	0.62

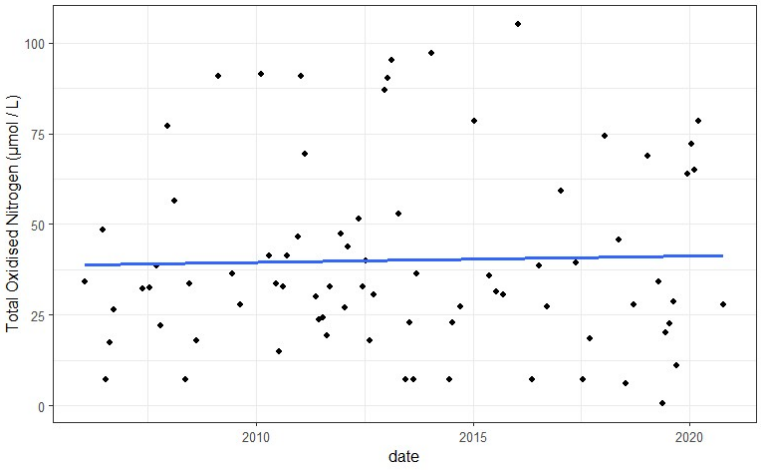

Figure 7.33 - TOxN concentrations as a function of time in the Thames Coastal South assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

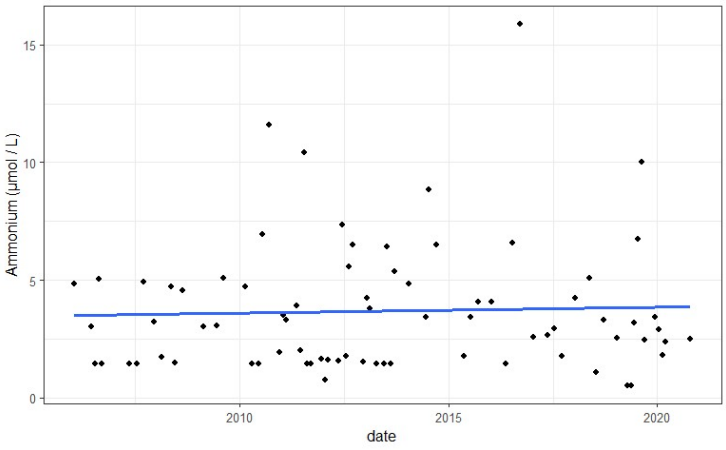

Figure 7.34 - Ammonium concentrations as a function of time in the Thames Coastal South assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

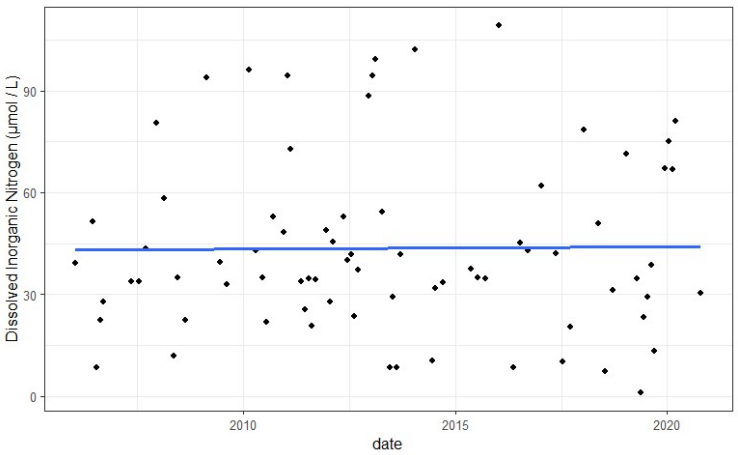

Figure 7.35 - DIN concentrations as a function of time in the Thames Coastal South assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

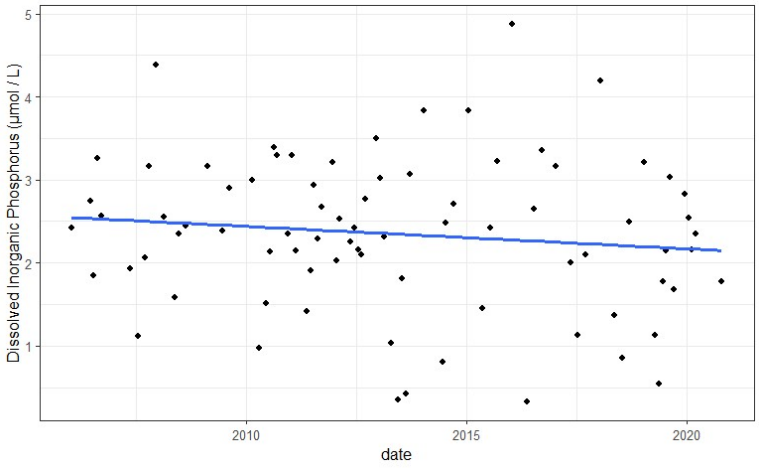

Figure 7.36 – DIP concentrations as a function of time in the Thames Coastal South assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

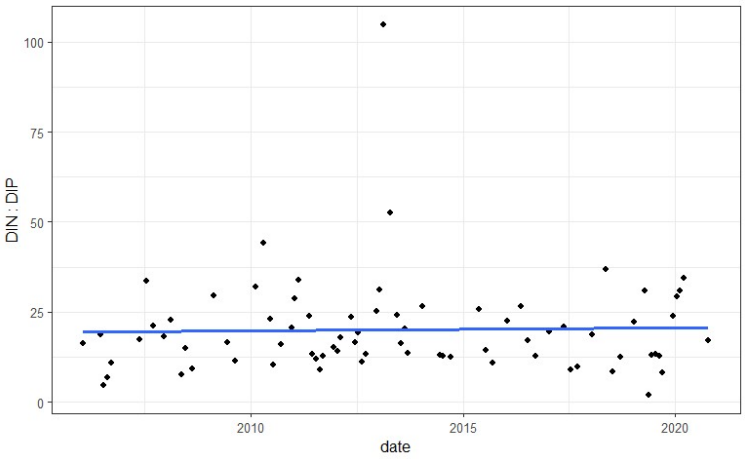

Figure 7.37 – DIN : DIP as a function of time in the Thames Coastal South assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN: DIP.

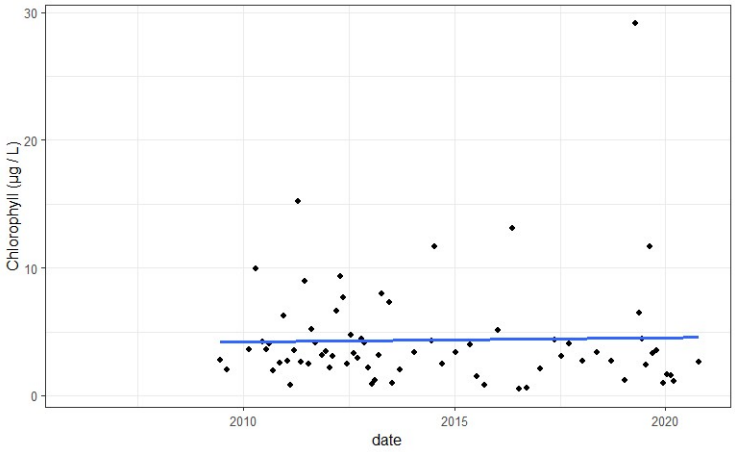

Figure 7.38 - Chlorophyll concentrations as a function of time in the Thames Coastal South assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

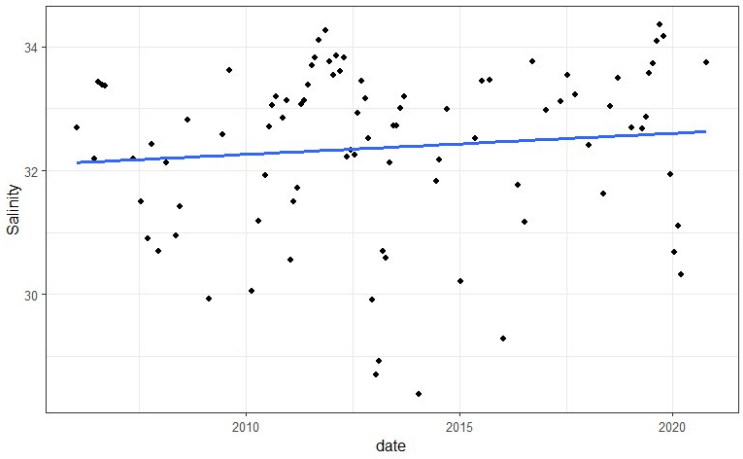

Figure 7.39 – Salinity as a function of time in the Thames Coastal South assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

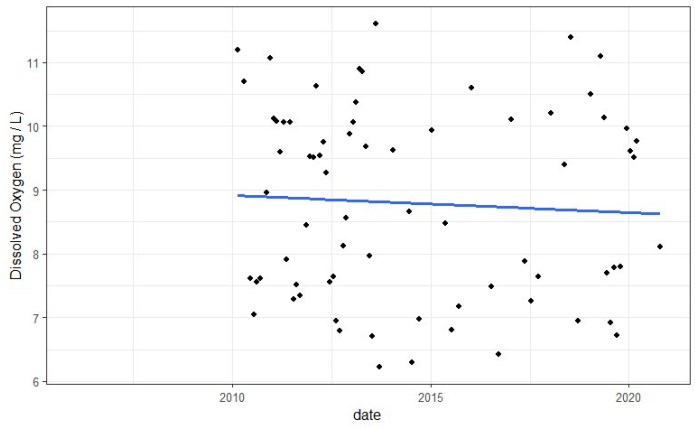

Figure 7.40 – Dissolved oxygen concentrations as a function of time in the Thames Coastal South assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.6 Whitstable Bay

Table 7.6 - *p* Values from the results of the linear models in the Whitstable Bay assessment area.

Variable	***P* value**
Chlorophyll	0.35
DIP	0.67
Ammonium	0.16
TOxN	0.62
Salinity	0.12
DIN : DIP	0.68
DIN	0.67
Dissolved Oxygen	0.89

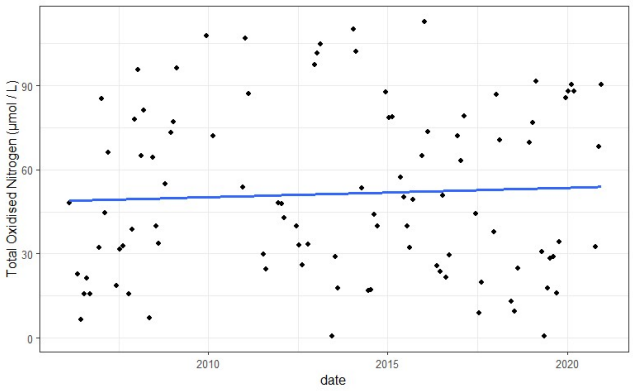

Figure 7.41 - TOxN concentrations as a function of time in the Whitstable Bay assessment are. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

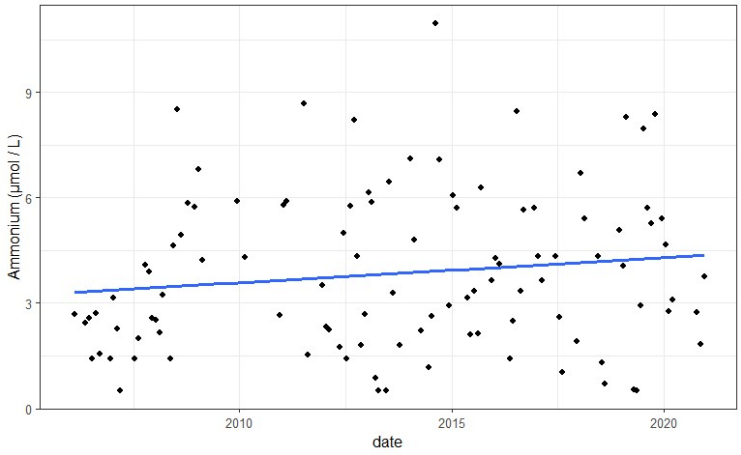

Figure 7.42 - Ammonium concentrations as a function of time in the Whitstable Bay assessment are. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

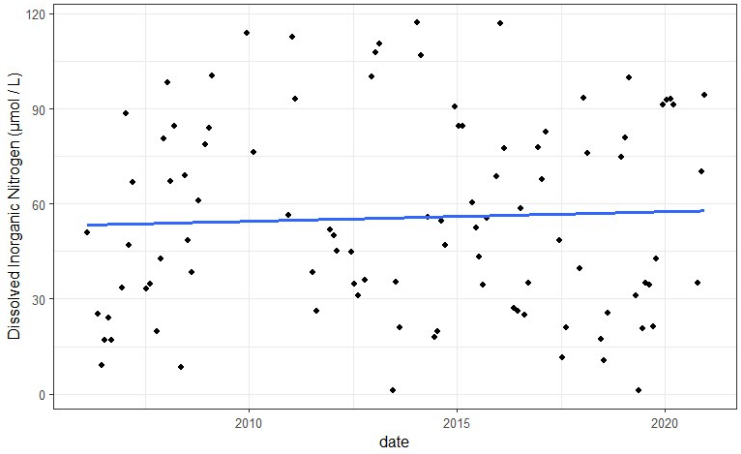

Figure 7.43 - DIN concentrations as a function of time in the Whitstable Bay assessment are. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

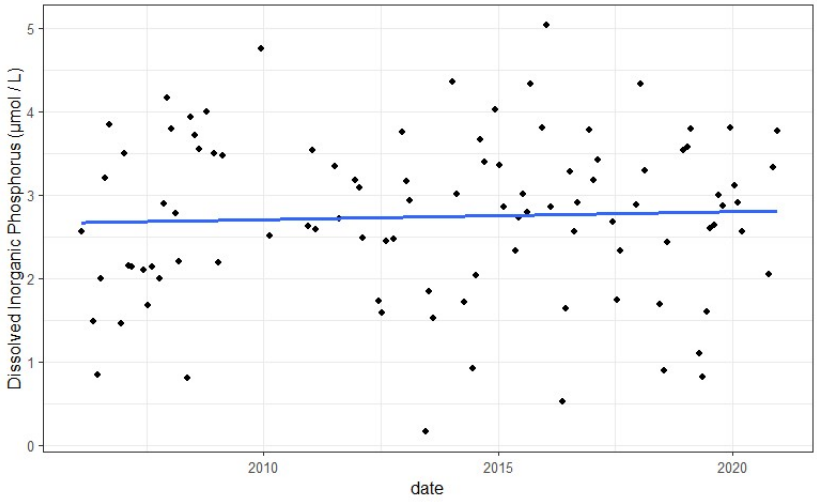

Figure 7.44 – DIP concentrations as a function of time in the Whitstable Bay assessment are. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

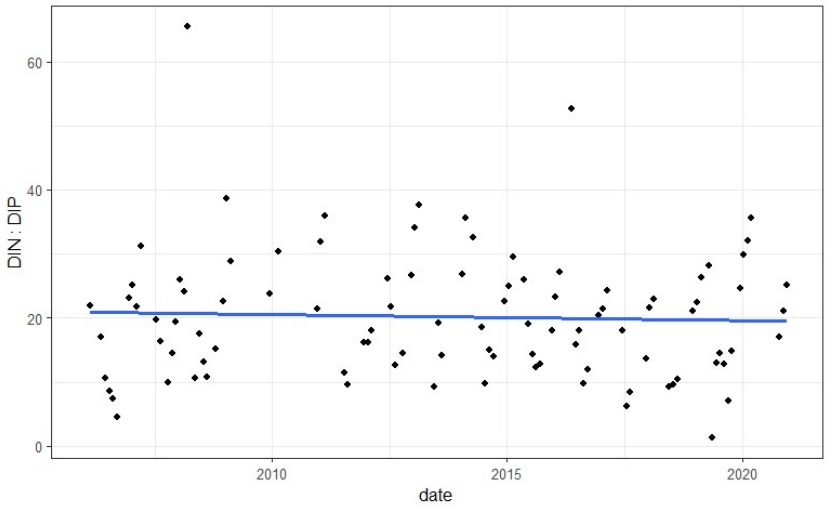

Figure 7.45 – DIN : DIP concentrations as a function of time in the Whitstable Bay assessment are. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

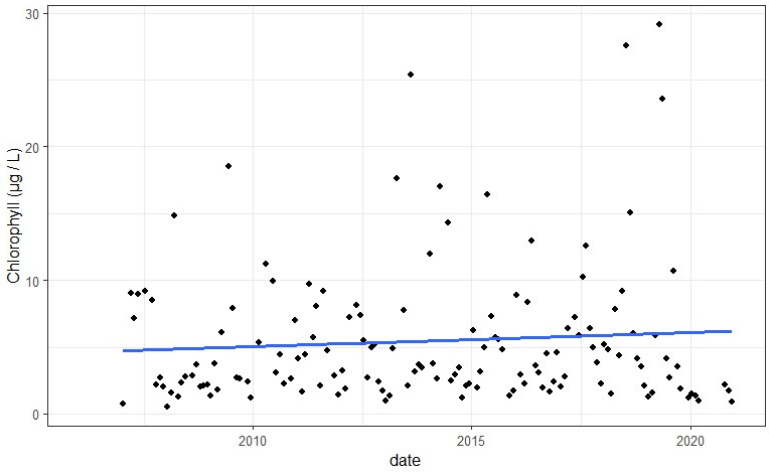

Figure 7.46 - Chlorophyll concentrations as a function of time in the Whitstable Bay assessment are. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

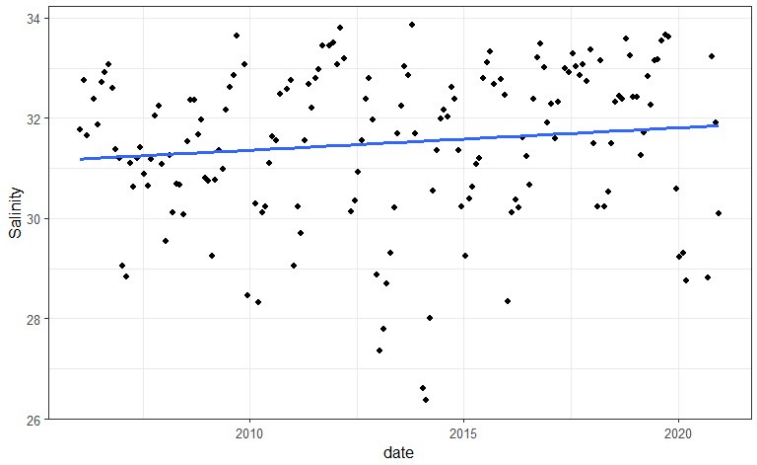

Figure 7.47 - Salinity as a function of time in the Whitstable Bay assessment are. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

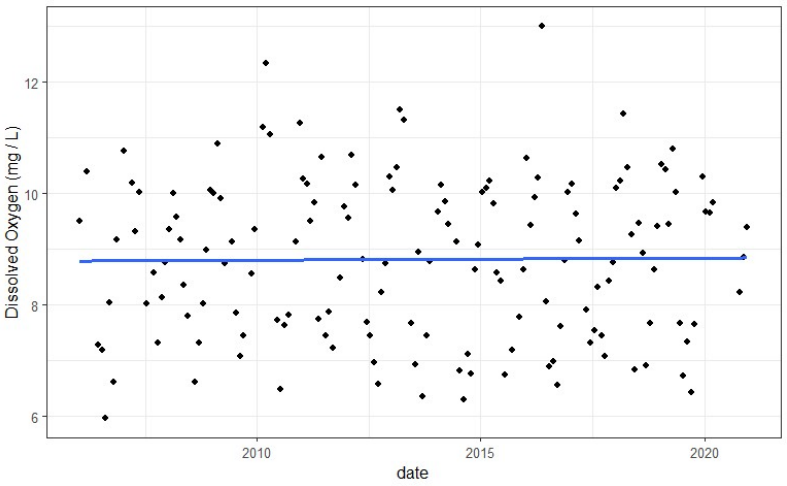

Figure 7.48 – Dissolved oxygen concentrations as a function of time in the Whitstable Bay assessment are. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.7 Kent North

Table 7.7 - *p* Values from the results of the linear models in the Kent North assessment area.

Variable	***P* value**
Chlorophyll	0.86
DIP	0.00
Ammonium	0.00
TOxN	0.08
Salinity	0.00
DIN : DIP	0.47
DIN	0.05
Dissolved Oxygen	0.30

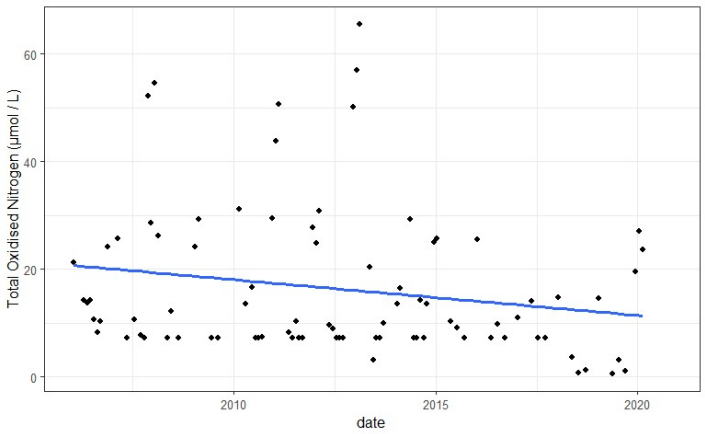

Figure 7.49 - TOxN concentrations as a function of time in the Kent north assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

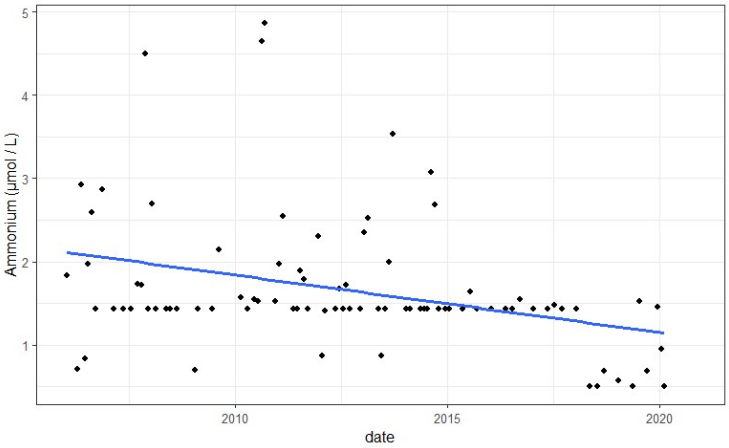

Figure 7.50 - Ammonium concentrations as a function of time in the Kent north assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

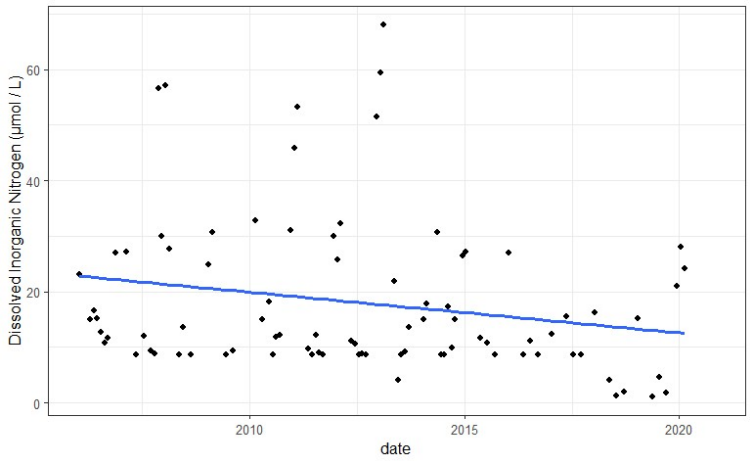

Figure 7.51 - DIN concentrations as a function of time in the Kent north assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

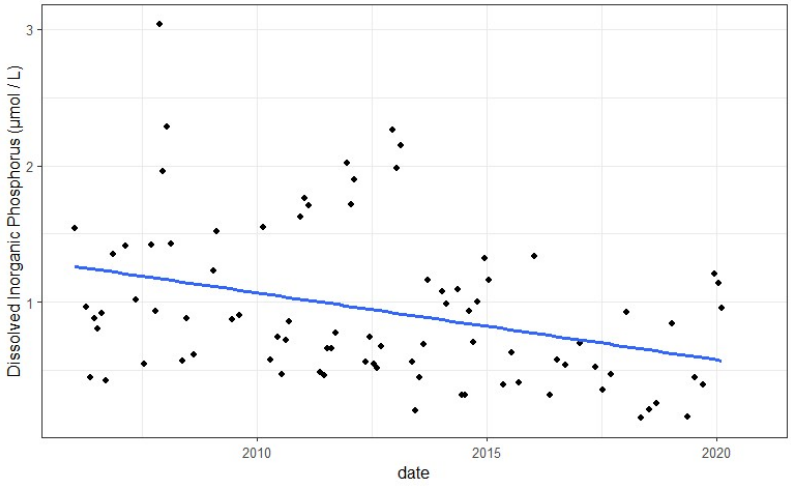

Figure 7.52 - DIP concentrations as a function of time in the Kent north assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

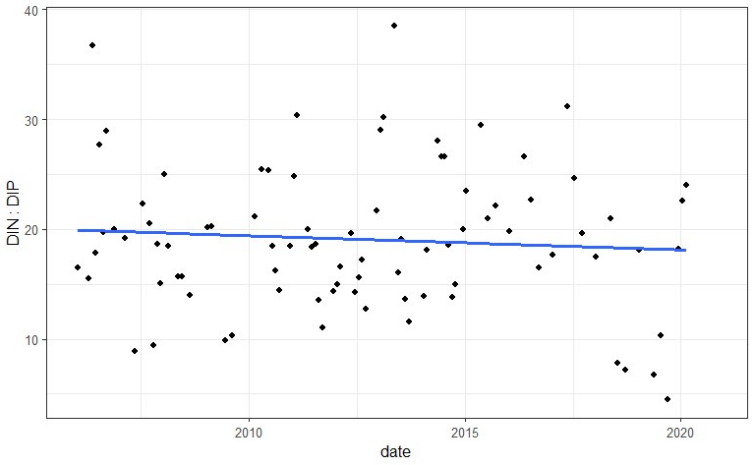

Figure 7.53 – DIN : DIP as a function of time in the Kent north assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

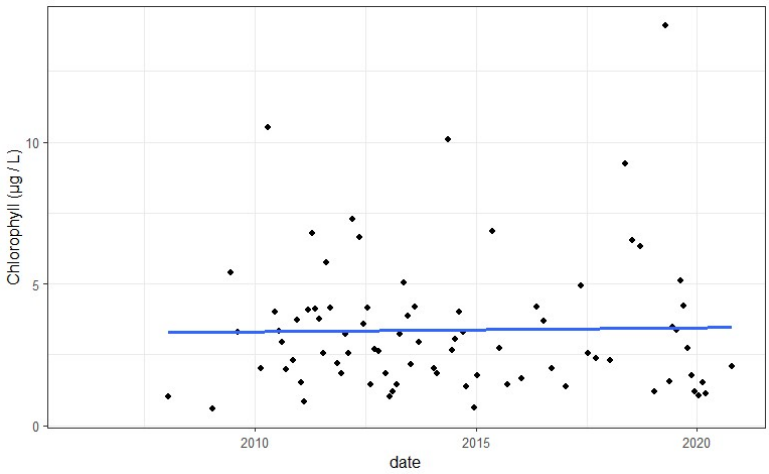

Figure 7.54 - Chlorophyll concentrations as a function of time in the Kent north assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

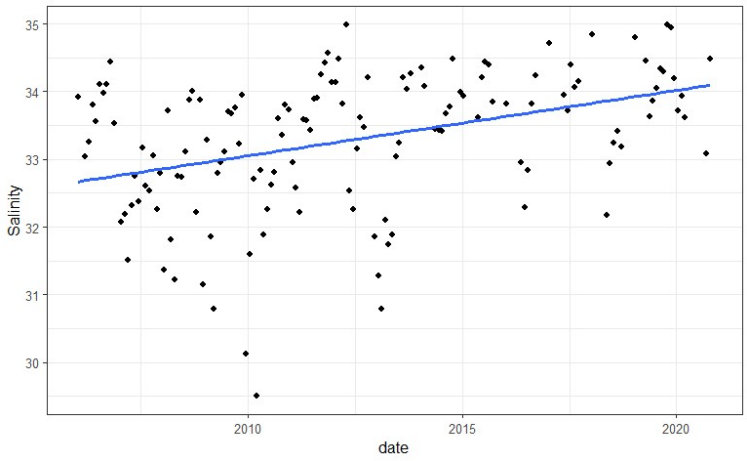

Figure 7.55 - Salinity as a function of time in the Kent north assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

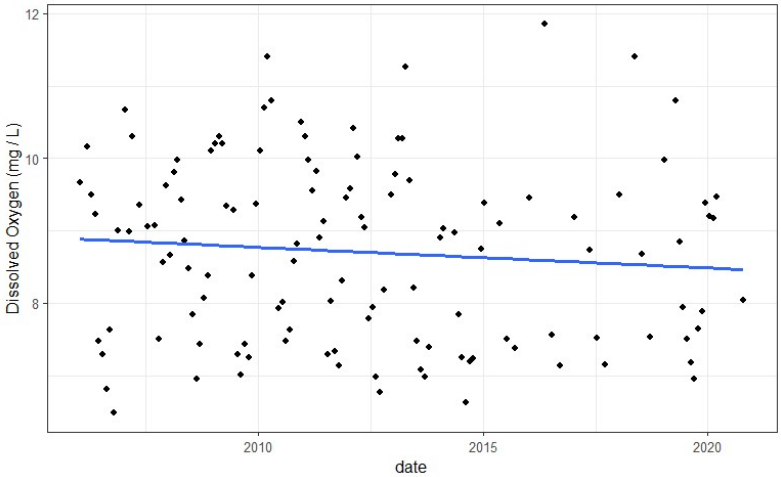

Figure 7.56 – Dissolved oxygen concentrations as a function of time in the Kent north assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.8 Blackwater Outer

Table 7.8 - *p* Values from the results of the linear models in the Blackwater Outer assessment area.

Variable	*P* value
Chlorophyll	0.19
DIP	0.55
Ammonium	0.96
TOxN	0.72
Salinity	0.01
DIN : DIP	0.50
DIN	0.73
Dissolved Oxygen	0.42

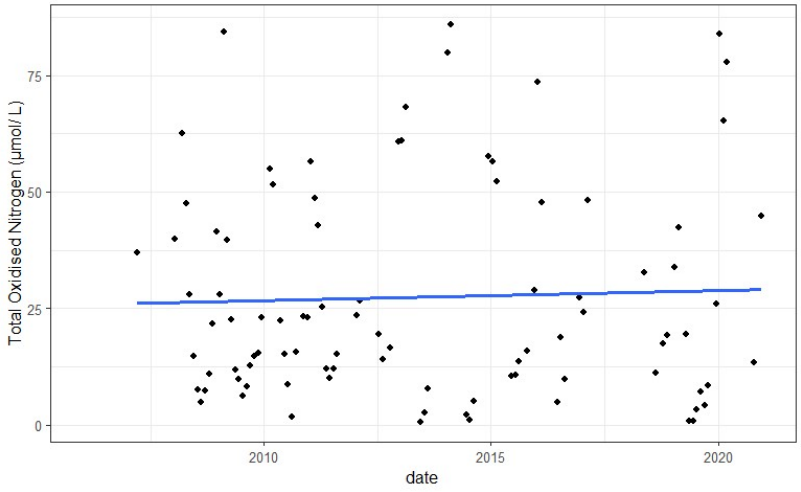

Figure 7.57 - TOxN concentrations as a function of time in the Blackwater Outer assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

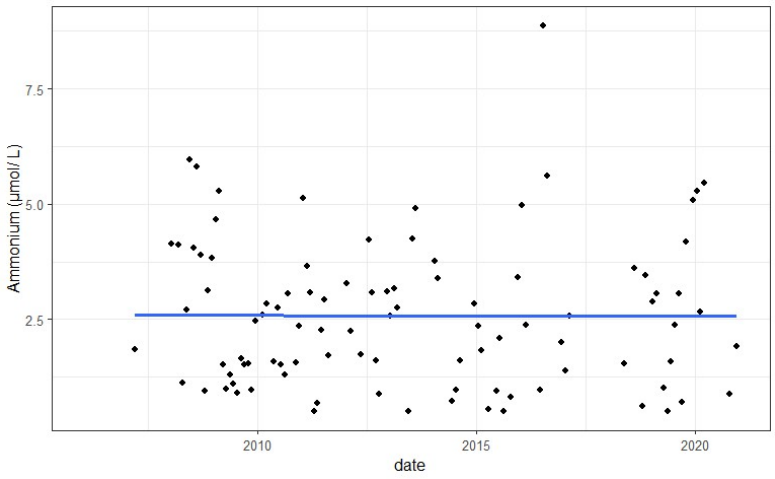

Figure 7.58 - Ammonium concentrations as a function of time in the Blackwater Outer assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

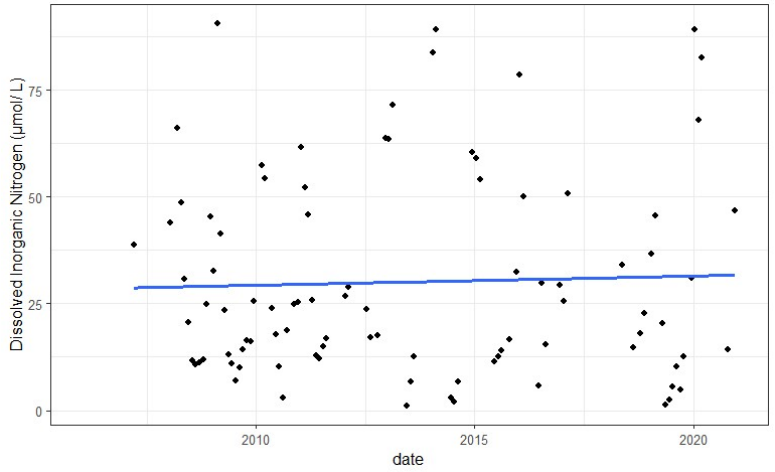

Figure 7.59 – DIN concentrations as a function of time in the Blackwater Outer assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

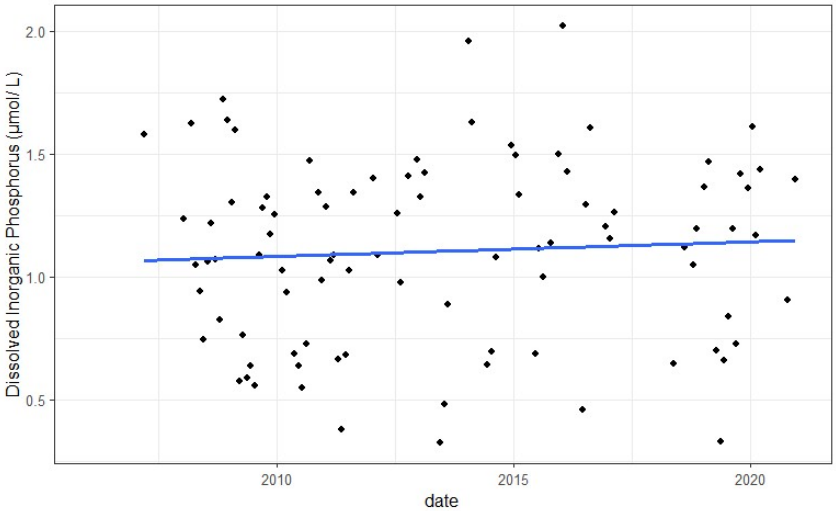

Figure 7.60 - DIP concentrations as a function of time in the Blackwater Outer assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

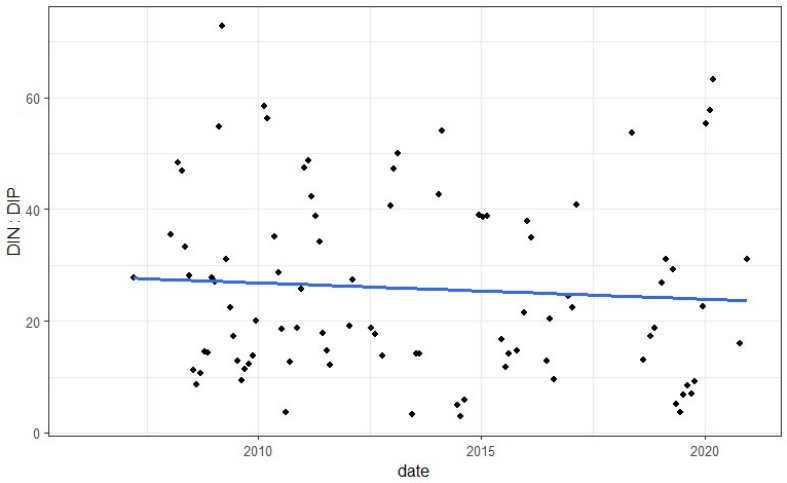

Figure 7.61 – DIN : DIP as a function of time in the Blackwater Outer assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

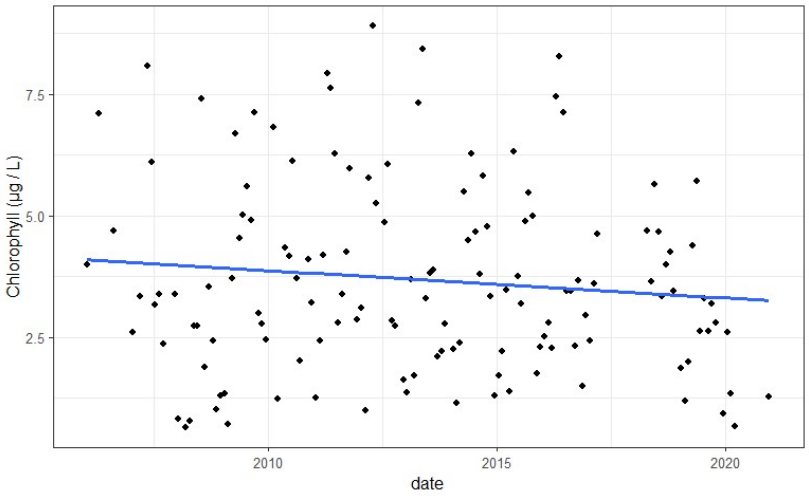

Figure 7.62 – Chlorophyll concentrations as a function of time in the Blackwater Outer assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

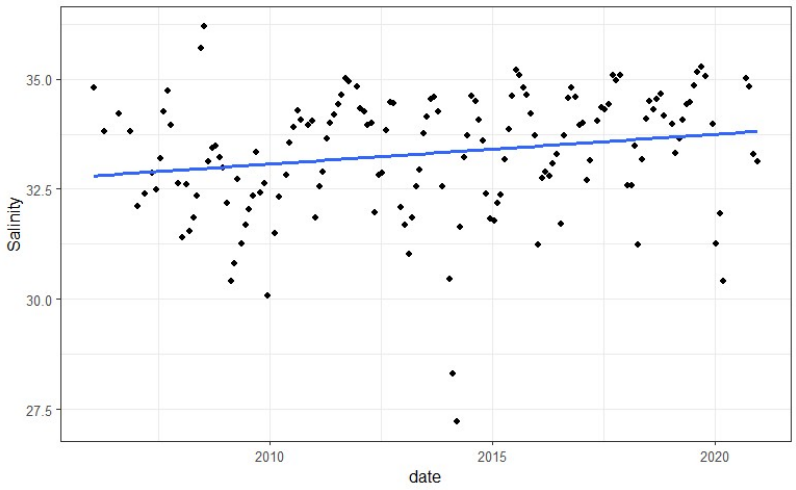

Figure 7.63 - Salinity as a function of time in the Blackwater Outer assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

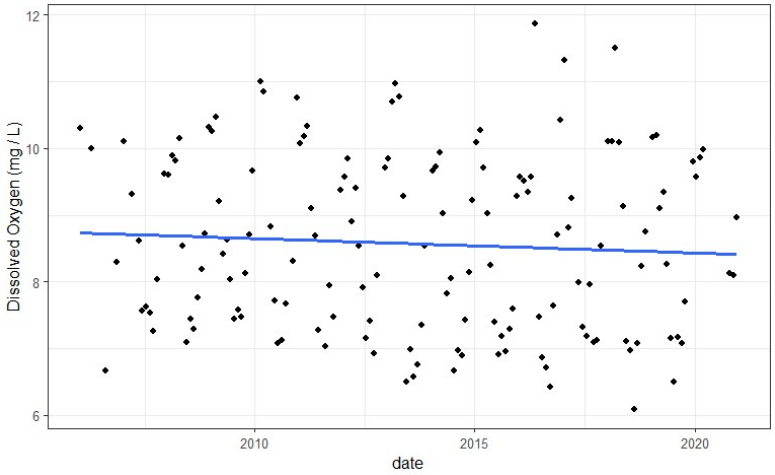

Figure 7.64 – Dissolved oxygen concentrations as a function of time in the Blackwater Outer assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.9 Medway

Table 7.9 - *p* Values from the results of the linear models in the Medway assessment area.

Variable	***P* value**
Chlorophyll	0.15
DIP	0.00
Ammonium	0.03
TOxN	0.00
Salinity	0.00
DIN : DIP	0.80
DIN	0.00
Dissolved Oxygen	0.82

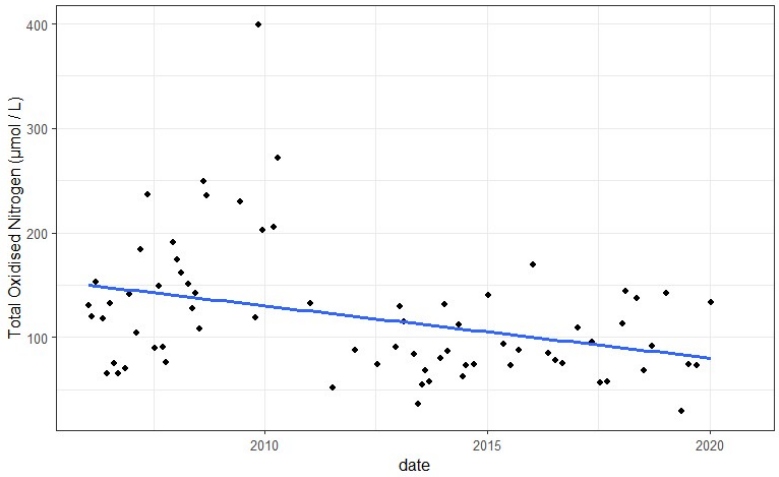

Figure 7.65 - TOxN concentrations as a function of time in the Medway assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

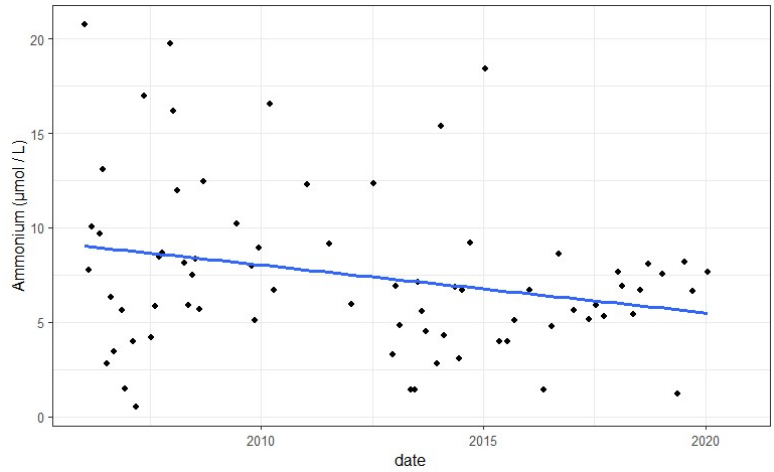

Figure 7.66 - Ammonium concentrations as a function of time in the Medway assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

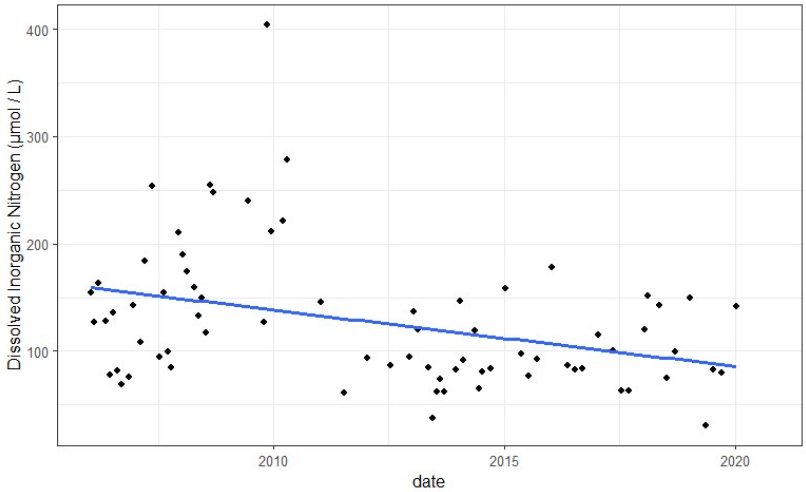

Figure 7.67 - DIN concentrations as a function of time in the Medway assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

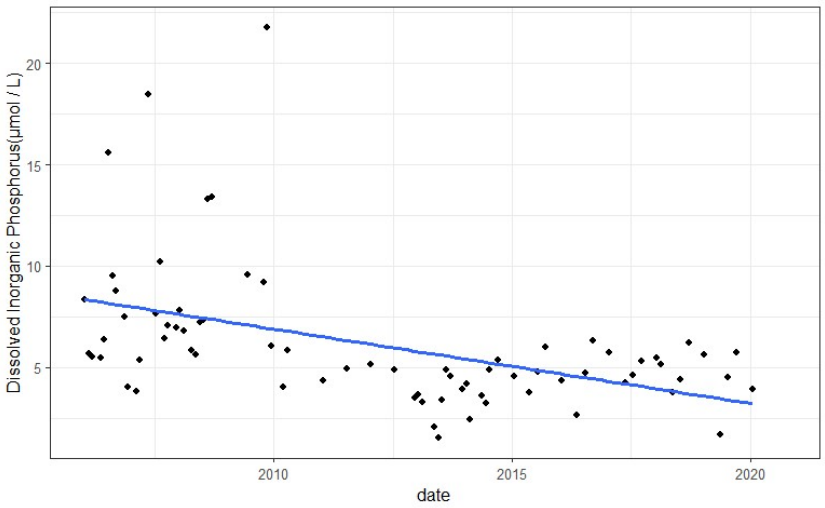

Figure 7.68 - DIP concentrations as a function of time in the Medway assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

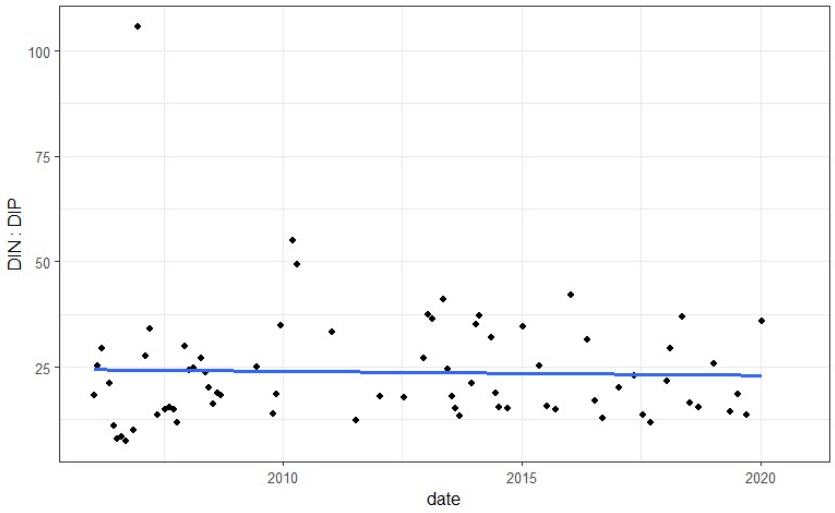

Figure 7.69 – DIN : DIP as a function of time in the Medway assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

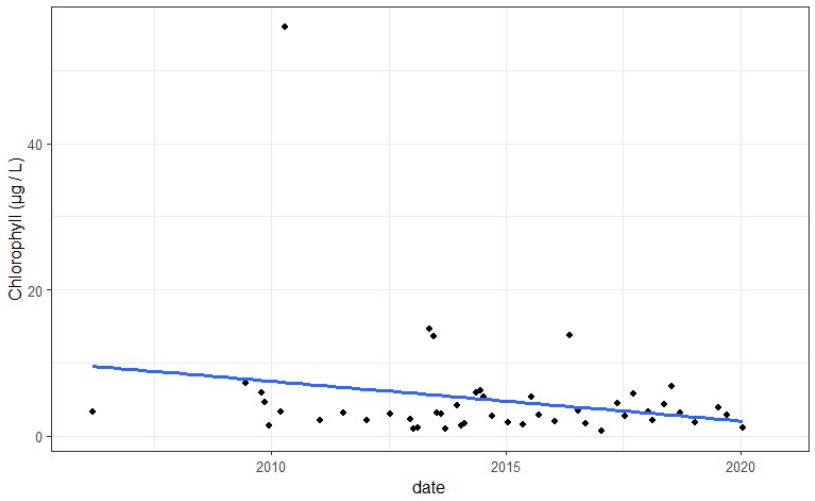

Figure 7.70 – Chlorophyll concentrations as a function of time in the Medway assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

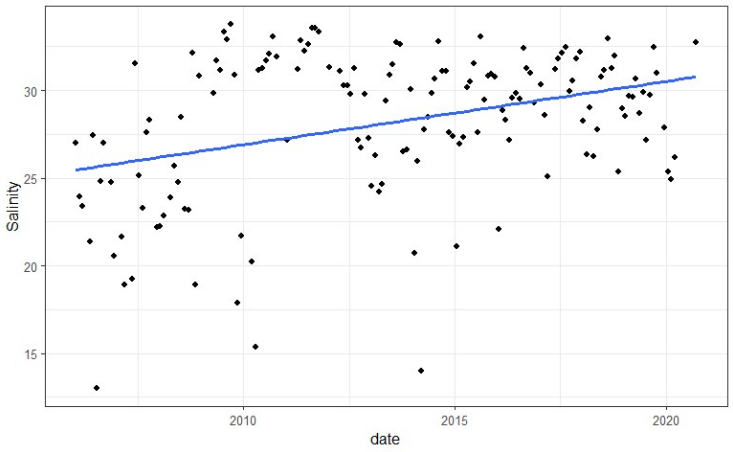

Figure 7.71 - Salinity as a function of time in the Medway assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

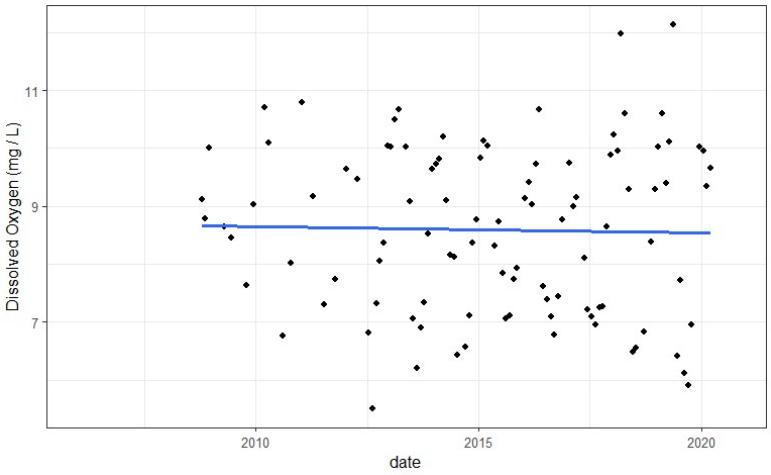

Figure 7.72 – Dissolved oxygen concentrations as a function of time in the Medway assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.10 Blackwater

Table 7.10 - *p* Values from the results of the linear models in the Blackwater assessment area.

Variable	***P* value**
Chlorophyll	0.00
DIP	0.06
Ammonium	0.00
TOxN	0.21
Salinity	0.86
DIN : DIP	0.45
DIN	0.16
Dissolved Oxygen	0.11

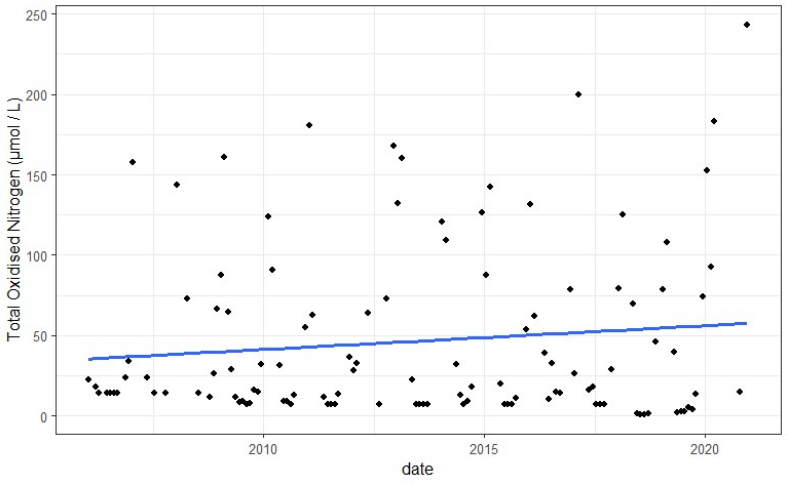

Figure 7.73 - TOxN concentrations as a function of time in the Blackwater assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

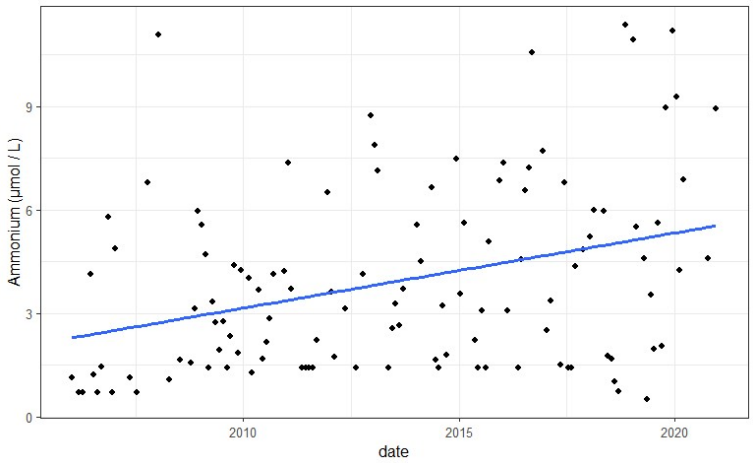

Figure 7.74 - Ammonium concentrations as a function of time in the Blackwater assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

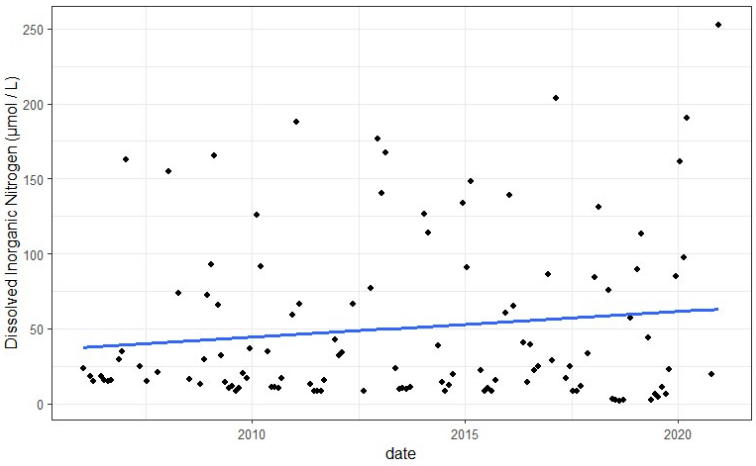

Figure 7.75 – DIN concentrations as a function of time in the Blackwater assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

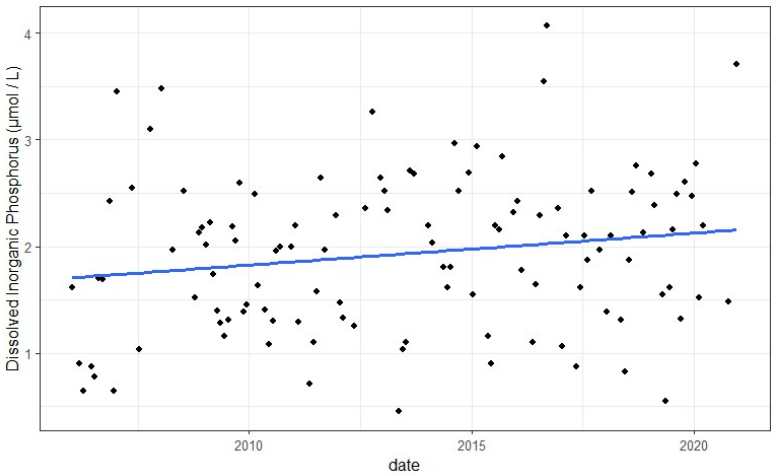

Figure 7.76 - DIP concentrations as a function of time in the Blackwater assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

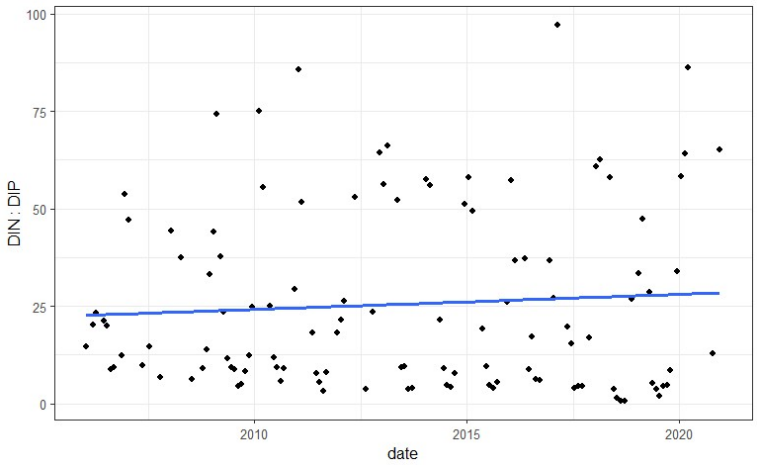

Figure 7.77 – DIN : DIP as a function of time in the Blackwater assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

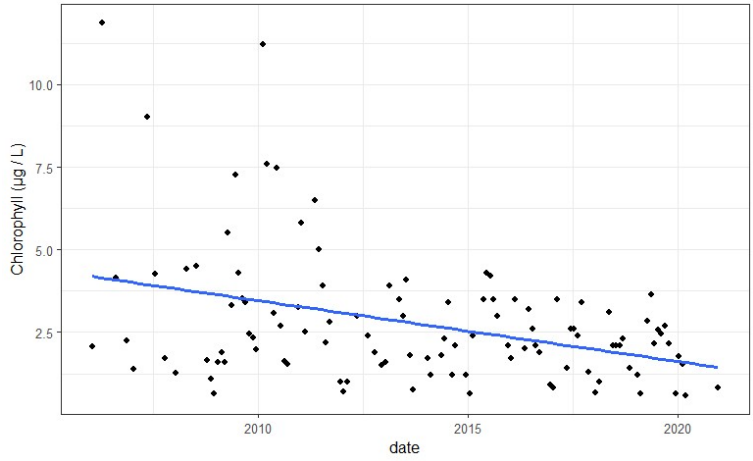

Figure 7.78 – Chlorophyll concentrations as a function of time in the Blackwater assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

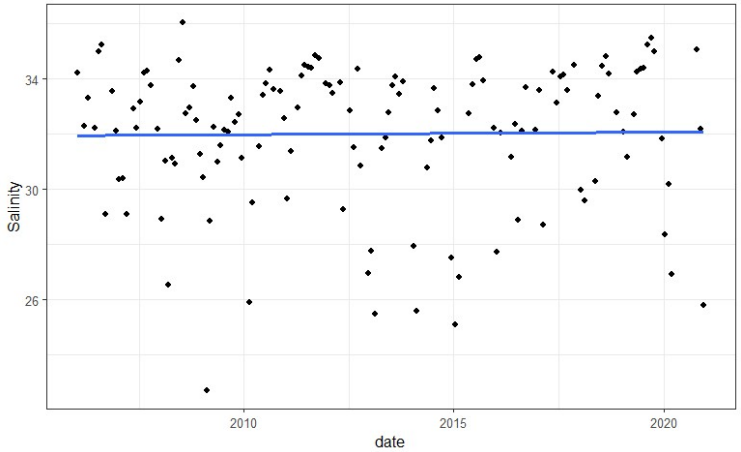

Figure 7.79 - Salinity as a function of time in the Blackwater assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

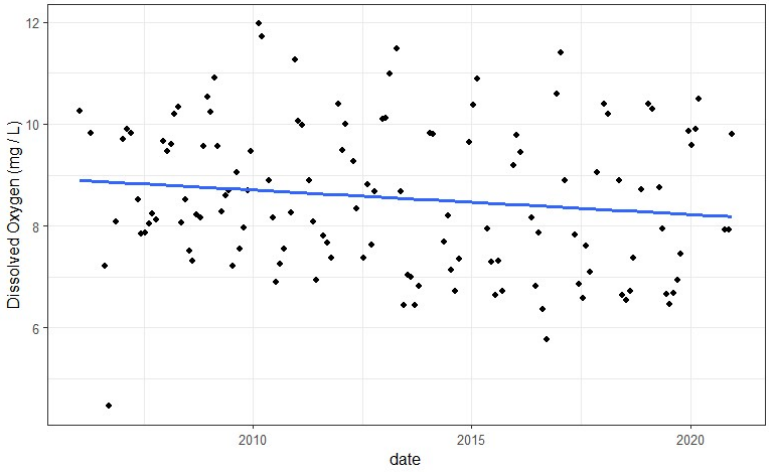

Figure 7.80 – Dissolved oxygen concentrations as a function of time in the Blackwater assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.11 Swale

Table 7.11 - *p* Values from the results of the linear models in the Swale assessment area.

Variable	***P* value**
Chlorophyll	0.00
DIP	0.79
Ammonium	0.40
TOxN	0.25
Salinity	0.09
DIN : DIP	0.10
DIN	0.20
Dissolved Oxygen	0.89

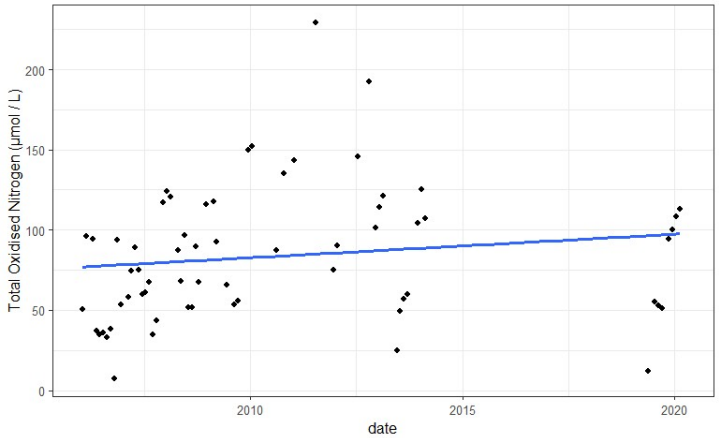

Figure 7.81 – TOxN concentrations as a function of time in the Swale assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

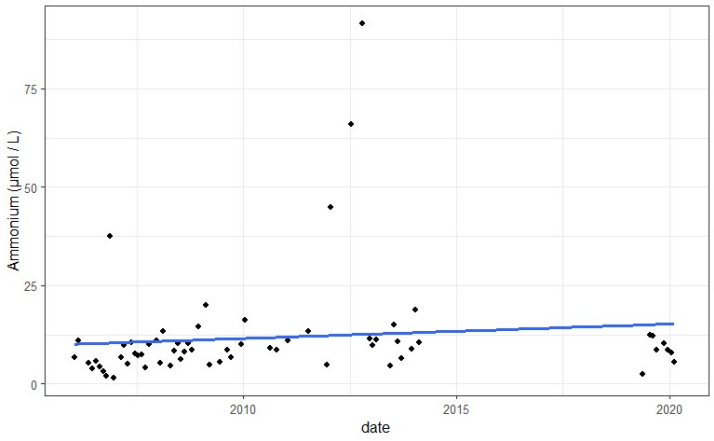

Figure 7.82 - Ammonium concentrations as a function of time in the Swale assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

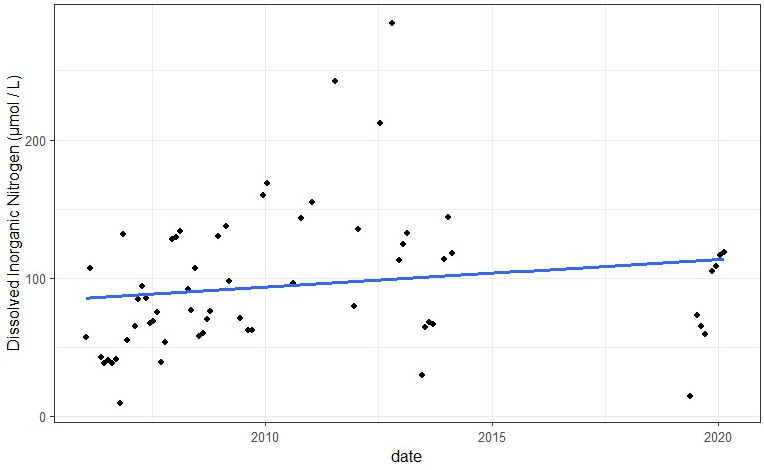

Figure 7.83 - DIN concentrations as a function of time in the Swale assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

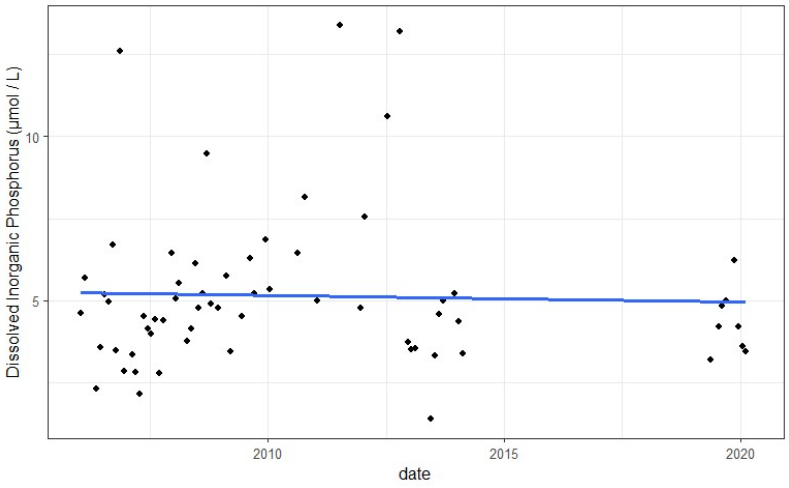

Figure 7.84 - DIP concentrations as a function of time in the Swale assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

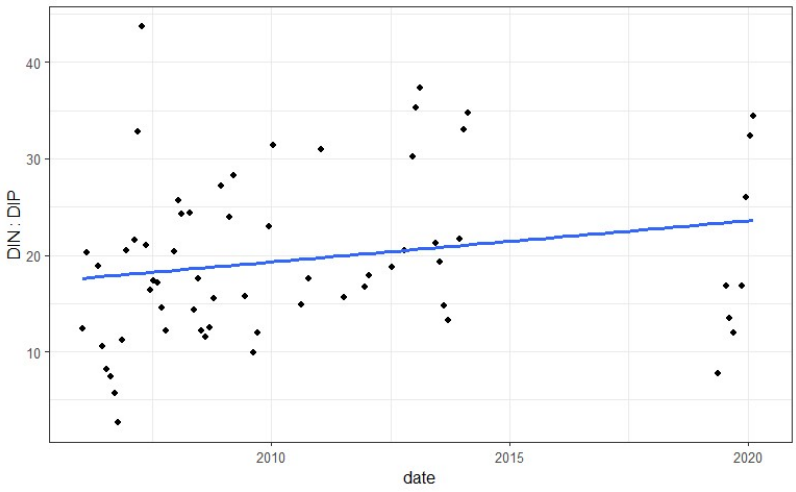

Figure 7.85 – DIN : DIP as a function of time in the Swale assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

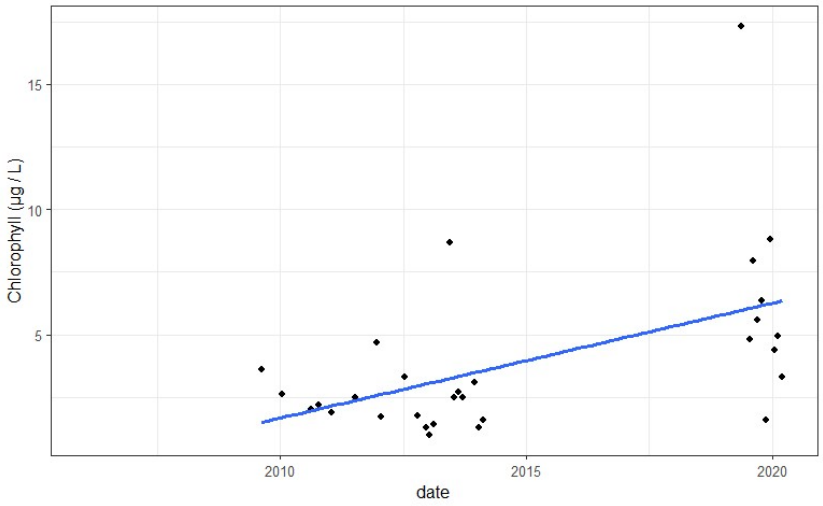

Figure 7.86 - Chlorophyll concentrations as a function of time in the Swale assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

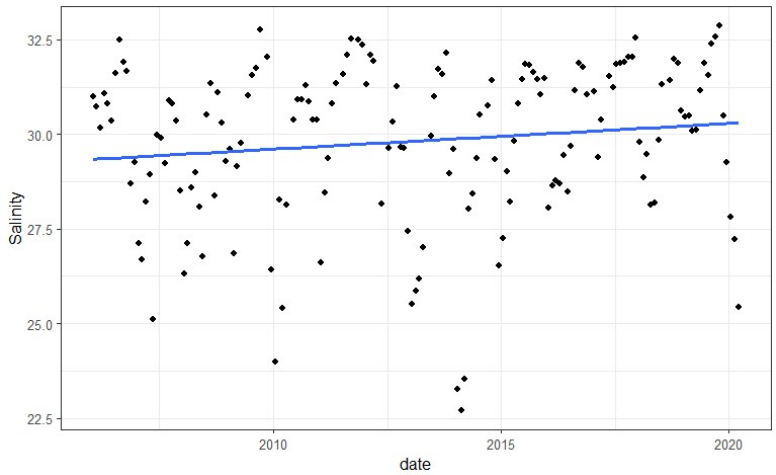

Figure 7.87- Salinity as a function of time in the Swale assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

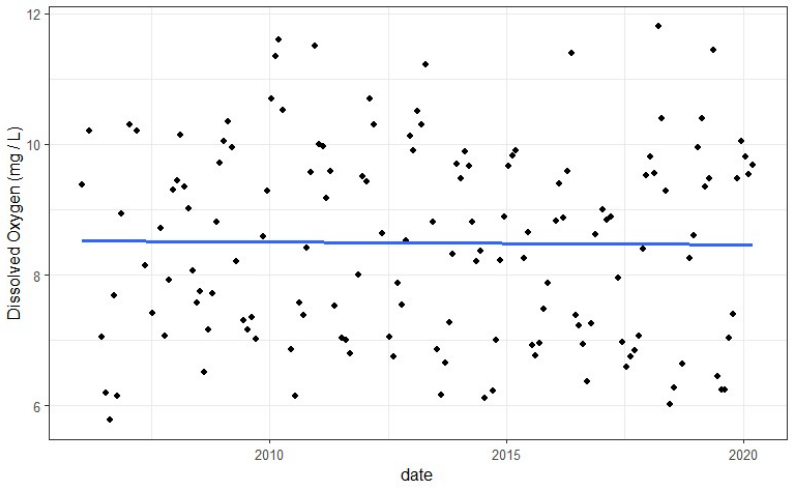

Figure 7.88 – Dissolved oxygen concentrations as a function of time in the Swale assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.12 Hamford Water

Table 7.12 - *p* Values from the results of the linear models in the Hamford Water assessment area.

Variable	***P* value**
Salinity	0.00
Dissolved oxygen	0.81

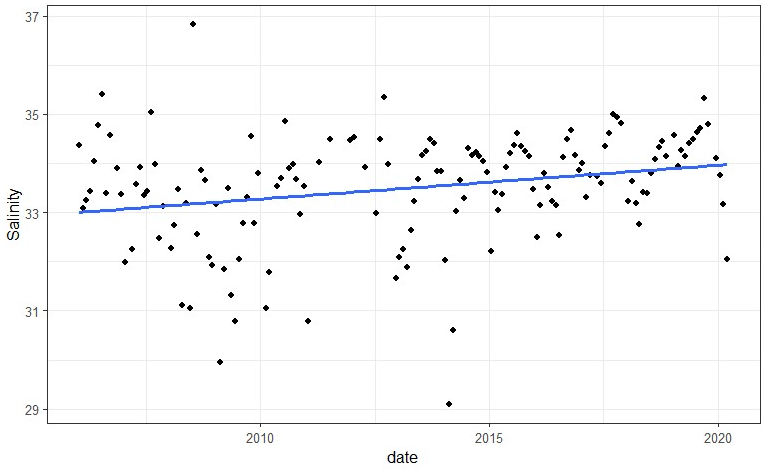

Figure 7.89 - Salinity as a function of time in the Hamford Water assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

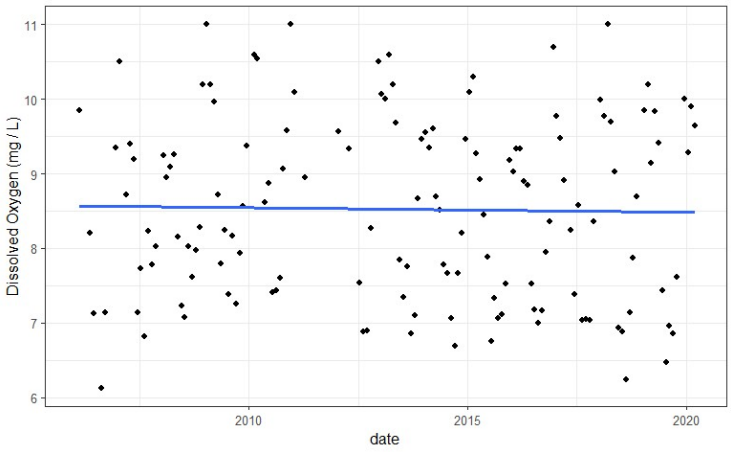

Figure 7.90 – Dissolved oxygen as a function of time in the Hamford Water assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.13 Harwich Approaches

Variable	***P* value**
Salinity	0.02

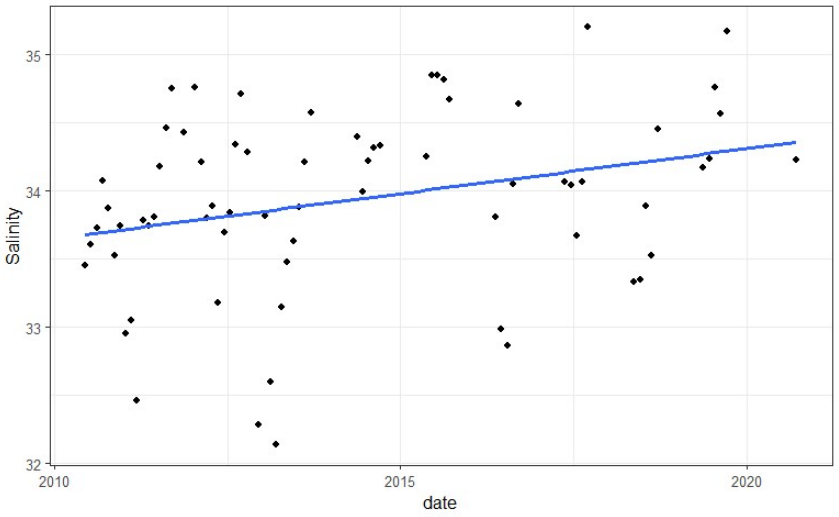

Figure 7.91 - Salinity as a function of time in the Harwich Approaches assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

Stour (Essex)

Table 7.13 - *p* Values from the results of the linear models in the Stour (Essex) assessment area.

Variable	***P* value**
Chlorophyll	0.00
DIP	0.78
Ammonium	0.00
TOxN	0.01
Salinity	0.01
DIN : DIP	0.40
DIN	0.01
Dissolved Oxygen	0.31

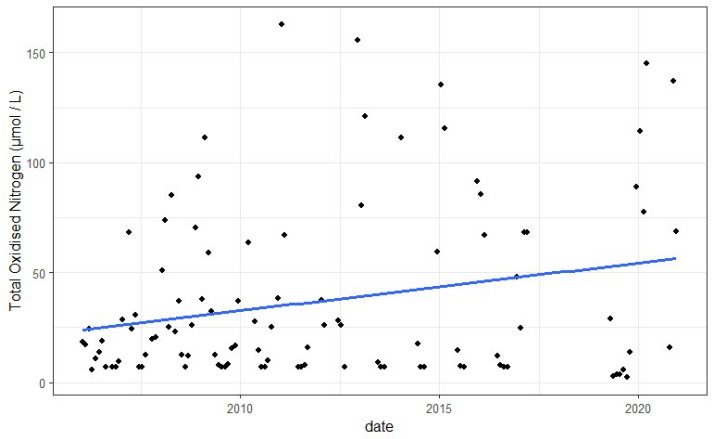

Figure 7.92 - TOxN concentrations as a function of time in the Stour (Essex) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

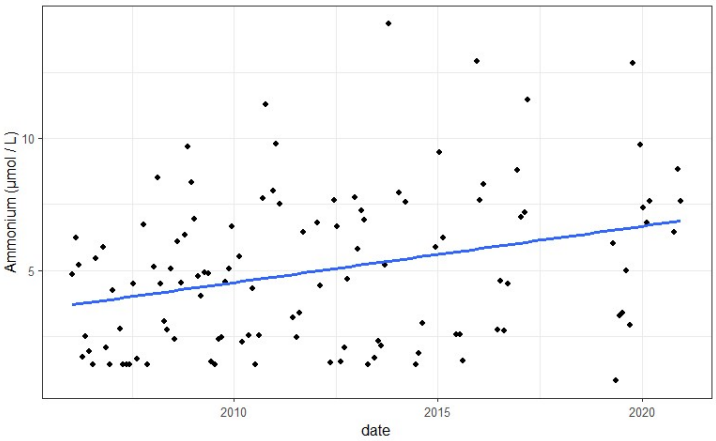

Figure 7.93 - Ammonium concentrations as a function of time in the Stour (Essex) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

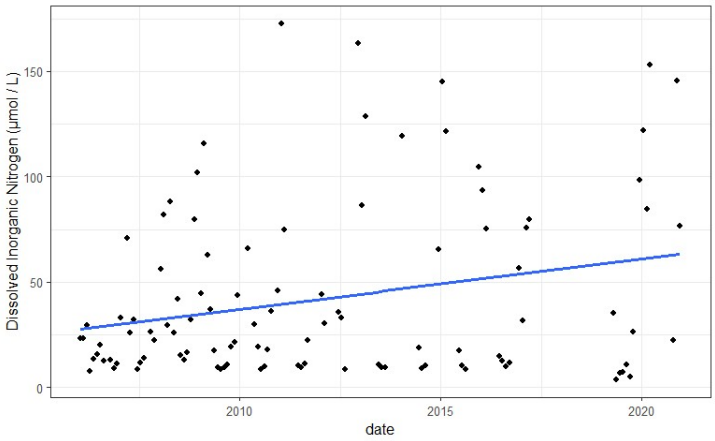

Figure 7.94 - DIN concentrations as a function of time in the Stour (Essex) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

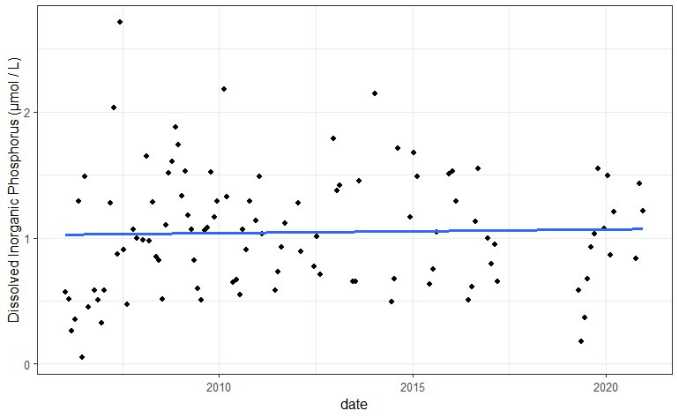

Figure 7.95 – DIP concentrations as a function of time in the Stour (Essex) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

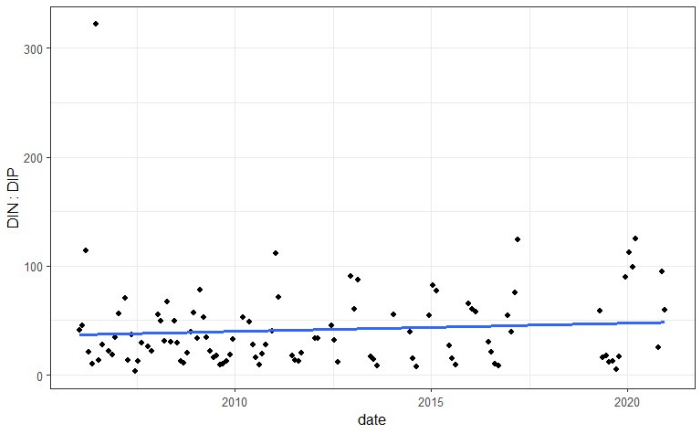

Figure 7.96 - DIN : DIP as a function of time in the Stour (Essex) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

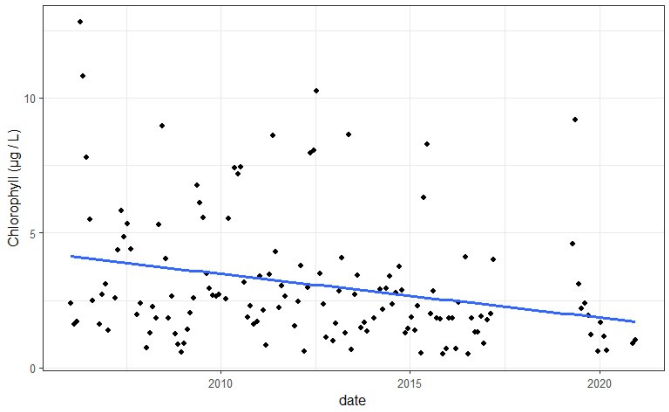

Figure 7.97 - Chlorophyll concentrations as a function of time in the Stour (Essex) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

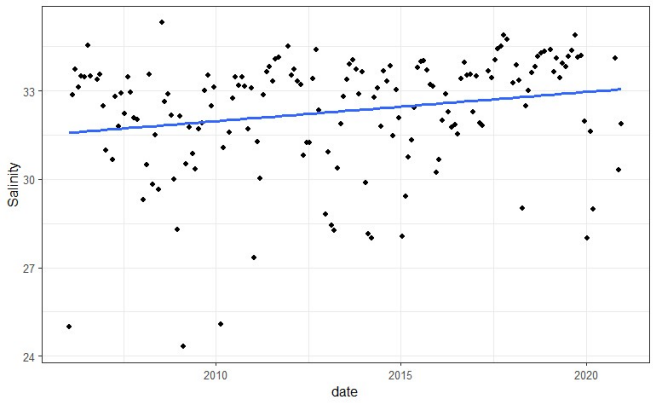

Figure 7.98 - Salinity as a function of time in the Stour (Essex) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity

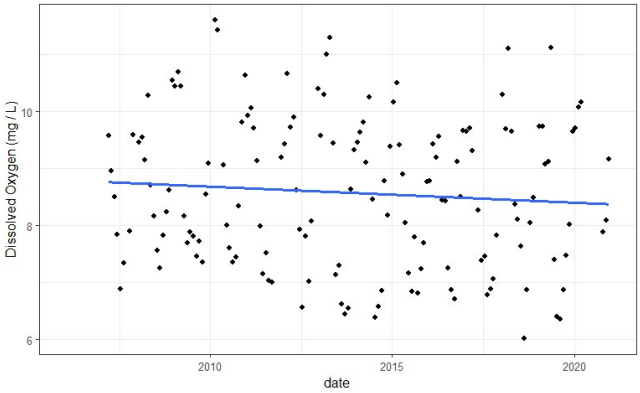

Figure 7.99 – Dissolved oxygen as a function of time in the Stour (Essex) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.14Orwell

Table 7.14 - *p* Values from the results of the linear models in the Orwell assessment area.

Variable	***P* value**
Chlorophyll	0.44
DIP	0.00
Ammonium	0.01
TOxN	0.66
Salinity	0.02
DIN : DIP	0.00
DIN	0.21
Dissolved Oxygen	0.30

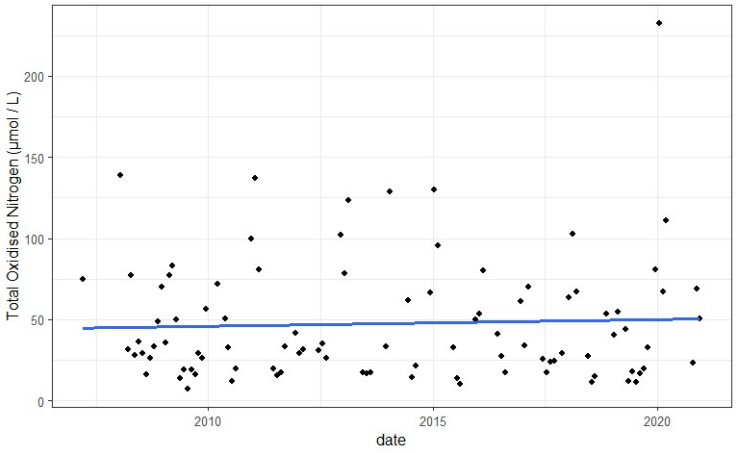

Figure 7.100 - TOxN concentrations as a function of time in the Orwell assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

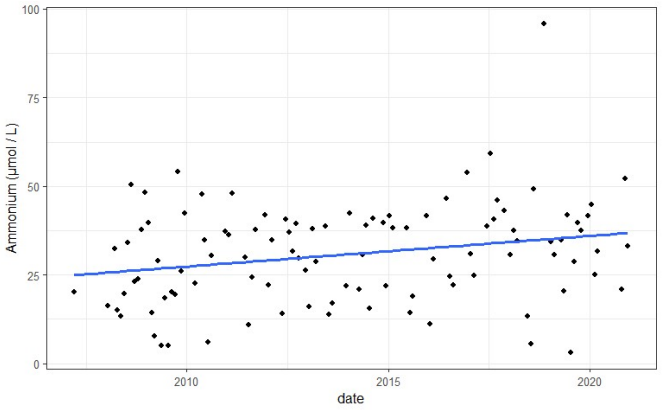

Figure 7.101 - Ammonium concentrations as a function of time in the Orwell assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

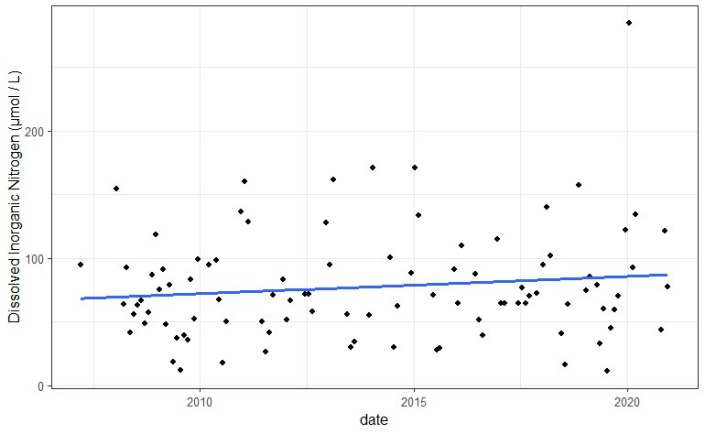

Figure 7.102 - DIN concentrations as a function of time in the Orwell assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

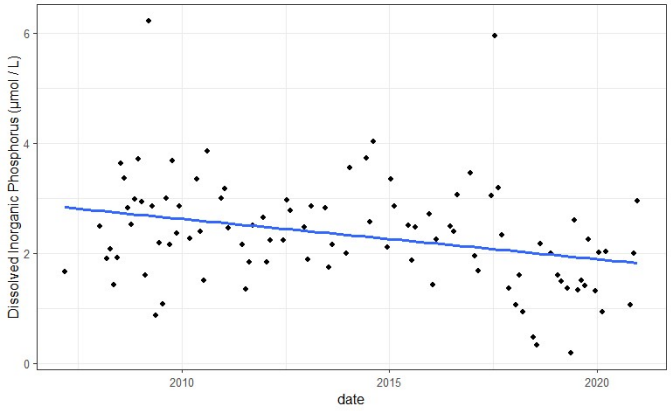

Figure 7.103 - DIP concentrations as a function of time in the Orwell assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

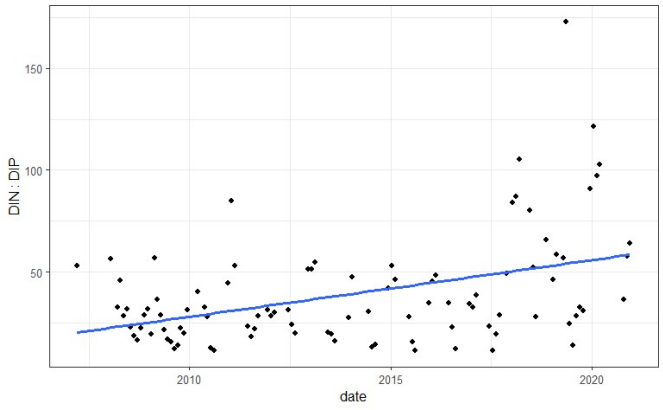

Figure 7.104 – DIN : DIP as a function of time in the Orwell assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

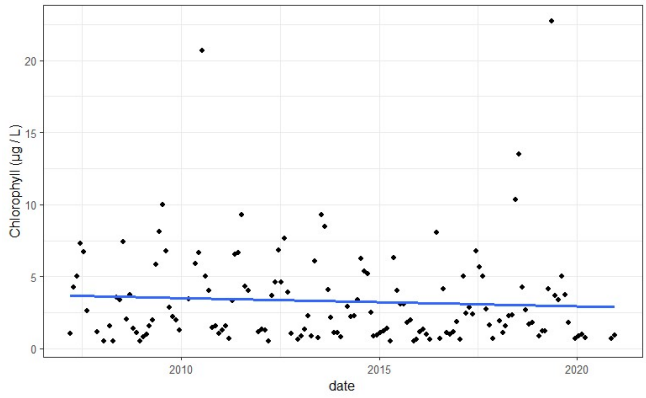

Figure 7.105 - Chlorophyll concentrations as a function of time in the Orwell assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

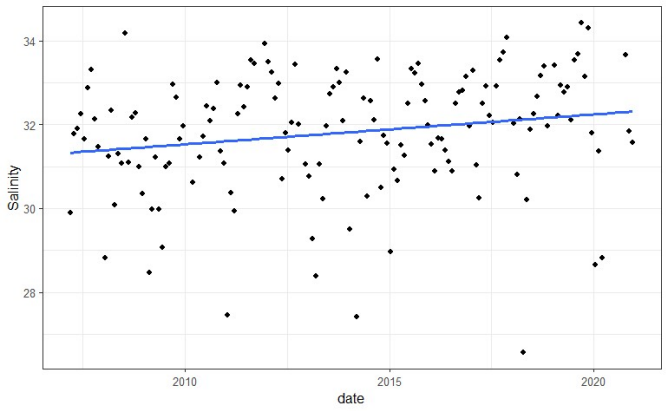

Figure 7.106 - Salinity as a function of time in the Orwell assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

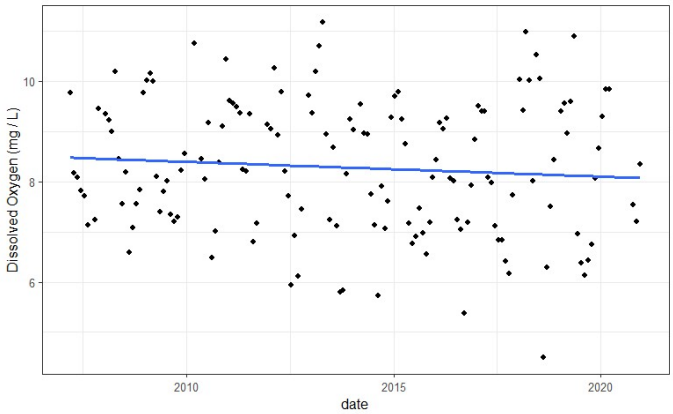

Figure 7.107 - Dissolved oxygen concentrations as a function of time in the Orwell assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.15 Colne

Table 7.15 - *p* Values from the results of the linear models in the Colne assessment area.

Variable	*P* value
Chlorophyll	0.01
DIP	0.00
Ammonium	0.00
NO2	0.00
TOxN	0.00
Salinity	0.00
Si	0.00
DIN : DIP	0.21
DIN	0.00
Dissolved Oxygen	0.95

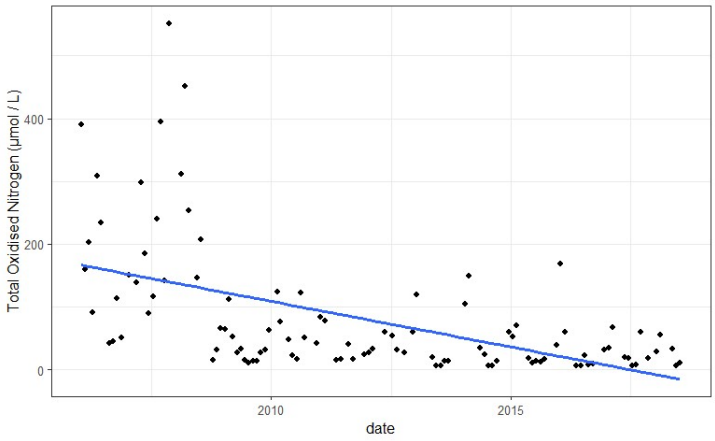

Figure 7.108 - TOxN concentrations as a function of time in the Colne assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

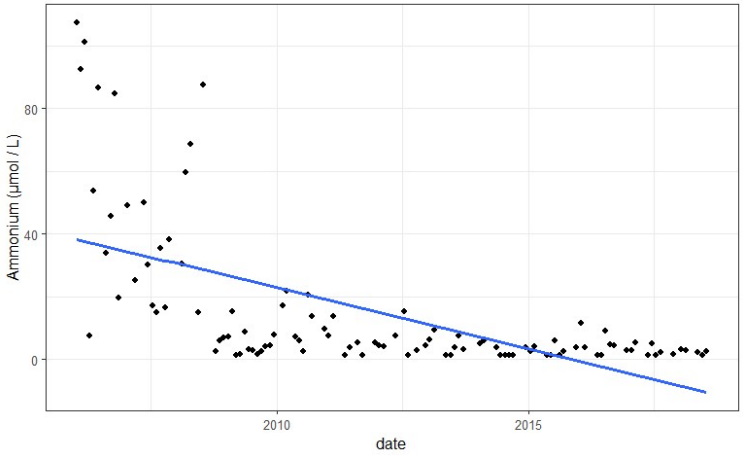

Figure 7.109 - Ammonium concentrations as a function of time in the Colne assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

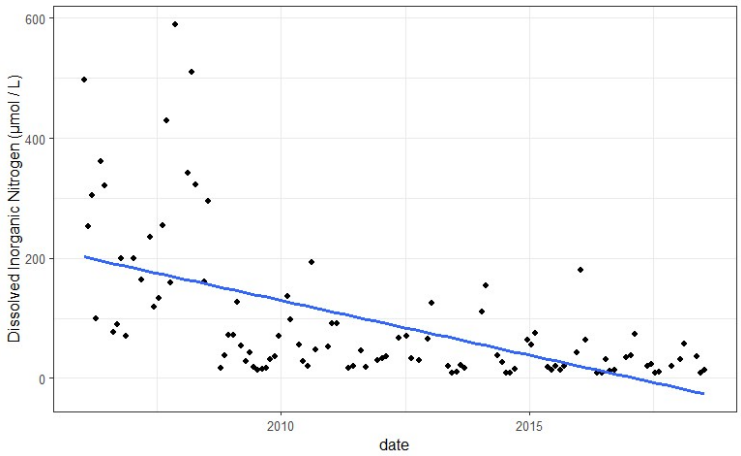

Figure 7.110 - DIN concentrations as a function of time in the Colne assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

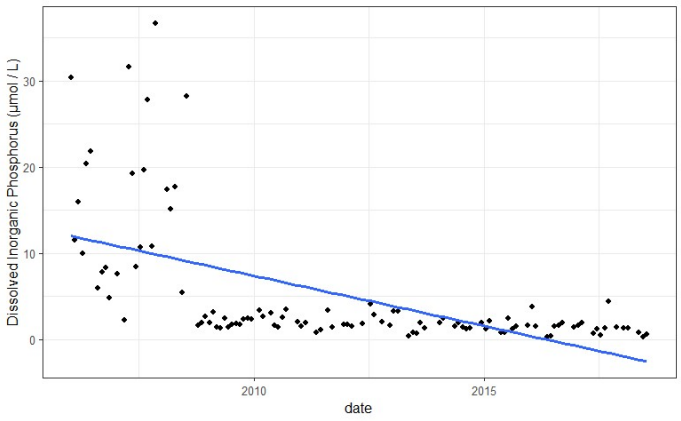

Figure 7.111 - DIP concentrations as a function of time in the Colne assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

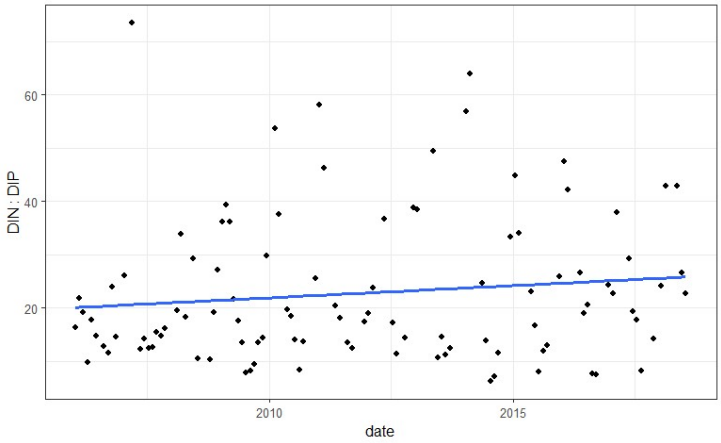

Figure 7.112 - DIN : DIP as a function of time in the Colne assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

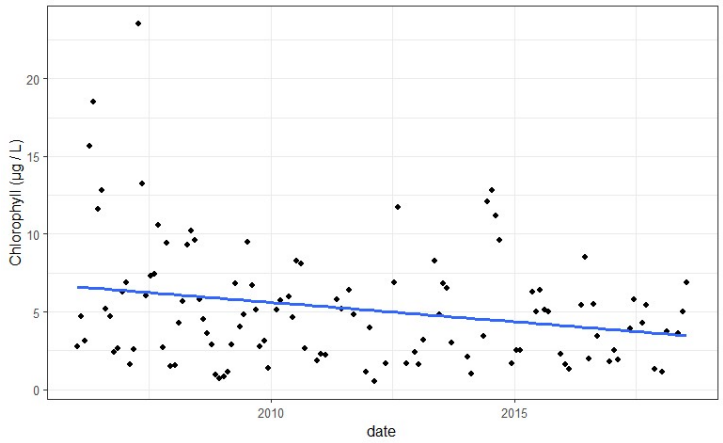

Figure 7.113 - Chlorophyll concentrations as a function of time in the Colne assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

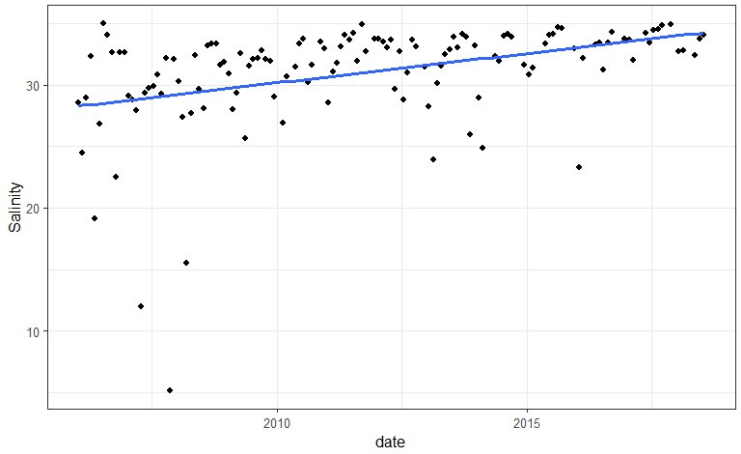

Figure 7.114 - Salinity as a function of time in the Colne assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

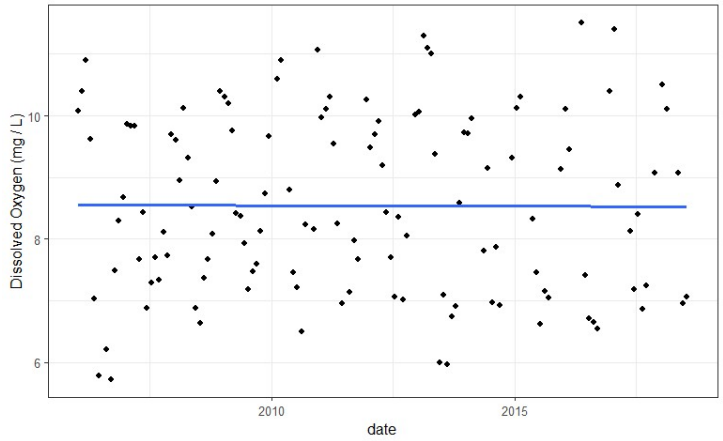

Figure 7.115 – Dissolved oxygen concentrations as a function of time in the Colne assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.16 Crouch

Table 7.16 - *p* Values from the results of the linear models in the Crouch assessment area.

Variable	***P* value**
Salinity	0.01
Dissolved Oxygen	0.13

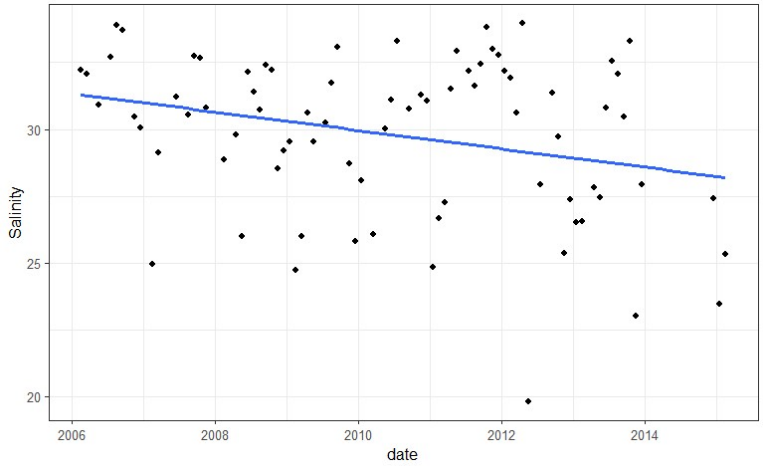

Figure 7.116 - Salinity as a function of time in the Crouch assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

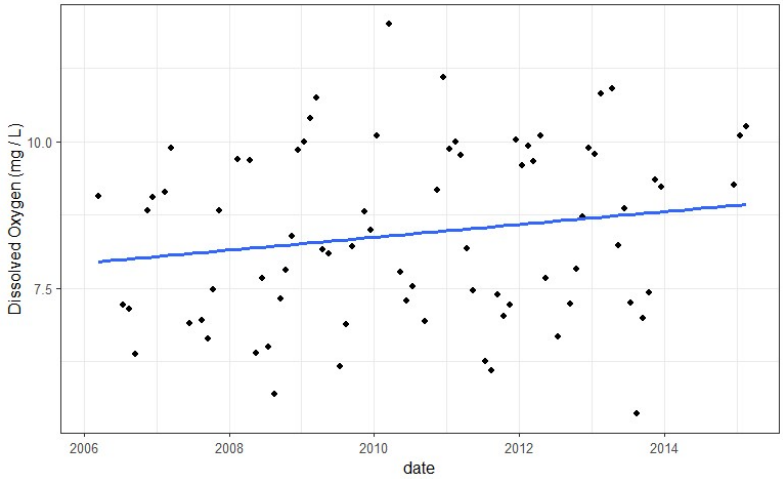

Figure 7.117 – Dissolved oxygen concentrations as a function of time in the Crouch assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.17 Stour (Kent)

Table 7.17 - *p* Values from the results of the linear models in the Stour (Kent) assessment area.

Variable	*P* value
Chlorophyll	0.07
DIP	0.00
Ammonium	0.00
TOxN	0.00
Salinity	0.00
DIN : DIP	0.00
DIN	0.00
Dissolved Oxygen	0.14

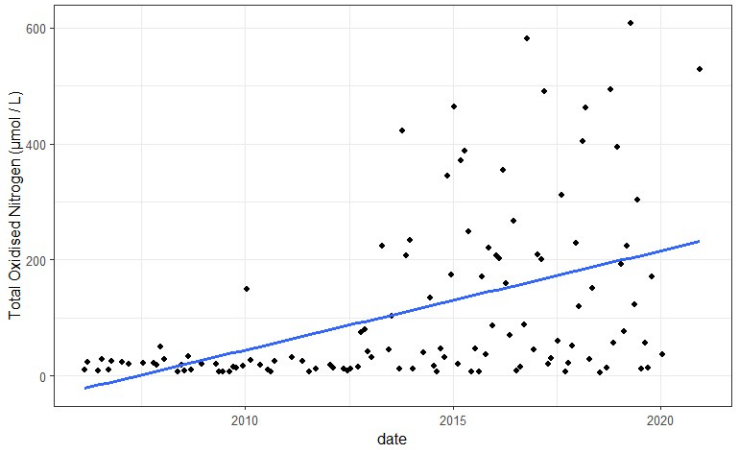

Figure 7.118 - TOxN concentrations as a function of time in the Stour (Kent) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

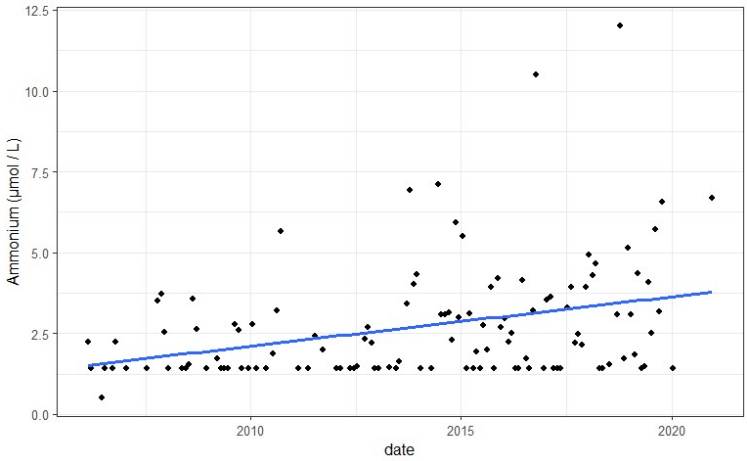

Figure 7.119 - Ammonium concentrations as a function of time in the Stour (Kent) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

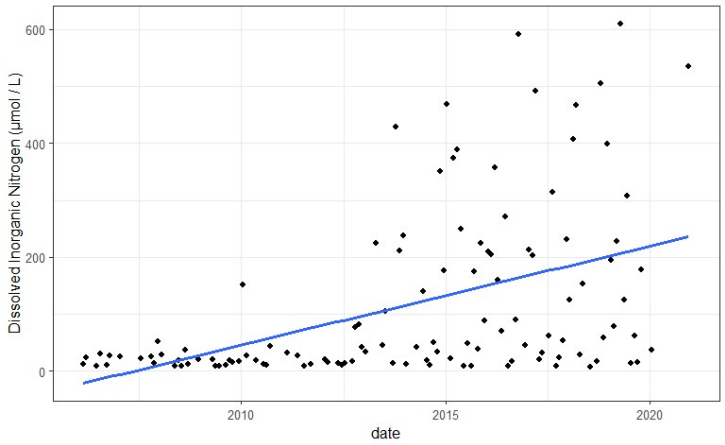

Figure 7.120 - DIN concentrations as a function of time in the Stour (Kent) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

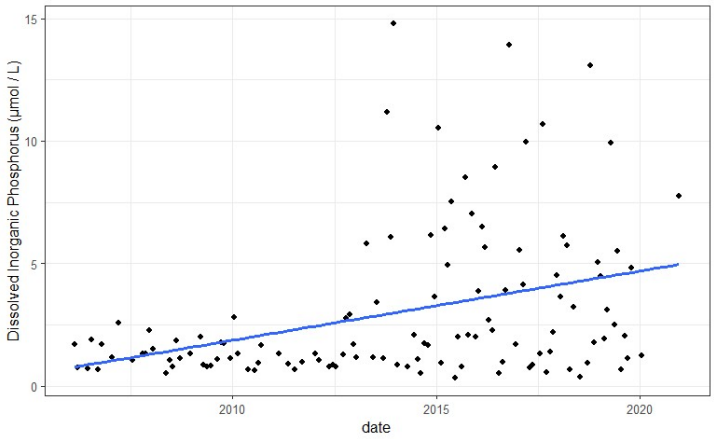

Figure 7.121 - DIP concentrations as a function of time in the Stour (Kent) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

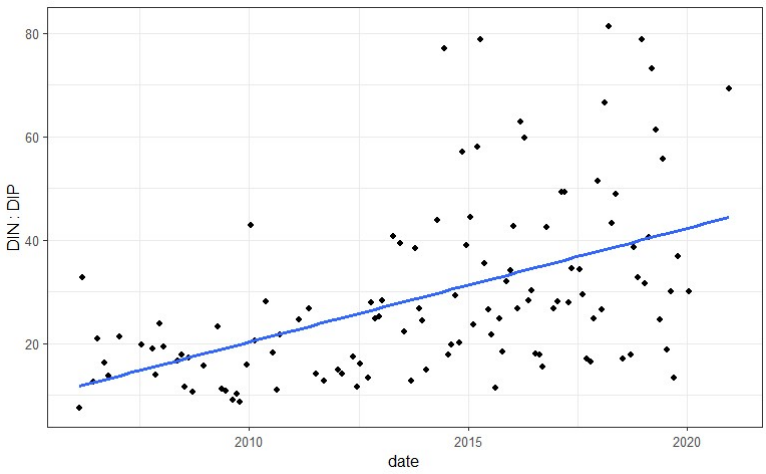

Figure 7.122 – DIN : DIP as a function of time in the Stour (Kent) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

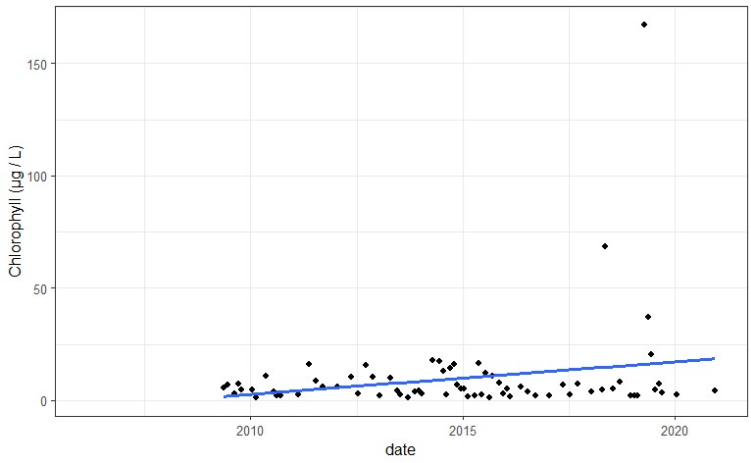

Figure 7.123 - Chlorophyll concentrations as a function of time in the Stour (Kent) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

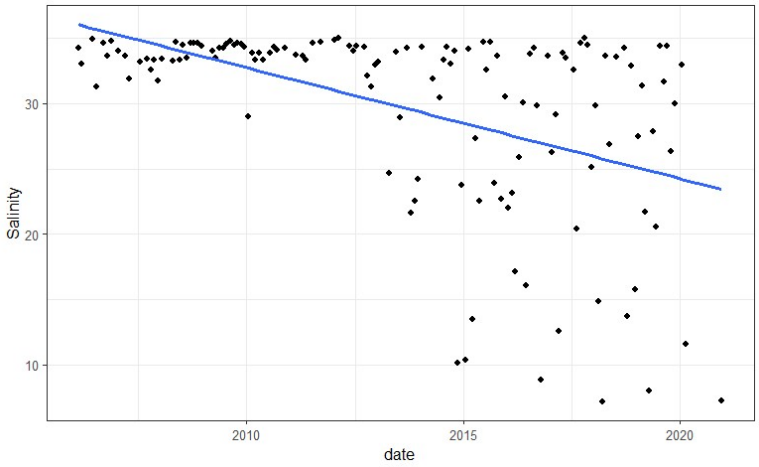

Figure 7.124 - Salinity as a function of time in the Stour (Kent) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

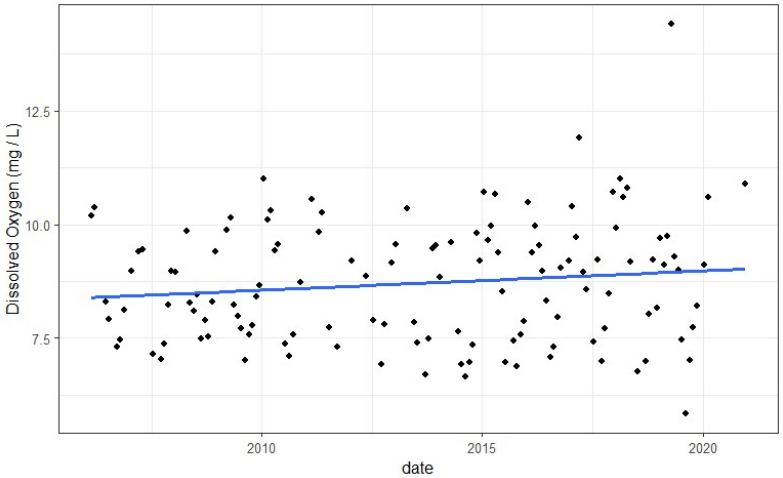

Figure 7.125- Dissolved oxygen concentrations as a function of time in the Stour (Kent) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.1.18 Deben

Table 7.18 - *p* Values from the results of the linear models in the Deben assessment area.

Variable	*P* value
Chlorophyll	0.11
DIP	0.00
Ammonium	0.00
TOxN	0.00
Salinity	0.00
DIN : DIP	0.02
DIN	0.00
Dissolved Oxygen	1.00

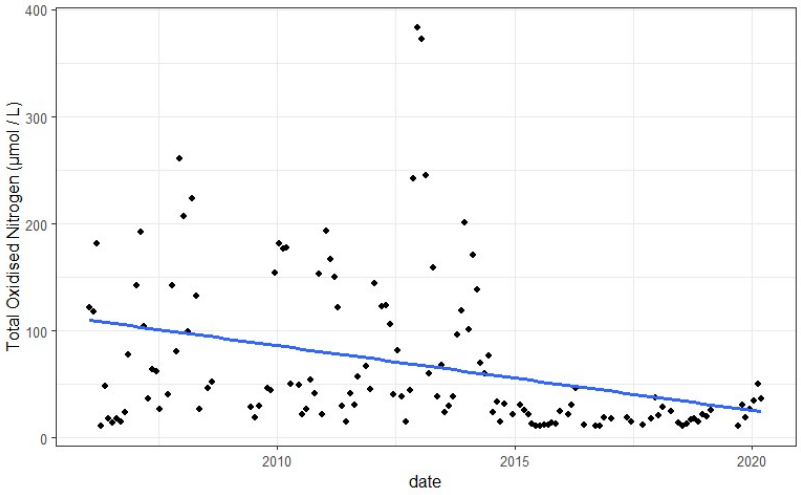

Figure 7.126 - TOxN concentrations as a function of time in the Deben assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

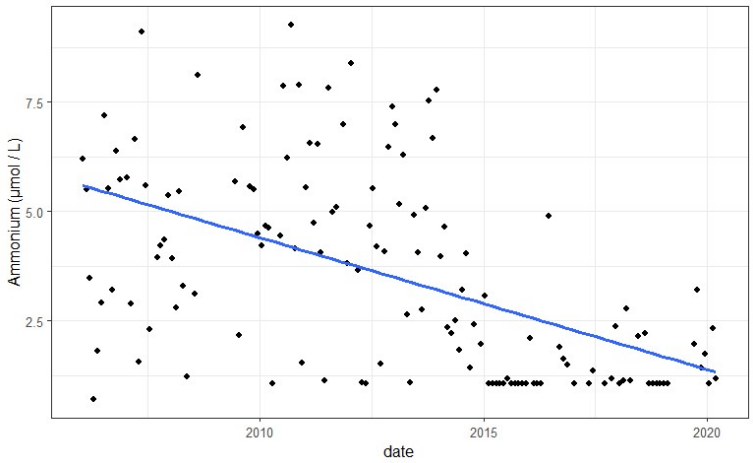

Figure 7.127 - Ammonium concentrations as a function of time in the Deben assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

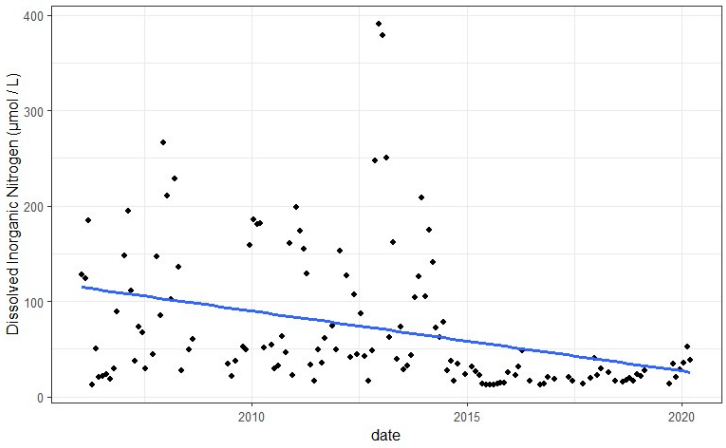

Figure 7.128 - DIN concentrations as a function of time in the Deben assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

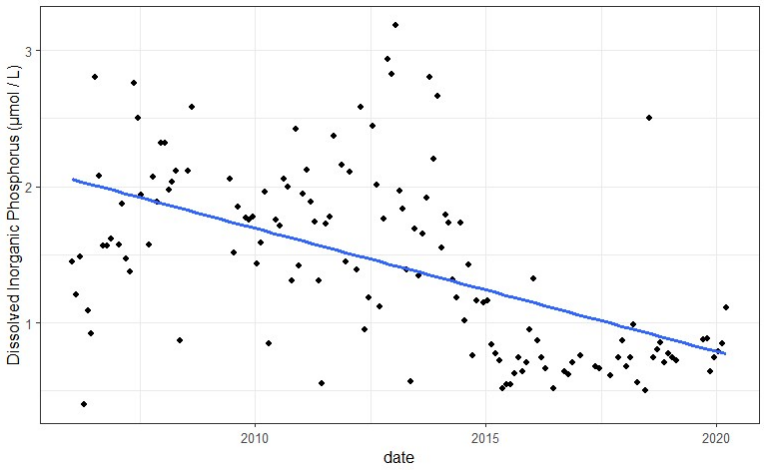

Figure 7.129 - DIP concentrations as a function of time in the Deben assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

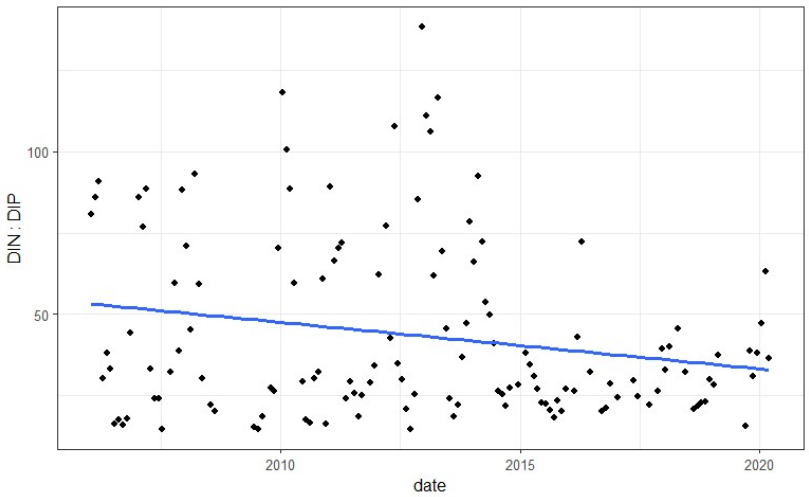

Figure 7.130 – DIN : DIP as a function of time in the Deben assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

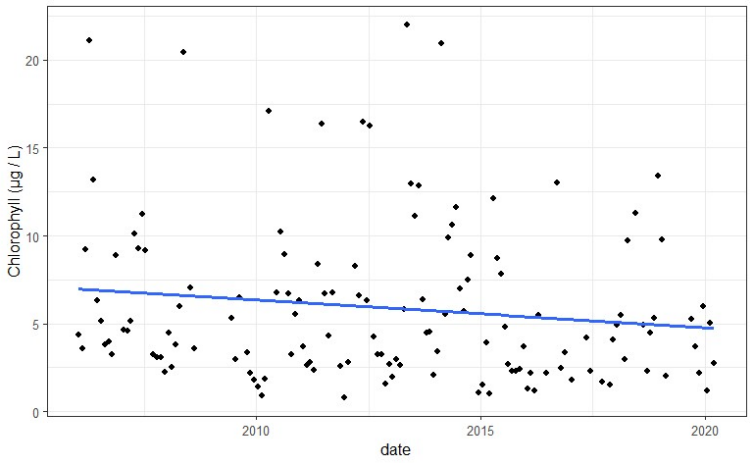

Figure 7.131 - Chlorophyll concentrations as a function of time in the Deben assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

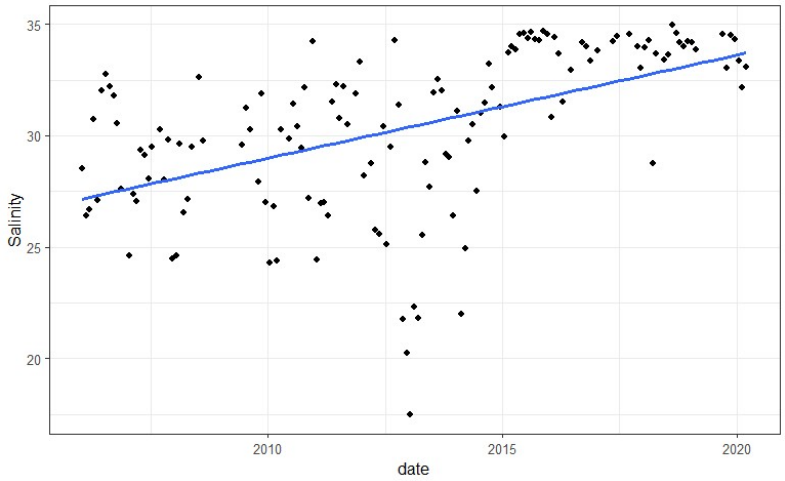

Figure 7.132 - Salinity as a function of time in the Deben assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

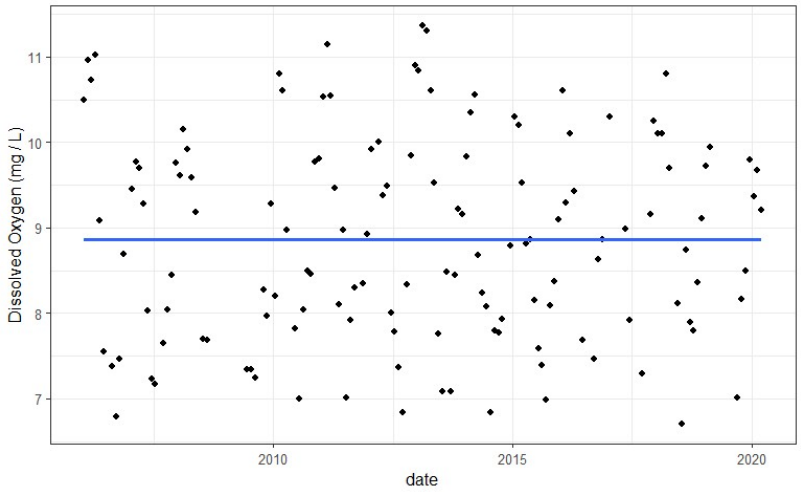

Figure 7.133 – Dissolved oxygen concentrations as a function of time in the Deben assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.2 Liverpool Bay long term trend results

7.2.1 Liverpool Bay plume

Table 7.19 – *p* Values from the results of the linear models in the Liverpool Bay plume assessment area.

Variable	*P* value
Chlorophyll	0.01
DIP	0.05
Ammonium	0.09
TOxN	0.52
Salinity	0.01
DIN : DIP	0.33
DIN	0.45
Dissolved Oxygen	0.28

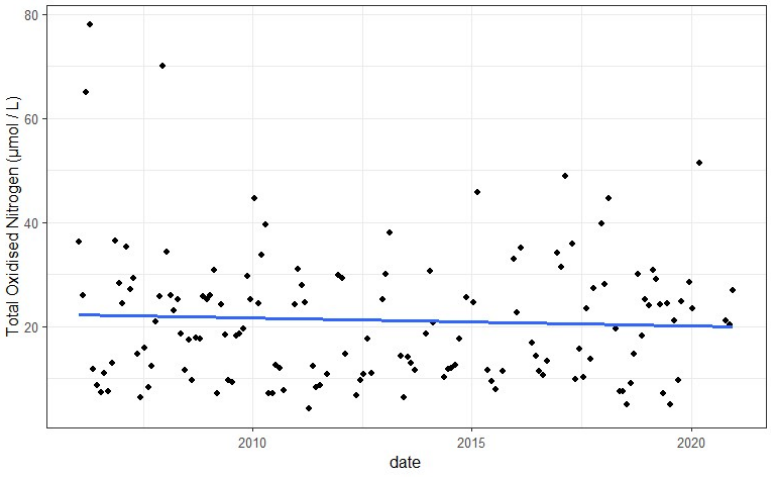

Figure 7.134 – TOxN concentrations as a function of time in the Liverpool Bay plume. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

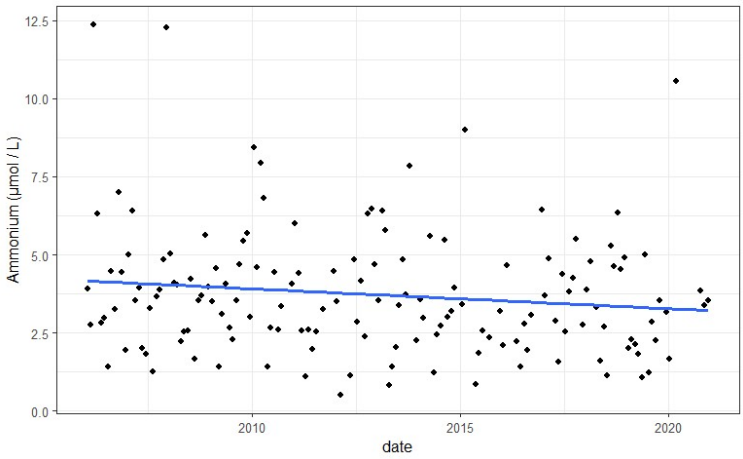

Figure 7.135 – Ammonium concentrations as a function of time in the Liverpool Bay plume. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

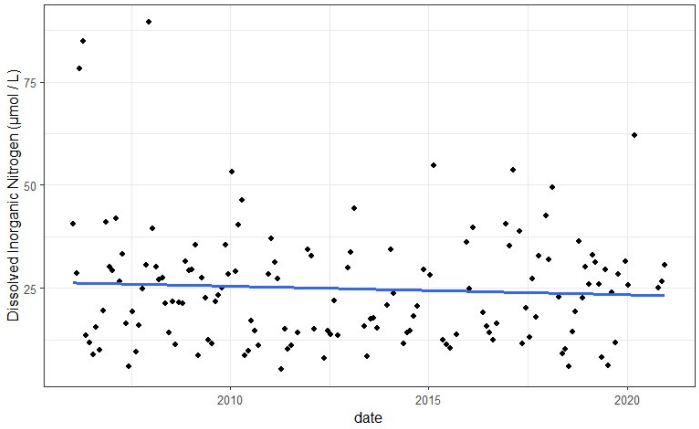

Figure 7.136 – Dissolved Inorganic Nitrogen concentrations as a function of time in the Liverpool Bay plume. The blue line represents a linear regression of date ~ concentration for the mean monthly Dissolved Inorganic Nitrogen.

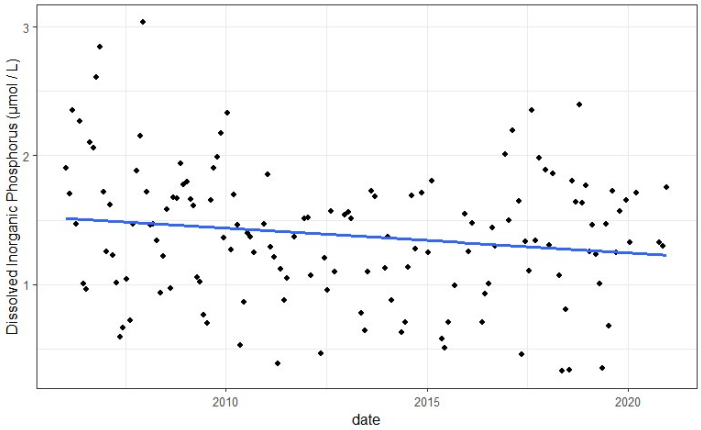

Figure 7.137 - DIP concentrations as a function of time in the Liverpool Bay plume. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

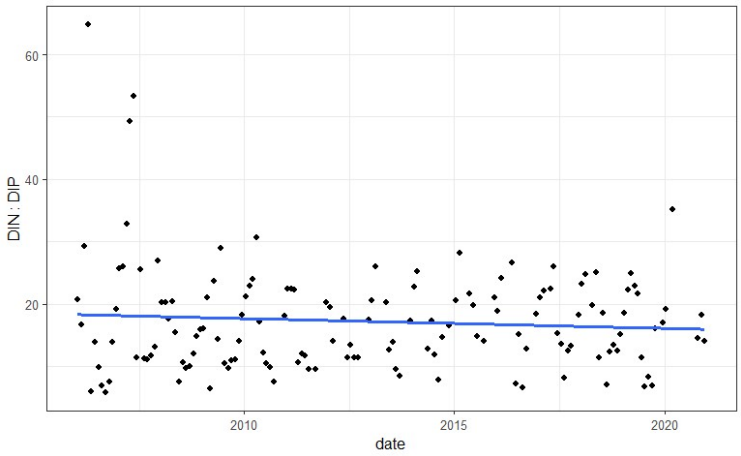

Figure 7.138 – DIN : DIP concentrations as a function of time in the Liverpool Bay plume. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

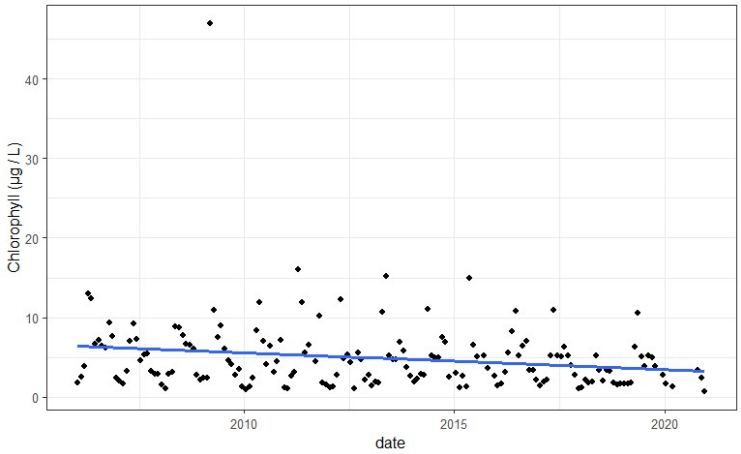

Figure 7.139 - Chlorophyll concentrations as a function of time in the Liverpool Bay plume. The blue line represents a linear regression of date ~ concentration for the mean monthly Chlorophyll.

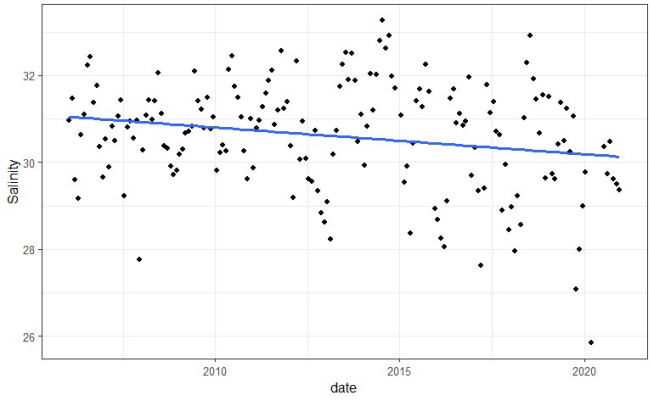

Figure 7.140 - Salinity as a function of time in the Liverpool Bay plume. The blue line represents a linear regression of date ~ concentration for the mean monthly Salinity.

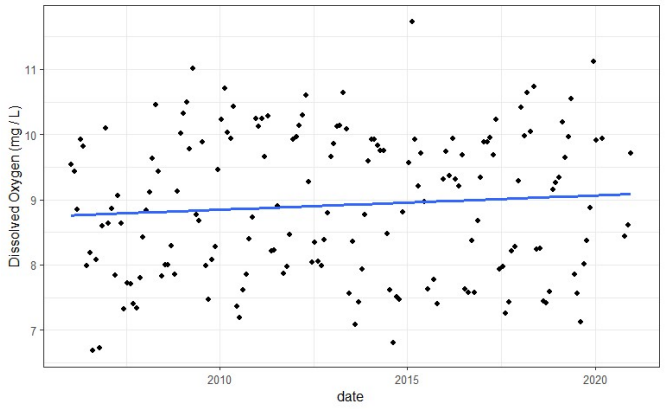

Figure 7.141 – Dissolved Oxygen concentrations as a function of time in the Liverpool Bay plume. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.2.2 Mersey Mouth

Table 7.20 - *p* Values from the results of the linear models in the Mersey Mouth assessment area.

Variable	***P* value**
Chlorophyll	0.01
DIP	0.94
Ammonium	0.47
TOxN	0.48
Salinity	0.00
DIN : DIP	0.39
DIN	0.56
Dissolved Oxygen	0.20

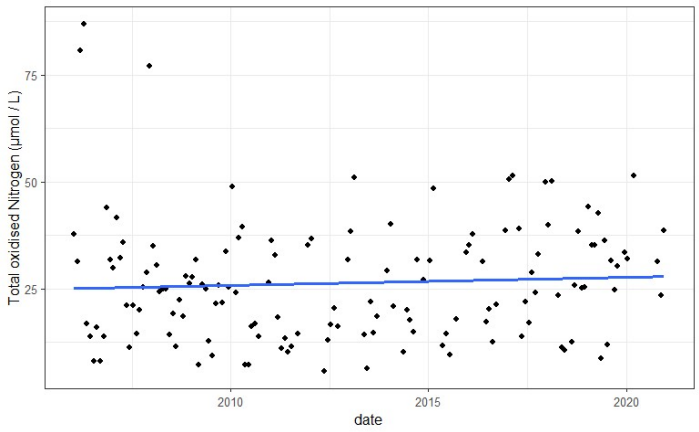

Figure 7.142 - TOxN concentrations as a function of time in the Mersey Mouth. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

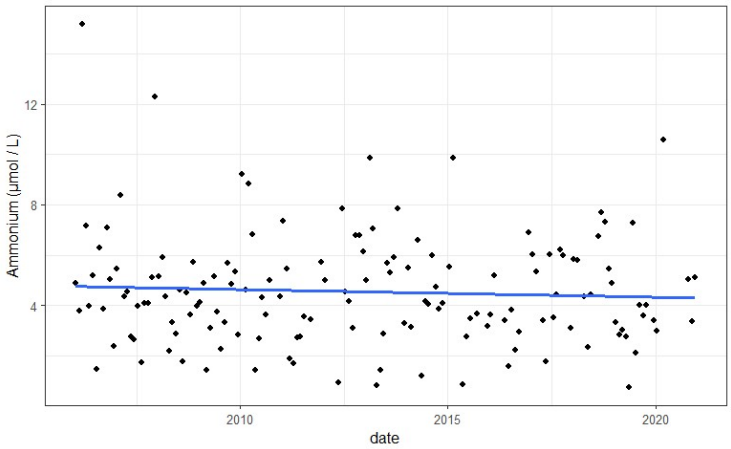

Figure 7.143 - Ammonium concentrations as a function of time in the Mersey Mouth. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

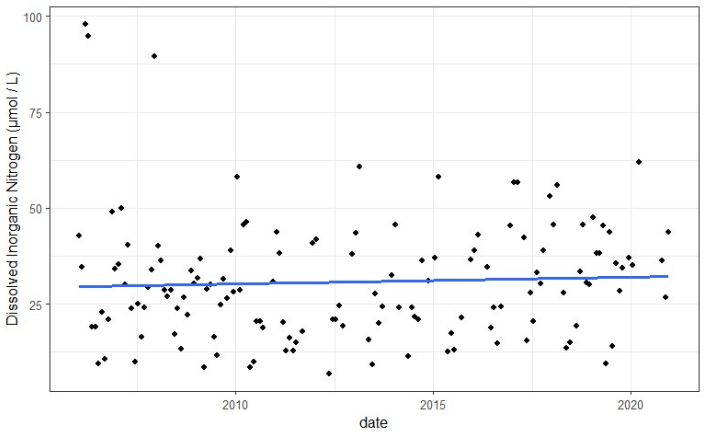

Figure 7.144 - DIN concentrations as a function of time in the Mersey Mouth. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

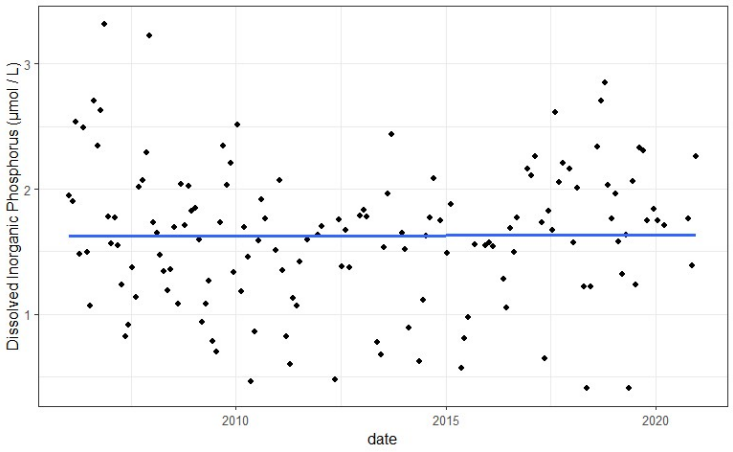

Figure 7.145 – DIP concentrations as a function of time in the Mersey Mouth. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

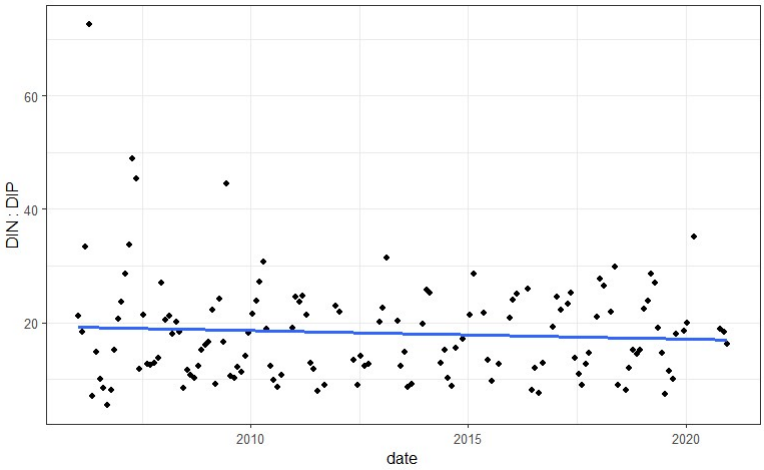

Figure 7.146 – DIN : DIP concentrations as a function of time in the Mersey Mouth. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

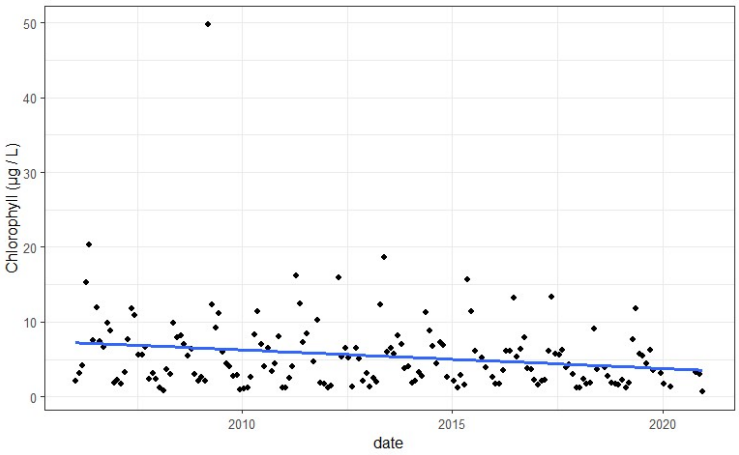

Figure 7.147 - Chlorophyll concentrations as a function of time in the Mersey Mouth. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

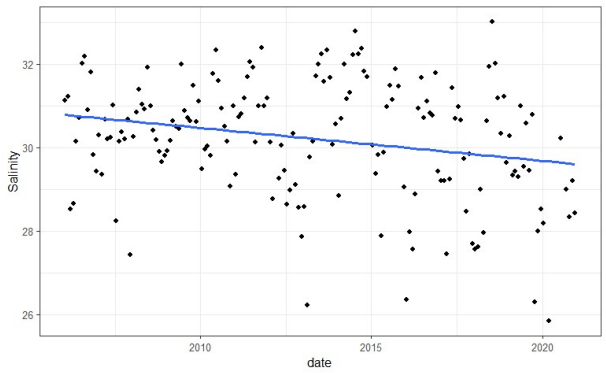

Figure 7.148 - Salinity as a function of time in the Mersey Mouth. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

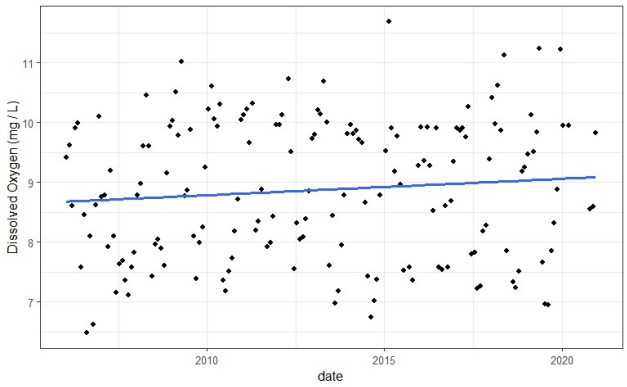

Figure 7.149 – Dissolved oxygen concentrations as a function of time in the Mersey Mouth. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.2.3 Dee (N. Wales)

Table 7.21 - *p* Values from the results of the linear models in the Liverpool Bay plume assessment area.

Variable	***P* value**
Chlorophyll	0.60
DIP	0.00
Ammonium	0.97
TOxN	0.05
Salinity	0.14
DIN : DIP	0.69
DIN	0.06
Dissolved Oxygen	0.89

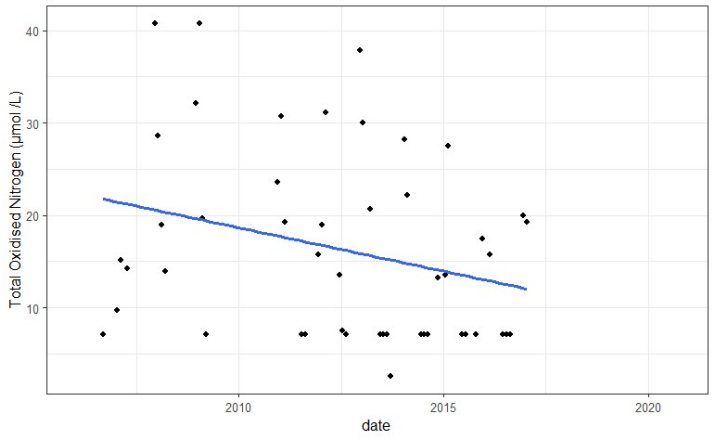

Figure 7.150 – TOxN concentrations as a function of time in the Dee (N. Wales) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

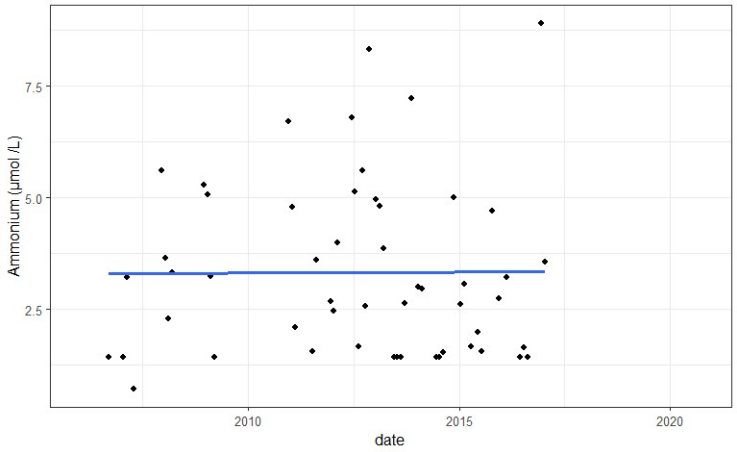

Figure 7.151 – Ammonium concentrations as a function of time in the Dee (N. Wales) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly Ammonium.

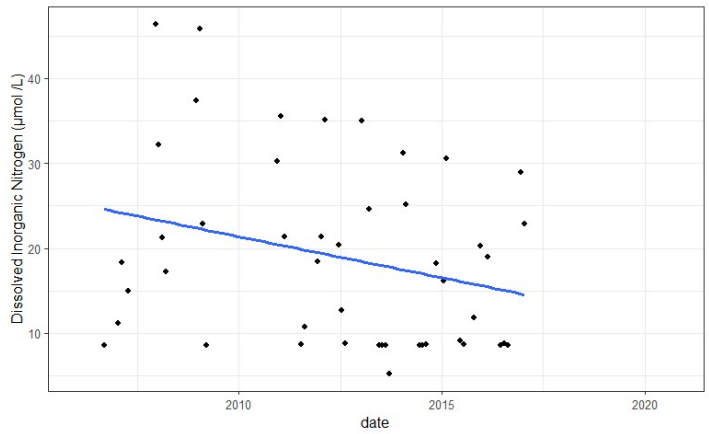

Figure 7.152 – DIN concentrations as a function of time in the Dee (N. Wales) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

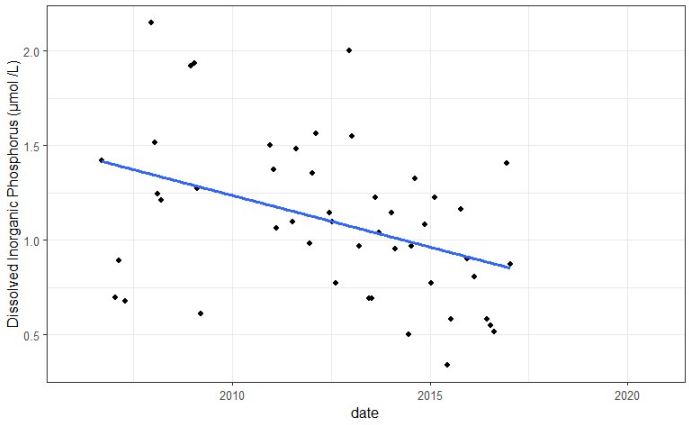

Figure 7.153 - Dissolved Inorganic phosphorus concentrations as a function of time in the Dee (N. Wales) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

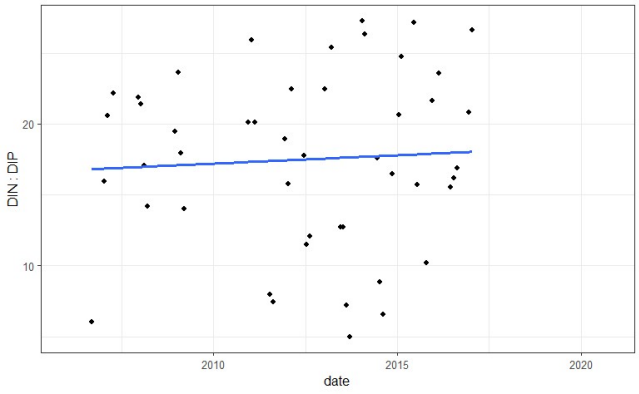

Figure 7.154 – DIN : DIP concentrations as a function of time in the Dee (N. Wales) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

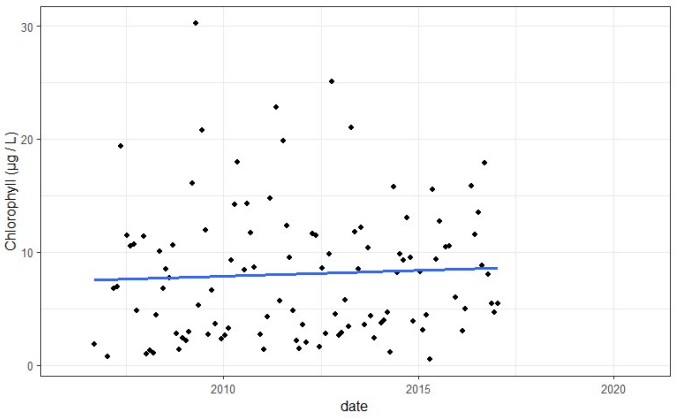

Figure 7.155 - Chlorophyll concentrations as a function of time in the Dee (N. Wales) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

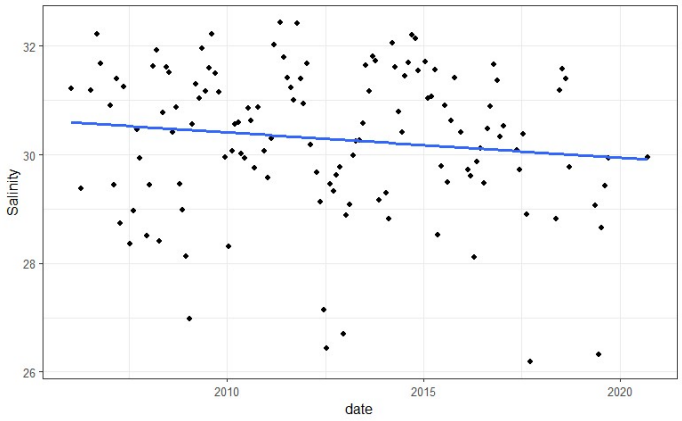

Figure 7.156 - Salinity concentrations as a function of time in the Dee (N. Wales) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly Salinity.

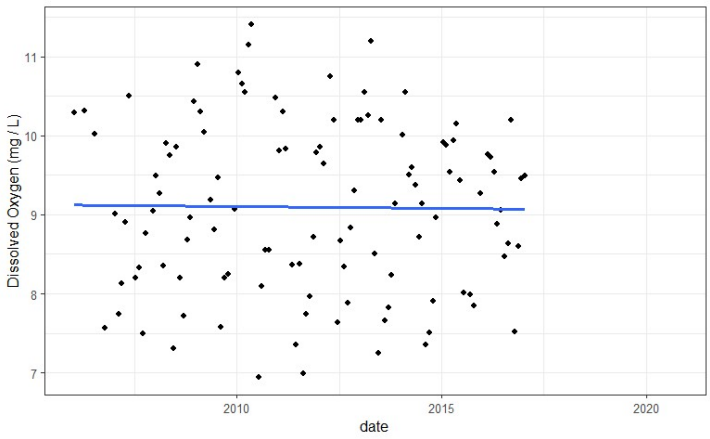

Figure 7.157 – Dissolved oxygen concentrations as a function of time in the Dee (N. Wales) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.2.4 Mersey

Table 7.22 - *p* Values from the results of the linear models in the Mersey assessment area.

Variable	***P* value**
Chlorophyll	0.00
DIP	0.18
Ammonium	0.00
TOxN	0.66
Salinity	0.00
DIN : DIP	0.01
DIN	0.33
Dissolved Oxygen	0.80

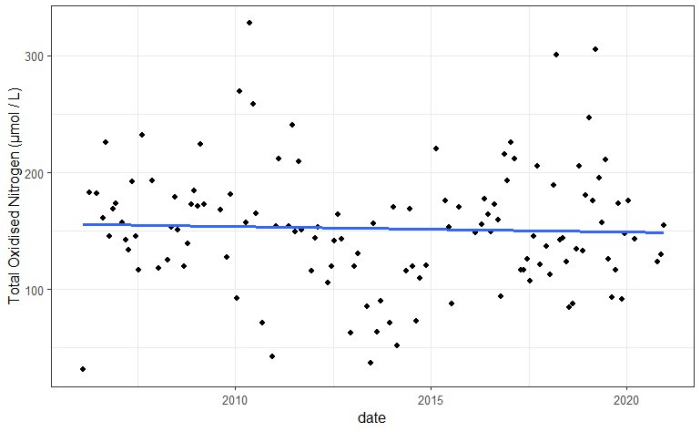

Figure 7.158 – TOxN concentrations as a function of time in the Mersey assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

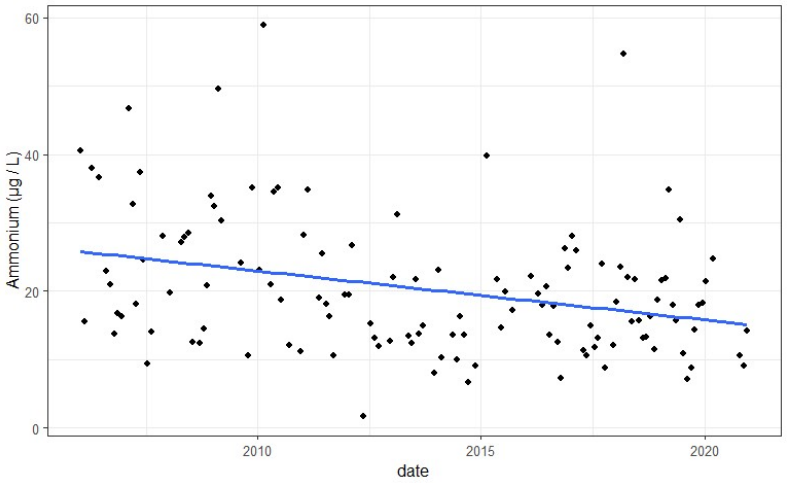

Figure 7.159 - Ammonium concentrations as a function of time in the Mersey assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly Ammonium.

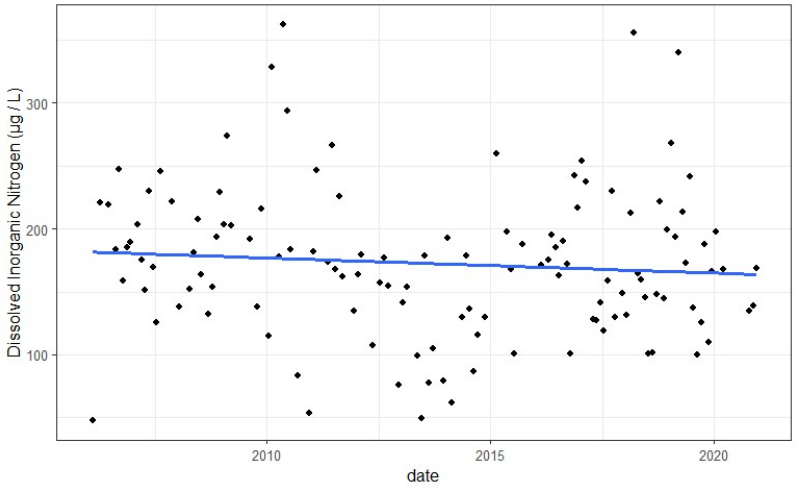

Figure 7.160 - DIN concentrations as a function of time in the Mersey assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

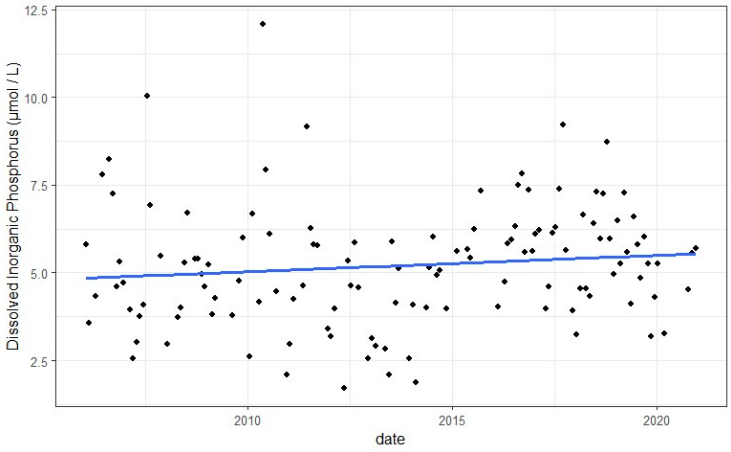

Figure 7.161 - DIP concentrations as a function of time in the Dee (N. Wales) assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

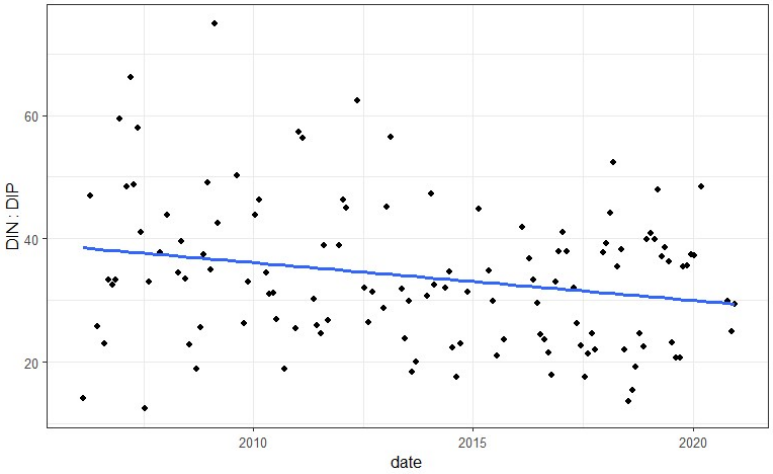

Figure 7.162 – DIN : DIP as a function of time in the Mersey assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

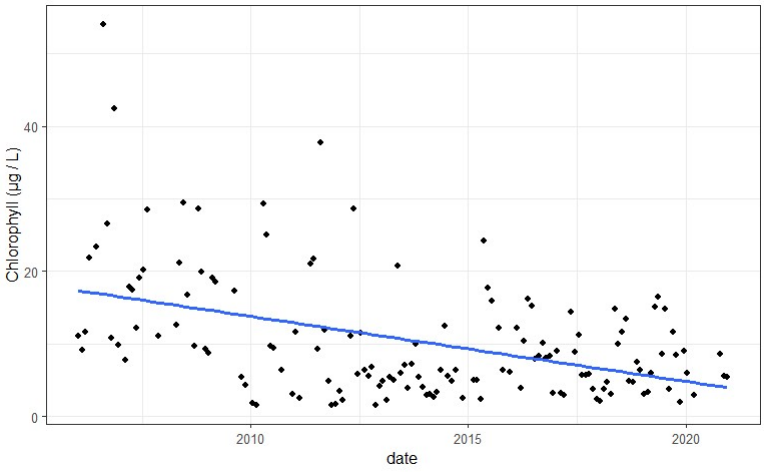

Figure 7.163 - Chlorophyll concentrations as a function of time in the Mersey assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly Chlorophyll.

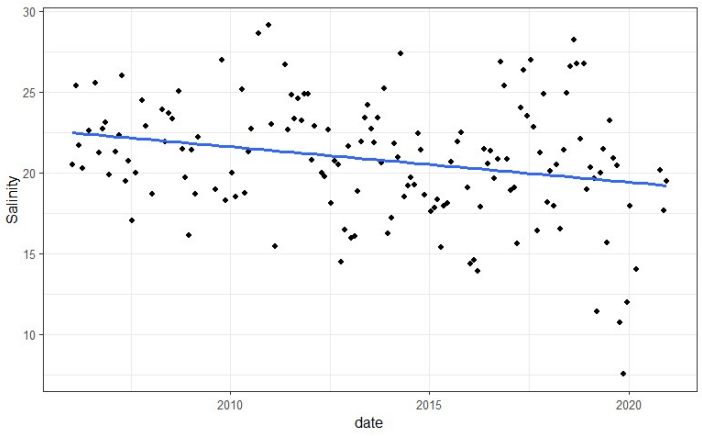

Figure 7.164 – Salinity as a function of time in the Mersey assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly Salinity.

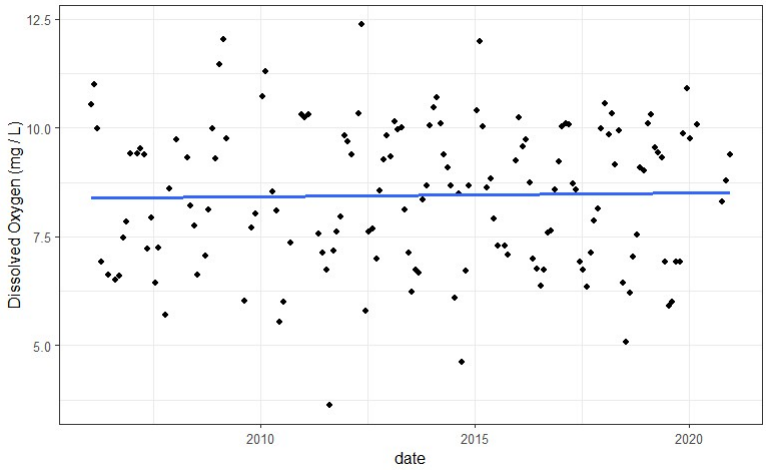

Figure 7.165 – Dissolved oxygen concentrations as a function of time in the Mersey assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.2.5 Ribble

Table 7.23 - *p* Values from the results of the linear models in the Ribble assessment area.

Variable	***P* value**
Chlorophyll	0.07
DIP	0.23
Ammonium	0.23
TOxN	0.01
Salinity	0.00
DIN : DIP	0.09
DIN	0.02
Dissolved Oxygen	0.49

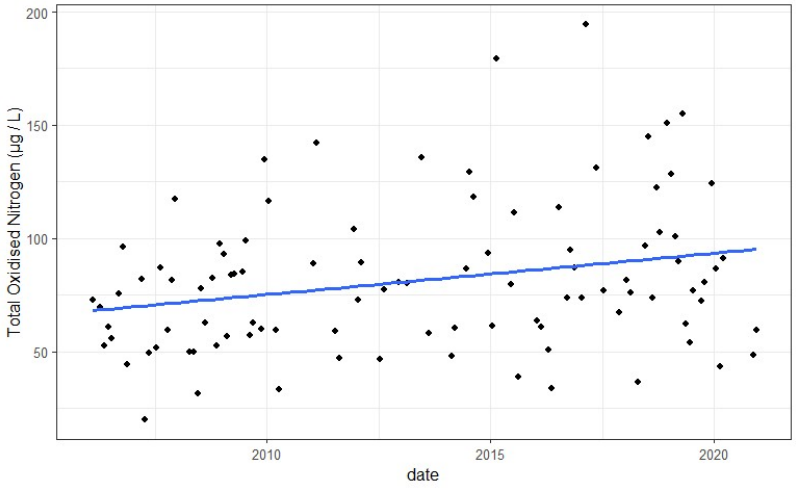

Figure 7.166 - TOxN concentrations as a function of time in the Ribble assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

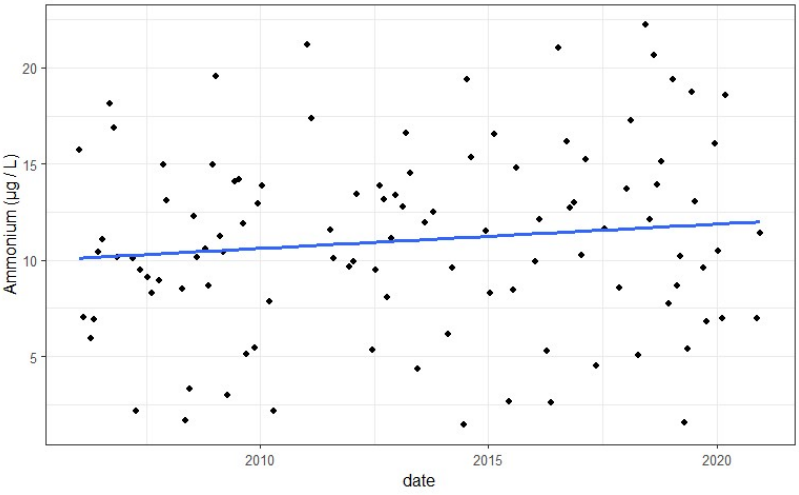

Figure 7.167 – Ammonium concentrations as a function of time in the Ribble assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

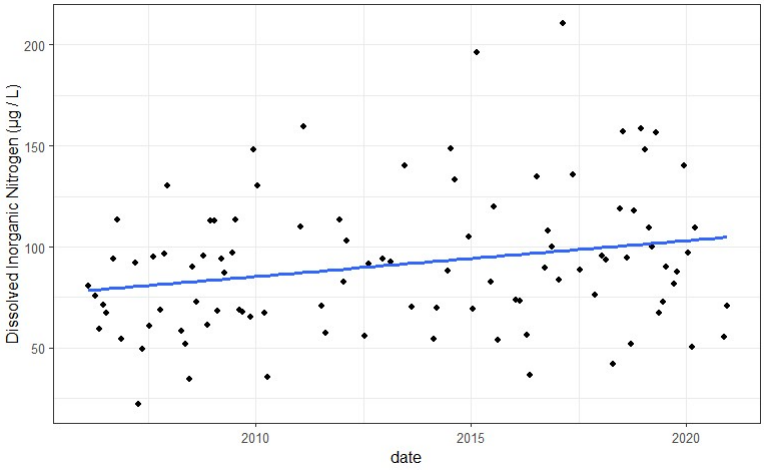

Figure 7.168 - DIN concentrations as a function of time in the Ribble assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

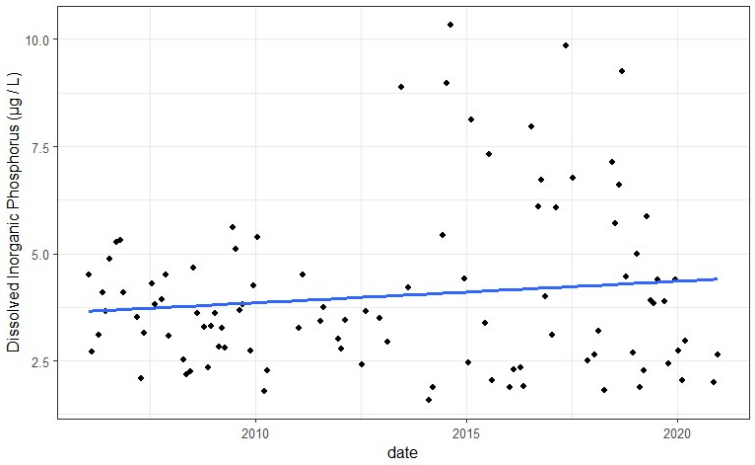

Figure 7.169 - DIP concentrations as a function of time in the Ribble assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

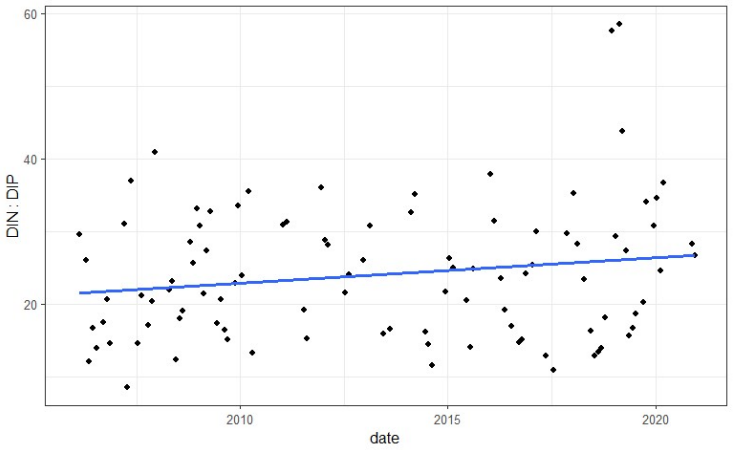

Figure 7.170 – DIN : DIP as a function of time in the Ribble assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

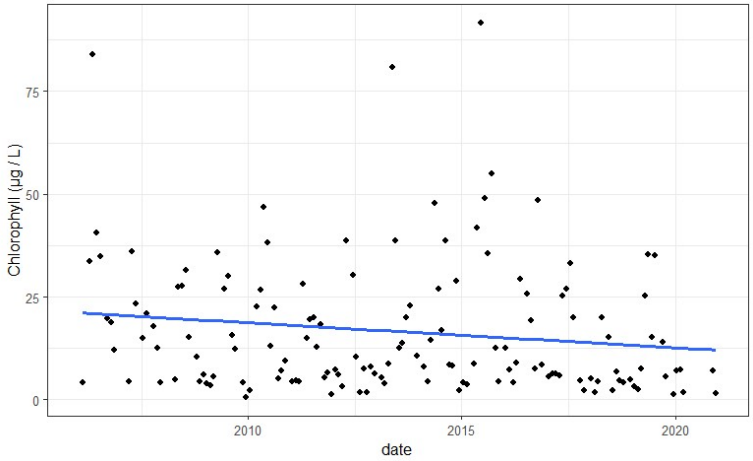

Figure 7.171- Chlorophyll concentrations as a function of time in the Ribble assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

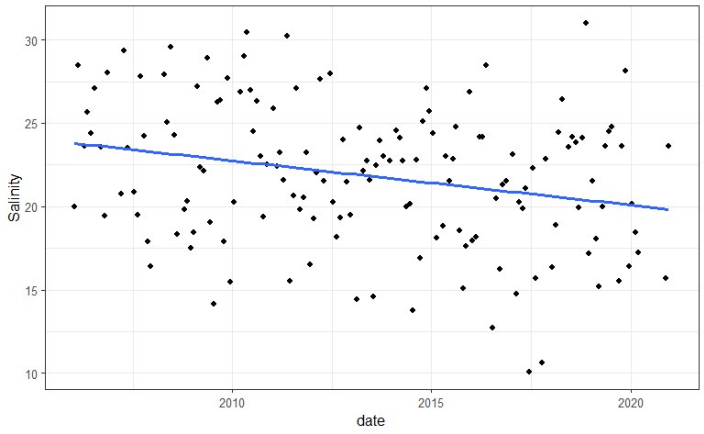

Figure 7.172 - Salinity as a function of time in the Ribble assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

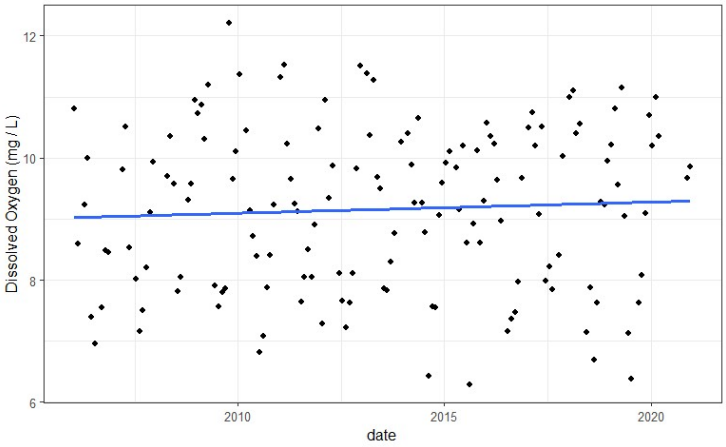

Figure 7.173 – Dissolved oxygen concentration as a function of time in the Ribble assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.2.6 Cumbria

Table 7.24 - *p* Values from the results of the linear models in the Cumbria assessment area.

Variable	***P* value**
Chlorophyll	0.58
DIP	0.00
Ammonium	0.00
TOxN	0.00
Salinity	0.00
DIN : DIP	0.00
DIN	0.00
Dissolved Oxygen	0.52

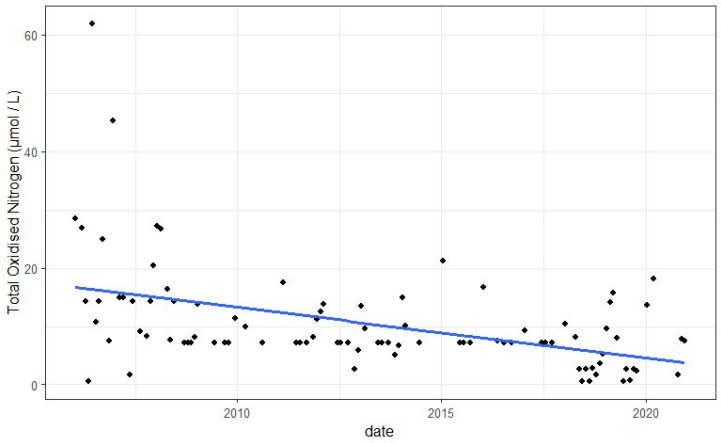

Figure 7.174 - TOxN concentrations as a function of time in the Cumbria assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

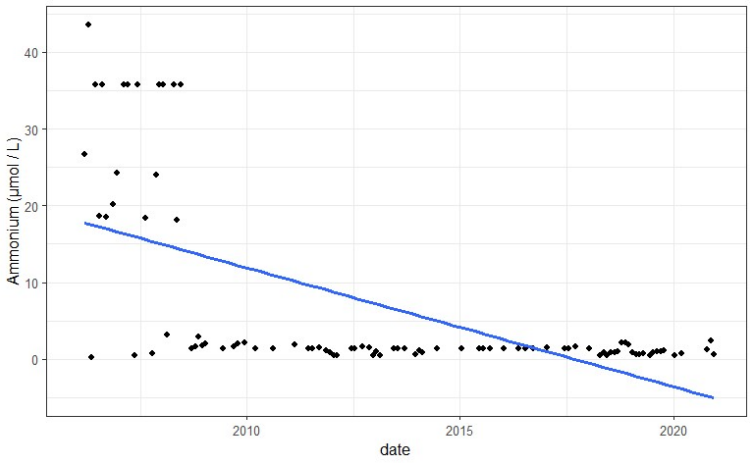

Figure 7.175 – Ammonium concentrations as a function of time in the Cumbria assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

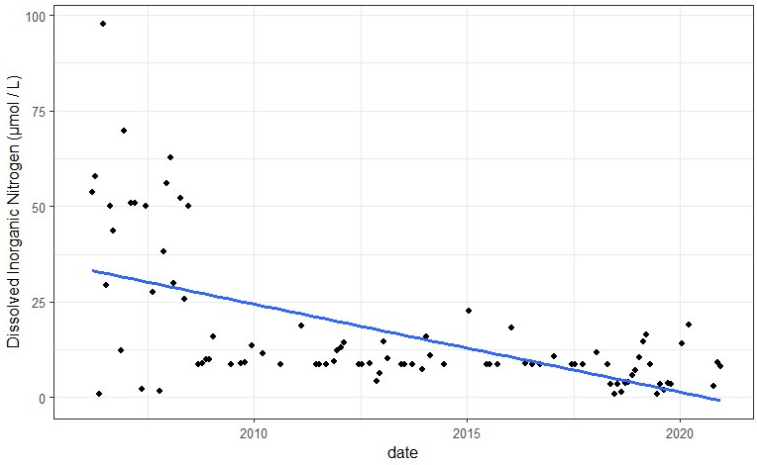

Figure 7.176 – DIN concentrations as a function of time in the Cumbria assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

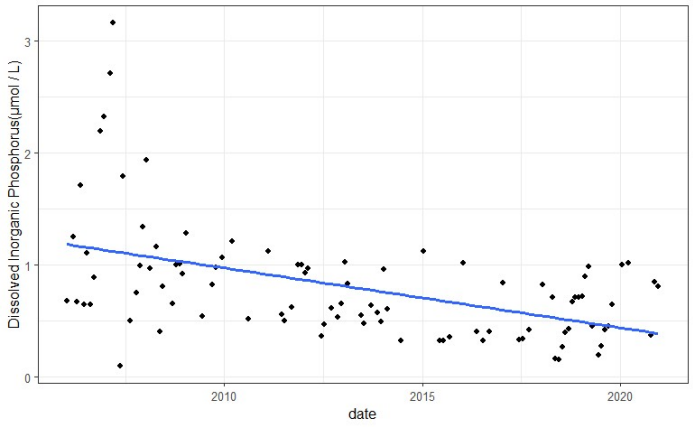

Figure 7.177 - DIP concentrations as a function of time in the Cumbria assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

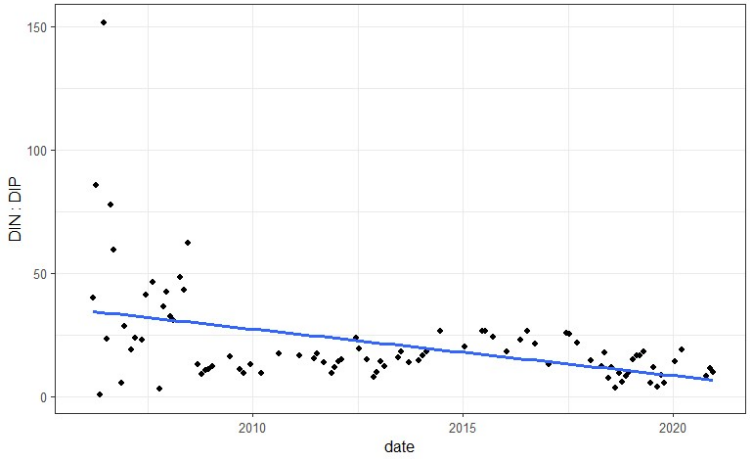

Figure 7.178 – DIN : DIP as a function of time in the Cumbria assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

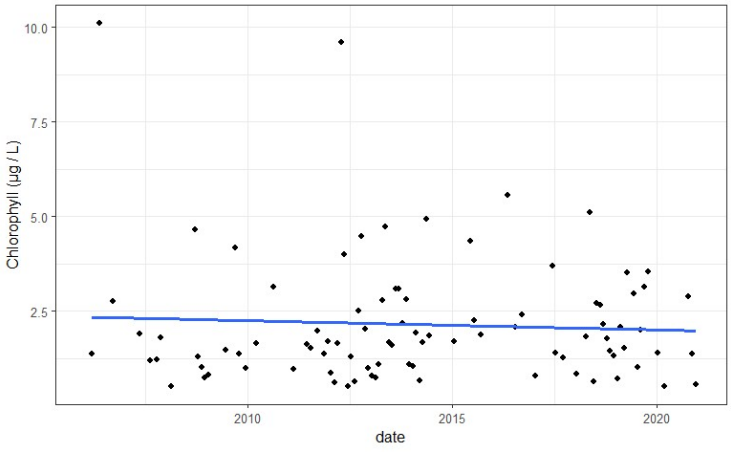

Figure 7.179 – Chlorophyll concentrations as a function of time in the Cumbria assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

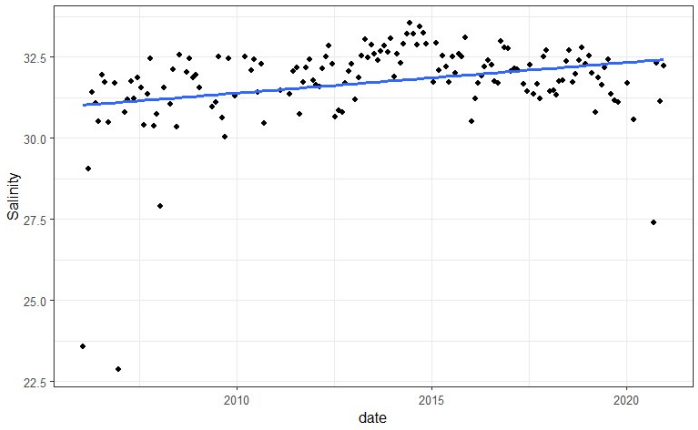

Figure 7.180 - Salinity as a function of time in the Cumbria assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

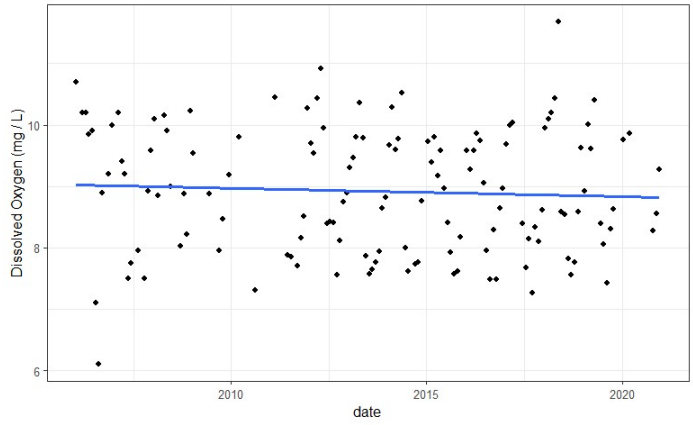

Figure 7.181 – Dissolved oxygen concentrations as a function of time in the Cumbria assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.2.7 Morecambe Bay

Table 7.25 - *p* Values from the results of the linear models in the Morecambe Bay assessment area.

Variable	***P* value**
Chlorophyll	0.00
DIP	0.00
Ammonium	0.00
TOxN	0.01
Salinity	0.28
DIN : DIP	0.02
DIN	0.00
Dissolved Oxygen	0.78

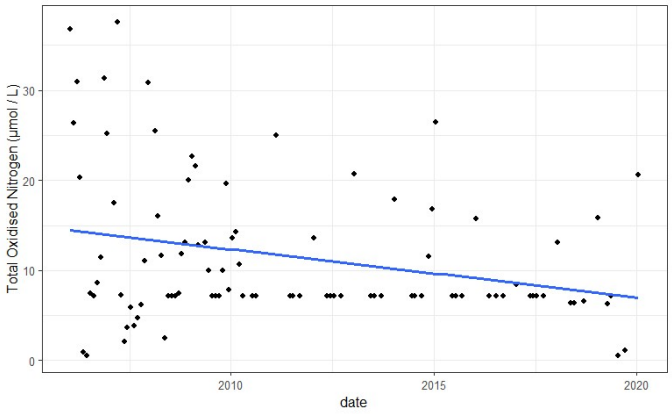

Figure 7.182 - TOxN concentrations as a function of time in the Morecambe Bay assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

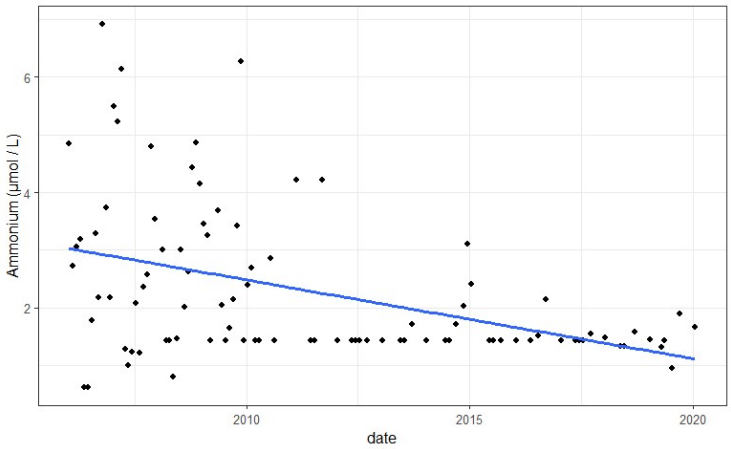

Figure 7.183 - Ammonium concentrations as a function of time in the Morecambe Bay assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

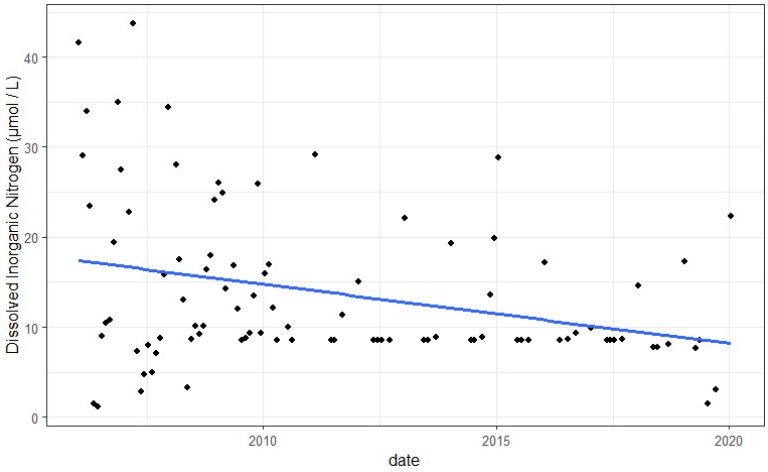

Figure 7.184 – DIN concentrations as a function of time in the Morecambe Bay assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

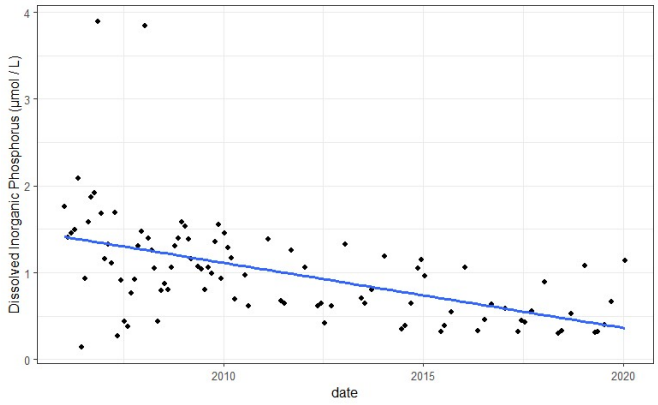

Figure 7.185 - DIP concentrations as a function of time in the Morecambe Bay assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

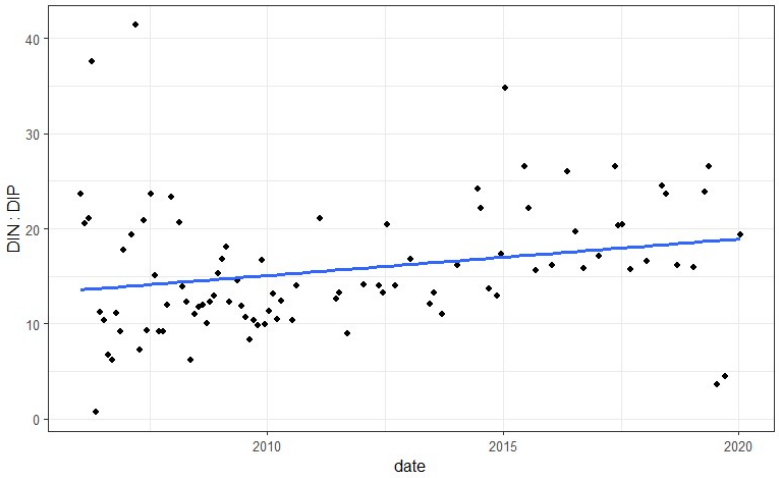

Figure 7.186 – DIN : DIP as a function of time in the Morecambe Bay assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN : DIP.

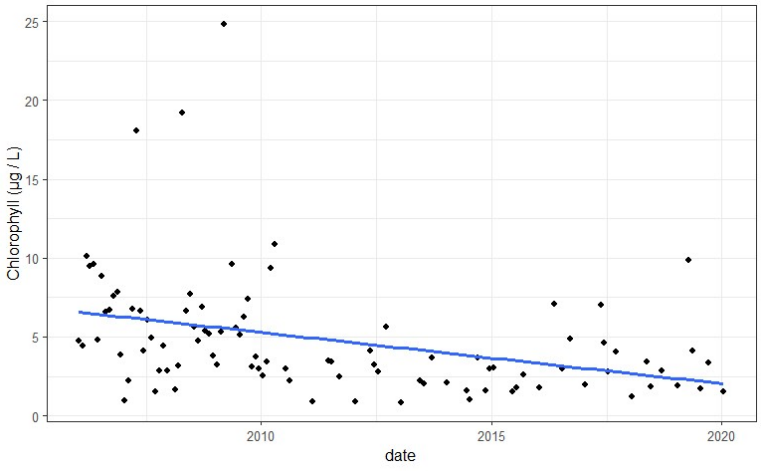

Figure 7.187 - Chlorophyll concentrations as a function of time in the Morecambe Bay assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

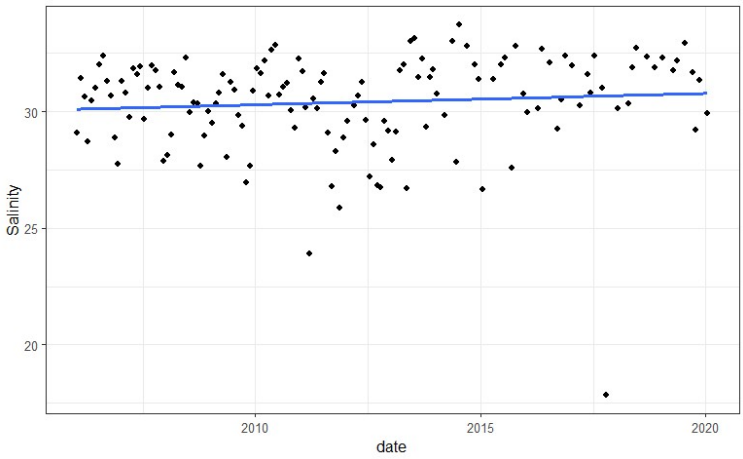

Figure 7.188 - Salinity as a function of time in the Morecambe Bay assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

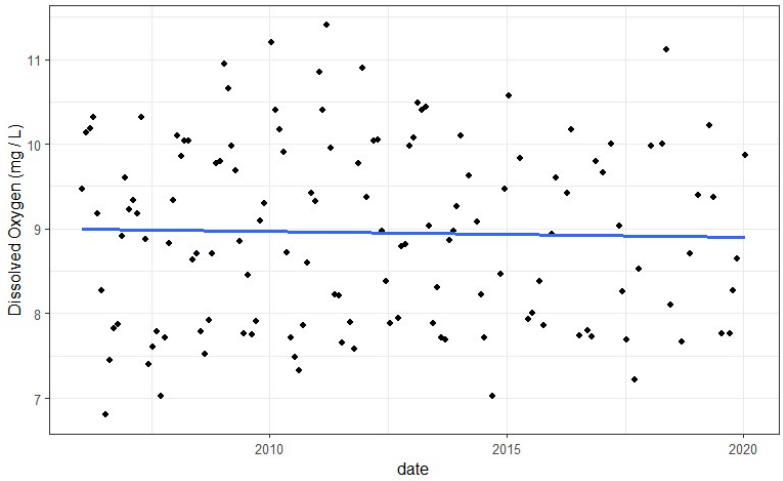

Figure 7.189 – Dissolved oxygen concentrations as a function of time in the Morecambe Bay assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.2.8 Kent

Table 7.26 - *p* Value from the result of the linear models in the Kent assessment area.

Variable	***P* value**
Salinity	0.00

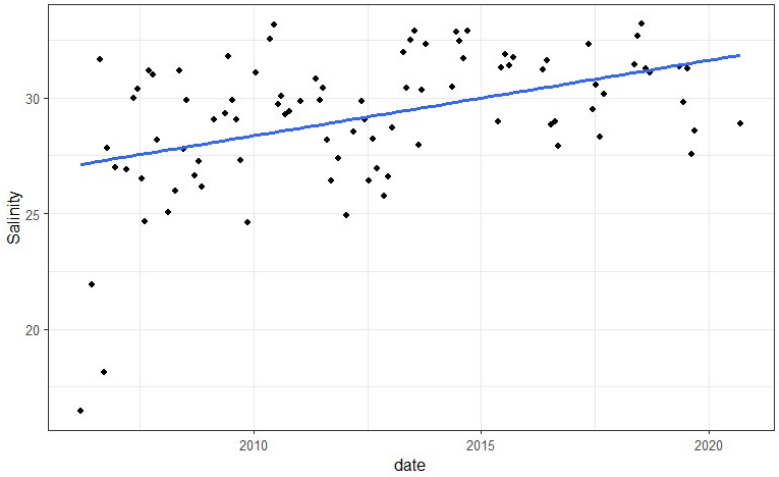

Figure 7.190 - Salinity as a function of time in the Kent assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

7.2.9 Leven

Table 7.27 - *p* values from the result of the linear models in the Leven assessment area.

Variable	***P* value**
Salinity	0.69
Dissolved Oxygen	0.09

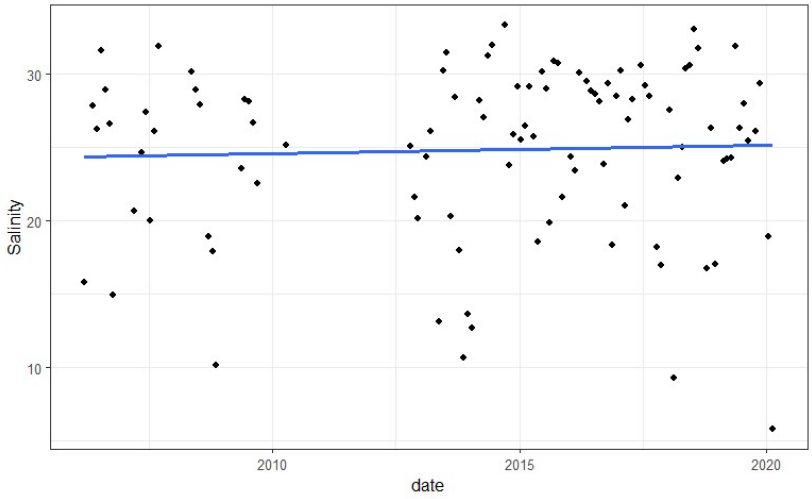

Figure 7.191 - Salinity as a function of time in the Leven assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

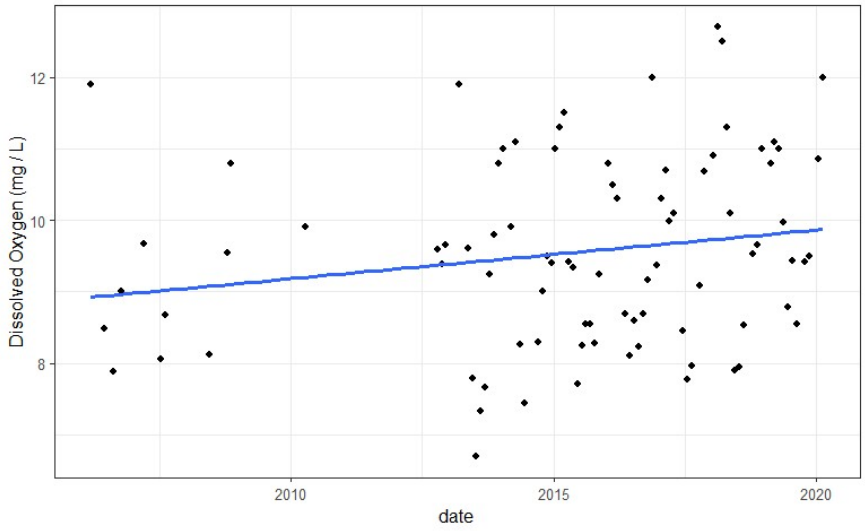

Figure 7.192 – Dissolved oxygen concentration as a function of time in the Leven assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.2.10 Wyre

Table 7.28 - *p* values from the result of the linear models in the Wyre assessment area.

Variable	***P* value**
Chlorophyll	0.53
DIP	0.00
Ammonium	0.08
TOxN	0.76
Salinity	0.85
DIN : DIP	0.92
DIN	0.61
Dissolved Oxygen	0.44

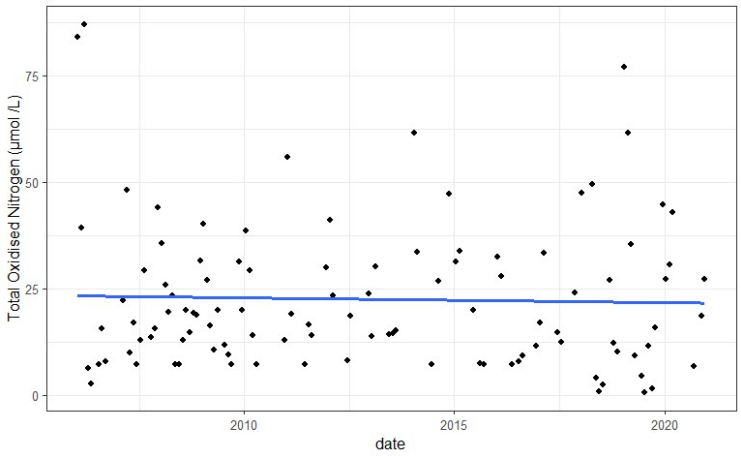

Figure 7.193 – TOxN concentration as a function of time in the Wyre assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly TOxN.

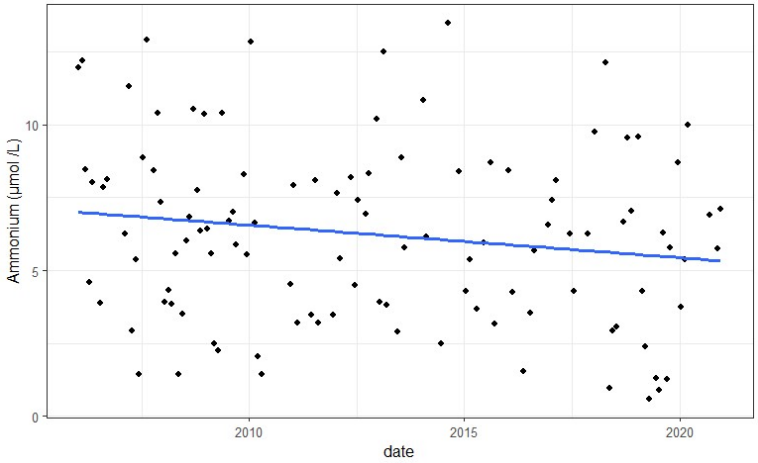

Figure 7.194 – Ammonium concentration as a function of time in the Wyre assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly ammonium.

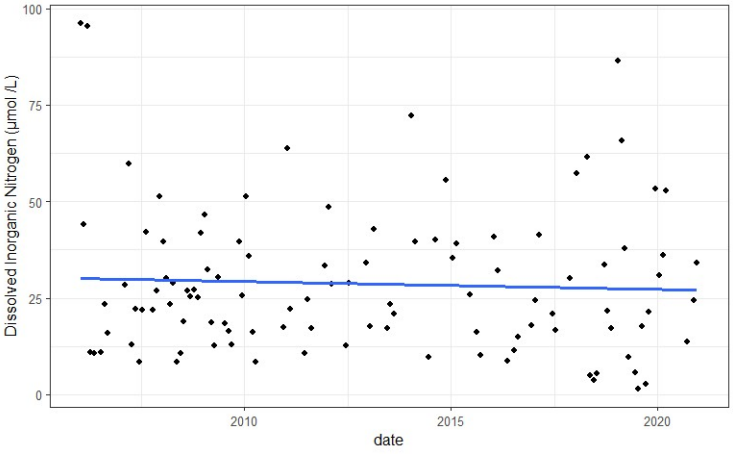

Figure 7.195 - DIN concentration as a function of time in the Wyre assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN.

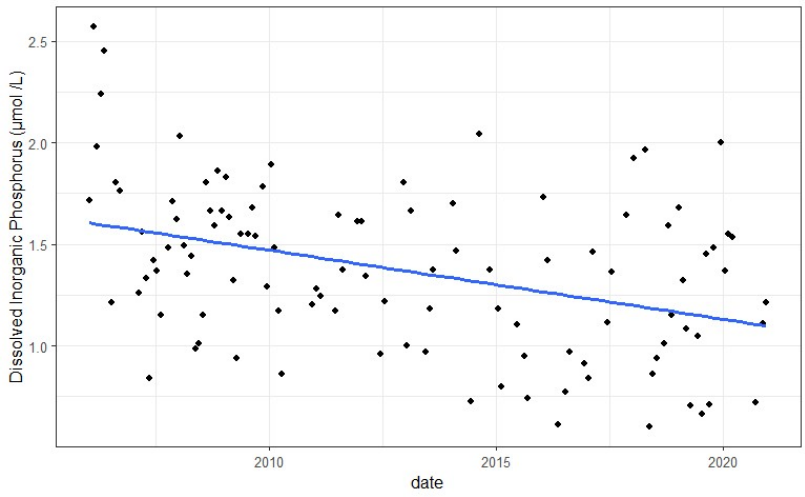

Figure 7.196 - DIP concentration as a function of time in the Wyre assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIP.

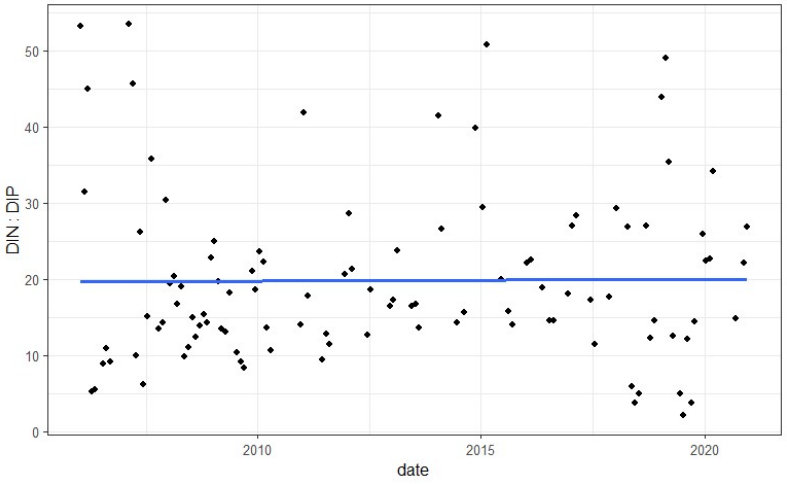

Figure 7.197 – DIN : DIP as a function of time in the Wyre assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly DIN :DIP.

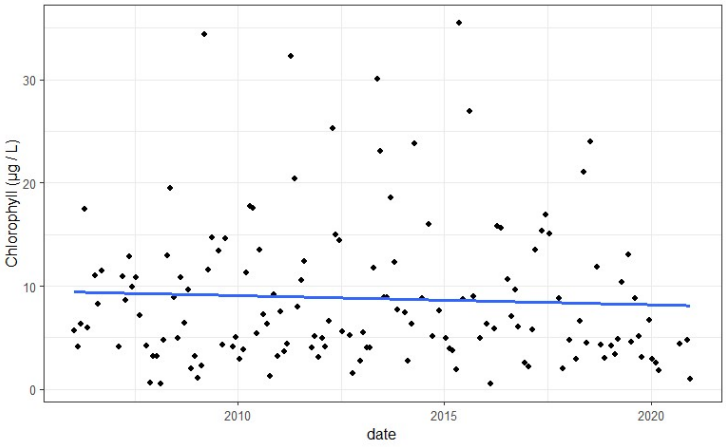

Figure 7.198 - Chlorophyll concentration as a function of time in the Wyre assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

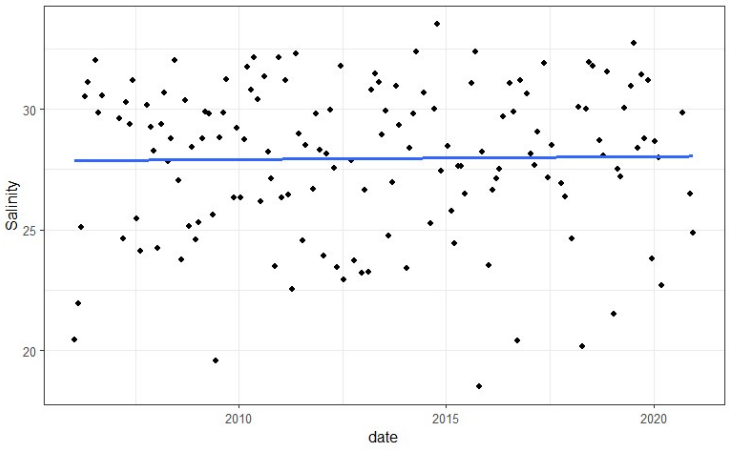

Figure 7.199 - Salinity as a function of time in the Wyre assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

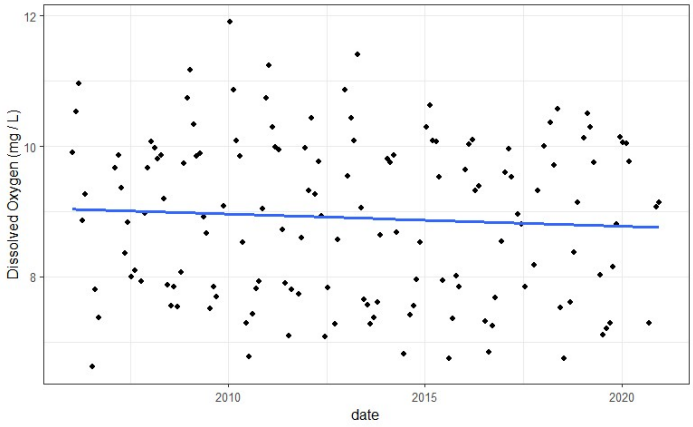

Figure 7.200 – Dissolved oxygen concentration as a function of time in the Wyre assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.2.11 Lune

Table 7.29 - *p* values from the result of the linear models in the Lune assessment area.

Variable	***P* value**
Chlorophyll	0.02
DIP	0.00
Ammonium	0.60
TOxN	0.52
Salinity	0.00
DIN : DIP	0.00
DIN	0.41
Dissolved Oxygen	0.63

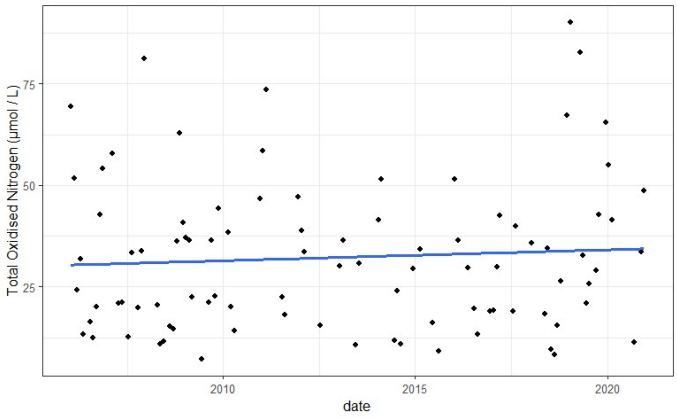

Figure 7.201 - TOxN concentration as a function of time in the Lune assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved TOxN.

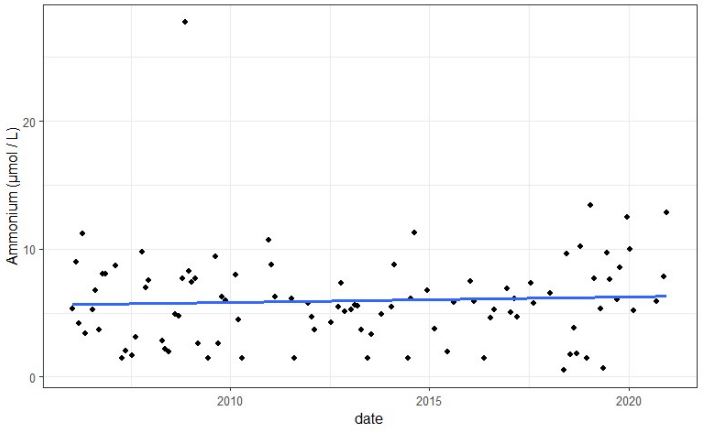

Figure 7.202 - Ammonium concentration as a function of time in the Lune assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved ammonium.

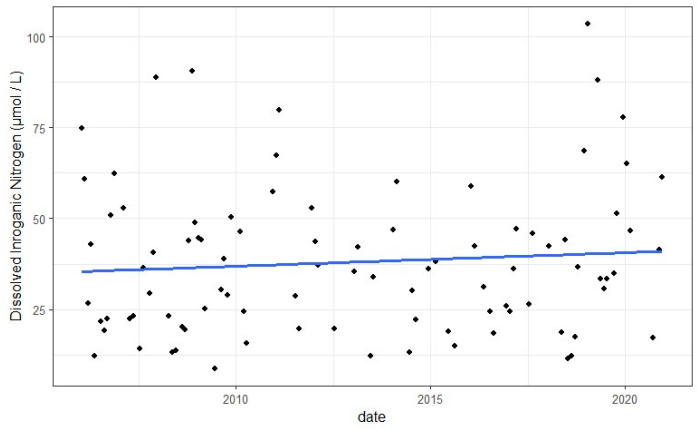

Figure 7.203 - DIN concentration as a function of time in the Lune assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved DIN.

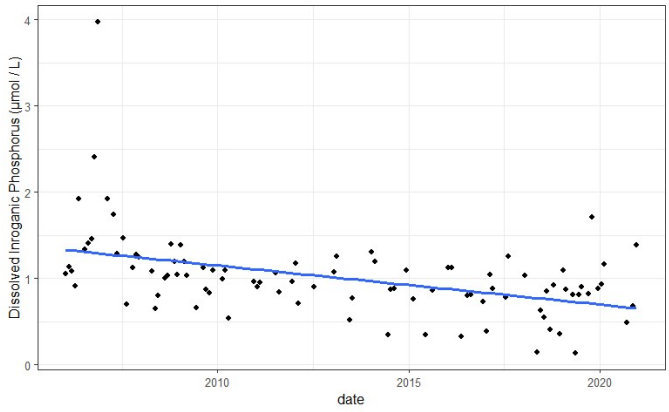

Figure 7.204 – DIP concentration as a function of time in the Lune assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved DIP.

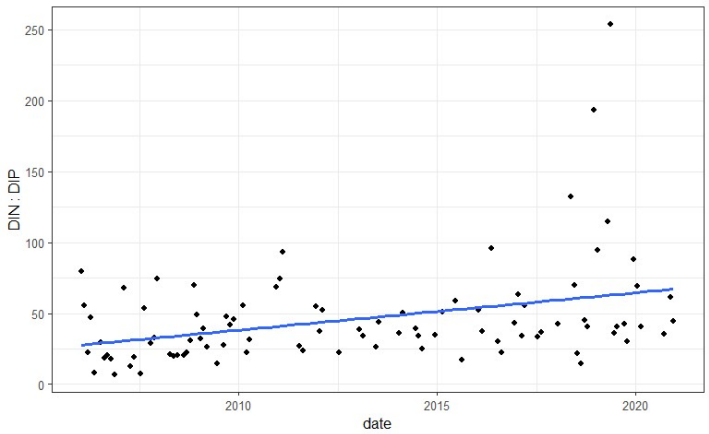

Figure 7.205 – DIN : DIP as a function of time in the Lune assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved DIN : DIP.

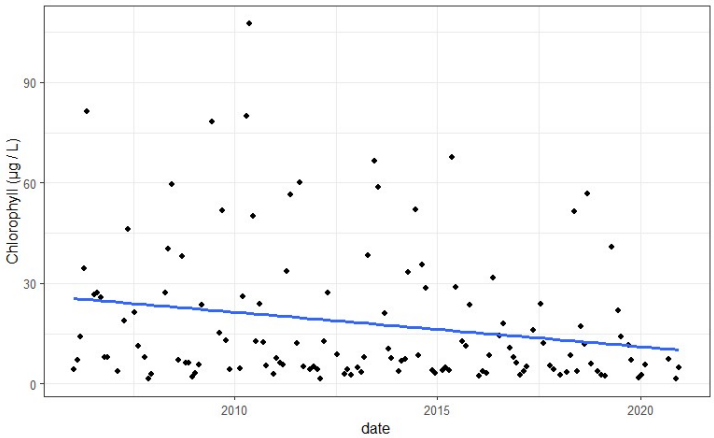

Figure 7.206 – Chlorophyll concentration as a function of time in the Lune assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly chlorophyll.

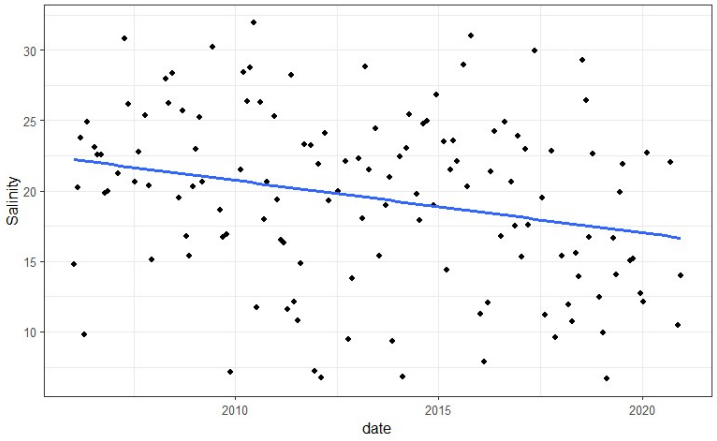

Figure 7.207 - Salinity as a function of time in the Lune assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly salinity.

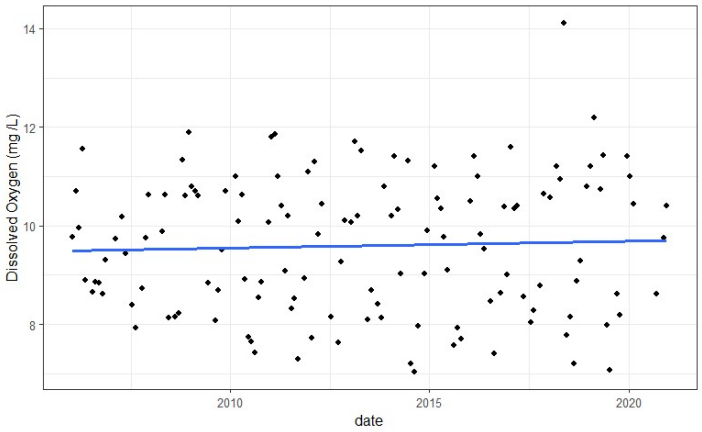

Figure 7.208 – Dissolved oxygen concentrations as a function of time in the Lune assessment area. The blue line represents a linear regression of date ~ concentration for the mean monthly dissolved oxygen.

7.3 Sampling occasions and data availability in the Thames estuary and Liverpool Bay between April 2022 and June 2024

7.3.1 Thames estuary

Table 7.30 - Available salinity data at each station in each sampling occasion in the Thames estuary.

	July 22	Aug 22	Sep 22	Oct 22	Jan 23	Jun 23	Jul 23	Sep 23	Oct 23	Dec 23	Jan 24	Feb 24	Jun 24
Essex Coast	x												
Black water 2	x												
Off Black Water	x												
South Black Water 2	x												
Warp SB	x	x	x	x		x	x	x	x	x	x	x	x
MA1		x	x	x	x	x	x	x	x	x	x		x
MA2		x	x	x	x	x	x	x	x	x	x		x
MA3		x	x	x		x	x	x	x	x	x		x
MA4		x	x	x		x	x	x	x	x	x		x
East of Warp		x	x	x	x	x	x	x	x	x	x	x	x
Graves end		x	x	x		x	x	x	x	x	x		x

Table 7.31 - Available nutrient data at each station in each sampling occasion in the Thames estuary.

	July 22	Aug 22	Sep 22	Oct 22	Jan 23	Jun 23	Jul 23	Sep 23	Oct 23	Dec 23	Jan 24	Feb 24	Jun 24
Essex Coast	X												
Black water 2	X												
Off Black Water	X												
South Black Water 2	X												
Warp SB	X	X	X	X	X	X	X	X	X	X	X	X	X
MA1		X	X	X		X	X	X	X	X	X		X
MA2		X	X	X	X	X	X	X	X	X	X		X
MA3		X	X	X		X	X	X	X	X	X		X
MA4		X	X	X		X	X	X	X	X	X		X
East of Warp		X	X	X	X	X	X	X	X	X	X	X	X
Graves end		X	X	X		X	X	X	X	X	X		X

Table 7.32 - Available suspended particulate matter data at each station in each sampling occasion in the Thames estuary.

	July 22	Aug 22	Sep 22	Oct 22	Jan 23	Jun 23	Jul 23	Sep 23	Oct 23	Dec 23	Jan 24	Feb 24	Jun 24
Essex Coast	X												
Black water 2	X												
Off Black Water	X												
South Black Water 2	X												
Warp SB	X	X	X	X	X	X	X	X	X	X	X	X	X
MA1		X	X	X		X	X	X	X	X	X		X
MA2		X	X	X	X	X	X	X	X	X	X		X
MA3		X	X	X		X	X	X	X	X	X		X
MA4		X	X	X		X	X	X	X	X	X		X
East of Warp		X	X	X	X	X	X	X	X	X	X	X	X
Graves end		X	X	X		X	X	X	X	X	X		X

Table 7.33 - Available RBR salinity data at each station in each sampling occasion within the Thames estuary.

	July 22	Aug 22	Sep 22	Oct 22	Jan 23	Jun 23	Jul 23	Sep 23	Oct 23	Dec 23	Jan 24	Feb 24	Jun 24	Sep24
Essex Coast														
Black water 2														
Off Black Water														
South Black Water 2														
Warp SB				X	X	X		X	X	X	X	X	X	X
MA1			X	X	X	X		X	X	X	X		X	X
MA2			X	X	X	X		X	X	X	X		X	X
MA3			X	X		X		X	X	X	X		X	X
MA4			X	X		X		X	X	X	X		X	X
East of Warp				X	X	X		X	X	X	X	X	X	X
Graves end			X	X		X		X	X	X	X		X	X

Table 7.34 - Available RBR turbidity data at each station in each sampling occasion within the Thames estuary.

	July 22	Aug 22	Sep 22	Oct 22	Jan 23	Jun 23	Jul 23	Sep 23	Oct 23	Dec 23	Jan 24	Feb 24	Jun 24	Sep24
Essex Coast														
Black water 2														
Off Black Water														
South Black Water 2														
Warp SB				X	X	X		X	X	X	X	X	X	X
MA1			X	X	X	X		X	X	X	X		X	X
MA2			X	X	X	X		X	X	X	X		X	X
MA3			X	X		X		X	X	X	X		X	X
MA4			X	X		X		X	X	X	X		X	X
East of Warp				X	X	X		X	X	X	X	X	X	X
Graves end			X	X		X		X	X	X	X		X	X

Table 7.35 - Available RBR PAR data at each station in each sampling occasion in the Thames estuary.

	July 22	Aug 22	Sep 22	Oct 22	Jan 23	Jun 23	Jul 23	Sep 23	Oct 23	Dec 23	Jan 24	Feb 24	Jun 24	Sep24
Essex Coast														
Black water 2														
Off Black Water														
South Black Water 2														
Warp SB						X		X		X	X	X	X	X
MA1						X		X		X	X		X	X
MA2						X		X		X	X		X	X
MA3						X		X		X	X		X	X
MA4						X		X		X	X		X	X
East of Warp						X		X		X	X	X	X	X
Graves end						X		X		X	X		X	X

Table 7.36 - Available phytoplankton data at each station in each sampling occasion in the Thames estuary.

	July 22	Aug 22	Sep 22	Oct 22	Jan 23	Jun 23	Jul 23	Sep 23	Oct 23	Dec 23	Jan 24	Feb 24	Jun 24
Essex Coast													
Black water 2													
Off Black Water													
South Black Water 2													
Warp SB		X	X	X			X	X	X	X	X	X	
MA1		X	X	X	X		X	X	X	X	X		
MA2		X	X	X	X		X	X	X	X	X		
MA3		X	X	X			X	X	X	X	X		
MA4		X	X	X			X	X	X		X		
East of Warp		X	X	X	X		X	X	X	X	X	X	
Graves end		X	X	X			X	X	X		X		

7.3.2 Liverpool Bay

Table 7.37 - Available salinity data at each station in each sampling occasion in Liverpool Bay.

	Apr-22	Aug-22	Sep-22	Dec-22	Apr-23	May-23	Jun-23	Oct-23	Feb-24	Mar-24	May-24	Jun-24
LBSB					x	x	x	x	x	x	x	x
LV1	x				x	x	x	x	x	x	x	x
LV10	x	x			x	x	x	x	x	x	x	x
LV11												
LV12	x											
LV13	x											
LV14	x											
LV15	x											
LV16	x			x	x	x	x	x	x	x	x	x
LV17	x											
LV18	x											
LV19	x											
LV2												
LV20	x			x	x	x	x	x	x	x	x	x
LV22	x				x	x	x	x	x	x	x	x
LV23	x			x	x	x	x	x	x	x	x	x
LV24	x											
LV4	x											
LV5	x											
LV6	x				x		x	x	x	x	x	x
LV7	x				x	x	x	x	x	x	x	x
LV8	x				x	x	x	x	x	x	x	x
LV9	x											
LVWRT												x
LVX										x		

Table 7.38 – Available nutrient data at each station in each sampling location in Liverpool Bay.

	Apr-22	Aug-22	Sep-22	Dec-22	Apr-23	May-23	Jun-23	Oct-23	Feb-24	Mar-24	May-24	Jun-24
LBSB			X		X	X	X	X	X	X	X	X
LV1	X		X		X	X	X	X	X	X	X	X
LV10	X		X		X	X	X	X	X	X	X	X
LV11	X											
LV12	X											
LV13	X											
LV14	X											
LV15	X											
LV16	X				X	X	X	X	X	X	X	X
LV17	X											
LV18	X											
LV19	X											
LV2	X											
LV20					X	X	X	X	X	X	X	X
LV22	X		X		X	X	X	X	X	X	X	X
LV23	X				X	X	X	X	X	X	X	X
LV24	X											
LV4	X											
LV5	X											
LV6	X				X		X	X	X	X	X	X
LV7	X				X	X	X	X	X	X	X	X
LV8	X				X	X	X	X	X	X	X	X
LV9	X											
LVWRT												X
LVX												

Table 7.39 – Available suspended particulate matter concentrations at each station in each sampling occasion in Liverpool Bay.

	Apr-22	Aug-22	Sep-22	Dec-22	Apr-23	May-23	Jun-23	Oct-23	Feb-24	Mar-24	May-24	Jun-24
LBSB			x		x	x	x	x	x	x	x	x
LV1			x		x	x	x	x	x	x	x	x
LV10			x		x		x	x	x	x	x	x
LV11												
LV12												
LV13												
LV14												
LV15												
LV16				x	x	x	x	x	x	x	x	x
LV17												
LV18												
LV19												
LV2												
LV20				x	x	x	x	x	x	x	x	x
LV22		x			x	x	x	x	x	x	x	x
LV23				x	x	x	x	x	x	x	x	x
LV24												
LV4												
LV5												
LV6					x	x	x	x	x	x	x	x
LV7					x	x	x	x	x	x	x	x
LV8					x	x	x	x	x	x	x	x
LV9												
LVWRT												x
LVX										x		

Table 7.40– Available Chlorophyll data at each station in each sampling occasion in Liverpool Bay.

	Apr-22	Aug-22	Sep-22	Dec-22	Apr-23	May-23	Jun-23	Oct-23	Feb-24	Mar-24	May-24	Jun-24
LBSB			X	X	X	X	X	X	X	X	X	X
LV1	X		X	X	X	X	X	X	X	X	X	X
LV10	X		X	X	X	X	X	X	X	X	X	X
LV11	X											
LV12	X											
LV13	X											
LV14	X											
LV15	X											
LV16	X			X	X	X	X	X	X	X	X	X
LV17	X											
LV18	X											
LV19	X											
LV2	X											
LV20	X			X	X	X	X	X	X	X	X	X
LV22	X	X	X	X	X	X	X	X	X	X	X	X
LV23	X			X	X	X	X	X	X	X	X	X
LV24	X											
LV4	X											
LV5	X											
LV6	X			X	X	X	X	X	X	X	X	X
LV7	X			X	X	X	X	X	X	X	X	X
LV8	X			X	X	X	X	X	X	X	X	X
LV9	X											
LVWRT												X
LVX										X		

Table 7.41 – Available phytoplankton data at each station in each sampling occasion in Liverpool Bay.

	Apr-22	Aug-22	Sep-22	Dec-22	Apr-23	May-23	Jun-23	Oct-23	Feb-24	Mar-24	May-24	Jun-24
LBSB				x	x			x	x	x		
LV1	x	x		x	x	x		x	x	x		
LV10	x	x		x	x	x		x	x	x		
LV11	x											
LV12	x											
LV13	x											
LV14	x											
LV15	x											
LV16	x			x	x	x		x	x	x		
LV17	x											
LV18	x											
LV19	x											
LV2	x											
LV20	x			x	x	x		x	x	x		
LV22	x	x		x	x			x	x	x		
LV23	x			x	x			x	x	x		
LV24	x											
LV4	x											
LV5	x											
LV6	x			x	x	x		x	x	x		
LV7	x			x	x	x		x	x	x		
LV8	x			x		x		x	x	x		
LV9	x											
LVWRT												
LVX										x		

Table 7.42 – Available RBR Salinity data at each station in each sampling occasion in Liverpool Bay

	Apr-22	Aug-22	Sep-22	Dec-22	Apr-23	May-23	Jun-23	Oct-23	Feb-24	Mar-24	May-24	Jun-24
LBSB		X	X				X	X		X	X	X
LV1		X	X				X	X		X	X	X
LV10		X	X				X	X		X	X	X
LV11												
LV12												
LV13												
LV14												
LV15												
LV16							X	X		X	X	X
LV17												
LV18												
LV19												
LV2												
LV20							X	X		X	X	X
LV22		X	X				X	X		X	X	X
LV23							X	X		X	X	X
LV24												
LV4												
LV5												
LV6							X	X		X	X	X
LV7								X		X	X	X
LV8							X	X		X	X	X
LV9												
LVWRT												X
LVX										X		

Table 7.43– Available RBR turbidity data at each station in each sampling occasion in Liverpool Bay

	Apr-22	Aug-22	Sep-22	Dec-22	Apr-23	May-23	Jun-23	Oct-23	Feb-24	Mar-24	May-24	Jun-24
LBSB		x	x				x	x		x	x	x
LV1	x	x	x				x	x		x	x	x
LV10	x	x	x				x	x		x	x	x
LV11	x											
LV12	x											
LV13	x											
LV14	x											
LV15	x											
LV16	x						x	x		x	x	x
LV17	x											
LV18	x											
LV19	x											
LV2	x											
LV20	x						x	x		x	x	x
LV22	x	x	x				x	x		x	x	x
LV23	x						x	x		x	x	x
LV24	x											
LV4	x											
LV5	x											
LV6	x						x	x		x	x	x
LV7	x						x	x		x	x	x
LV8	x						x	x		x	x	x
LV9	x											
LVWRT												x
LVX										x		

Table 7.44 – Available RBR PAR data at each station in each sampling occasion in Liverpool Bay

	Apr-22	Aug-22	Sep-22	Dec-22	Apr-23	May-23	Jun-23	Oct-23	Feb-24	Mar-24	May-24	Jun-24
LBSB							X	X		X	X	X
LV1							X	X		X	X	X
LV10							X	X		X	X	X
LV11												
LV12												
LV13												
LV14												
LV15												
LV16							X	X		X	X	X
LV17												
LV18												
LV19												
LV2												
LV20							X	X		X	X	X
LV22							X	X		X	X	X
LV23							X	X		X	X	X
LV24												
LV4												
LV5												
LV6							X	X		X	X	X
LV7								X		X	X	X
LV8							X	X		X	X	X
LV9												
LVWRT												X
LVX										X		

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References

Adolf, J.E., Stoecker, D.K., Harding, L.W., 2006. The Balance of Autotrophy and Heterotrophy During Mixotrophic Growth of *Karlodinium Micrum* (Dinophyceae). *Journal of Plankton Research*, 28, 737-751.doi:10.1093/plankt/fbl007

Alldred, F.C., Gröcke, D.R., Jackson, S.E., Reid, G., 2024. Nitrogen Isotopes in Herbaria Document Historical Nitrogen Sewage Pollution in the Mersey Estuary, England. *Environmental Science-Advances*, 3.doi:10.1039/d4va00015c

Allen, J.R., Slinn, D.J., Shammon, T.M., Hartnoll, R.G., Hawkins, S.J., 1998. Evidence for Eutrophication of the Irish Sea over Four Decades. *Limnology and Oceanography*, 43, 1970-1974.doi:10.4319/lo.1998.43.8.1970

Alvarez-Fernandez, S., Riegman, R., 2014. Chlorophyll in North Sea Coastal and Offshore Waters Does Not Reflect Long Term Trends of Phytoplankton Biomass. *Journal of Sea Research*, 91, 35-44.doi:10.1016/j.seares.2014.04.005

Andersen, I.M., Williamson, T.J., González, M.J., Vanni, M.J., 2020. Nitrate, Ammonium, and Phosphorus Drive Seasonal Nutrient Limitation of Chlorophytes, Cyanobacteria, and Diatoms in a Hyper-Eutrophic Reservoir. *Limnology and Oceanography*, 65, 962-978.doi:10.1002/lno.11363

Anderson, D.M., Burkholder, J.M., Cochlan, W.P., Glibert, P.M., Gobler, C.J., Heil, C.A., Kudela, R.M., Parsons, M.L., Rensel, J.E.J., Townsend, D.W., Trainer, V.L., Vargo, G.A., 2008. Harmful Algal Blooms and Eutrophication: Examining Linkages from Selected Coastal Regions of the United States. *Harmful Algae*, 8, 39-53.doi:10.1016/j.hal.2008.08.017

Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful Algal Blooms and Eutrophication: Nutrient Sources, Composition, and Consequences. *Estuaries*, 25, 704-726.doi:10.1007/bf02804901

Anning, T., MacIntyre, H.L., Pratt, S.M., Sammes, P.J., Gibb, S., Geider, R.J., 2000. Photoacclimation in the Marine Diatom Skeletonema Costatum. *Limnology and Oceanography*, 45, 1807-1817.doi:10.4319/lo.2000.45.8.1807

Ansari, A.A., Gill, S.S., Khan, F.A., 2010. Eutrophication: Threat to Aquatic Ecosystems. In: Ansari, A., Singh Gill, S., Lanza, G., Rast, W. (Eds) Eutrophication: Causes, Consequences and Control edited by. Springer, Dordrecht. 143-170.https://doi.org/10.1007/978-90-481-9625-8_7

Arar, E.J., Collins, G.B., 1997. Method 445.0 in Vitro Determination of Chlorophyll a and Pheophytin Ain Marine and Freshwater Algae by Fluorescence. edited by. U.S. Environmental Protection Agency, Washington, DC

Atkinson, A., Lilley, M.K.S., Hirst, A.G., McEvoy, A.J., Tarran, G.A., Widdicombe, C., Fileman, E.S., Woodward, E.M.S., Schmidt, K., Smyth, T.J., Somerfield, P.J., 2021. Increasing Nutrient Stress Reduces the Efficiency of Energy Transfer through Planktonic Size Spectra. *Limnology and Oceanography*, 66, 422-437.doi:10.1002/lno.11613

Baek, S.H., Sun, X.X., Lee, Y.J., Wang, S.Y., Han, K.N., Choi, J.K., Noh, J.H., Kim, E.K., 2003. Mitigation of Harmful Algal Blooms by Sophorolipid. *Journal of Microbiology and Biotechnology*, 13, 651-659

Barçante, B., Nascimento, N.O., Silva, T.F.G., Reis, L.A., Giani, A., 2020. Cyanobacteria Dynamics and Phytoplankton Species Richness as a Measure of Waterbody Recovery: Response to

Phosphorus Removal Treatment in a Tropical Eutrophic Reservoir. *Ecological Indicators*, 117, 11.doi:10.1016/j.ecolind.2020.106702

Barney, S., 2022. Earth's Other Lung: Phytoplankton. *The Synapse: Intercollegiate science magazine*, 30, 13

Beaugrand, G., 2005. Monitoring Pelagic Ecosystems Using Plankton Indicators. *Ices Journal of Marine Science*, 62, 333-338.doi:10.1016/j.icesjms.2005.01.002

Becker, S., Aoyama, M., Woodward, E.M.S., Bakker, K., Coverly, S., Mahaffey, C., Tanhua, T., 2020. Go-Ship Repeat Hydrography Nutrient Manual: The Precise and Accurate Determination of Dissolved Inorganic Nutrients in Seawater, Using Continuous Flow Analysis Methods. *Frontiers in marine science*, 7, 581 - 790.doi:10.3389/fmars.2020.581790

Bedford, J., Ostle, C., Johns, D.G., Atkinson, A., Best, M., Bresnan, E., Machairopoulou, M., Graves, C.A., Devlin, M., Milligan, A., Pitois, S., Mellor, A., Tett, P., McQuatters-Gollop, A., 2020. Lifeform Indicators Reveal Large-Scale Shifts in Plankton across the North-West European Shelf. *Global Change Biology*, 26, 3482-3497.doi:10.1111/gcb.15066

Berdalet, E., Peters, F., Koumandou, V.L., Roldan, C., Guadayol, O., Estrada, M., 2007. Species-Specific Physiological Response of Dinoflagellates to Quantified Small-Scale Turbulence. *Journal of Phycology*, 43, 965-977.doi:10.1111/j.1529-8817.2007.00392.x

Best, M.A., Wither, A.W., Coates, S., 2007. Dissolved Oxygen as a Physico-Chemical Supporting Element in the Water Framework Directive. *Marine Pollution Bulletin*, 55, 53-64.doi:10.1016/j.marpolbul.2006.08.037

Beusen, A., Doelman, J., Van Beek, L., Van Puijenbroek, P., Mogollón, J., Van Grinsven, H., Stehfest, E., Van Vuuren, D., Bouwman, A., 2022. Exploring River Nitrogen and Phosphorus Loading and Export to Global Coastal Waters in the Shared Socio-Economic Pathways. *Global Environmental Change*, 72, 102426.doi:10.1016/j.gloenvcha.2021.102426

Bi, R., Cao, Z., Ismar-Rebitz, S.M.H., Sommer, U., Zhang, H.L., Ding, Y., Zhao, M.X., 2021. Responses of Marine Diatom-Dinoflagellate Competition to Multiple Environmental Drivers: Abundance, Elemental, and Biochemical Aspects. *Front. Microbiol.*, 12.doi:10.3389/fmicb.2021.731786

Bieroza, M., Acharya, S., Benisch, J., Ter Borg, R.N., Hallberg, L., Negri, C., Pruitt, A., Pucher, M., Saavedra, F., Staniszewska, K., 2023. Advances in Catchment Science, Hydrochemistry, and Aquatic Ecology Enabled by High-Frequency Water Quality Measurements. *Environmental Science & Technology*, 57, 4701-4719.doi:10.1021/acs.est.2c07798

Billen, G., Garnier, J., Deligne, C., Billen, C., 1999. Estimates of Early-Industrial Inputs of Nutrients to River Systems: Implication for Coastal Eutrophication. *Science of the Total Environment*, 243, 43-52.doi:10.1016/s0048-9697(99)00327-7

Blomqvist, P., Pettersson, A., Hyenstrand, P., 1994. Ammonium-Nitrogen - a Key Regulatory Factor Causing Dominance of Non-Nitrogen-Fixing Cyanobacteria in Aquatic Systems. *Archiv Fur Hydrobiologie*, 132, 141-164

Boersma, M., 2000. The Nutritional Quality of P-Limited Algae for Daphnia. *Limnology and Oceanography*, 45, 1157-1161.doi:10.4319/lo.2000.45.5.1157

Boesch, D.F., 2019. Barriers and Bridges in Abating Coastal Eutrophication. *Frontiers in Marine Science*, 6.doi:10.3389/fmars.2019.00123

Bowes, M.J., Armstrong, L.K., Harman, S.A., Wickham, H.D., Nicholls, D.J., Scarlett, P.M., Roberts, C., Jarvie, H.P., Old, G.H., Gozzard, E., 2018. Weekly Water Quality Monitoring Data for the River Thames (Uk) and Its Major Tributaries (2009–2013): The Thames Initiative Research Platform. *Earth System Science Data*, 10, 1637-1653.doi:10.5194/essd-10-1637-2018

Bowes, M.J., Gozzard, E., Johnson, A.C., Scarlett, P.M., Roberts, C., Read, D.S., Armstrong, L.K., Harman, S.A., Wickham, H.D., 2012. Spatial and Temporal Changes in Chlorophyll-a Concentrations in the River Thames Basin, Uk: Are Phosphorus Concentrations Beginning to Limit Phytoplankton Biomass? *Science of the Total Environment*, 426, 45-55.doi:10.1016/j.scitotenv.2012.02.056

Bowes, M.J., Ings, N.L., McCall, S.J., Warwick, A., Barrett, C., Wickham, H.D., Harman, S.A., Armstrong, L.K., Scarlett, P.M., Roberts, C., Lehmann, K., Singer, A.C., 2012. Nutrient and Light Limitation of Periphyton in the River Thames: Implications for Catchment Management. *Science of the Total Environment*, 434, 201-212.doi:10.1016/j.scitotenv.2011.09.082

Branco, P., Egas, M., Elser, J.J., Huisman, J., 2018. Eco-Evolutionary Dynamics of Ecological Stoichiometry in Plankton Communities. *American Naturalist*, 192, E1-E20.doi:10.1086/697472

Breitburg, D., Levin, L.A., Oschlies, A., Grégoire, M., Chavez, F.P., Conley, D.J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G.S., Limburg, K.E., Montes, I., Naqvi, S.W.A., Pitcher, G.C., Rabalais, N.N., Roman, M.R., Rose, K.A., Seibel, B.A., Telszewski, M., Yasuhara, M., Zhang, J., 2018. Declining Oxygen in the Global Ocean and Coastal Waters. *Science*, 359, eaam7240.doi:10.1126/science.aam7240

Brzezinski, M.A., 1985. The Si-C-N Ratio of Marine Diatoms - Interspecific Variability and the Effect of Some Environmental Variables. *Journal of Phycology*, 21, 347-357

Burkholder, J.M., Glibert, P.M., Skelton, H.M., 2008. Mixotrophy, a Major Mode of Nutrition for Harmful Algal Species in Eutrophic Waters. *Harmful Algae*, 8, 77-93.doi:10.1016/j.hal.2008.08.010

Burson, A., Stomp, M., Akil, L., Brussaard, C.P.D., Huisman, J., 2016. Unbalanced Reduction of Nutrient Loads Has Created an Offshore Gradient from Phosphorus to Nitrogen Limitation in the North Sea. *Limnology and Oceanography*, 61, 869-888.doi:10.1002/lno.10257

Burson, A., Stomp, M., Greenwell, E., Grosse, J., Huisman, J., 2018. Competition for Nutrients and Light: Testing Advances in Resource Competition with a Natural Phytoplankton Community. *Ecology*, 99, 1108-1118.doi:10.1002/ecy.2187

Cabral, A., Bercovich, M.V., Fonseca, A., 2019. Implications of Poor-Regulated Wastewater Treatment Systems in the Water Quality and Nutrient Fluxes of a Subtropical Coastal Lagoon. *Regional Studies in Marine Science*, 29.doi:10.1016/j.rsma.2019.100672

Capuzzo, E., Lynam, C.P., Barry, J., Stephens, D., Forster, R.M., Greenwood, N., McQuatters-Gollop, A., Silva, T., van Leeuwen, S.M., Engelhard, G.H., 2018. A Decline in Primary Production in the North Sea over 25 Years, Associated with Reductions in Zooplankton Abundance and Fish Stock Recruitment. *Global Change Biology*, 24, E352-E364.doi:10.1111/gcb.13916

Capuzzo, E., Stephens, D., Silva, T., Barry, J., Forster, R.M., 2015. Decrease in Water Clarity of the Southern and Central North Sea During the 20th Century. *Global Change Biology*, 21, 2206-2214.doi:10.1111/gcb.12854

Carter, C.M., Ross, A.H., Schiel, D.R., Howard-Williams, C., Hayden, B., 2005. In Situ Microcosm Experiments on the Influence of Nitrate and Light on Phytoplankton Community Composition. *Journal of Experimental Marine Biology and Ecology*, 326, 1-13.doi:10.1016/j.jembe.2005.05.006

Charalampous, E., Matthiessen, B., Sommer, U., 2021. Grazing Induced Shifts in Phytoplankton Cell Size Explain the Community Response to Nutrient Supply. *Microorganisms*, 9.doi:10.3390/microorganisms9122440

Chen, K., Li, K.Q., Gao, P.Y., Wang, P., Hang, X.R., Chen, Y.A., Wang, X.L., 2022. Was Dissolved Nitrogen Regime Driving Diatom to Dinoflagellate Shift in the Bohai Sea? Evidences from Microcosm Experiment and Modeling Reproduction. *Journal of Geophysical Research-Biogeosciences*, 127.doi:10.1029/2021jg006737

Chu, T.V., Torréton, J.-P., Mari, X., Nguyen, H.M.T., Pham, K.T., Pham, T.T., Bouvier, T., Bettarel, Y., Pringault, O., Bouvier, C., 2014. Nutrient Ratios and the Complex Structure of Phytoplankton Communities in a Highly Turbid Estuary of Southeast Asia. *Environmental monitoring and assessment*, 186, 8555-8572.doi:10.1007/s10661-014-4024-y

Cloern, J., Alpine, A., 1988. Phytoplankton Growth Rates in a Light-Limited Environment, San Francisco Bay. *Marine Ecology Progress Series*, 44, 167-173.doi:10.3354/meps044167

Cloern, J.E., 1987. Turbidity as a Control on Phytoplankton Biomass and Productivity in Estuaries. *Continental Shelf Research*, 7, 1367-1381.doi:10.1016/0278-4343(87)90042-2

Cloern, J.E., 1999. The Relative Importance of Light and Nutrient Limitation of Phytoplankton Growth: A Simple Index of Coastal Ecosystem Sensitivity to Nutrient Enrichment. *Aquatic ecology*, 33, 3-15.doi:10.1023/A:1009952125558

Cloern, J.E., 2001. Our Evolving Conceptual Model of the Coastal Eutrophication Problem. *Marine Ecology Progress Series*, 210, 223-253.doi:10.3354/meps210223

Cloern, J.E., Foster, S., Kleckner, A., 2014. Phytoplankton Primary Production in the World's Estuarine-Coastal Ecosystems. *Biogeosciences*, 11, 2477-2501.doi:10.5194/bg-11-2477-2014

Cloern, J.E., Jassby, A.D., 2008. Complex Seasonal Patterns of Primary Producers at the Land-Sea Interface. *Ecology Letters*, 11, 1294-1303.doi:10.1111/j.1461-0248.2008.01244.x

Cloern, J.E., Jassby, A.D., 2010. Patterns and Scales of Phytoplankton Variability in Estuarine-Coastal Ecosystems. *Estuaries and Coasts*, 33, 230-241.doi:10.1007/s12237-009-9195-3

Cole, B.E., Cloern, J.E., 1984. Significance of Biomass and Light Availability to Phytoplankton Productivity in San Francisco Bay. *Marine Ecology Progress Series*, 17, 15-24

Cole, J.J., Caraco, N.F., Peierls, B.L., 1992. Can Phytoplankton Maintaina Positive Carbon Balance in a Turbid, Freshwater, Tidal Estuary? *Limnology and Oceanography*, 37, 1608-1617.doi:10.4319/lo.1992.37.8.1608

Colijn, F., Cadée, G.C., 2003. Is Phytoplankton Growth in the Wadden Sea Light or Nitrogen Limited? *Journal of Sea Research*, 49, 83-93.doi:10.1016/S1385-1101(03)00002-9

Conley, D.J., Humborg, C., Rahm, L., Savchuk, O.P., Wulff, F., 2002. Hypoxia in the Baltic Sea and Basin-Scale Changes in Phosphorus Biogeochemistry. *Environmental Science & Technology*, 36, 5315-5320.doi:10.1021/es025763w

Cooper, R.J., Hiscock, K.M., 2023. Two Decades of the Eu Water Framework Directive: Evidence of Success and Failure from a Lowland Arable Catchment (River Wensum, Uk). *Science of the Total Environment*, 869.doi:10.1016/j.scitotenv.2023.161837

da Costa, M.D., Gast, R.J., Millette, N.C., 2024. Temporal and Spatial Variability of Constitutive Mixotroph Abundance and Proportion. *Fems Microbiology Ecology*, 100.doi:10.1093/femsec/fiae015

Dashkova, V., Malashenkov, D.V., Baishulakova, A., Davidson, T.A., Vorobjev, I.A., Jeppesen, E., Barteneva, N.S., 2022. Changes in Phytoplankton Community Composition and Phytoplankton Cell Size in Response to Nitrogen Availability Depend on Temperature. *Microorganisms*, 10.doi:10.3390/microorganisms10071322

Davidson, K., Gowen, R.J., Tett, P., Bresnan, E., Harrison, P.J., McKinney, A., Milligan, S., Mills, D.K., Silke, J., Crooks, A.M., 2012. Harmful Algal Blooms: How Strong Is the Evidence That Nutrient Ratios and Forms Influence Their Occurrence? *Estuarine Coastal and Shelf Science*, 115, 399-413.doi:10.1016/j.ecss.2012.09.019

Davis, T.R., Harasti, D., Smith, S.D.A., Kelaher, B.P., 2016. Using Modelling to Predict Impacts of Sea Level Rise and Increased Turbidity on Seagrass Distributions in Estuarine Embayments. *Estuarine Coastal and Shelf Science*, 181, 294-301.doi:10.1016/j.ecss.2016.09.005

De Swart, H., Schuttelaars, H., Talke, S., 2009. Initial Growth of Phytoplankton in Turbid Estuaries: A Simple Model. *Continental Shelf Research*, 29, 136-147.doi:10.1016/j.csr.2007.09.006

Defra, 2019. Marine Strategy Part One: Uk Updated Assessment and Good Environmental Status.

Defra, 2022. Natural Capital and Ecosystem Assessment Programme. https://www.gov.uk/government/publications/natural-capital-and-ecosystem-assessment-programme/natural-capital-and-ecosystem-assessment-programme,

Defra, 2024. Agricultural Facts: Summary. Updated 31 October 2024. https://www.gov.uk/government/statistics/agricultural-facts-england-regional-profiles/agricultural-facts-summary Accessed 11th December 2024, Accessed 11th December 2024

Deininger, A., Faithfull, C.L., Lange, K., Bayer, T., Vidussi, F., Liess, A., 2016. Simulated Terrestrial Runoff Triggered a Phytoplankton Succession and Changed Seston Stoichiometry in Coastal Lagoon Mesocosms. *Marine Environmental Research*, 119, 40-50.doi:10.1016/j.marenvres.2016.05.001

Desmit, X., Nohe, A., Borges, A.V., Prins, T., De Cauwer, K., Lagring, R., Van der Zande, D., Sabbe, K., 2020. Changes in Chlorophyll Concentration and Phenology in the North Sea in Relation to De-Eutrophication and Sea Surface Warming. *Limnology and Oceanography*, 65, 828-847.doi:10.1002/lno.11351

Devlin, M., Barry, J., Painting, S., Best, M., 2009. Extending the Phytoplankton Tool Kit for the Uk Water Framework Directive: Indicators of Phytoplankton Community Structure. *Hydrobiologia*, 633, 151-168.doi:10.1007/s10750-009-9879-5

Devlin, M., Best, M., Coates, D., Bresnan, E., O'Boyle, S., Park, R., Silke, J., Cusack, C., Skeats, J., 2007a. Establishing Boundary Classes for the Classification of Uk Marine Waters Using Phytoplankton Communities. *Marine Pollution Bulletin*, 55, 91-103.doi:10.1016/j.marpolbul.2006.09.018

Devlin, M., Le Quesne, W., Greenwood, N., Garcia, C., Parker, R., 2023. One Year On: Cefas Generates New Tools and Evidence to Support Marine Natural Capital Programme. https://marinescience.blog.gov.uk/2023/08/07/one-year-on-cefas-generates-new-tools-and-evidence-to-support-marine-natural-capital-programme/, Marine Science Blog,

Devlin, M., Painting, S., Best, M., 2007b. Setting Nutrient Thresholds to Support an Ecological Assessment Based on Nutrient Enrichment, Potential Primary Production and Undesirable Disturbance. *Marine Pollution Bulletin*, 55, 65-73.doi:10.1016/j.marpolbul.2006.08.030

Devlin, M., Smith, A., Graves, C.A., Petus, C., Tracey, D., Maniel, M., Hooper, E., Kotra, K., Samie, E., Loubser, D., Lyons, B.P., 2020. Baseline Assessment of Coastal Water Quality, in Vanuatu, South Pacific: Insights Gained from in-Situ Sampling. *Marine Pollution Bulletin*, 160.doi:10.1016/j.marpolbul.2020.111651

Devlin, M.J., Barry, J., Mills, D.K., Gowen, R.J., Foden, J., Sivyer, D., Tett, P., 2008. Relationships between Suspended Particulate Material, Light Attenuation and Secchi Depth in Uk Marine Waters. *Estuarine Coastal and Shelf Science*, 79, 429-439.doi:10.1016/j.ecss.2008.04.024

Devlin, M.J., Breckels, M., Graves, C.A., Barry, J., Capuzzo, E., Huerta, F.P., Al Ajmi, F., Al-Hussain, M.M., LeQuesne, W.J.F., Lyons, B.P., 2019. Seasonal and Temporal Drivers Influencing Phytoplankton Community in Kuwait Marine Waters: Documenting a Changing Landscape in the Gulf. *Frontiers in Marine Science*, 6.doi:10.3389/fmars.2019.00141

Devlin, M.J., Graves, C.A., Greenwood, N., Allerton, M., Bresnan, E., Holland, M., McQuatters-Gollop, A., Tett, P., Tracey, D., Best, M.A., 2025. Shifting Sands of Marine Eutrophication Assessments: Building a Future Approach for Uk Marine Waters. *Frontiers in Ocean Sustainability*, 3, 1561741.doi:10.3389/focsu.2025.1561741

Devlin, M.J., Prins, T.C., Enserink, L., Leujak, W., Heyden, B., Axe, P.G., Ruiter, H., Blauw, A., Bresnan, E., Collingridge, K., 2023. A First Ecological Coherent Assessment of Eutrophication across the North-East Atlantic Waters (2015–2020). *Frontiers in Ocean Sustainability*, 1, 1253923.doi:10.3389/focsu.2023.1253923

Diaz, R.J., Rosenberg, R., 2008. Spreading Dead Zones and Consequences for Marine Ecosystems. *Science*, 321, 926-929.doi:10.1126/science.1156401

Diaz, R.J., Rosenberg, R., 2011. Introduction to Environmental and Economic Consequences of Hypoxia. *International Journal of Water Resources Development*, 27, 71-82.doi:10.1080/07900627.2010.531379

Diehl, S., Berger, S., Ptacnik, R., Wild, A., 2002. Phytoplankton, Light, and Nutrients in a Gradient of Mixing Depths: Field Experiments. *Ecology*, 83, 399-411.doi:10.1890/0012-9658(2002)083[0399:PLANIA]2.0.CO;2

Diehl, S., Berger, S.A., Uszko, W., Stibor, H., 2022. Stoichiometric Mismatch Causes a Warming-Induced Regime Shift in Experimental Plankton Communities. *Ecology*, 103.doi:10.1002/ecy.3674

Dijkstra, Y.M., Chant, R.J., Reinfelder, J.R., 2019. Factors Controlling Seasonal Phytoplankton Dynamics in the Delaware River Estuary: An Idealized Model Study. *Estuaries and Coasts*, 42, 1839-1857.doi:10.1007/s12237-019-00612-3

Ding, X.K., Li, X.Y., Wang, A.B., Guo, X.Y., Xu, X.T., Liu, C.L., Qin, X.H., Xie, Y.X., Wei, Y.Q., Cui, Z.G., Jiang, T., 2024. Unprecedented Phytoplankton Blooms in Autumn/Winter in the Southern Bohai Sea

(China) Due to High Yellow River Discharge: Implications of Extreme Rainfall Events. *Journal of Environmental Management*, 351.doi:10.1016/j.jenvman.2023.119901

Dobbertin da Costa, M., Gast, R.J., Millette, N.C., 2024. Temporal and Spatial Variability of Constitutive Mixotroph Abundance and Proportion. *FEMS Microbiology Ecology*, 100.doi:10.1093/femsec/fiae015

Domingues, R.B., Anselmo, T.P., Barbosa, A.B., Sommer, U., Galvao, H.M., 2011. Light as a Driver of Phytoplankton Growth and Production in the Freshwater Tidal Zone of a Turbid Estuary. *Estuarine Coastal and Shelf Science*, 91, 526-535.doi:10.1016/j.ecss.2010.12.008

Domingues, R.B., Barbosa, A.B., Sommer, U., Galvao, H.M., 2011. Ammonium, Nitrate and Phytoplankton Interactions in a Freshwater Tidal Estuarine Zone: Potential Effects of Cultural Eutrophication. *Aquatic Sciences*, 73, 331-343.doi:10.1007/s00027-011-0180-0

Donald, D.B., Bogard, M.J., Finlay, K., Bunting, L., Leavitt, P.R., 2013. Phytoplankton-Specific Response to Enrichment of Phosphorus-Rich Surface Waters with Ammonium, Nitrate, and Urea. *PloS one*, 8, e53277.doi:10.1371/journal.pone.0053277

Dorgham, M.M., 2014. Effects of Eutrophication. In: Ansari, A., Gill, S. (Eds) Eutrophication: Causes, Consequences and Control edited by. Springer, Dordrecht. 29-44.https://doi.org/10.1007/978-94-007-7814-6_3

Duarte, C.M., Krause-Jensen, D., 2018. Intervention Options to Accelerate Ecosystem Recovery from Coastal Eutrophication. *Frontiers in Marine Science*, Volume 5 - 2018.doi:10.3389/fmars.2018.00470

Dugdale, R., Wilkerson, F., Parker, A.E., Marchi, A., Taberski, K., 2012. River Flow and Ammonium Discharge Determine Spring Phytoplankton Blooms in an Urbanized Estuary. *Estuarine, Coastal and Shelf Science*, 115, 187-199.doi:10.1016/j.ecss.2012.08.025

Dupont, N., Aksnes, D.L., 2013. Centennial Changes in Water Clarity of the Baltic Sea and the North Sea. *Estuarine Coastal and Shelf Science*, 131, 282-289.doi:10.1016/j.ecss.2013.08.010

Earl, T.J., Upton, G.J.G., Nedwell, D.B., 2014. Uk Catchment Nutrient Loads 1993-2003, a New Approach Using Harmonised Monitoring Scheme Data: Temporal Changes, Geographical Distribution, Limiting Nutrients and Loads to Coastal Waters. *Environmental Science-Processes & Impacts*, 16, 1646-1658.doi:10.1039/c4em00021h

Edwards, M., Atkinson, A., Bresnan, E., Helaouet, P., McQuatters-Gollop, A., Ostle, C., Pitois, S., Widdicombe, C., 2020. Plankton, Jellyfish and Climate in the North-East Atlantic. *MCCIP Sci. Rev*, 2020, 322-353.doi:10.14465/2020.arc15.plk

Edwards, M., Richardson, A.J., 2004. Impact of Climate Change on Marine Pelagic Phenology and Trophic Mismatch. *Nature*, 430, 881-884.doi:10.1038/nature02808

Egge, J.K., 1998. Are Diatoms Poor Competitors at Low Phosphate Concentrations? *Journal of Marine Systems*, 16, 191-198.doi:10.1016/s0924-7963(97)00113-9

Egge, J.K., Aksnes, D.L., 1992. Silicate as Regulating Nutrient in Phytoplankton Competition. *Marine Ecology Progress Series*, 83, 281-289.doi:10.3354/meps083281

Eiane, K., Aksnes, D.L., BagØien, E., Kaartvedt, S., 1999. Fish or Jellies—a Question of Visibility? *Limnology and Oceanography*, 44, 1352-1357.doi:10.4319/lo.1999.44.5.1352

Elser, J.J., Hayakawa, K., Urabe, J., 2001. Nutrient Limitation Reduces Food Quality for Zooplankton: Daphnia Response to Seston Phosphorus Enrichment. *Ecology*, 82, 898-903.doi:10.2307/2680208

Environment Agency, 2023a. Northwest River Basin District - Summary. Last Modified 03 August 2023. https://environment.data.gov.uk/catchment-planning/RiverBasinDistrict/12/print Accessed 11th December 2024, Accessed 11th December 2024

Environment Agency, 2023b. Thames River Basin District - Summary. Last Modified 03 August 2023. https://environment.data.gov.uk/catchment-planning/RiverBasinDistrict/6/print Accessed 11th December 2024, Accessed 11th December 2024

Environment Agency, 2024. River Basin Planning Programmes of Measures: Mechanisms Summary.

European Commission, E., 1991. Council Directive of 21. May 1991 Concerning Urban Waste Water Treatment (91/271/Eec). *J. Eur. Commun*, 34, 40

European Commission, E., 2000. Directive 2000/60/Ec of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. *Official Journal of the European Communities*, 327, 1-72

European Parliament, 2008. Directive 2008/56/Ec of the European Parliament and of the Council of 17 June 2008 Establishing a Framework for Community Action in the Field of Marine Environmental Policy (Marine Strategy Framework Directive).

Falkowski, P.G., 2002. The Ocean's Invisible Forest. *Scientific American*, 287, 54-61.doi:10.1038/scientificamerican0802-54

Fernández-Barba, M., Almaraz, P., Huertas, I.E., Navarro, G., 2025. Climate Variability Induces Changes in Phytoplankton Phenology across Spanish Marine Ecoregions. *Progress in Oceanography*,103583.doi:10.1016/j.pocean.2025.103583

Ferreira, J., Wolff, W., Simas, T., Bricker, S., 2005. Does Biodiversity of Estuarine Phytoplankton Depend on Hydrology? *Ecological modelling*, 187, 513-523.doi:10.1016/j.ecolmodel.2005.03.013

Fichez, R., Jickells, T., Edmunds, H., 1992. Algal Blooms in High Turbidity, a Result of the Conflicting Consequences of Turbulence on Nutrient Cycling in a Shallow Water Estuary. *Estuarine, Coastal and Shelf Science*, 35, 577-592.doi:10.1016/S0272-7714(05)80040-X

Field, C.B., Behrenfeld, M.J., Randerson, J.T., Falkowski, P., 1998. Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. *Science*, 281, 237-240.doi:10.1126/science.281.5374.237

Fisher, T.R., Harding Jr, L.W., Stanley, D.W., Ward, L.G., 1988. Phytoplankton, Nutrients, and Turbidity in the Chesapeake, Delaware, and Hudson Estuaries. *Estuarine, Coastal and Shelf Science*, 27, 61-93.doi:10.1016/0272-7714(88)90032-7

Fitzsimons, M.F., Probert, I., Gaillard, F., Rees, A.P., 2020. Dissolved Organic Phosphorus Uptake by Marine Phytoplankton Is Enhanced by the Presence of Dissolved Organic Nitrogen. *Journal of Experimental Marine Biology and Ecology*, 530.doi:10.1016/j.jembe.2020.151434

Foden, J., Devlin, M.J., Mills, D.K., Malcolm, S.J., 2011. Searching for Undesirable Disturbance: An Application of the Ospar Eutrophication Assessment Method to Marine Waters of England and Wales. *Biogeochemistry*, 106, 157-175.doi:10.1007/s10533-010-9475-9

Friedland, R., Macias, D., Cossarini, G., Daewel, U., Estournel, C., Garcia-Gorriz, E., Grizzetti, B., Gregoire, M., Gustafson, B., Kalaroni, S., Kerimoglu, O., Lazzari, P., Lenhart, H., Lessin, G., Maljutenko, I., Miladinova, S., Muller-Karulis, B., Neumann, T., Parn, O., Patsch, J., Piroddi, C., Raudsepp, U., Schrum, C., Stegert, C., Stips, A., Tsiaras, K., Ulses, C., Vandenbulcke, L., 2021. Effects of Nutrient Management Scenarios on Marine Eutrophication Indicators: A Pan-European, Multi-Model Assessment in Support of the Marine Strategy Framework Directive. *Frontiers in Marine Science*, 8.doi:10.3389/fmars.2021.596126

Fronkova, L., Greenwood, N., Martinez, R., Graham, J.A., Harrod, R., Graves, C.A., Devlin, M.J., Petus, C., 2022. Can Forel-Ule Index Act as a Proxy of Water Quality in Temperate Waters? Application of Plume Mapping in Liverpool Bay, Uk. *Remote Sensing*, 14.doi:10.3390/rs14102375

Gameiro, C., Zwolinski, J., Brotas, V., 2011. Light Control on Phytoplankton Production in a Shallow and Turbid Estuarine System. *Hydrobiologia*, 669, 249-263.doi:10.1007/s10750-011-0695-3

Garmendia, M., Borja, A., Franco, J., Revilla, M., 2013. Phytoplankton Composition Indicators for the Assessment of Eutrophication in Marine Waters: Present State and Challenges within the European Directives. *Marine Pollution Bulletin*, 66, 7-16.doi:10.1016/j.marpolbul.2012.10.005

Gazeau, F., Gattuso, J.-P., Middelburg, J.J., Brion, N., Schiettecatte, L.-S., Frankignoulle, M., Borges, A.V., 2005. Planktonic and Whole System Metabolism in a Nutrient-Rich Estuary (the Scheldt Estuary). *Estuaries*, 28, 868-883.doi:10.1007/BF02696016

Geider, R.J., La Roche, J., 2002. Redfield Revisited: Variability of C : N : P in Marine Microalgae and Its Biochemical Basis. *European Journal of Phycology*, 37, 1-17.doi:10.1017/s0967026201003456

Gilpin, L.C., Davidson, K., Roberts, E., 2004. The Influence of Changes in Nitrogen: Silicon Ratios on Diatom Growth Dynamics. *Journal of Sea Research*, 51, 21-35.doi:10.1016/j.seares.2003.05.005

Glibert, P.M., 2020. Harmful Algae at the Complex Nexus of Eutrophication and Climate Change. *Harmful Algae*, 91.doi:10.1016/j.hal.2019.03.001

Glibert, P.M., Burford, M.A., 2017. Globally Changing Nutrient Loads and Harmful Algal Blooms Recent Advances, New Paradigms, and Continuing Challenges. *Oceanography*, 30, 58-69.doi:10.5670/oceanog.2017.110

Glibert, P.M., Burkholder, J.M., 2011. Harmful Algal Blooms and Eutrophication: "Strategies" for Nutrient Uptake and Growth Outside the Redfield Comfort Zone. *Chinese Journal of Oceanology and Limnology*, 29, 724-738.doi:10.1007/s00343-011-0502-z

Glibert, P.M., Wilkerson, F.P., Dugdale, R.C., Raven, J.A., Dupont, C.L., Leavitt, P.R., Parker, A.E., Burkholder, J.M., Kana, T.M., 2016. Pluses and Minuses of Ammonium and Nitrate Uptake and Assimilation by Phytoplankton and Implications for Productivity and Community Composition, with Emphasis on Nitrogen-Enriched Conditions. *Limnology and Oceanography*, 61, 165-197.doi:10.1002/lno.10203

Gowen, R.J., Collos, Y., Tett, P., Scherer, C., Bec, B., Abadie, E., Allen, M., O'Brien, T., 2015. Response of Diatom and Dinoflagellate Lifeforms to Reduced Phosphorus Loading: A Case Study in

the Thau Lagoon, France. *Estuarine Coastal and Shelf Science*, 162, 45-52.doi:10.1016/j.ecss.2015.03.033

Gowen, R.J., Tett, P., Bresnan, E., Davidson, K., McKinney, A., Harrison, P.J., Milligan, S., Mills, D.K., Silke, J., Crooks, A.M., 2012. Anthropogenic Nutrient Enrichment and Blooms of Harmful Phytoplankton. *Oceanography and Marine Biology: an Annual Review*, 50, 65-126.doi:10.1016/j.jenvman.2014.07.002

Gowen, R.J., Tett, P., Jones, K.J., 1992. Predicting Marine Eutrophication - the Yield of Chlorophyll from Nitrogen in Scottish Coastal Waters. *Marine Ecology Progress Series*, 85, 153-161.doi:10.3354/meps085153

Graves, C.A., Best, M., Atkinson, A., Bear, B., Bresnan, E., Holland, M., Johns, D.G., Machairopoulou, M., McQuatters-Gollop, A., Mellor, A., Ostle, C., Paxman, K., Pitois, S., Tett, P., Devlin, M., 2023. At What Scale Should We Assess the Health of Pelagic Habitats? Trade-Offs between Small-Scale Manageable Pressures and the Need for Regional Upscaling. *Ecological Indicators*, 154.doi:10.1016/j.ecolind.2023.110571

Greenwood, N., Devlin, M.J., Best, M., Fronkova, L., Graves, C.A., Milligan, A., Barry, J., van Leeuwen, S.M., 2019. Utilizing Eutrophication Assessment Directives from Transitional to Marine Systems in the Thames Estuary and Liverpool Bay, Uk. *Frontiers in Marine Science*, 6.doi:10.3389/fmars.2019.00116

Greenwood, N., Forster, R.M., Creach, V., Painting, S.J., Dennis, A., Cutchey, S.J., Silva, T., Sivyer, D.B., Jickells, T., 2012. Seasonal and Interannual Variation of the Phytoplankton and Copepod Dynamics in Liverpool Bay. *Ocean Dynamics*, 62, 307-320.doi:10.1007/s10236-011-0500-x

Greenwood, N., Hydes, D.J., Mahaffey, C., Wither, A., Barry, J., Sivyer, D.B., Pearce, D.J., Hartman, S.E., Andres, O., Lees, H.E., 2011. Spatial and Temporal Variability in Nutrient Concentrations in Liverpool Bay, a Temperate Latitude Region of Freshwater Influence. *Ocean Dynamics*, 61, 2181-2199.doi:10.1007/s10236-011-0463-y

Grigoratou, M., Menden-Deuer, S., McQuatters-Gollop, A., Arhonditsis, G., Artigas, L.F., Ayata, S.-D., Bedikoğlu, D., Beisner, B.E., Chen, B., Davies, C., 2025. The Immeasurable Value of Plankton to Humanity. *BioScience*,biaf049.doi:10.1093/biosci/biaf049

Grizzetti, B., Bouraoui, F., Aloe, A., 2012. Changes of Nitrogen and Phosphorus Loads to European Seas. *Global Change Biology*, 18, 769-782.doi:10.1111/j.1365-2486.2011.02576.x

Grizzetti, B., Vigiak, O., Udias, A., Aloe, A., Zanni, M., Bouraoui, F., Pistocchi, A., Dorati, C., Friedland, R., De Roo, A., Benitez Sanz, C., Leip, A., Bielza, M., 2021. How Eu Policies Could Reduce Nutrient Pollution in European Inland and Coastal Waters. *Global Environmental Change*, 69, 102281.doi:10.1016/j.gloenvcha.2021.102281

Grobbelaar, J.U., 1985. Phytoplankton Productivity in Turbid Waters. *Journal of Plankton Research*, 7, 653-663.doi:10.1093/plankt/7.5.653

Grosse, J., Burson, A., Stomp, M., Huisman, J., Boschker, H.T.S., 2017. From Ecological Stoichiometry to Biochemical Composition: Variation in N and P Supply Alters Key Biosynthetic Rates in Marine Phytoplankton. *Front. Microbiol.*, 8, 11.doi:10.3389/fmicb.2017.01299

Halliday, S.J., Skeffington, R.A., Wade, A.J., Bowes, M.J., Gozzard, E., Newman, J.R., Loewenthal, M., Palmer-Felgate, E.J., Jarvie, H.P., 2015. High-Frequency Water Quality Monitoring in an Urban

Catchment: Hydrochemical Dynamics, Primary Production and Implications for the Water Framework Directive. *Hydrological Processes*, 29, 3388-3407.doi:10.1002/hyp.10453

Hansen, P.J., 2011. The Role of Photosynthesis and Food Uptake for the Growth of Marine Mixotrophic Dinoflagellates. *Journal of Eukaryotic Microbiology*, 58, 203-214.doi:10.1111/j.1550-7408.2011.00537.x

Hart, R., 1988. Zooplankton Feeding Rates in Relation to Suspended Sediment Content: Potential Influences on Community Structure in a Turbid Reservoir. *Freshwater Biology*, 19, 123-139.doi:10.1111/j.1365-2427.1988.tb00334.x

He, H., Hu, E., Yu, J.L., Luo, X.G., Li, K.Y., Jeppesen, E., Liu, Z.W., 2017. Does Turbidity Induced by *Carassius Carassius* Limit Phytoplankton Growth? A Mesocosm Study. *Environmental Science and Pollution Research*, 24, 5012-5018.doi:10.1007/s11356-016-8247-z

Heslenfeld, P., Enserink, E.L., 2008. Ospar Ecological Quality Objectives: The Utility of Health Indicators for the North Sea. *Ices Journal of Marine Science*, 65, 1392-1397.doi:10.1093/icesjms/fsn159

Hessen, D.O., Færøvig, P.J., Andersen, T., 2002. Light, Nutrients, and P: C Ratios in Algae: Grazer Performance Related to Food Quality and Quantity. *Ecology*, 83, 1886-1898.doi:10.1890/0012-9658(2002)083[1886:LNAPCR]2.0.CO;2

HM Government, 2012. 'Marine Strategy Part One: Uk Initial Assessment and Good Environmental Status.

Holland, M.M., Artigas, L.F., Atkinson, A., Best, M., Bresnan, E., Devlin, M., Eerkes-Medrano, D., Johansen, M., Johns, D.G., Machairopoulou, M., 2025. Mind the Gap-the Need to Integrate Novel Plankton Methods Alongside Ongoing Long-Term Monitoring. *Ocean & Coastal Management*,107542.doi:10.1016/j.ocecoaman.2025.107542

Hopkins, J., Polton, J.A., 2012. Scales and Structure of Frontal Adjustment and Freshwater Export in a Region of Freshwater Influence. *Ocean Dynamics*, 62, 45-62.doi:10.1007/s10236-011-0475-7

Howarth, R., Paerl, H.W., 2008. Coastal Marine Eutrophication: Control of Both Nitrogen and Phosphorus Is Necessary. *Proceedings of the National Academy of Sciences of the United States of America*, 105, E103-E103.doi:10.1073/pnas.0807266106

Howarth, R.W., Marino, R., 2006. Nitrogen as the Limiting Nutrient for Eutrophication in Coastal Marine Ecosystems: Evolving Views over Three Decades. *Limnology and Oceanography*, 51, 364-376.doi:10.4319/lo.2006.51.1_part_2.0364

Irigoien, X., Castel, J., 1997. Light Limitation and Distribution of Chlorophyll Pigments in a Highly Turbid Estuary: The Gironde (Sw France). *Estuarine Coastal and Shelf Science*, 44, 507-517.doi:10.1006/ecss.1996.0132

Jafar-Sidik, M., Gohin, F., Bowers, D., Howarth, J., Hull, T., 2017. The Relationship between Suspended Particulate Matter and Turbidity at a Mooring Station in a Coastal Environment: Consequences for Satellite-Derived Products. *Oceanologia*, 59, 365-378.doi:10.1016/j.oceano.2017.04.003

Jarvie, H.P., Pallett, D.W., Schäfer, S.M., Macrae, M.L., Bowes, M.J., Farrand, P., Warwick, A.C., King, S.M., Williams, R.J., Armstrong, L., 2020. Biogeochemical and Climate Drivers of Wetland

Phosphorus and Nitrogen Release: Implications for Nutrient Legacies and Eutrophication Risk.https://doi.org/10.1002/jeq2.20155

Jeong, H.J., 1999. The Ecological Roles of Heterotrophic Dinoflagellates in Marine Planktonic Community. *Journal of Eukaryotic Microbiology*, 46, 390-396.doi:10.1111/j.1550-7408.1999.tb04618.x

Jeong, H.J., Lee, K.H., Du Yoo, Y., Kang, N.S., Song, J.Y., Kim, T.H., Seong, K.A., Kim, J.S., Potvin, E., 2018. Effects of Light Intensity, Temperature, and Salinity on the Growth and Ingestion Rates of the Red-Tide Mixotrophic Dinoflagellate Paragymnodinium Shiwhaense. *Harmful Algae*, 80, 46-54.doi:10.1016/j.hal.2018.09.005

Jiang, Z.P., Tong, Y.Q., Tong, M.M., Yuan, J.J., Cao, Q., Pan, Y.W., 2021. The Effects of Suspended Particulate Matter, Nutrient, and Salinity on the Growth of Amphidinium Carterae under Estuary Environmental Conditions. *Frontiers in Marine Science*, 8.doi:10.3389/fmars.2021.690764

Jickells, T.D., Andrews, J.E., Parkes, D.J., Suratman, S., Aziz, A.A., Hee, Y.Y., 2014. Nutrient Transport through Estuaries: The Importance of the Estuarine Geography. *Estuarine Coastal and Shelf Science*, 150, 215-229.doi:10.1016/j.ecss.2014.03.014

Jickells, T.D., Buitenhuis, E., Altieri, K., Baker, A.R., Capone, D., Duce, R.A., Dentener, F., Fennel, K., Kanakidou, M., LaRoche, J., Lee, K., Liss, P., Middelburg, J.J., Moore, J.K., Okin, G., Oschlies, A., Sarin, M., Seitzinger, S., Sharples, J., Singh, A., Suntharalingam, P., Uematsu, M., Zamora, L.M., 2017. A Reevaluation of the Magnitude and Impacts of Anthropogenic Atmospheric Nitrogen Inputs on the Ocean. *Global Biogeochemical Cycles*, 31, 289-305.doi:10.1002/2016gb005586

Joint, I., Pomroy, A., 1981. Primary Production in a Turbid Estuary. *Estuarine, Coastal and Shelf Science*, 13, 303-316.doi:10.1016/S0302-3524(81)80028-X

Kelly, N.E., Guijarro-Sabaniel, J., Zimmerman, R., 2021. Anthropogenic Nitrogen Loading and Risk of Eutrophication in the Coastal Zone of Atlantic Canada. *Estuarine, Coastal and Shelf Science*, 263, 107630.doi:10.1016/j.ecss.2021.107630

Khan, M.N., Mohammad, F., 2014. Eutrophication: Challenges and Solutions, Eutrophication: Causes, Consequences and Control: Volume 2, pp. 1-15.

Kim, B., Park, N.Y., Kang, S.U., Choi, H.S., Park, M.J., Du Yoo, Y., Lim, A.S., 2025. Mixotrophic Abilities of Dinof Lagellate Gonyaulax Species: Compensation for Low Growth Rates under Low-Light Conditions. *Algae*, 40, 211-225.doi:10.4490/algae.2025.40.8.11

Kinniburgh, J.H., Barnett, M., 2010. Orthophosphate Concentrations in the River Thames: Reductions in the Past Decade. *Water and Environment Journal*, 24, 107-115.doi:10.1111/j.1747-6593.2008.00161.x

Kiørboe, T., 2011. How Zooplankton Feed: Mechanisms, Traits and Trade-Offs. *Biological reviews*, 86, 311-339.doi:10.1111/j.1469-185X.2010.00148.x

Kocum, E., Underwood, G.J., Nedwell, D.B., 2002. Simultaneous Measurement of Phytoplanktonic Primary Production, Nutrient and Light Availability Along a Turbid, Eutrophic Uk East Coast Estuary (the Colne Estuary). *Marine ecology progress series*, 231, 1-12.doi:10.3354/meps231001

Kromkamp, J., Peene, J., Vanrijswijk, P., Sandee, A., Goosen, N., 1995. Nutrients, Light and Primary Production by Phytoplankton and Microphytobenthos in the Eutrophic, Turbid Westerschelde Estuary (the Netherlands). *Hydrobiologia*, 311, 9-19.doi:10.1007/bf00008567

Lagus, A., Suomela, J., Weithoff, G., Heikkilä, K., Helminen, H., Sipura, J., 2004. Species-Specific Differences in Phytoplankton Responses to N and P Enrichments and the N: P Ratio in the Archipelago Sea, Northern Baltic Sea. *Journal of Plankton Research*, 26, 779-798.doi:10.1093/plankt/fbh070

Laurent, A., Fennel, K., Ko, D.S., Lehrter, J., 2018. Climate Change Projected to Exacerbate Impacts of Coastal Eutrophication in the Northern Gulf of Mexico. *Journal of Geophysical Research: Oceans*, 123, 3408-3426.doi:10.1002/2017JC013583

Lazar, A.N., Wade, A.J., Whitehead, P.G., Neal, C., Loewenthal, M., 2012. Reconciling Observed and Modelled Phytoplankton Dynamics in a Major Lowland Uk River, the Thames. *Hydrology Research*, 43, 576-588.doi:10.2166/nh.2012.029

Lewis, W.M., Wurtsbaugh, W.A., Paerl, H.W., 2011. Rationale for Control of Anthropogenic Nitrogen and Phosphorus to Reduce Eutrophication of Inland Waters. *Environmental Science & Technology*, 45, 10300-10305.doi:10.1021/es202401p

Li, J., Glibert, P.M., Alexander, J.A., 2011. Effects of Ambient Din:Dip Ratio on the Nitrogen Uptake of Harmful Dinoflagellate Prorocentrum Minimum and Prorocentrum Donghaiense in Turbidistat. *Chinese Journal of Oceanology and Limnology*, 29, 746-761.doi:10.1007/s00343-011-0504-x

Li, J., Glibert, P.M., Gao, Y., 2015. Temporal and Spatial Changes in Chesapeake Bay Water Quality and Relationships to Prorocentrum Minimum, Karlodinium Veneficum, and Cyanohab Events, 1991–2008. *Harmful Algae*, 42, 1-14.doi:10.1016/j.hal.2014.11.003

Liess, A., Faithfull, C., Reichstein, B., Rowe, O., Guo, J., Pete, R., Thomsson, G., Uszko, W., Francoeur, S.N., 2015. Terrestrial Runoff May Reduce Microbenthic Net Community Productivity by Increasing Turbidity: A Mediterranean Coastal Lagoon Mesocosm Experiment. *Hydrobiologia*, 753, 205-218.doi:10.1007/s10750-015-2207-3

Liu, B., de Swart, H.E., de Jonge, V.N., 2018. Phytoplankton Bloom Dynamics in Turbid, Well-Mixed Estuaries: A Model Study. *Estuarine Coastal and Shelf Science*, 211, 137-151.doi:10.1016/j.ecss.2018.01.010

Longphuirt, S.N., Mockler, E.M., O'Boyle, S., Wynne, C., Stengel, D.B., 2016. Linking Changes in Nutrient Source: Load to Estuarine Responses: An Irish Perspective. *Biology and Environment-Proceedings of the Royal Irish Academy*, 116B, 295-311.doi:10.3318/bioe.2016.21

Lu, D., Huang, X., Yang, B., Dan, S.F., Kang, Z., Zhou, J., Lao, Y., Zhong, Q., Wu, H., 2021. Composition and Distributions of Nitrogen and Phosphorus and Assessment of Eutrophication Status in the Maowei Sea. *Journal of Ocean University of China*, 20, 361-371.doi:10.1007/s11802-021-4557-y

Lueangthuwapranit, C., Sampantarak, U., Wongsai, S., 2011. Distribution and Abundance of Phytoplankton: Influence of Salinity and Turbidity Gradients in the Na Thap River, Songkhla Province, Thailand. *Journal of Coastal Research*, 27, 585-594.doi:10.2112/jcoastres-d-10-00123.1

MacIntyre, H., Geider, R., 1997. Regulation of Rubisco Activity and Its Potential Effect on Photosynthesis During Mixing in a Turbid Estuary. *Oceanographic Literature Review*, 7, 720.doi:10.3354/meps144247

Mahaffey, C., Hull, T., Hunter, W., Greenwood, N., Palmer, M., Sharples, J., Wakelin, S., Williams, C., 2023. Climate Change Impacts on Dissolved Oxygen Concentration in Marine and Coastal Waters around the Uk and Ireland. *MCCIP Sci. Rev*, 31.doi:10.14465/2023.reu07.oxy

Maier, G., Nimmo-Smith, R.J., Glegg, G.A., Tappin, A.D., Worsfold, P.J., 2009. Estuarine Eutrophication in the Uk: Current Incidence and Future Trends. *Aquatic Conservation-Marine and Freshwater Ecosystems*, 19, 43-56.doi:10.1002/aqc.982

Malone, T.C., Newton, A., 2020. The Globalization of Cultural Eutrophication in the Coastal Ocean: Causes and Consequences. *Frontiers in Marine Science*, 7.doi:10.3389/fmars.2020.00670

Marshall, H.G., Lacouture, R.V., Buchanan, C., Johnson, J.M., 2006. Phytoplankton Assemblages Associated with Water Quality and Salinity Regions in Chesapeake Bay, USA. *Estuarine Coastal and Shelf Science*, 69, 10-18.doi:10.1016/j.ecss.2006.03.019

Martiny, A.C., Vrugt, J.A., Primeau, F.W., Lomas, M.W., 2013. Regional Variation in the Particulate Organic Carbon to Nitrogen Ratio in the Surface Ocean. *Global Biogeochemical Cycles*, 27, 723-731.doi:10.1002/gbc.20061

May, C.L., Koseff, J.R., Lucas, L.V., Cloern, J.E., Schoellhamer, D.H., 2003. Effects of Spatial and Temporal Variability of Turbidity on Phytoplankton Blooms. *Marine Ecology Progress Series*, 254, 111-128.doi:10.3354/meps254111

McCrackin, M.L., Jones, H.P., Jones, P.C., Moreno-Mateos, D., 2017. Recovery of Lakes and Coastal Marine Ecosystems from Eutrophication: A Global Meta-Analysis. *Limnology and Oceanography*, 62, 507-518.doi:10.1002/lno.10441

McQuatters-Gollop, A., Atkinson, A., Aubert, A., Bedford, J., Best, M., Bresnan, E., Cook, K., Devlin, M., Gowen, R., Johns, D.G., Machairopoulou, M., McKinney, A., Mellor, A., Ostle, C., Scherer, C., Tett, P., 2019. Plankton Lifeforms as a Biodiversity Indicator for Regional-Scale Assessment of Pelagic Habitats for Policy. *Ecological Indicators*, 101, 913-925.doi:10.1016/j.ecolind.2019.02.010

McQuatters-Gollop, A., Gilbert, A.J., Mee, L.D., Vermaat, J.E., Artioli, Y., Humborg, C., Wulff, F., 2009. How Well Do Ecosystem Indicators Communicate the Effects of Anthropogenic Eutrophication? *Estuarine Coastal and Shelf Science*, 82, 583-596.doi:10.1016/j.ecss.2009.02.017

McQuatters-Gollop, A., Raitsos, D.E., Edwards, M., Attrill, M.J., 2007a. Spatial Patterns of Diatom and Dinoflagellate Seasonal Cycles in the Ne Atlantic Ocean. *Marine Ecology Progress Series*, 339, 301-306.doi: 10.3354/meps339301

McQuatters-Gollop, A., Raitsos, D.E., Edwards, M., Pradhan, Y., Mee, L.D., Lavender, S.J., Attrill, M.J., 2007b. A Long-Term Chlorophyll Data Set Reveals Regime Shift in North Sea Phytoplankton Biomass Unconnected to Nutrient Trends. *Limnology and Oceanography*, 52, 635-648.doi:10.4319/lo.2007.52.2.0635

McSweeney, J.M., Chant, R.J., Wilkin, J.L., Sommerfield, C.K., 2017. Suspended-Sediment Impacts on Light-Limited Productivity in the Delaware Estuary. *Estuaries and Coasts*, 40, 977-993.doi:10.1007/s12237-016-0200-3

Mena, C., Long, M., Lorand, O., Malestroit, P., Rabiller, E., Maguer, J.-F., L'helguen, S., Regaudie De Gioux, A., 2025. Impact of Light and Nutrient Availability on the Phagotrophic Activity of Harmful Bloom-Forming Dinoflagellates. *Journal of Plankton Research*, 47, fbae038.doi:10.1093/plankt/fbae038

Mészáros, L., van der Meulen, F., Jongbloed, G., El Serafy, G., 2021. Climate Change Induced Trends and Uncertainties in Phytoplankton Spring Bloom Dynamics. *Frontiers in Marine Science*, Volume 8 - 2021.doi:10.3389/fmars.2021.669951

Middelburg, J.J., Nieuwenhuize, J., 2001. Nitrogen Isotope Tracing of Dissolved Inorganic Nitrogen Behaviour in Tidal Estuaries. *Estuarine Coastal and Shelf Science*, 53, 385-391.doi:10.1006/ecss.2001.0805

Mitra, A., Caron, D.A., Faure, E., Flynn, K.J., Leles, S.G., Hansen, P.J., McManus, G.B., Not, F., Gomes, H.D., Santoferrara, L.F., Stoecker, D.K., Tillmann, U., 2023. The Mixoplankton Database (Mdb): Diversity of Photo-Phago-Trophic Plankton in Form, Function, and Distribution across the Global Ocean. *Journal of Eukaryotic Microbiology*.doi:10.1111/jeu.12972

Mitra, A., Flynn, K.J., Tillmann, U., Raven, J.A., Caron, D., Stoecker, D.K., Not, F., Hansen, P.J., Hallegraeff, G., Sanders, R., Wilken, S., McManus, G., Johnson, M., Pitta, P., Vage, S., Berge, T., Calbet, A., Thingstad, F., Jeong, H.J., Burkholder, J., Glibert, P.M., Graneli, E., Lundgren, V., 2016. Defining Planktonic Protist Functional Groups on Mechanisms for Energy and Nutrient Acquisition: Incorporation of Diverse Mixotrophic Strategies. *Protist*, 167, 106-120.doi:10.1016/j.protis.2016.01.003

Monbet, Y., 1992. Control of Phytoplankton Biomass in Estuaries: A Comparative Analysis of Microtidal and Macrotidal Estuaries. *Estuaries*, 15, 563-571.doi:10.2307/1352398

Moss, B., Kosten, S., Meerhoff, M., Battarbee, R.W., Jeppesen, E., Mazzeo, N., Havens, K., Lacerot, G., Liu, Z.W., De Meester, L., Paerl, H., Scheffer, M., 2011. Allied Attack: Climate Change and Eutrophication. *Inland Waters*, 1, 101-105.doi:10.5268/iw-1.2.359

Neal, C., Jarvie, H.P., 2005. Agriculture, Community, River Eutrophication and the Water Framework Directive. *Hydrological Processes*, 19, 1895-1901.doi:10.1002/hyp.5903

Neal, C., Jarvie, H.P., Williams, R., Love, A., Neal, M., Wickham, H., Harman, S., Armstrong, L., 2010. Declines in Phosphorus Concentration in the Upper River Thames (Uk): Links to Sewage Effluent Cleanup and Extended End-Member Mixing Analysis. *Science of the Total Environment*, 408, 1315-1330.doi:10.1016/j.scitotenv.2009.10.055

Nedwell, D.B., Dong, L.F., Sage, A., Underwood, G.J.C., 2002. Variations of the Nutrients Loads to the Mainland Uk Estuaries: Correlation with Catchment Areas, Urbanization and Coastal Eutrophication. *Estuarine Coastal and Shelf Science*, 54, 951-970.doi:10.1006/ecss.2001.0867

Neukermans, G., Ruddick, K., Loisel, H., Roose, P., 2012. Optimization and Quality Control of Suspended Particulate Matter Concentration Measurement Using Turbidity Measurements. *Limnology and Oceanography-Methods*, 10, 1011-1023.doi:10.4319/lom.2012.10.1011

Nixon, S.W., 1995. Coastal Marine Eutrophication: A Definition, Social Causes, and Future Concerns. *Ophelia*, 41, 199-219.doi:10.1080/00785236.1995.10422044

Nohe, A., Goffin, A., Tyberghein, L., Lagring, R., De Cauwer, K., Vyverman, W., Sabbe, K., 2020. Marked Changes in Diatom and Dinoflagellate Biomass, Composition and Seasonality in the Belgian Part of the North Sea between the 1970s and 2000s. *Science of the Total Environment*, 716.doi:10.1016/j.scitotenv.2019.136316

Nunes, P., Roland, F., Amado, A.M., Resende, N.d.S., Cardoso, S.J., 2022. Responses of Phytoplanktonic Chlorophyll-a Composition to Inorganic Turbidity Caused by Mine Tailings. *Frontiers in Environmental Science*, 9, 605838.doi:10.3389/fenvs.2021.605838

O'Donohue, M.J., Dennison, W.C., 1997. Phytoplankton Productivity Response to Nutrient Concentrations, Light Availability and Temperature Along an Australian Estuarine Gradient. *Estuaries*, 20, 521-533.doi:10.2307/1352611

Ok, J.H., Jeong, H.J., Lim, A.S., You, J.H., Kang, H.C., Kim, S.J., Lee, S.Y., 2019. Effects of Light and Temperature on the Growth of Takayama Helix (Dinophyceae): Mixotrophy as a Survival Strategy against Photoinhibition(1). *Journal of Phycology*, 55, 1181-1195.doi:10.1111/jpy.12907

Opdal, A.F., Lindemann, C., Aksnes, D.L., 2019. Centennial Decline in North Sea Water Clarity Causes Strong Delay in Phytoplankton Bloom Timing. *Global Change Biology*, 25, 3946-3953.doi:10.1111/gcb.14810

Örnólfsdóttir, E.B., Lumsden, S.E., Pinckney, J.L., 2004. Phytoplankton Community Growth-Rate Response to Nutrient Pulses in a Shallow Turbid Estuary, Galveston Bay, Texas. *Journal of plankton research*, 26, 325-339.doi:10.1093/plankt/fbh035

OSPAR, 2003. Ospar Integrated Report 2003 on the Eutrophication Status of the Ospar Maritime Area Based Upon the First Application of the Comprehensive Procedure.

OSPAR, 2008. Second Ospar Integrated Report on the Eutrophication Status of the Ospar Maritime Area, edited by. OSPAR Commission

OSPAR, 2017. Third Integrated Report on the Eutrophication Status of the Ospar Maritime Area edited by:

OSPAR, C., 2005. Common Procedure for the Identification of the Eutrophication Status of the Ospar Maritime Area. *OSPAR Commission*, 3,

Ostle, C., Paxman, K., Graves, C.A., Arnold, M., Artigas, L.F., Atkinson, A., Aubert, A., Baptie, M., Bear, B., Bedford, J., Best, M., Bresnan, E., Brittain, R., Broughton, D., Budria, A., Cook, K., Devlin, M., Graham, G., Halliday, N., Helaouet, P., Johansen, M., Johns, D.G., Lear, D., Machairopoulou, M., McKinney, A., Mellor, A., Milligan, A., Pitois, S., Rombouts, I., Scherer, C., Tett, P., Widdicombe, C., McQuatters-Gollop, A., 2021. The Plankton Lifeform Extraction Tool: A Digital Tool to Increase the Discoverability and Usability of Plankton Time-Series Data. *Earth System Science Data*, 13, 5617-5642.doi:10.5194/essd-13-5617-2021

Paerl, H.W., 2009. Controlling Eutrophication Along the Freshwater-Marine Continuum: Dual Nutrient (N and P) Reductions Are Essential. *Estuaries and Coasts*, 32, 593-601.doi:10.1007/s12237-009-9158-8

Paerl, H.W., Hall, N.S., Peierls, B.L., Rossignol, K.L., 2014. Evolving Paradigms and Challenges in Estuarine and Coastal Eutrophication Dynamics in a Culturally and Climatically Stressed World. *Estuaries and coasts*, 37, 243-258.doi:10.1007/s12237-014-9773-x

Paerl, H.W., Otten, T.G., Kudela, R., 2018. Mitigating the Expansion of Harmful Algal Blooms across the Freshwater-to-Marine Continuum. *Environmental Science & Technology*, 52, 5519-5529.doi:10.1021/acs.est.7b05950

Paerl, H.W., Scott, J.T., McCarthy, M.J., Newell, S.E., Gardner, W.S., Havens, K.E., Hoffman, D.K., Wilhelm, S.W., Wurtsbaugh, W.A., 2016. It Takes Two to Tango: When and Where Dual Nutrient (N & P) Reductions Are Needed to Protect Lakes and Downstream Ecosystems. *Environmental Science & Technology*, 50, 10805-10813.doi:10.1021/acs.est.6b02575

Pahlow, M., Riebesell, U., Wolf-Gladrow, D.A., 1997. Impact of Cell Shape and Chain Formation on Nutrient Acquisition by Marine Diatoms. *Limnology and Oceanography*, 42, 1660-1672.doi:10.4319/lo.1997.42.8.1660

Painting, S.J., Devlin, M.J., Malcolm, S.J., Parker, E.R., Mills, D.K., Mills, C., Tett, P., Wither, A., Burt, J., Jones, R., Winpenny, K., 2007. Assessing the Impact of Nutrient Enrichment in Estuaries: Susceptibility to Eutrophication. *Marine Pollution Bulletin*, 55, 74-90.doi:10.1016/j.marpolbul.2006.08.020

Palmer, M.R., 2010. The Modification of Current Ellipses by Stratification in the Liverpool Bay Rofi. *Ocean Dynamics*, 60, 219-226.doi:10.1007/s10236-009-0246-x

Palmer, M.R., Polton, J.A., 2011. A Strain-Induced Freshwater Pump in the Liverpool Bay Rofi. *Ocean Dynamics*, 61, 1905-1915.doi:10.1007/s10236-011-0430-7

Pan, C.W., Chuang, Y.L., Chou, L.S., Chen, M.H., Lin, H.J., 2016. Factors Governing Phytoplankton Biomass and Production in Tropical Estuaries of Western Taiwan. *Continental Shelf Research*, 118, 88-99.doi:10.1016/j.csr.2016.02.015

Panton, A., Purdie, D.A., 2022. Dinophysis Spp. Abundance and Toxicity Events in South Cornwall, Uk: Interannual Variability and Environmental Drivers at Three Coastal Sites. *Harmful Algae*, 112.doi:10.1016/j.hal.2021.102169

Paredes, I., Ramírez, F., Aragonés, D., Bravo, M.Á., Forero, M.G., Green, A.J., 2021. Ongoing Anthropogenic Eutrophication of the Catchment Area Threatens the Doñana World Heritage Site (South-West Spain). *Wetlands Ecology and Management*, 29, 41-65.doi:10.1007/s11273-020-09766-5

Pennock, J.R., Sharp, J.H., 1986. Phytoplankton Production in the Delaware Estuary - Temporal and Spatial Variability. *Marine Ecology Progress Series*, 34, 143-155.doi:10.3354/meps034143

Philippart, C.J.M., Beukema, J.J., Cadee, G.C., Dekker, R., Goedhart, P.W., van Iperen, J.M., Leopold, M.F., Herman, P.M.J., 2007. Impacts of Nutrient Reduction on Coastal Communities. *Ecosystems*, 10, 95-118.doi:10.1007/s10021-006-9006-7

Philippart, C.J.M., Cadee, G.C., van Raaphorst, W., Riegman, R., 2000. Long-Term Phytoplankton-Nutrient Interactions in a Shallow Coastal Sea: Algal Community Structure, Nutrient Budgets, and Denitrification Potential. *Limnology and Oceanography*, 45, 131-144.doi:10.4319/lo.2000.45.1.0131

Pinckney, J.L., Paerl, H.W., Harrington, M.B., 1999. Responses of the Phytoplankton Community Growth Rate to Nutrient Pulses in Variable Estuarine Environments. *Journal of Phycology*, 35, 1455-1463.doi:10.1046/j.1529-8817.1999.3561455.x

Piroddi, C., Akoglu, E., Andonegi, E., Bentley, J.W., Celic, I., Coll, M., Dimarchopoulou, D., Friedland, R., de Mutsert, K., Girardin, R., Garcia-Gorriz, E., Grizzetti, B., Hernvann, P.Y., Heymans, J.J., Muller-Karulis, B., Libralato, S., Lynam, C.P., Macias, D., Miladinova, S., Moullec, F., Palialexis, A., Parn, O., Serpetti, N., Solidoro, C., Steenbeek, J., Stips, A., Tomczak, M.T., Travers-Trolet, M., Tsikliras, A.C., 2021. Effects of Nutrient Management Scenarios on Marine Food Webs: A Pan-European Assessment in Support of the Marine Strategy Framework Directive. *Frontiers in Marine Science*, 8.doi:10.3389/fmars.2021.596797

Plew, D.R., Zeldis, J.R., Dudley, B.D., Whitehead, A.L., Stevens, L.M., Robertson, B.M., Robertson, B., 2020. Assessing the Eutrophic Susceptibility of New Zealand Estuaries. *Estuaries and Coasts*, 43, 2015-2033.doi:10.1007/s12237-020-00729-w

Poikane, S., Kelly, M.G., Herrero, F.S., Pitt, J.A., Jarvie, H.P., Claussen, U., Leujak, W., Solheim, A.L., Teixeira, H., Phillips, G., 2019. Nutrient Criteria for Surface Waters under the European Water Framework Directive: Current State-of-the-Art, Challenges and Future Outlook. *Science of the Total Environment*, 695.doi:10.1016/j.scitotenv.2019.133888

Polton, J.A., Palmer, M.R., Howarth, M.J., 2011. Physical and Dynamical Oceanography of Liverpool Bay. *Ocean Dynamics*, 61, 1421-1439.doi:10.1007/s10236-011-0431-6

Poulton, A.J., Davis, C.E., Daniels, C.J., Mayers, K.M.J., Harris, C., Tarran, G.A., Widdicombe, C.E., Woodward, E.M.S., 2019. Seasonal Phosphorus and Carbon Dynamics in a Temperate Shelf Sea (Celtic Sea). *Progress in Oceanography*, 177.doi:10.1016/j.pocean.2017.11.001

Preisner, M., Neverova-Dziopak, E., Kowalewski, Z., 2020. Analysis of Eutrophication Potential of Municipal Wastewater. *Water Science and Technology*, 81, 1994-2003.doi:10.2166/wst.2020.254

Pretty, J.N., Mason, C.F., Nedwell, D.B., Hine, R.E., Leaf, S., Dils, R., 2003. Environmental Costs of Freshwater Eutrophication in England and Wales.https://doi.org/10.1021/es020793k

Ptacnik, R., Andersen, T., Tamminen, T., 2010. Performance of the Redfield Ratio and a Family of Nutrient Limitation Indicators as Thresholds for Phytoplankton N Vs. P Limitation. *Ecosystems*, 13, 1201-1214.doi:10.1007/s10021-010-9380-z

Ptacnik, R., Lepisto, L., Willen, E., Brettum, P., Andersen, T., Rekolainen, S., Solheim, A.L., Carvalho, L., 2008. Quantitative Responses of Lake Phytoplankton to Eutrophication in Northern Europe. *Aquatic Ecology*, 42, 227-236.doi:10.1007/s10452-008-9181-z

Quigg, A., Irwin, A.J., Finkel, Z.V., 2011. Evolutionary Inheritance of Elemental Stoichiometry in Phytoplankton. *Proceedings of the Royal Society B-Biological Sciences*, 278, 526-534.doi:10.1098/rspb.2010.1356

R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Https://Www.R-Project.Org/.

Rabalais, N.N., Turner, R.E., Diaz, R.J., Justic, D., 2009. Global Change and Eutrophication of Coastal Waters. *Ices Journal of Marine Science*, 66, 1528-1537.doi:10.1093/icesjms/fsp047

Randall, J., Day Jr, J., 1987. Effects of River Discharge and Vertical Circulation on Aquatic Primary Production in a Turbid Louisiana (USA) Estuary. *Netherlands Journal of Sea Research*, 21, 231-242.doi:10.1016/0077-7579(87)90015-9

Redfield, A.C., 1958. The Biological Control of Chemical Factors in the Environment. *American Scientist*, 46, 205-221

Rhodes, C., Bingham, A., Heard, A.M., Hewitt, J., Lynch, J., Waite, R., Bell, M.D., 2017. Diatoms to Human Uses: Linking Nitrogen Deposition, Aquatic Eutrophication, and Ecosystem Services. *Ecosphere*, 8.doi:10.1002/ecs2.1858

Rios, A.F., Fraga, F., Figueiras, F.G., Perez, F.F., 1998. A Modelling Approach to the Redfield Ratio Deviations in the Ocean. *Scientia Marina*, 62, 169-176.doi:10.3989/scimar.1998.62n1-2169

Rombouts, I., Simon, N., Aubert, A., Cariou, T., Feunteun, E., Guerin, L., Hoebeke, M., McQuatters-Gollop, A., Rigaut-Jalabert, F., Artigas, L.F., 2019. Changes in Marine Phytoplankton Diversity: Assessment under the Marine Strategy Framework Directive. *Ecological Indicators*, 102, 265-277.doi:10.1016/j.ecolind.2019.02.009

Rozemeijer, J., Jordan, P., Hooijboer, A., Kronvang, B., Glendell, M., Hensley, R., Rinke, K., Stutter, M., Bieroza, M., Turner, R., 2025. Best Practice in High-Frequency Water Quality Monitoring for Improved Management and Assessment; a Novel Decision Workflow. *Environmental Monitoring and Assessment*, 197, 353.doi:10.1021/acs.est.2c07798

Rumyantseva, A., Henson, S., Martin, A., Thompson, A.F., Damerell, G.M., Kaiser, J., Heywood, K.J., 2019. Phytoplankton Spring Bloom Initiation: The Impact of Atmospheric Forcing and Light in the Temperate North Atlantic Ocean. *Progress in Oceanography*, 178.doi:10.1016/j.pocean.2019.102202

Sanders, R., Jickells, T., Mills, D., 2001. Nutrients and Chlorophyll at Two Sites in the Thames Plume and Southern North Sea. *Journal of Sea Research*, 46, 13-28.doi:10.1016/s1385-1101(01)00068-5

Savchuk, O.P., 2018. Large-Scale Nutrient Dynamics in the Baltic Sea, 1970-2010. *Frontiers in Marine Science*, 5.doi:10.3389/fmars.2018.00095

Shangguan, Y., Glibert, P.M., Alexander, J., Madden, C.J., Murasko, S., 2017. Phytoplankton Assemblage Response to Changing Nutrients in Florida Bay: Results of Mesocosm Studies. *Journal of Experimental Marine Biology and Ecology*, 494, 38-53.doi:10.1016/j.jembe.2017.05.006

Shao, N.F., Yang, S.T., Sun, Y., Gai, Y., Zhao, C.S., Wang, F., Yin, X., Dong, B., 2019. Assessing Aquatic Ecosystem Health through the Analysis of Plankton Biodiversity. *Marine and Freshwater Research*, 70, 647-655.doi:10.1071/mf18342

Sharma, A., 2020. The Wicked Problem of Diffuse Nutrient Pollution from Agriculture. *Journal of Environmental Law*, 32, 471-501.doi:10.1093/jel/eqaa017

Sharples, J., Holt, J., Wakelin, S., 2020. Impacts of Climate Change on Shelf Sea Stratification, Relevant to the Coastal and Marine Environment around the Uk. *MCCIP Science Review 2020*,103-115.doi:10.14465/2020.arc05.str

Shaw, P.J., Purdie, D.A., de Frietas, P.S., Rees, A.P., Joint, I., 1998. Nutrient Uptake in a Highly Turbid Estuary (the Humber, United Kingdom) and Adjacent Coastal Waters. *Estuaries*, 21, 507-517.doi:10.2307/1353290

Shen, P.P., Li, G., Huang, L.M., Zhang, J.L., Tan, Y.H., 2011. Spatio-Temporal Variability of Phytoplankton Assemblages in the Pearl River Estuary, with Special Reference to the Influence of Turbidity and Temperature. *Continental Shelf Research*, 31, 1672-1681.doi:10.1016/j.csr.2011.07.002

Shi, P.L., Shen, H., Wang, W.J., Yang, Q., Xie, P., 2016. Habitat-Specific Differences in Adaptation to Light in Freshwater Diatoms. *Journal of Applied Phycology*, 28, 227-239.doi:10.1007/s10811-015-0531-7

Shi, S.Y., Xu, Y., Li, W.Q., Ge, J.Z., 2022. Long-Term Response of an Estuarine Ecosystem to Drastic Nutrients Changes in the Changjiang River During the Last 59 Years: A Modeling Perspective. *Frontiers in Marine Science*, 9.doi:10.3389/fmars.2022.1012127

Shilova, I.N., Mills, M.M., Robidart, J.C., Turk-Kubo, K.A., Björkman, K.M., Kolber, Z., Rapp, I., van Dijken, G.L., Church, M.J., Arrigo, K.R., Achterberg, E.P., Zehr, J.P., 2017. Differential Effects of Nitrate, Ammonium, and Urea as N Sources for Microbial Communities in the North Pacific Ocean. *Limnology and Oceanography*, 62, 2550-2574.doi:10.1002/lno.10590

Sinha, E., Michalak, A.M., Balaji, V., 2017. Eutrophication Will Increase During the 21st Century as a Result of Precipitation Changes. *Science*, 357, 405-408.doi:10.1126/science.aan2409

Skarbovik, E., Stålnacke, P., Kaste, O., Austnes, K., 2014. Trends in Nutrients and Metals in Norwegian Rivers and Point Sources 1990-2009. *Hydrology Research*, 45, 441-454.doi:10.2166/nh.2013.233

Sobolev, D., Moore, K., Morris, A.L., 2009. Nutrients and Light Limitation of Phytoplankton Biomass in a Turbid Southeastern Reservoir: Implications for Water Quality. *Southeastern Naturalist*, 8, 255-266.doi:10.1656/058.008.0205

Song, Y.Y., Guo, Y.Y., Liu, H.J., Zhang, G.C., Zhang, X.D., Thangaraj, S., Sun, J., 2022. Water Quality Shifts the Dominant Phytoplankton Group from Diatoms to Dinoflagellates in the Coastal Ecosystem of the Bohai Bay. *Marine Pollution Bulletin*, 183.doi:10.1016/j.marpolbul.2022.114078

Spilling, K., Olli, K., Lehtoranta, J., Kremp, A., Tedesco, L., Tamelander, T., Klais, R., Peltonen, H., Tamminen, T., 2018. Shifting Diatom-Dinoflagellate Dominance During Spring Bloom in the Baltic

Sea and Its Potential Effects on Biogeochemical Cycling. *Frontiers in Marine Science*, 5.doi:10.3389/fmars.2018.00327

Stoecker, D.K., Hansen, P.J., Caron, D.A., Mitra, A., Annual, R., 2017. Mixotrophy in the Marine Plankton. *Annual Review of Marine Science, Vol 9*, 9, 311-335.doi:10.1146/annurev-marine-010816-060617

Stoecker, D.K., Li, A.S., Coats, D.W., Gustafson, D.E., Nannen, M.K., 1997. Mixotrophy in the Dinoflagellate Prorocentrum Minimum. *Marine Ecology Progress Series*, 152, 1-12.doi:10.3354/meps152001

Stolte, W., Riegman, R., 1995. Effect of Phytoplankton Cell-Size on Transient-State Nitrate and Ammonium Uptake Kinetics. *Microbiology-Sgm*, 141, 1221-1229.doi:10.1099/13500872-141-5-1221

Strom, S.L., Bright, K.J., Fredrickson, K.A., 2024. Widespread Ciliate and Dinoflagellate Mixotrophy May Contribute to Ecosystem Resilience in a Subarctic Sea: The Northern Gulf of Alaska. *Aquatic Microbial Ecology*, 90, 1-21.doi:10.3354/ame02005

Sutton, M., Raghuram, N., Adhya, T.K., Baron, J., Cox, C., de Vries, W., Hicks, K., Howard, C., Ju, X., Kanter, D., 2019. The Nitrogen Fix: From Nitrogen Cycle Pollution to Nitrogen Circular Economy, Frontiers 2018/2019. United Nations Environment Programme, pp. 52-64.

Swan, S., Davidson, K., 2007. Monitoring Programme for the Presence of Toxin Producing Plankton in Shellfish Production Areas in Scotland: April 2006-March 2007.

Taipale, S.J., Vuorio, K., Aalto, S.L., Peltomaa, E., Tiirola, M., 2019. Eutrophication Reduces the Nutritional Value of Phytoplankton in Boreal Lakes. *Environmental Research*, 179.doi:10.1016/j.envres.2019.108836

Tett, P., 2021. Guide to the Plankton Index Method and Software, V4.02. *Scottish Association for Marine Science.*,

Tett, P., Baker, J.M., Wolff, W.J., 1987. Plankton, In: Biological Surveys of Estuaries and Coasts edited by: Baker, J.M., Wolff, W.J. Cambridge University Press. 280 - 341

Tett, P., Carreira, C., Mills, D.K., van Leeuwen, S., Foden, J., Bresnan, E., Gowen, R.J., 2008. Use of a Phytoplankton Community Index to Assess the Health of Coastal Waters. *Ices Journal of Marine Science*, 65, 1475-1482.doi:10.1093/icesjms/fsn161

Tett, P., Gowen, R., Mills, D., Fernandes, T., Gilpin, L., Huxham, M., Kennington, K., Read, P., Service, M., Wilkinson, M., Malcolm, S., 2007. Defining and Detecting Undesirable Disturbance in the Context of Marine Eutrophication. *Marine Pollution Bulletin*, 55, 282-297.doi:10.1016/j.marpolbul.2006.08.028

Thackeray, S.J., Jones, I.D., Maberly, S.C., 2008. Long-Term Change in the Phenology of Spring Phytoplankton: Species-Specific Responses to Nutrient Enrichment and Climatic Change. *Journal of Ecology*, 96, 523-535.doi:10.1111/j.1365-2745.2008.01355.x

Tomkins, M., Martin, A.P., Nurser, A.J.G., Anderson, T.R., 2020. Phytoplankton Acclimation to Changing Light Intensity in a Turbulent Mixed Layer: A Lagrangian Modelling Study. *Ecological Modelling*, 417.doi:10.1016/j.ecolmodel.2019.108917

Trommer, G., Leynaert, A., Klein, C., Naegelen, A., Beker, B., 2013. Phytoplankton Phosphorus Limitation in a North Atlantic Coastal Ecosystem Not Predicted by Nutrient Load. *Journal of Plankton Research*, 35, 1207-1219.doi:10.1093/plankt/fbt070

Turner, A.D., Dhanji-Rapkova, M., Dean, K., Milligan, S., Hamilton, M., Thomas, J., Poole, C., Haycock, J., Spelman-Marriott, J., Watson, A., Hughes, K., Marr, B., Dixon, A., Coates, L., 2018. Fatal Canine Intoxications Linked to the Presence of Saxitoxins in Stranded Marine Organisms Following Winter Storm Activity. *Toxins*, 10.doi:10.3390/toxins10030094

Turner, R.E., Rabalais, N.N., Justic, D., Dortch, Q., 2003. Global Patterns of Dissolved N, P and Si in Large Rivers. *Biogeochemistry*, 64, 297-317.doi:10.1023/a:1024960007569

Tye, A.M., Jarvie, H.P., Spears, B.M., Dise, N.B., Williamson, J.L., Lapworth, D.J., Monteith, D., Sanders, R., Mayor, D.J., Bowes, M.J., 2024. Riverine Concentrations and Export of Dissolved Silicon, and Potential Controls on Nutrient Stoichiometry, across the Land–Ocean Continuum in Great Britain. *Journal of Hydrology*, 640, 131738.doi:10.1016/j.jhydrol.2024.131738

UK Parliament, 2017. The Water Environment (Water Framework Directive)(England and Wales) Regulations 2017.

Ulen, B., Bechmann, M., Folster, J., Jarvie, H.P., Tunney, H., 2007. Agriculture as a Phosphorus Source for Eutrophication in the North-West European Countries, Norway, Sweden, United Kingdom and Ireland: A Review. *Soil Use and Management*, 23, 5-15.doi:10.1111/j.1475-2743.2007.00115.x

UNESCO, I., 1981. The Practical Salinity Scale1978 and the International Equation of State of Seawater 1980. *Tenth Report of the Joint Panel on Oceanographic Tables and Standards (JPOTS)*, 25,

Utermohl, H., 1958. Zur Ver Vollkommung Der Quantitativen Phytoplankton-Methodik. *Mitteilung Internationale Vereinigung Fuer Theoretische unde Amgewandte Limnologie*, 9,

van de Waal, D.B., Eberlein, T., Bublitz, Y., John, U., Rost, B., 2014. Shake It Easy: A Gently Mixed Continuous Culture System for Dinoflagellates. *Journal of Plankton Research*, 36, 889-894.doi:10.1093/plankt/fbt138

Van Meerssche, E., Pinckney, J.L., 2019. Nutrient Loading Impacts on Estuarine Phytoplankton Size and Community Composition: Community-Based Indicators of Eutrophication. *Estuaries and Coasts*, 42, 504-512.doi:10.1007/s12237-018-0470-z

Vigouroux, G., Kari, E., Beltran-Abaunza, J.M., Uotila, P., Yuan, D.K., Destouni, G., 2021. Trend Correlations for Coastal Eutrophication and Its Main Local and Whole-Sea Drivers-Application to the Baltic Sea. *Science of the Total Environment*, 779.doi:10.1016/j.scitotenv.2021.146367

Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M., 1997. Human Domination of Earth's Ecosystems. *Science*, 277, 494-499.doi:10.1126/science.277.5325.494

Vrede, T., Ballantyne, A., MILLE-LINDBLOM, C., Algesten, G., Gudasz, C., Lindahl, S., Brunberg, A.K., 2009. Effects of N: P Loading Ratios on Phytoplankton Community Composition, Primary Production and N Fixation in a Eutrophic Lake. *Freshwater Biology*, 54, 331-344.doi:10.1111/j.1365-2427.2008.02118.x

Wang, Y., Wu, H., Lin, J., Zhu, J., Zhang, W., Li, C., 2019. Phytoplankton Blooms Off a High Turbidity Estuary: A Case Study in the Changjiang River Estuary. *Journal of Geophysical Research: Oceans*, 124, 8036-8059.doi:10.1029/2019JC015343

Wang, Y.S., Xie, X., Liu, C., Wang, Y.Y., Li, M., 2020. Variation of Net Anthropogenic Phosphorus Inputs (Napi) and Riverine Phosphorus Fluxes in Seven Major River Basins in China. *Science of the Total Environment*, 742.doi:10.1016/j.scitotenv.2020.140514

Wang, Y.Z., Liu, Y., Chen, X.Y., Cui, Z.G., Qu, K.M., Wei, Y.Q., 2022. Exploring the Key Factors Affecting the Seasonal Variation of Phytoplankton in the Coastal Yellow Sea. *Frontiers in Marine Science*, 9.doi:10.3389/fmars.2022.1076975

Wasmund, N., 2017. The Diatom/Dinoflagellate Index as an Indicator of Ecosystem Changes in the Baltic Sea. 2. Historical Data for Use in Determination of Good Environmental Status. *Frontiers in Marine Science*, 4.doi:10.3389/fmars.2017.00153

Wasmund, N., Kownacka, J., Gobel, J., Jaanus, A., Johansen, M., Jurgensone, I., Lehtinen, S., Powilleit, M., 2017. The Diatom/Dinoflagellate Index as an Indicator of Ecosystem Changes in the Baltic Sea 1. Principle and Handling Instruction. *Frontiers in Marine Science*, 4.doi:10.3389/fmars.2017.00022

Webber, M., Edwards-Myers, E., Campbell, C., Webber, D., 2005. Phytoplankton and Zooplankton as Indicators of Water Quality in Discovery Bay, Jamaica. *Hydrobiologia*, 545, 177-193.doi:10.1007/s10750-005-2676-x

Weston, K., Greenwood, N., Fernand, L., Pearce, D.J., Sivyer, D.B., 2008. Environmental Controls on Phytoplankton Community Composition in the Thames Plume, Uk. *Journal of Sea Research*, 60, 262-270.doi:10.1016/j.seares.2008.09.003

Westphal, K., Musolff, A., Graeber, D., Borchardt, D., 2020. Controls of Point and Diffuse Sources Lowered Riverine Nutrient Concentrations Asynchronously, Thereby Warping Molar N:P Ratios. *Environmental Research Letters*, 15.doi:10.1088/1748-9326/ab98b6

Wilkerson, F., Dugdale, R., 2016. The Ammonium Paradox of an Urban High-Nutrient Low-Growth Estuary, Aquatic Microbial Ecology and Biogeochemistry: A Dual Perspective, pp. 117-126.

Willén, E., 2000. Phytoplankton in Water Quality Assessment–an Indicator Concept. *Hydrological and limnological aspects of lake monitoring*, 2, 58-80.doi:10.1002/9780470511121.ch6

Winder, M., Cloern, J.E., 2010. The Annual Cycles of Phytoplankton Biomass. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 365, 3215-3226.doi:10.1098/rstb.2010.0125

Withers, P.J.A., Neal, C., Jarvie, H.P., Doody, D.G., 2014. Agriculture and Eutrophication: Where Do We Go from Here? *Sustainability*, 6, 5853-5875.doi:10.3390/su6095853

Wofsy, S.C., 1983. A Simple Model to Predict Extinction Coefficients and Phytoplankton Biomass in Eutrophic Waters 1. *Limnology and Oceanography*, 28, 1144-1155.doi:10.4319/lo.1983.28.6.1144

Wood, D., Capuzzo, E., Kirby, D., Mooney-McAuley, K., Kerrison, P., 2017. Uk Macroalgae Aquaculture: What Are the Key Environmental and Licensing Considerations? *Marine policy*, 83, 29-39.doi:10.1016/j.marpol.2017.05.021

You, J.H., Ok, J.H., Kang, H.C., Park, S.A., Eom, S.H., Jeong, H.J., 2023. Five Phototrophic *Scrippsiella* Species Lacking Mixotrophic Ability and the Extended Prey Spectrum of *Scrippsiella Acuminata* (Thoracosphaerales, Dinophyceae). *Algae*, 38, 111-126.doi:10.4490/algae.2023.38.6.6

Zhang, W.Z., Liu, J., Xiao, Y.X., Zhang, Y.M., Yu, Y.J.Z., Zheng, Z., Liu, Y.F., Li, Q., 2022. The Impact of Cyanobacteria Blooms on the Aquatic Environment and Human Health. *Toxins*, 14.doi:10.3390/toxins14100658

Zhang, X., Qi, M., Chen, L., Wu, T., Zhang, W., Wang, X., Tong, Y., 2020. Recent Change in Nutrient Discharge from Municipal Wastewater in China's Coastal Cities and Implication for Nutrient Balance in the Nearshore Waters. *Estuarine, Coastal and Shelf Science*, 242, 106856.doi:10.1016/j.ecss.2020.106856

Zhang, X.F., Mei, X.Y., Gulati, R.D., 2017. Effects of Omnivorous Tilapia on Water Turbidity and Primary Production Dynamics in Shallow Lakes: Implications for Ecosystem Management. *Reviews in Fish Biology and Fisheries*, 27, 245-254.doi:10.1007/s11160-016-9458-6

Zhang, Y.Q., Lin, X., Shi, X.G., Lin, L.X., Luo, H., Li, L., Lin, S.J., 2019. Metatranscriptomic Signatures Associated with Phytoplankton Regime Shift from Diatom Dominance to a Dinoflagellate Bloom. *Front. Microbiol.*, 10.doi:10.3389/fmicb.2019.00590

Zhou, L., Yang, X., Li, K., Xiang, C., Wu, Y., Huang, X., Huang, L., Tan, Y., 2024. Regime Shift in a Coastal Pelagic Ecosystem with Increasing Human-Induced Nutrient Inputs over Decades. *Water Research*, 263, 122147.doi:10.1016/j.watres.2024.122147

Zhou, Y.P., Zhang, Y.M., Li, F.F., Tan, L.J., Wang, J.T., 2017a. Nutrients Structure Changes Impact the Competition and Succession between Diatom and Dinoflagellate in the East China Sea. *Science of the Total Environment*, 574, 499-508.doi:10.1016/j.scitotenv.2016.09.092

Zhou, Z.X., Yu, R.C., Zhou, M.J., 2017b. Seasonal Succession of Microalgal Blooms from Diatoms to Dinoflagellates in the East China Sea: A Numerical Simulation Study. *Ecological Modelling*, 360, 150-162.doi:10.1016/j.ecolmodel.2017.06.027

Zhu, K., Zhang, H., Zhang, P., Wang, P., Li, H., Feng, M., Wang, H., Wang, H., Zhang, M., Xu, J., 2023. Interactive Effects of Warming and Eutrophication on Zooplankton Could Reverse the Stoichiometric Mismatch with Phytoplankton. *Water Biology and Security*, 2, 100205.doi:10.1016/j.watbs.2023.100205