Using nitrogen isotopes in macroalgae to determine and monitor sources of nitrogen pollution in estuarine and coastal areas: an in vitro study and case study in North East England, UK.

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Using nitrogen isotopes in macroalgae to determine and monitor sources of nitrogen pollution in estuarine and coastal areas: an *in vitro* study and case study in North East England, UK.

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Abstract

The growing human population in coastal areas, combined with the intensification of agricultural activity have increased the amount of dissolved inorganic nitrogen (DIN) delivered to estuaries and coastal waters. These high concentrations of DIN can cause eutrophication which in turn causes the growth of opportunistic macroalgae (seaweed) and anoxic conditions both in the water column and in the sediment. Stable nitrogen isotope ratios ($\delta^{15}N$) can be used to discern sources of excess nitrogen pollution in water. The $\delta^{15}N$ values of nitrate in water often do not reflect the true $\delta^{15}N$ source value owing to high temporal variation, and there are high analytical costs associated with obtaining $\delta^{15}N$ values from water nitrate. As such, $\delta^{15}N$ values can be measured in macroalgae samples to identify the bioavailable nitrogen inputs in an area. Macroalgae have been previously reported to be accurate biomonitors for sources of nitrogen in the environment, with fractionation assumed to be negligible; however, some workers report varying environmental and biological conditions can cause fractionation between the water column and macroalgal $\delta^{15}N$ values. Tips of *Fucus vesiculosus* (*F. vesiculosus*) were incubated in isotopically-labelled artificial seawater solutions with varying temperatures and salinities to determine whether these factors affected equilibration of macroalgal $\delta^{15}N$ values with $\delta^{15}N$-DIN values. Temperature and salinity were found to have a significant effect on the uptake of nitrogen isotopes by *F. vesiculosus* tips, suggesting varying these environmental conditions is likely to cause fractionation of nitrogen isotopes between the water column and macroalgal tissue. After 14 days of incubation there was a greater than 1 ‰ difference between $\delta^{15}N$ and $\delta^{15}N$-DIN. Tips of *F. vesiculosus* were also collected from around the North East of England coastline every 2-3 months from October 2020 to July 2021. The tips were analysed for $\delta^{15}N$ values. There was a significant difference in macroalgal $\delta^{15}N$ values between sampling locations for each collection period in both the River Wear and Tyne and also along the North East coastline. For *F. vesiculosus* tips collected from the River Wear, the dominant source of nitrogen pollution was found to be treated sewage or manure with point source pollution from untreated sewage discharged from Combined Sewage Overflows (CSO's). The River Tyne macroalgal tips appear to be impacted by untreated sewage, given the low $\delta^{15}N$ values and the temporal variations. Tips collected from the North East coastline look to be affected by wastewater pollution, i.e., treated sewage or manure but are regulated by the unpolluted marine nitrogen. Tips from all sites record $\delta^{15}N$ values in the unpolluted range in the winter months, potentially as a result of increased river discharges diluting any pollution $\delta^{15}N$

values with lower terrestrial nitrogen δ^{15}N values. There are also environmental and physiological factors that could influence the δ^{15}N values recorded by macroalgae such as biogeochemical cycles, temperature and salinity. All sites followed the same temporal trend of δ^{15}N values, suggesting an impact from environmental factors, rather than source changes. Therefore, δ^{15}N values in *F. vesiculosus* tips can be used as an indicator of sources of nitrogen loading in the environment, including for point source pollution. However, δ^{15}N values in *F. vesiculosus* tips are significantly affected by changes in temperature and salinity, and so more work is needed to quantify fractionation between water column and macroalgal δ^{15}N caused by different factors. These data demonstrate that macroalgae can provide an efficient, low-cost alternative to current analytical methods for determining and monitoring nitrogen pollution.

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Statement of copyright

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Dedication

For Granda Dunn, Uncle Kevin, Nanna Peg, Gran and Granda Bailes.

Introduction

The modern nitrogen cycle has been heavily influenced by human activity. The growing human population in coastal areas, combined with the intensification of agricultural activity have increased the amount of dissolved inorganic nitrogen (DIN) delivered to estuaries and coastal waters (Raimonet et al. 2013). These high concentrations of DIN can cause eutrophication which in turn causes environmental disturbances such as the growth of opportunistic macroalgae (i.e., seaweed) and anoxic conditions both in the water column and in the sediment (Fujita 1985; Howarth et al. 2011; Dailer et al. 2010; Teichberg et al. 2010). Traditional monitoring techniques, such as measuring DIN concentrations in the water column, fail to offer any information regarding the origin of pollutants (García-Seoane et al. 2018). Furthermore, DIN loads in the water column are rapidly diluted by hydrodynamic forces and/or are removed by plant and microbial uptake, rendering these techniques of limited use (Lemesle et al. 2016). To add to this, direct DIN measurements do not tell us the amount of nitrogen that is bioavailable in the water column (Dailer et al. 2010; Orlandi et al. 2014).

Nitrogen isotopes can offer a unique perspective on nitrogen pollution because different sources of pollution have different isotopic signatures. The proportion of the two nitrogen isotopes, ^{15}N and ^{14}N, can vary in the environment depending on fractionation processes (Viana & Bode 2013). $\delta^{15}N$ is calculated by considering the ratio of ^{14}N to ^{15}N in reference to an international standard. This standard is atmospheric N_2 which has a ^{15}N:^{14}N ratio of 0.3663% (Junk & Svec 1958). $\delta^{15}N$ of a sample is defined as the deviation away from this standard (air) with units of per mil (‰) and is calculated using Equation 1:

$$\delta^{15}\text{N (‰)} = \frac{^{15}N/^{14}N_{\text{sample}} - {}^{15}N/^{14}N_{\text{air}}}{^{15}N/^{14}N_{\text{air}}} \times 1000 \qquad (1)$$

Anthropogenically derived sources of nitrogen e.g., treated sewage, manure, terrestrial runoff, and fish farm effluent tend to be more enriched in ^{15}N and therefore have higher $\delta^{15}N$ values, relative to seawater (Figure 1) (Heaton 1986; Vizzini & Mazzola 2004; Viana et al. 2015; Savage 2005; Xue et al. 2009). Chemical fertilisers, used in agriculture, tend to be more enriched in ^{14}N compared to seawater, and therefore have lower $\delta^{15}N$ values because they are synthesised from atmospheric N_2

which has a $\delta^{15}N$ value of 0‰ (Figure 1) (Heaton 1986). Organic fertilisers, however, have higher and more variable $\delta^{15}N$ values (Figure 1) (Heaton 1986; Curt et al. 2004; Bateman & Kelly 2007). $\delta^{15}N$ values can also vary as a result of biogeochemical processes occurring in the water column, such as nitrification and denitrification (Sebilo et al. 2006). Nitrification is the dominant process to be considered in this project, because denitrification occurs only in anaerobic environments, and rivers, estuaries and coastlines tend to be well oxygenated. Therefore, nitrification, the biologically-mediated process by which ammonium (NH_4^+) is oxygenated to nitrate (NO_3^-), will decrease the $\delta^{15}N$ values of product nitrate (Sebilo et al. 2006). Hence, even if ammonium fertilisers are oxygenated to nitrate in the water column, the $\delta^{15}N$ values will remain characteristically low.

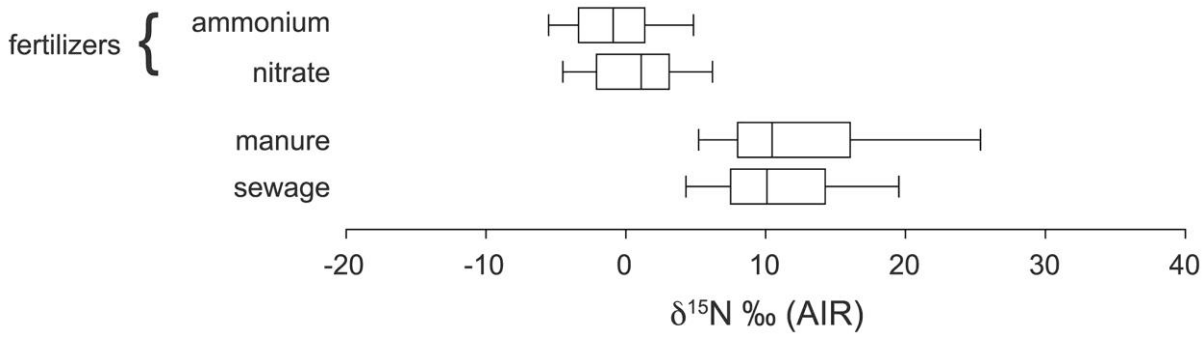


Figure 1: Box-and-whisker plots of $\delta^{15}N$ values from various nitrogen sources clearly showing the distinct difference between effluent sources and industrial fertilizers (adapted from Xue, et al. (2009) (Bailes & Gröcke 2020).

Increasingly, $\delta^{15}N$ values are being utilised in macroalgae samples to identify the bioavailable nitrogen inputs in an area (Savage & Elmgren 2004; Raimonet et al. 2013; Ochoa-Izaguirre & Soto-Jiménez 2014). Macroalgae have been proven to be accurate bioindicators of nitrogen loading in the natural environment (García-Seoane, et al., 2018; Costanzo, et al., 2001; Savage & Elmgren, 2004; Gartner, et al., 2002) for a number of reasons: they absorb nitrogen directly from the water column and assimilate the particles into their tissue; depending on the species, they can reflect the $\delta^{15}N$ values of the source nitrogen over a period ranging from days to weeks (Gartner et al. 2002; Deutsch & Voss, 2006); fractionation is assumed to be negligible during uptake and assimilation of nitrogen at concentrations found in the natural environment (or environmentally relevant concentrations) (Naldi & Wheeler, 2002; Cohen & Fong, 2005; Deutsch & Voss, 2006; Dudley, et al., 2010; Swart, et al., 2014; McClelland, et al., 1997); the range of $\delta^{15}N$ values in macroalgae is higher

than in other biotic samples, ranging from 0.2 to 50.1 ‰ (Dailer et al. 2010) which means that macroalgae can better discriminate against different types of nitrogen sources (Lemesle et al. 2016).

Generally, δ^{15}N values in primary producers found in built-up areas that are lower than 4‰ indicate an input of anthropogenic N sources derived from agriculture (fertilisers) and the chemical industry (Heaton 1986; Dailer et al. 2010). Untreated sewage can also have δ^{15}N values below 4‰ (Barr, et al., 2013). In pristine areas, low δ^{15}N values can be indicative of riverine nitrate that has been derived from soil nitrification processes (Mayer, et al., 2002). δ^{15}N values ranging between 4 and 8‰ in marine macroalgae indicate unaffected natural conditions (Riera 1998; Riera et al., 2000; Savage & Elmgren 2004; Orlandi et al. 2014). δ^{15}N values in macroalgae above 8‰ are indicative of sewage and aquaculture inputs (Heaton 1986; Vizzini & Mazzola, 2004; Viana et al. 2015; Savage 2005; Xue, et al. 2009).

However, fractionation processes in the water column, or during nitrogen uptake could mean the assumption that macroalgae reflects the δ^{15}N values of the nitrogen source, is invalid (Viana et al. 2011; Swart et al. 2014). Few studies have investigated fractionation between source δ^{15}N values and macroalgal tissue δ^{15}N values as a result of external conditions (Cohen & Fong 2005; Cornelisen et al. 2007; Dudley et al. 2010; Swart et al. 2014; Howarth et al. 2011; Howarth et al. 2020; Naldi & Wheeler 2002; Gröcke et al. 2017; Viana & Bode 2015). Viana and Bode (2015) argue that *Fucus* species can be used only to monitor nitrogen sources over long-term periods, given the fact the δ^{15}N values in macroalgal tissue did not reflect the δ^{15}N-DIN values. Some studies did find evidence of fractionation at high concentrations of external nitrogen, however these concentrations are unlikely to be environmentally relevant concentrations (Gröcke et al. 2017; Swart et al. 2014; Viana & Bode 2015). Therefore, it is generally believed that δ^{15}N values in macroalgae are representative of biologically available nitrogen in estuaries, and for *Fucus* macroalgae, over longer periods of weeks to allow for integration time (Dudley et al. 2010; Viana & Bode, 2015).

Monitoring and identifying nitrogen pollution in coastal environments and estuaries is essential because of the need to conform to legislation such as the European Water Framework Directive (WFD, 2000/60/EC) (European Commission, 2000). The WFD was developed to assess the ecological status of water bodies. Looking forward and given the departure of the UK from the European Union, the UK may develop its own legislation to assess pollution levels in coastal areas and estuaries.

Imogen Bailes

There has been particular controversy in the UK recently, surrounding water companies releasing untreated sewage into rivers when flow is too high for the sewage system to cope. As such, there has been a wide range of media coverage and public outrage at this issue (BBC, 2022). Given the public interest in these issues surrounding water quality, it is important to be able to quickly and accurately determine the sources of nitrogen pollution in water bodies, in theory so that interventions can be taken to reduce pollution from these sources.

Macroalgal selection

Perennial macroalgae species, are those which live for more than two years and slowly take up and store nutrients as to sustain growth. Opportunistic/ephemeral macroalgae species tend to rapidly incorporate nitrogen and only survive for around a year. They are generally considered stressors in the estuarine/coastal environment caused by eutrophication. Perennial macroalgae are considered indicators of ecosystem health, as they provide a wealth of services, including sequestering carbon into their tissues, the carbon stored by algae is sometimes referred to as 'blue carbon'. This study will utilise perennial species, specifically brown macroalgae to measure δ^{15}N values.

Brown macroalgae are photoautotrophic, meaning they use light and carbon dioxide in photosynthesis to create chemical energy. They're composed mainly of sulphated fucans and alginates, with cellulose accounting for a small fraction of the dry weight (1-8%) (Deniaud-Bouët, et al., 2014). *Fucus vesiculosus* (hereafter *F. vesiculosus*) is a perennial, intertidal brown macroalga with growth rates of approximately 4.5 mm per week (Knight & Parke 1950). The species is widespread around Europe, is robust and is believed to integrate variations in δ^{15}N values in DIN over a period of 2-3 weeks (Viana et al. 2015; Gartner et al. 2002; Deutsch & Voss 2006; Bailes & Gröcke 2020). The meristematic tissue of the vegetative tips of *F. vesiculosus* have a greater uptake of nitrogen compared to fertile tips (that release gametes) and older parts of the macroalgae tissue (Savage & Elmgren 2004; Viana et al. 2015). Given these reasons, and the species' use in research, the non-fertile tips of *F. vesiculosus* have been chosen to be the main species studies throughout this thesis.

Study area

A large number of catchments areas in North East England, UK are in nitrate vulnerable zones (Environment Agency 2021) and fail to achieve good overall status (Environment Agency & DEFRA 2009). In particular, The Heritage Coast, which runs from Sunderland to Hartlepool, is an area of

significant interest due to the cultural heritage of former industry and also the beauty of the natural landscape. Significant environmental improvements have been made to the Heritage Coast over the last few decades, but additional research and monitoring is required to assess increased population on sewage and coastal landfills, as well as legacy pollutants and mine water (The Heritage Coast Partnership 2017). Additionally, diffuse pollution from inland catchments areas through agricultural fertilisers and chemical effluents can impact the coastal environment; especially through hydrologic transfer through the magnesian limestone aquifer that dominates the Durham Heritage Coast and has now been assessed as having poor water quality (Environment Agency & DEFRA 2009). This study aims to assess the pollution status of the North East coastline in the UK by collecting native samples of macroalgae from around coastal areas and estuaries, with the aim being to identify sources of nitrogen pollution in specific areas by using nitrogen isotopes to determine the source.

Collections of macroalgae were done at several points in the year to generate isoscape maps of $\delta^{15}N$ values along the coastline seasonally. This is important because precipitation/runoff rates, as well as nitrogen sources, change seasonally and hence nitrogen loading into the coastal environment. Seasonal studies of nitrogen isotopes in native macroalgae have been conducted globally (Lemesle et al. 2016; García-Seoane et al. 2018), however this type of seasonal assessment has not been conducted in this study area.

In vitro studies

In addition to the *in situ* studies, *in vitro* studies were also done to assess fractionation of nitrogen isotopes under varying environmental conditions. It is generally thought there is minimal fractionation during the uptake and assimilation of nitrogen into macroalgal tissue (Dudley et al. 2010), however constraining this would be beneficial so as to contribute to the debate within the literature. Several laboratory experiments were conducted, with a specific focus on varying temperature and salinity because these conditions vary considerably in the environment and so studying these *in vitro* will better our understanding of how they could affect fractionation.

Research questions

1. What evidence is there in the literature to suggest that macroalgal $\delta^{15}N$ values are/are not reflective of $\delta^{15}N$-DIN values?
2. Do nitrogen isotopes in *F. vesiculosus* show a seasonal cycle?

3. To what extent do environmental conditions cause fractionation of nitrogen isotopes?
4. Is the North East coastline subject to nitrogen pollution?

Aims and objectives

- Conduct a literature review on the fractionation of $\delta^{15}N$ in macroalgae. Addresses research questions 1, 2 and 3.
- Determine the fractionation of nitrogen isotopes in macroalgae under varying conditions *in vitro*. Addresses research question 3.
- Produce seasonal nitrogen isoscape maps for the North East of England coastline and therefore identify areas of excess nitrogen pollution. Addresses research questions 2 and 4.

Thesis structure

Chapter One summarises the literature on the effects of environmental and biological factors on the potential fractionation of $\delta^{15}N$ between macroalgal tissue and DIN. Chapter Two of this thesis addresses the effect of different temperatures and salinities on macroalgal $\delta^{15}N$ values. Chapter Three evaluates the seasonal and spatial differences in $\delta^{15}N$ values of *F. vesiculosus* in North East England.

Chapter 1: Factors affecting fractionation of nitrogen isotopes in macroalgae

It is important to consider the factors that can cause fractionation of $\delta^{15}N$ values between DIN in the water column and the resultant $\delta^{15}N$ values measured in macroalgal tissue. As such, this chapter reviews the current literature where studies have investigated potential factors in the environment affecting fractionation, as well as the mechanisms of nitrogen uptake to better understand the process of potential fractionation.

1.1 Nitrogen uptake

Macroalgae requires nitrogen, phosphorus and a whole host of micronutrients found in the oceans, but in general it is thought that nitrogen is the most important nutrient source for macroalgae, it is N-limited (Hurd et al. 2014). Therefore, it is useful to understand the kinetics and isotopic fractionation effects that can manifest as a result of the uptake of different forms of nitrogen and varying concentrations in the environment.

1.1.1 Mechanisms of nutrient uptake and potential fractionation

Firstly, it is important to consider the mechanisms by which nutrients are taken up from the water column and consider any isotopic effects here. Gases may be taken up by macroalgae passively if there is a favourable concentration gradient; for example, CO_2, O_2 and NH_3 may be diffused through cell membranes (Harrison & Hurd 2001). These gases will cross the lipid biolayers by dissolving within the lipid portion of the membrane, then diffusing to the lipid-water interface before finally dissolving in the aqueous phase on the other side of the membrane (Hurd et al. 2014). For carbon uptake, the most abundant carbon species in the ocean, HCO_3^- is dehydrated by extracellular carbonic anhydrase, which is accessible from outside the cell membrane, following which the CO_2 is then taken up through the membrane (Axelsson et al. 2000). For macroalgae to uptake inorganic nitrogen and phosphorus, it is assumed active transport is the responsible mechanism, given that the Michaelis-Menten equation often describes the kinetics of nitrogen uptake in macroalgae (Harrison & Hurd 2001). However, passive uptake of ammonium can occur due to the molecule

being converted to ammonia at the thallus surface, caused by a higher pH at the cell surface which is in turn caused by the passive uptake of carbon dioxide (Hurd, et al., 2014). The vast majority of time, active uptake is used to assimilate ammonium (Harrison & Hurd 2001). Ammonium can be transported across cell membranes and be assimilated directly into macromolecules and later to be used for growth (Alwyn and Rees 2007).

In microalgae and bacteria, the uptake and fractionation of nitrate is a three step process (Granger et al. 2004; Hoch et al. 1992; Karsh et al. 2012, 2014; Mariotti et al. 1982; Shearer et al. 1991). To begin with the molecule needs to be transported across the cellular membrane, it is then reduced by the nitrate reductase (NR) enzyme and possibly further by nitrite reductase before it is fluxed out of the cell (Swart et al. 2014). It has been assumed by Swart et al. (2014) that macroalgae behave similarly to microalgae in that during the nitrate reductase step, fractionation of nitrate has the potential to be the greatest.

1.1.2 Concentration dependant fractionation

The external concentration of nitrate is thought to have an effect on the extent of the fractionation when being processed by macroalgae (Swart, et al., 2014). At lower external concentrations of nitrate, when it is limiting, most nitrate is consumed and efflux from the cell is minimal meaning that the $\delta^{15}N$ signature of the solution doesn't change, however the fractionation during the NR step could still occur so the $\delta^{15}N$ of the macroalgae potentially changes (Swart et al. 2014). At high external concentrations of nitrate, the opposite phenomenon occurs, whereby the fractionation that takes place during the NR step is translated into the solution because nitrogen isn't limiting so there will be efflux from the cell (Swart et al. 2014). This fractionation during the NR step creates isotopically light macroalgae in comparison to the initial solution $\delta^{15}N$.

This potential fractionation at higher concentrations could have consequences for interpreting $\delta^{15}N$ in macroalgae that has been transplanted. If there are high external concentrations of nitrate, the macroalgae will initially become isotopically light due to the preference for ^{14}N (Swart et al. 2014). However, as the NO_3^- is consumed the residual pool will become isotopically more positive, meaning that eventually the macroalgae will in turn become isotopically positive (Swart et al. 2014).

Resultingly the $\delta^{15}N$ of the solution could be wrongly interpreted to be more positive than it actually is, as the macroalgae accumulates more ^{15}N as a result of fractionation during assimilation (Swart et al. 2014).

Several studies have investigated concentration dependant fractionation. Cohen and Fong (2005) concluded concentration dependant isotopic fractionation does not occur when *Ulva intestinalis* is exposed to nitrate and ammonia concentrations of 50 – 500 mM over a period of 12 hours. They found that there isn't a relationship between nitrogen concentration and macroalgal $\delta^{15}N$, demonstrating that no selection for ^{14}N over ^{15}N occurred. This demonstrated that *U. intestinalis*, and perhaps more broadly green macroalgae, can be used to determine the availability of nitrogen sources in estuaries and coastal areas. On the other hand, Swart et al. (2014) conducted further *in vitro* studies and documented evidence of concentration dependant isotopic fractionation. They found that under nitrogen limiting conditions (< 2 mM), *Ulva sp*. and the red macroalgae *Agardhiella sp*. had the same isotopic composition as the nitrate in solution. But at higher external nitrate concentrations (> 10-50 mM), these species will fractionate the nitrate pool and therefore form biomass which is isotopically lighter than the nitrate in solution by 4 – 6 ‰. This study argued that Cohen and Fong (2005) did not find evidence of isotopic fractionation in *Ulva sp*. because relatively high concentrations of nitrate were used, which Swart et al. (2014) disputed on the basis that fractionation wouldn't be constant at these environmentally irrelevant concentrations. Teichberg et al. (2007; 2008) conducted *in situ* experiments, where *Ulva lactuca* and a red macroalga, *Gracilaria tikvahia*, were placed in cages enriched with various nutrients. Fractionation was assumed to be very small between the nitrogen source $\delta^{15}N$ and the macroalgal $\delta^{15}N$ (0 – 1.5 ‰).

When investigating brown macroalgae, Umezawa et al. (2007) found that $\delta^{15}N$ values in *Padina australis* increased when exposed to isotopically heavy nitrate. This enrichment, however, was less than what was predicted by a simple mixing model for the macroalgae incubated in a high nitrate concentration of 40 mM with low light levels and temperature. Consequentially, the authors concluded that isotopic discrimination was occurring in these conditions due to the selective uptake of isotopically lighter nitrate in concentration saturating conditions. At high light levels and temperature, but the same concentration, significant amounts of isotopically light dissolved organic nitrogen (DON) were excreted from the cells meaning $\delta^{15}N$ enrichment occurred in line with expected levels from the mixing model, demonstrating that some isotopic dilution occurred. This study demonstrates the fact that varying concentration and other external factors such as light and

temperature can affect isotopic fractionation, this fractionation though, was determined to be generally in the range of 1 ‰ which is smaller than the variation between nitrogen sources in the environment (Umezawa, et al., 2007).

Orlandi et al. (2017) used inorganic and organic nitrogen sources, and a mixture of these solutions at high and low concentrations (40 and 10 mgN/L respectively) to culture *Ulva lactuca*. The macroalgal tissue integrated the $\delta^{15}N$ of the solutions most effectively (greatest $\delta^{15}N$ variation) at the higher concentration solution used. Concentration dependant fractionation was not observed in this study. Gröcke et al. (2017) found that the $\delta^{15}N$ of *F. vesiculosus* was fractionated *in vitro* when incubated in a nitrate solution, but not an ammonium solution.

1.1.3 Ammonium preference

Macroalgae will preferentially take up ammonium solutions over those containing solely nitrate (Cohen & Fong, 2004; Phillips & Hurd, 2003; Pritchard, et al., 2015; Robertson & Savage, 2018). Uptake and assimilation of ammonia by macroalgae tends to occur at a higher rate than for nitrate regardless of the species, although the extent to which this preference occurs could be dependent on the species (Phillips & Hurd, 2003). Alwyn and Rees (2007) report that K_m (the concentration of a nutrient that gives half of the maximum rate of uptake) and V_{max} (the maximum rate of uptake achieved at saturating conditions of the nutrient) are greater for ammonium uptake than nitrate uptake for a range of macroalgal species studied.

The nitrate and nitrite reductase enzymes are redundant in ammonium assimilation and hence the fractionation manifested in these steps does not occur during ammonium uptake by macroalgae (Swart et al. 2014; Gröcke et al. 2017; Ross et al. 2018). Contrasting evidence to demonstrate fractionation occurs during ammonium uptake is documented by Dudley et al. (2010) in green macroalga *Ulva pertusa*. To add to this, nitrate and nitrite reductases are repressible enzymes, meaning ammonium assimilation can inhibit nitrate assimilation (Syrett and Leftley 1976; Ross et al. 2018). Macroalgae could therefore preferentially assimilate the $\delta^{15}N$ signal of ammonium present in an area over the nitrate which would lead to inaccurate interpretations of nitrogen sources present (Cohen and Fong 2005). Orlandi et al. (2017) found that a mixed solution containing organic nitrogen, nitrate and ammonium, was integrated most effectively (greatest $\Delta^{15}N$ variation)

by *Ulva lactuca* as compared with the solutions containing solely either organic nitrogen, nitrate or ammonium.

1.1.4 Nutritional history

Some studies culturing macroalgae with various nutrient sources first acclimated the macroalgae to low nutrient seawater before the actual experiments (Cohen & Fong, 2006; Dailer, et al., 2010; for a review see García-Seoane, et al., 2018). The aim of the pre-exposure treatments is to homogenise the macroalgae, as well as deplete the internal nutrient stores so that the initial tissue nutrient content is reduced (García-Seoane et al. 2018). In addition to this, Naldi and Wheeler (2002) and Umezawa et al. (2007) document the release of nitrogen from macroalgae during nutrient enrichment, emphasising the need for acclimation to deplete the internal nutrient stores and prevent isotopic dilution due to this release.

Macroalgae that have been acclimated in high nutrient conditions (particularly perennial species), such as seen in Swart et al. (2014) and Bailes and Gröcke (2020), could retain the internal nutrient reserves which could prove problematic if the aim is to use these macroalgae in transplantation experiments (Gröcke et al. 2017; Bailes and Gröcke 2020). In this case, the macroalgae could preferentially release ^{14}N or ^{15}N as efflux, whilst integrating the $\delta^{15}N$ signal from the DIN, and hence the isotopic signature of the macroalgae will not be true of the environment. Several more studies need to be conducted, for example carrying out replicate nutrient enrichment experiments with and without acclimation.

1.1.5 Phosphate enrichment

Macroalgae are usually N-limited and hence most studies concentrate on nitrogen enrichment to study nutrient uptake. Some studies have investigated whether adding phosphate will increase the rate of nitrogen uptake and consequentially affect the $\delta^{15}N$ ratio in macroalgae. Teichberg et al. (2008) concluded in their *in situ* study that adding phosphate does not increase the uptake of nitrate, or the growth rate of *Ulva lactuca* and hence has no impact on $\delta^{15}N$. Other studies (Naldi and Wheeler 2002; Umezawa et al. 2007; Swart et al. 2014) have added phosphate into nitrogen

solutions to culture macroalgae but haven't explicitly determined whether this phosphate has any effect on nitrogen uptake or fractionation.

1.2 Temperature

Few studies have explicitly investigated how temperature affects the fractionation of nitrogen isotopes (Howarth et al. 2020; Umezawa et al. 2007). Other studies have looked at how temperature affects various biological characteristic such as the uptake and assimilation of nutrients, which can in turn affect $\delta^{15}N$ ratios but this has not been directly commented on (Pedersen et al. 2004; Young et al. 2007; Lehvo et al. 2001; Graiff et al. 2015). Most reach the conclusion that increasing temperatures will increase the uptake of nitrogen by macroalgae, until a limit whereby the macroalgae starts to become heat stressed. One hypothesis is that increases in temperature could enhance macroalgae metabolism and therefore lead to a lower selection of light isotopes, increasing $\delta^{15}N$ values (Dudley, et al., 2010; Raimonet, et al., 2013).

Young et al. (2007) reported that temperature had a very minimal effect on nitrate reductase activity (NRA) which is thought to be an indicator of nitrate uptake, in several species of macroalgae, including *F. vesiculosus, Fucus spiralis, Fucus serratus* and *Laminara digitata*. Laboratory cultured *Padina australis* (a brown macroalga) samples had $\delta^{15}N$ values more reflective of the $\delta^{15}N$ source values at higher temperatures (29-32°C) compared to lower temperatures (21-23°C) (Umezawa et al. 2007). This observed difference between temperatures in $\delta^{15}N$ values, however, was approximately 1.0 ‰, which is smaller than the variation of nitrogen isotopes from various sources of nitrogen pollution, and therefore the authors argue changes in temperature do not affect the ability of macroalgae to reflect $\delta^{15}N$ values of nitrogen sources in the environment. In this experiment, the $\delta^{15}N$ values of the macroalgal tissue were more reflective of source $\delta^{15}N$ at higher temperature, which corresponded to higher rates of nitrogen uptake, therefore these findings do not support the hypothesis presented by that greater fractionation occurs at higher rates of uptake (Umezawa et al. 2007).

On the other hand, the red macroalga, *Chondrus crispus*, better incorporates the external nitrogen isotopic signature when cultured *in vitro* at 7 ℃ rather than 14 ℃ (Howarth et al. 2020) which is an unexpected result given the fact previous studies found optimum nitrogen uptake rates for this species of macroalgae at a temperature of 15 ℃ (Kübler & Davison 1995). This finding supports the

hypothesis that fractionation of nitrogen isotopes between the water column and macroalgal tissue is more likely to occur when the macroalgae is experiencing optimal nitrogen uptake, which could also explain why fractionation has been observed at high external concentrations of nitrate (Swart et al. 2014; Gröcke et al. 2017). This study will seek to address the lack of literature investigating the effect of temperature on nitrogen isotopes in macroalgae. In *F. vesiculosus* optimal temperatures for growth have been found to be between 10 and 20°C (Graiff et al. 2015).

1.2.1 Indirect effects from temperature changes

Changes in temperature in the natural environment could cause $\delta^{15}N$ values in macroalgae to change indirectly. Denitrification often occurs in higher temperatures and produces nitrate with higher $\delta^{15}N$ values, hence the $\delta^{15}N$ values rise when temperatures rise, as heavier nitrate is present in the water column due to this process (Baeta, et al., 2009). Raimonet et al. (2013) assume that denitrification and nitrification are limited by low temperatures in the winter, and therefore discrimination between different sources of nitrogen pollution may be much higher in the summer when bacterial processes are enhanced by higher temperatures. Another indirect effect of temperature is the fact conditions are more preferable for growth in the warmer summer months, but DIN concentrations are generally lower, and so macroalgae may mobilise stored nitrogen for growth resulting in fractionation (Viana & Bode, 2015).

1.3 Nitrogen in the environment

The concentration of nitrogen in estuaries may depend on the magnitude of N loads, seasonal biogeochemical processes such as uptake by producers, denitrification and regeneration, and any effects associated with hydrodynamic processes such as mixing, flushing rate and water residence time (Valiela, et al., 2021 and references therein). Nitrogen biogeochemistry is very dependent on redox reactions which are facilitated by microorganisms. Removal of nitrate by denitrification is thought to be the dominant loss pathway in shallow coastal and estuarine systems (Seitzinger, 1988). Denitrification can affect the $\delta^{15}N$ values in the water column if there is exchange between the sediment and the water column, as denitrification tends to occur in anoxic sediments. Denitrifying bacteria are abundant in coastal and estuarine environments (Mosier & Francis, 2010). This process of denitrification, which tends occur at greater rates in the spring and summer due to

higher temperatures, favours ^{14}N and so produces ^{14}N-enriched nitrous oxides and N_2 gas, resulting in an enrichment of the remaining nitrogen pool (Baeta, et al., 2009; Bergamino & Richoux, 2014). Increases in $\delta^{15}N$ values in macrophytes were evidenced by Baeta et al. (2009) in the Mondego estuary, Portugal, following periods of elevated temperatures and drought; which the authors concluded could be as a result of increased rates of denitrification. Nitrification occurs alongside denitrification in sediments and leaves the remaining ammonium pool enriched in ^{15}N (Brandes & Devol, 1997), and so if macroalgal tissue takes up ammonium from sediment it could have higher $\delta^{15}N$ values. This could explain why Raimonet et al. (2013) documented higher $\delta^{15}N$ values in macroalgae collected from intertidal mudflats compared with $\delta^{15}N$ values extracted from DIN. However, this phenomenon was not considered by the authors to have a large effect on $\delta^{15}N$ values on the macroalgae given the fact it was collected in low temperatures (section 1.2), suggesting in summer this effect could affect $\delta^{15}N$ in the water column (Raimonet, et al., 2013). Therefore, as a result of these transformations in the natural environment, macroalgal $\delta^{15}N$ values may not actually reflect source $\delta^{15}N$ values because the ratio of nitrogen isotopes has changed due to these bacterial processes.

Nitrate concentrations decrease with increasing flow due to dilution of point and diffuse sources by rainfall (Neal, et al., 2000). Therefore, concentrations also vary seasonally because discharge varies seasonally (Neal, et al., 2000). However, in large discharge events the nitrate concentrations can increase (Knapp, et al., 2020). Bioavailable nitrogen concentrations can decrease down-estuary as a result of the passive mixing with low-nitrogen seawater or from biogeochemical interception in the estuary, including nitrification and denitrification (Valiela, et al., 2020).

1.4 Irradiance

There has been limited research into whether varying light availability can cause fractionation of nitrogen isotopes during uptake by macroalgae, although several workers have identified fractionation caused by light in microalgae (Wada & Hattori, 1978; Needoba & Harrison, 2004). Macroalgae located in the intertidal zone experience regular exposure to atmospheric conditions (Hurd, et al., 2014). Direct solar radiation is the major source of heat during an ebb tide, although irradiance may be reduced because of shading by clouds, water, topography and other algae (Hurd, et al., 2014; Phillips & Hurd, 2003).

Cornelisen et al. (2007) and Barr (2007) report results for *in situ* studies on *Ulva pertusa*, finding a small average difference of 1 ‰ in $\delta^{15}N$ values between summer and winter, and measured changing light availabilities, although this effect can't be distinguished from other environmental parameters that change seasonally, most notably temperature. Dudley et al. (2010) found that light has minimal influence on the uptake of nitrogen isotopes by *Ulva pertusa* when treated *in vitro* with nitrate (0.8 ‰), however fractionation was documented between different light availability when treated with ammonium (3.7 ‰). The mechanism behind these differences between nitrate and ammonium uptake on fractionation caused by varying light levels is unclear, however the authors state that given ammonium does not generally comprise a large proportion of DIN in the natural environment (approximately 20 %), then it should have minimal effect on fractionation by macroalgae (Dudley, et al., 2010). In the brown macroalga, *Padina australis*, Umezawa et al. (2007) report that high light levels and temperatures can cause the cultured tissue to fractionate nitrogen isotopes, but this fractionation was in the range of 1 ‰. Overall, fractionation of nitrogen isotopes by macroalgae appears to be minimal and consensus within the limited literature is that fractionation is not greater than the variation in $\delta^{15}N$ values between different sources of nitrogen.

1.5 Salinity

Macroalgae is typically found in pools with salinities from around 10-77 S_A (absolute salinity, which is equivalent to mg/L salinity) (Hurd, et al., 2014), demonstrating how adaptable (phenotypically) macroalgae can be to survive in such varied conditions. Intertidal macroalgae, such as *Fucus* and *Ulva* can generally tolerate salinities of 10-100 mg/L salinity because of unpredictable changes in salinity during emersion, due to evaporation and any precipitation (Hurd, et al., 2014). There tends to be optimum salinities for growth, photosynthesis and therefore nutrient uptake in macroalgae, much the same as there are optimum temperatures (Hurd, et al., 2014). Many recent studies have examined the interactions between different environmental stressors, such as temperature, salinity and irradiance (Nygard & Dring, 2008; Takolander, et al., 2019; Schmid, et al., 2021; Lehvo, et al., 2001; Graiff, et al., 2015) on biological parameters such as mannitol production (a key product of photosynthesis) and growth rate. There have not been any studies that have examined the effects of changing salinity on nitrogen isotopes within macroalgae, although Viana and Bode (2015) suggest one reason for changes in $\delta^{15}N$ values of macroalgal tissue could be because of large

changes in salinity, which acts as a stressor and subsequently the macroalgae releases light nitrogen into the surrounding water.

Several studies have investigated $\delta^{15}N$ values of macroalgae along a salinity gradient, using salinity as a proxy for the mix of freshwater and therefore DIN loads with marine water (Deutsch & Voss, 2006; Cornelisen, et al., 2007; Abaya, et al., 2018). These studies have not considered the effect varying salinity could have on the uptake of nitrogen isotopes.

There have been several studies that have looked at long-term adaptation to varying salinity, commonly by investigating the difference between *F. vesiculosus* that has grown in the Baltic Sea, and the Atlantic, which have very different salinities, at approximately 5 and 35 mg/L respectively (Nygard & Ekelund, 2006; Nygard & Dring, 2008; Barboza, et al., 2019). In general, *Fucus* from the Baltic is better adapted to low salinities, and still shows growth at very low salinity, whereas Atlantic *Fucus* dies when cultured in low salinity seawater for too long. The optimum salinity for Atlantic *Fucus* is in the range 20-35 mg/L salinity, whereas Baltic *Fucus* is in the range 10-20 mg/L salinity; damage to cell structure occurred in salinities lower than 20 mg/L for Atlantic *Fucus* (Nygard & Dring, 2008). This study also found that high nutrient concentrations in the *in vitro* solutions increased both photosynthesis and growth for both types of *Fucus*, but also provided tolerance to low salinities (Nygard & Dring, 2008). Therefore, despite *Fucus* being generally euryhaline, individuals that have been grown at varying salinities long-term will have optimum salinities of similar values, showing adaptation to environmental conditions.

1.6 Other factors affecting macroalgal $\delta^{15}N$ fractionation

Bacterial processes at the surface of the frond (organic nitrogen mineralisation and nitrogen fixation) could increase $\delta^{15}N$-DIN values at the vicinity of the frond (Goecke, et al., 2010). The uptake of dissolved organic nitrogen (DON), such as urea and amino acids, could also control $\delta^{15}N$ values in macroalgae (Raimonet, et al., 2013). DON is rarely measured in routine monitoring of estuarine waters, because it is quickly mineralised during transport in the water column and DIN is more often than not, the dominant form of nitrogen (Seitzinger & Sanders, 1997).

1.7 Conclusion

There are many factors that could affect the difference between δ^{15}N values in macroalgal tissues and δ^{15}N values of nitrogen sources; some of these have been quantified, i.e. changes in light and temperature seem to generate an approximate 1 ‰ difference between δ^{15}N-DIN and macroalgal δ^{15}N values. Whereas other effects have only been proposed in the literature, such as salinity and bacterial processes like denitrification. Much more research is needed into these potential effects on fractionation in order for macroalgae to become a standardised biomonitor.

Chapter 2: Do temperature and salinity affect the uptake of nitrogen isotopes by *Fucus vesiculosus*?

2.1 Introduction

In order to use macroalgae as a biomonitor for nitrogen pollution sources, it is necessary to understand how environmental variables can affect the uptake of nitrogen isotopes, and therefore determine whether the $\delta^{15}N$ values measured in macroalgal tissue are representative of the $\delta^{15}N$ values in estuaries and around the coastline. Both temperature and salinity can vary in the natural environment, in particular around estuaries and coastlines. Due to their very nature, estuaries experience large changes in salinity with the tides, and this can vary throughout the year depending on currents and weather conditions. Particularly in temperate regions, temperature varies both daily and seasonally. There is debate in the literature as to the effect temperature can have on the uptake of nitrogen isotopes by macroalgae (Umezawa et al. 2007; Howarth et al. 2020). As far as the author is aware, there are no studies that have examined the effect of salinity on the uptake of nitrogen isotopes, although some studies have examined the physiological responses of macroalgae that have been cultured in varying salinities (Lehvo et al. 2001; Nygard & Dring 2008).

In order to understand the effect of varying temperatures and salinities on the uptake of nitrogen isotopes by macroalgae, several analogue laboratory experiments were conducted by culturing *F. vesiculosus* in solutions with different temperatures and salinities. In this study, *F. vesiculosus* tips were cultured in ^{15}N-enriched seawater, with a calculated $\delta^{15}N$ value of 1 ‰ ± 0.50 ‰ over three, two-week experiments at varying temperatures and salinities. The aim of this study was not to determine the amount of fractionation, although this should be studied in the future when there is funding to be able to analyse $\delta^{15}N$ values in water; but to determine whether the macroalgal $\delta^{15}N$ values are representative of the environment when temperature and salinity change.

2.2 Materials and Methodology

2.2.1 Artificial seawater

For the experiment it was decided to use artificial seawater over natural seawater so that the nitrogen content was controlled. An isotopically-labelled stock solution was prepared by dissolving sodium nitrate, $NaNO_3$ in distilled water. $NaNO_3$ fertiliser, with a $\delta^{15}N$ value of 0.84 ‰ (UK Nitrates Limited, King's Lynn, UK) and ^{15}N-enriched $NaNO_3$ (Sigma-Aldrich, Gillingham, UK; CAS: 31432-45-8; PubChem Substance ID: 24862444) were both dissolved in the solution with the aim of producing a solution with a $\delta^{15}N$ signal of 20‰ (Table 2.1). Despite adding ^{15}N-$NaNO_3$ salt to the stock solution of $NaNO_3$, as well as the fertiliser, it appears not to have dissolved into the solution, as the $\delta^{15}N$ of the stock solution measured to be 1.00‰ ± 0.50 ‰, when the aim was for the solution to be 20 ‰. It is unknown as to why this error has occurred; speculation includes human error, i.e. not measuring out the correct amount of salt or dissolving it completely in the solution; the initial calculations could be incorrect meaning not enough salt was added; or the salt may not have fully dissolved due to its chemical properties, although this is unlikely given it has a solubility in water at 25℃ of 91.2 g/100 g (Lide, 2005) which was accounted for. In order to avoid this in future, the ^{15}N-enriched salt perhaps should not have as high an isotopologue value so that a larger amount of salt can be measured out.

The following calculations describe how the stock solution was prepared with the aim of producing a solution with the $\delta^{15}N$ value of 20‰.

$d^hE(tracer) = f_1 \times d^hE(isotopologue1) + f_2 \times d^hE(isotopologue2)$

h = atomic mass

E = element

f = proportion

	^{14}N salt	^{15}N salt
Isotopologue (‰)	0.84	13376013.38
Proportion of $NaNO_3$ salt (%)	0.9999985	0.0000015
	$NaNO_3$ stock	
Desired concentration (NO_3^--mg/L)	68.5	

Table 2.1: values of ^{14}N- and ^{15}N- enriched salts and respective proportions used to create the stock solution.

2.2.1.1 Stock solution worked example

$$\delta 15N\ (‰) = (0.9999985 \times 0.84) + (0.0000015 \times 13376013.38)$$

$$\delta 15N\ (‰) = 20.9\ ‰$$

The desired concentration of $NaNO_3$ within the artificial seawater solutions is 68.5 mg/L, which represents a nitrate concentration of 50 mg/L, or nitrogen concentration of 11.3 mg/L. This concentration was chosen because it represents the limit for nitrate in drinking water, set by the Drinking Water Directive (98/83/EC). 6 % of all average nitrate concentrations measured in rivers and lakes annually exceeded this 50 mg/L limit (Environment Agency, 2019). This concentration, therefore, is an environmentally relevant concentration and means results from the study will represent the behaviour of the macroalgae at what is considered a polluted environmental concentration.

Varying amounts of artificial sea salt were dissolved in distilled water to prepare the artificial seawater solutions to make solution with salinities of 10, 20 and 35 mg/L. The sodium nitrate stock solutions were then added to each artificial seawater solution to produce a concentration of 68.5 mg/L, or 50 mg NO_3/L. One solution of artificial seawater was created without adding sodium nitrate as a control for each experiment.

2.2.2 Macroalgal collection

Non-fertile tips of *F. vesiculosus* were collected from Sandhaven Beach (55°00’17.4” N, 1°24’51.6” W), South Shields, Tyne and Wear, UK, in May and June 2021. The non-fertile tips were taken back to the laboratory in a container filled with local seawater, and then cleaned using distilled water; any visible epiphytes were physically removed.

2.2.3 Growth incubator

The non-fertile *F. vesiculosus* tips were added to specimen jars containing the different solutions of artificial seawater and sodium nitrate. Plastic mesh was placed in the jars to create four sections as

to prevent the tips from congregating as seen in Bailes and Gröcke (2020) each section contained three macroalga tips. The specimen jars were covered by stocking material to ensure exchange with the atmosphere in the growth incubator and so as not to limit the light reaching the tips. The growth incubator used was a MaxQÔ 6000 (Thermo Fisher Scientific, Bremen, Germany) housed in the Department of Biosciences, Durham University. The incubator ran for 14 days over the course of each experiment, with each 14-day interval having a set temperature of 5°C, 10°C and 15°C respectively. The incubator also was set to have a light/dark rhythm of 16 h/8 h, and a light intensity of 125 mmol photons m^{-2} s^{-2}; the same settings as used by Gröcke et al. (2017) and Bailes and Gröcke (2020). The specimen jars were arranged as shown in Table 2.2 . The macroalga tips were sampled at approximately the same time every day during the working days of each 14 day experiment (9 days cumulative due to access to the lab not given for 1 day a week due to Covid-19 restrictions). The solutions the macroalga tips were cultured in were changed twice weekly, every 3/4 days. Each day, 12 macroalgal tips were removed: a tip from each jar. Table 2.2 details the type of solution in each jar the macroalgal tips were cultured in.

Jar 1 10 mg/L artificial seawater + $NaNO_3$	Jar 4 20 mg/L artificial seawater + $NaNO_3$	Jar 7 35 mg/L artificial seawater + $NaNO_3$	Jar 10 35 mg/L artificial seawater
Jar 2 10 mg/L artificial seawater + $NaNO_3$	Jar 5 20 mg/L artificial seawater + $NaNO_3$	Jar 8 35 mg/L artificial seawater + $NaNO_3$	Jar 11 35 mg/L artificial seawater
Jar 3 10 mg/L artificial seawater + $NaNO_3$	Jar 6 20 mg/L artificial seawater + $NaNO_3$	Jar 9 35 mg/L artificial seawater + $NaNO_3$	Jar 12 35 mg/L artificial seawater

Table 2.2: Details of the type of solution each jar contained during the laboratory experiments. Each jar contained 12 macroalgal tips.

2.2.4 Sample preparation

Macroalgal tips were removed from the jars and dried using paper towels to remove any salt; following this the tips were placed into labelled, small brown envelopes. The envelopes were then placed into a drying oven set at 60°C for at least 24 h until completely dry. The tips were then removed and subsampled for stable isotope analysis by cutting a small fragment from the tip apex. This method was chosen over grinding the entire tip as it is not currently known how much of the tip region exchanges nitrogen with the seawater solution. Previous research did find a difference in $\delta^{15}N$ values between the tip apex, middle and torn base of macroalgal tips (Bailes and Gröcke, 2020). As such, whilst grinding the tips would reduce standard deviation in $\delta^{15}N$ values, this may not be representative of the isotopic uptake by the macroalgae as there could be isotopic dilution when homogenised with the rest of the tip. Macroalgal samples were weighed into 6 mm × 4 mm tin capsules with a weight range between 1.5 and 2.5 mg. Replicate analyses were not performed for the majority of the samples because there were 3 jars of each type of solution, meaning the tip from each jar of a type of solution forms a triplicate.

2.2.5 Isotopic analysis

Isotopic analysis was performed using a ECS 4010 elemental analyser (Costech, Valencia, CA, USA) connected to a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer. The mass spectrometer was calibrated using internal reference samples (e.g., glutamic acid, α-cellulose, IVA urea) and international reference standards (e.g., IAEA-600, IAEA-CH-3, IAEA-CH-6, IAEA-N-1, IAEA-N-2, NBS 19, USGS40, USGS24). All these standards were analysed in duplicate with every run. The standard deviation of the standards was better than ±0.1 ‰ (1σ) for carbon and nitrogen isotope ratios.

2.2.6 Nitrate concentrations

Samples of the solutions were taken throughout the experiments, including the isotopically-labelled stock solutions when prepared for each water change, and then the solutions the macroalgal tips had been cultured in for several days, just before each water change. Samples were not taken from each jar, instead samples were taken from jars with each different solution, i.e., 10 mg/L seawater +$NaNO_3$ to gain a representative understanding of the nitrate concentrations throughout the experiments. Water samples were then stored in the fridge (approximately 4°C) before being analysed at the James Hutton Institute (Aberdeen, Scotland, UK). Here, using colourimetric techniques to determine the concentration of total oxidised nitrogen (TON) and also nitrite (NO_2-

N), the NO_2-N concentration was then subtracted from the TON concentration to generate the NO_3-N concentration. Although this method didn't analyse the NO_3-N directly, it was chosen due to the fact other methods could not be guaranteed to be accurate as they had not been tested in saline solutions.

2.2.7 Data analysis

The data were analysed using Microsoft Excel, where average $\delta^{15}N$ values were generated by taking the mean $\delta^{15}N$ values of tips removed from each jar containing the same solution. I.e., the average $\delta^{15}N$ value for day 1 of the experiment at 10℃ with the solution 10 mg/L + $NaNO_3$ was done by calculating the mean $\delta^{15}N$ values of tips from jars 1, 2 and 3 removed on day 1. $\Delta^{15}N$ values were calculated by subtracting the mean $\delta^{15}N$ value of tips from one solution on a particular day from the mean $\delta^{15}N$ value of the collected *F. vesiculosus* tips that were sampled on the day of collection (Equation 2). Therefore, positive $\Delta^{15}N$ values represent a decrease in $\delta^{15}N$ values from the initial macroalgae $\delta^{15}N$ value, towards the $\delta^{15}N$ value of the solution. SPSS software was used to produce statistical data, using a two-way ANOVAs to examine the interactions between temperature and salinity on $\delta^{15}N$ values over the course of the experiments with a post-hoc Tukey test. Assumptions for ANOVA were tested for and met, including Levene's test for homogeneity of variances and the Shapiro-Wilk test for normally distributed data.

(2) $\Delta^{15}N$ (‰) = mean $\delta^{15}N$ (‰) value on collection – mean $\delta^{15}N$ (‰) value from jar on a given day

2.3 Results

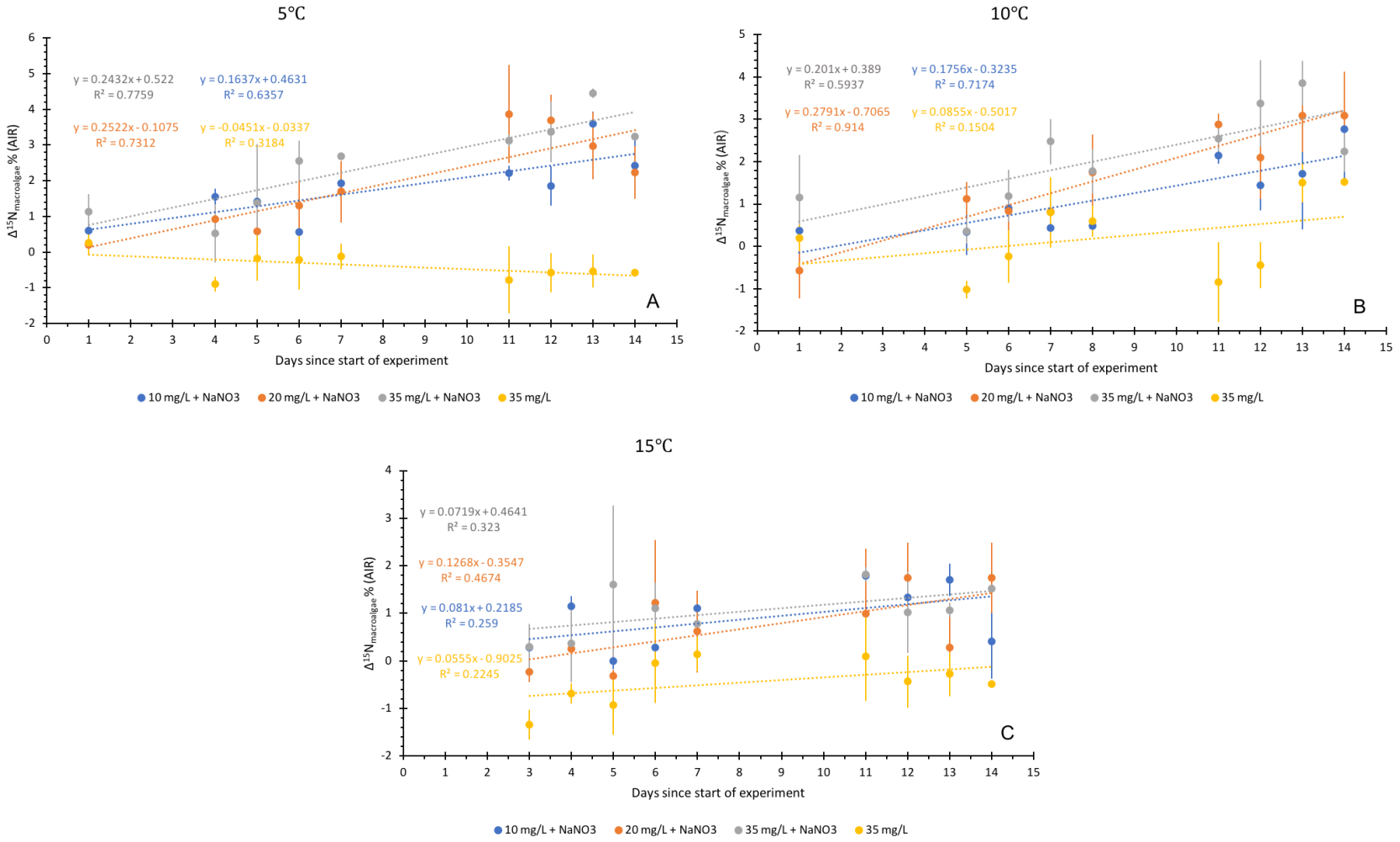


Figure 2.1: $\Delta^{15}N$ values of *Fucus vesiculosus* tips cultured in solutions of varying salinity containing $NaNO_3$ at a concentration of 11.3 mg NO_3^--N/L. One solution did not contain $NaNO_3$ to act as the control (35 mg/L). Three experiments were conducted at 5°C, 10°C, and 15°C, representing graphs A, B, and C respectively. Means and standard deviations are representative of three sampled tips. Note the y-axis does not begin at zero.

Data	Dependant variable	P	**df**	**F**
$\Delta^{15}N$ (‰) of macroalgae tips cultured with $NaNO_3$	Temperature	**0.003**	**2**	**8.034**
	Salinity	**0.028**	**2**	**4.454**
	Temperature*Salinity	0.110	4	2.218

Table 2.3: Results from two- way ANOVA analysis carried out using SPSS software. A significance level of 0.05 was used, meaning p values less than or equal to 0.05 represent a statistically significant result, i.e., there is less than a 5% probability the null hypothesis is correct. Significant values are highlighted in bold.

Temperature (℃)	**Temperature (℃)**	**P**
5	5	0.344
	15	**0.004**
10	5	0.344
	15	0.067
15	5	**0.004**
	10	0.067

Table 2.4: Post-hoc Tukey test results for the variable Temperature on $\Delta^{15}N$ values, generated using a two-way ANOVA in SPSS. Significant values are highlighted in bold.

Salinity (mg/L)	**Salinity (mg/L)**	**P**
10	20	0.126
	35	**0.038**
20	10	0.126
	35	0.847
35	10	**0.038**
	20	0.847

Table 2.5: Post-hoc Tukey test results for the variable Salinity on $\Delta^{15}N$ values, generated using a two-way ANOVA in SPSS. Significant values are highlighted in bold.

The calculated $\Delta^{15}N$ values describe the difference between the mean $\delta^{15}N$ value of the *F. vesiculosus* tips when first collected, and the mean $\delta^{15}N$ value of *F. vesiculosus* tips collected after being cultured in the solutions, on a certain day. Hence, to demonstrate the difference in $\delta^{15}N$ values between each experiment, $\Delta^{15}N$ values have been chosen so the starting $\delta^{15}N$ values of the *F. vesiculosus* tips does not affect interpretation.

Excluding the control *F. vesiculosus* tips, the $\Delta^{15}N$ values increased for each experiment, demonstrating the $\delta^{15}N$ values of the tips by the end of the 14 day experiment had changed. In this experiment, the $\delta^{15}N$ values generally decreased (shown as increases in $\Delta^{15}N$) because the solutions contained $NaNO_3$ with a $\delta^{15}N$ value of 1.00 ‰ ± 0.50 ‰ and the $\delta^{15}N$ values of the tips at the beginning of each experiment varied between 7.18 ‰ and 9.49 ‰. Hence, the $\delta^{15}N$ values of the tips converged toward the $\delta^{15}N$ value of the solutions.

2.3.1 Experiments at 5 and 10℃

For the experiments conducted at 5℃ and 10℃, there is not a large difference between the $\Delta^{15}N$ values of those *F. vesiculosus* tips cultured with $NaNO_3$ in varying salinities throughout the 14-day experiment (Figure 2.1). The regression lines for each of these solutions, excluding the control, are similar with comparable gradients, although the gradient for solution 10 mg/L + $NaNO_3$ is slightly lower in both experiment at 5℃ and 10℃ (Figure 2.1). The R^2 values are also high for the $\Delta^{15}N$ values of tips treated with $NaNO_3$, although slightly lower for solution 10 mg/L + $NaNO_3$ at 5℃ (Figure 2.1). Conversely, the tips in the solution 35 mg/L + $NaNO_3$ at 10℃ has the lowest R^2 value out of the $NaNO_3$ treated tips (Figure 2.1B) which could be as a result of the average $\Delta^{15}N$ value of the tips collected on the final day on the experiment, which is not as large a difference as previous days (Figure 2.1B). Following statistical analysis, a post-hoc Tukey test revealed there was a significant difference between tips cultured in solutions 10 mg/L + $NaNO_3$ and 35 mg/L + $NaNO_3$ (Table 2.5).

Although there isn't a large difference, it could be observed that for tips cultured at 5℃, tips in the solution 35 mg/L + $NaNO_3$ incorporated the $\delta^{15}N$ signal most effectively, closely followed by the 20 mg/L +$NaNO_3$ solution, however the $\delta^{15}N$ of these tips decreases for the last 4 days of the

experiment (note the high standard deviation on these data) (Figure 2.1A). Tips cultured in the 10 mg/L +$NaNO_3$ solution also effectively incorporated the $\delta^{15}N$ signal of the solution (Figure 2.1A), despite visible damage to the tips observed between days 1-5 (pigment released into the solution which is likely due to cell walls breaking down because of hypo-osmotic pressure). For the experiment conducted at 10℃, similar effects can be observed in that tips in solutions 35 mg/L +$NaNO_3$ and 20 mg/L +$NaNO_3$ incorporated the $\delta^{15}N$ signal most effectively; followed by the 10 mg/L+$NaNO_3$ tips which also showed signs of stress (Figure 2.1B).

The *F. vesiculosus* tips cultured in solutions 35 mg/L (the control solutions) show little change in $\delta^{15}N$ values from the initial average values (small $\Delta^{15}N$ values) (figures 2.1A and B). Over the course of the experiment at 5℃ (Figure 2.1A), there is a small decrease in $\Delta^{15}N$ values. This is reflected in the low but negative gradient of the regression line, and the low R^2 values, showing that in fact the $\delta^{15}N$ values have increased from the initial average (Figure 2.1A). It is difficult to pick out a clear trend in $\Delta^{15}N$ values for the tips in the control solution in the experiment at 10℃, this is reflected by the low R^2 value of the regression line, and the loosely positive gradient (Figure 2.1B).

2.3.2 Experiment at 15℃

F. vesiculosus tips treated with $NaNO_3$ and cultured at 15℃ converge towards the $\delta^{15}N$ signal of the solutions away from the initial $\delta^{15}N$ values of the tips, and hence show increasing $\Delta^{15}N$ values (Figure 2.1C). However, this incorporation of the $\delta^{15}N$ of the solutions is not as pronounced as the incorporation by the tips cultured in 5 and 10℃, respectively (Figure 2.1). For the $NaNO_3$ treated tips cultured at 5℃ and 10℃, there is a change in $\delta^{15}N$ values of around 1-5‰ at the end of the experiments, whereas for tips cultured at 15℃, there is only a change in $\delta^{15}N$ values of around 0-3‰ (Figure 2.1). Statistical analysis revealed a significant difference between tips cultured in different temperatures (Table 2.3), and following post-hoc Tukey tests, a significant difference was found in $\Delta^{15}N$ values cultured in 5℃ and 15℃, respectively (Table 2.4).

When considering the $NaNO_3$ treated tips there is little difference between the tips cultured in the varying salinity solutions and therefore it cannot be said whether tips in one solution incorporated the $\delta^{15}N$ signal better than the others, all gradients and R^2 values are relatively low, compared to experiments done at 5℃ and 10℃ (Figure 2.1C). Tips in this experiment also appeared to undergo hypo-osmotic shock in the solution 10 mg/L + $NaNO_3$. There does not appear to be a trend in the

$\Delta^{15}N$ values of the control tips, there is variability in the $\delta^{15}N$ values but overall, the lack of trend is reflected in the low gradient and R^2 value of the regression line (Figure 2.1C).

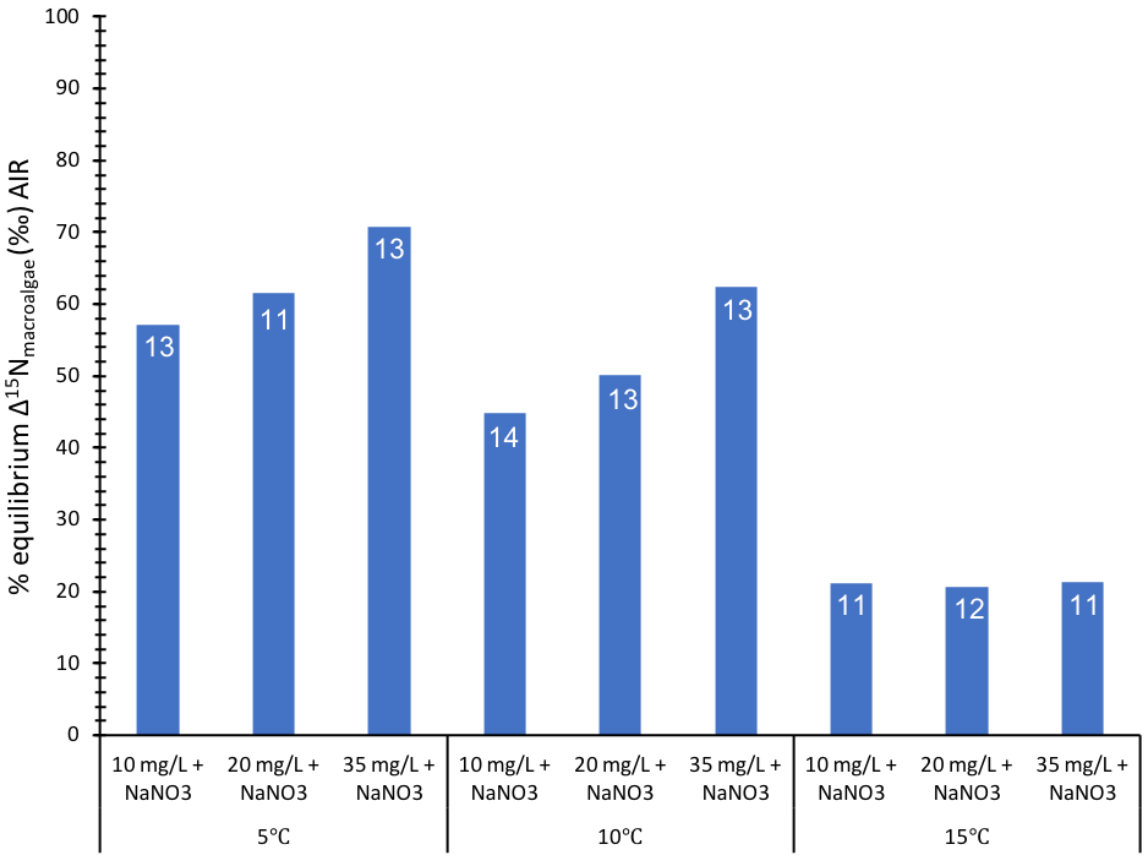


Figure 2.2: Highest average % equilibrium $\Delta^{15}N$ values for *F. vesiculosus* tips cultured in solutions of varying salinities (S_A) containing $NaNO_3$ at a concentration of approximately 11.3 mg NO_3^--N/L. Three experiments were conducted at 5°C, 10°C, and 15°C, representing the three clustered columns. The number on each bar represents the day from the beginning of the experiment on which the highest % equilibrium $\Delta^{15}N$ value was recorded.

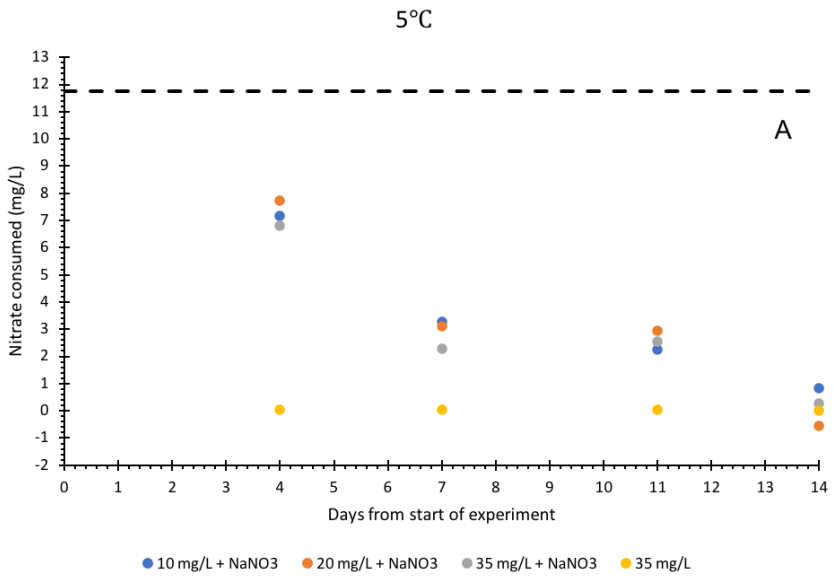
The % equilibrium $\Delta^{15}N$ values were calculated by dividing the $\Delta^{15}N$ values for the *F. vesiculosus* tips (Equation 2) by the theoretical $\Delta^{15}N$ value if the tips reached isotopic equilibrium with the solution (initial average $\delta^{15}N$ value of collected tips - $\delta^{15}N$ of the $NaNO_3$ solutions).

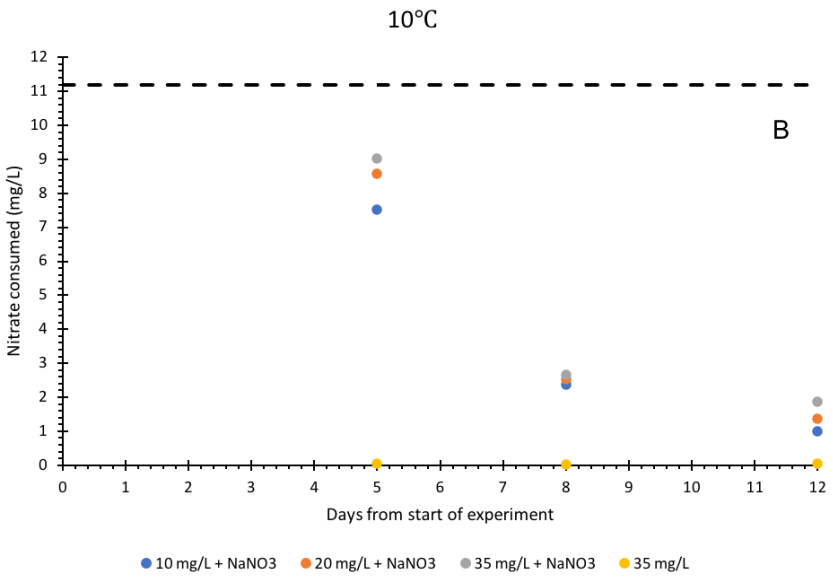
2.3.3 General trends

All of the $NaNO_3$ treated tips converge towards the $\delta^{15}N$ of the solution (i.e., increasing $\Delta^{15}N$) but none reach equilibrium with the solution (Figure 2.2). Despite this, the macroalgal tips integrated

the $NaNO_3$ solutions effectively (i.e., more than 50% difference from initial values (Gröcke, et al., 2017; Bailes & Gröcke, 2020)) with the highest % equilibrium $\Delta^{15}N$ value reported from tips cultured at 5℃, in 35 mg/L salinity and $NaNO_3$ solution on day 13, at a value of 70.8% (Figure 2.2). In contrast, the tips cultured in the nitrate solutions at 15℃, do not integrate the isotopic signal of the solutions as effectively, with tips from all solutions reporting % equilibrium $\Delta^{15}N$ values of around 20% (Figure 2.2). When comparing tips incubated in varying salinities, it can be observed that the tips cultured at higher salinities were better able to incorporate the isotopic value of the $NaNO_3$ solution, integration of the solution was most effective at 35 mg/L salinity, followed by 20 mg/L and was least effective at 10 mg/L (Figure 2.2). Apart from the tips cultured at 15℃, where salinity appears to have had no effect on the integration of the $NaNO_3$ solution (Figure 2.2). All tips had reached their highest isotopic difference by at least day 11 (Figure 2.2). It is unclear why some tips then decreased in $\Delta^{15}N$ values following day 11 but could be explained by damage to the tissue due to salinity stress, or natural variation in uptake between tips.

When comparing between the tips cultured with isotopically-labelled $NaNO_3$ at different temperatures, the tips incubated at 5℃ appear to incorporate the $\delta^{15}N$ of the solutions most effectively, demonstrated by the highest $\Delta^{15}N$ values and % equilibrium values by salinity, followed closely by the tips at 10℃ and then 15℃ (Figure 2.2). Temperature was found to be a statistically significant factor that influenced $\Delta^{15}N$ values in the tips at day 12, with post-hoc tests revealing a significant difference in values between tips cultured at 5℃ and tips cultured at 15℃ (Table 2.4). Salinity was also found to be a statistically significant factor on $\Delta^{15}N$ values in nitrate treated tips, with post-hoc tests indicating a significant difference between tips cultured in solutions with 10 mg/L and 35 mg/L salinity (tables 2.5). Temperature and salinity were not found to have an interaction effect on $\Delta^{15}N$ values in the *F. vesiculosus* tips (Table 2.3).





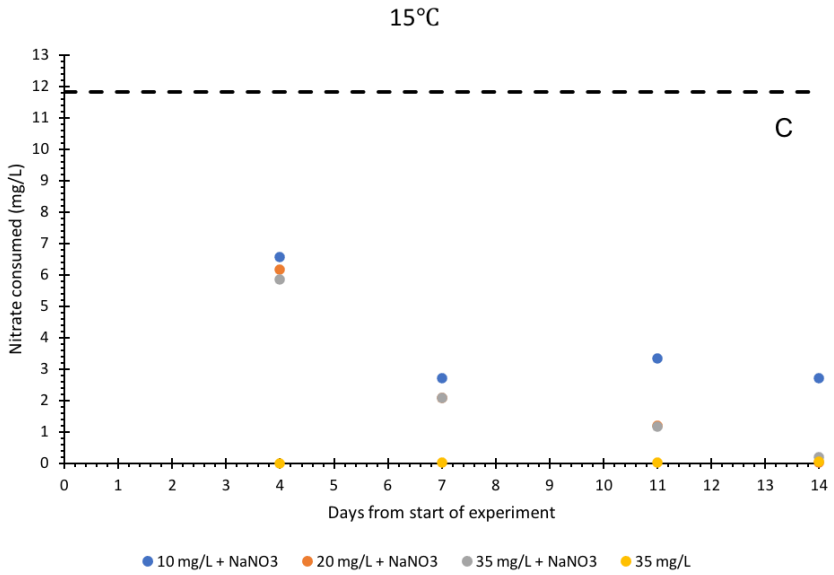


Figure 2.3: Amount of nitrate-N consumed by *F. vesiculosus* tips at various points over a 14 day incubation, calculated by subtracting the nitrate concentration in the incubation jars following several days of incubation, from the stock solution nitrate concentrations made up a few days earlier. One solution did not contain $NaNO_3$ to act as the control. Three experiments were conducted at 5°C, 10°C, and 15°C, representing graphs A, B, and C respectively. Tips were cultured in solutions of varying salinity (mg/L) containing $NaNO_3$ at a concentration of approximately 11.3 mg NO_3^--N/L, where the dotted line represents the average solution nitrate-N concentrations. Solutions were changed on days 4, 7, 11 and 14 for experiments conducted at 5°C and 15°C, and on days 5, 8, 12 and 14 for the experiment done at 10°C. Note day 14 concentrations aren't displayed for the experiment at 10, due to errors with the stock nitrate solutions not reaching concentrations around 11.3 mg NO_3^--N/L.

The amount of nitrate consumed by the macroalgae is high at the beginning of each experiment and then decreases throughout, as tips are sequentially removed each day there is less demand for nitrate as the biomass decreases (Figure 2.3). There is not a large variation in the amount of nitrate consumed between tips cultured in different temperatures and solutions with varying salinity (Figure 2.3). There is a deviation in the amount of nitrate consumed by tips that were cultured in the solution 10 mg/L + $NaNO_3$ and at 15℃, these tips appear to have consumed more nitrate towards the latter half of the experiment.

2.4 Discussion

2.4.1 Assimilation of isotopically-labelled nitrogen seawater solutions

Over the course of 14 days, *F. vesiculosus* tips cultured in isotopically-labelled solutions incorporated the $\delta^{15}N$ signal of these solutions to varying degrees depending on the temperature and salinity of the solutions (Figure 2.2). The macroalgal $\delta^{15}N$ values did not reach equilibrium with the $\delta^{15}N$-DIN values for any of the solutions at any culture temperature. Despite the fact that the macroalgal tips did not reach isotopic equilibrium with the solutions, some tips still integrated the isotopic signal effectively, i.e., more than 50% (Gröcke et al. 2017) (Figure 2.2). Tips cultured at 5℃ appeared to integrate the isotopic signal of the solution best, with tips cultured at 10℃ integrating the next most effectively, followed by tips at 15℃ (Figure 2.2). For tips cultured at 5℃ and 10℃, higher salinities increased the integration of the isotopic signal of the solutions, whereas salinity had no effect on $\delta^{15}N$ values in tips cultured at 15℃ (Figure 2.2).

F. vesiculosus tips cultured in ^{14}N- and ^{15}N-enriched 35 mg/L seawater over a period of 19 days varied in how effectively they incorporated the isotopic signal of the solutions (Bailes and Gröcke 2020). In this previous study, the ^{14}N-enriched tips did not integrate the $\delta^{15}N$ signal of the solution as quickly as the ^{15}N-enriched tips did (Bailes and Gröcke 2020). This could be as a result of the ^{15}N-enriched solution being further away isotopically from the starting $\delta^{15}N$ of the macroalgal tips than the ^{14}N-enriched solution; however, the $\Delta^{15}N$ values of the tips cultured in the ^{15}N-enriched solution were still greater than that of those in the ^{14}N-enriched solution (~69% equilibrium and 36% equilibrium, respectively) (Bailes and Gröcke 2020). Therefore, in this previous study the ^{14}N-enriched solution was not integrated by the macroalgal tips as effectively as the ^{15}N-enriched. When comparing to this study, tips in similar solutions to those used in the previous study (i.e., 35 mg/L) and a culture

temperature of 11℃, it can be observed that the tips in this study integrated the $\delta^{15}N$ values of the solutions more effectively than the ^{14}N-enriched tips did in the previous study (Figure 2.2) (Bailes and Gröcke 2020). Tips from one solution (35mg/L + $NaNO^3$) at 5℃, integrated the $\delta^{15}N$ value of the solution more effectively than the ^{15}N-enriched tips from the previous study (Bailes and Gröcke 2020).

Given that there was a much larger difference between the initial macroalgal $\delta^{15}N$ values and the solution values for the ^{15}N-enriched tips in the previous study (Bailes and Gröcke), it is surprising that similar % equilibrium values for $\Delta^{15}N$ have been reached in this current study for certain solutions (Figure 2.2). Furthermore, this result is unexpected because ammonium, used in the previous study, is thought to be taken up quicker by macroalgae than nitrate and therefore this study should show slower integration rates (Phillips & Hurd 2003; Cohen and Fong 2004; Pritchard et al. 2015; Robertson & Savage 2018). In addition to this, the tips were not cultured for as long in this experiment (14 days, as compared to 19 days previously) and so it would be expected that integration would not be as effective as the previous study as the tips were not given as long to equilibrate (Umezawa et al. 2007). Furthermore, these experiments were conducted at different times in the year, with the previous study collecting tips in November, and this study in May and June. Given that macroalgae will store nitrogen over the winter months and then mobilise it for growth in summer when conditions are more preferable (Viana and Bode 2015); it could be the case that the internal nitrogen stores of the tips collected in this study were depleted as they were collected in the summer months. Hence, the integration of the solution was more effective as the tips sought to take up more nitrogen, as compared with the previous study where tips were collected in winter and therefore internal reserves were high. This current study used much higher concentrations of nitrogen than the previous study (0.7 mg/L vs 11.3 mg/L approximately) (Bailes and Gröcke, 2020). Higher concentrations could've meant the macroalgae was able to take up more nitrogen molecules, and therefore assimilate the $\delta^{15}N$ values of the solution more effectively, and at a faster rate. However, given integration of the solutions was much more effective in this current study, it is unclear whether the tips would have integrated a solution with higher $\delta^{15}N$ values more effectively, as they did in the previous study, and so this in theory would suggest a discrimination against ^{14}N. This potential discrimination against ^{14}N could also explain why there was some overshooting whereby the macroalgal tips has higher $\delta^{15}N$ values than the $\delta^{15}N$ signal of the ^{15}N-enriched solution (Bailes and Gröcke 2020). This should be investigated further in future studies.

Despite the fact that a smaller amount of nitrate was consumed by the *F. vesiculosus* tips as each experiment continued, the rate of the integration of the nitrogen solutions appeared to remain constant (Figure 2.3). This could be explained by the fact that the biomass in each jar decreased as tips were removed for analysis throughout the experiment. On the other hand, the *F. vesiculosus* tips could have taken up the nitrogen in surge uptake, a phenomenon that ephemeral macroalgae show in order to assimilate nitrogen quickly and therefore grow quickly (Pedersen & Borum 1997; Harrison & Hurd 2001). This has not, however, been reported in *F. vesiculosus* as far as the author is aware. Therefore, it is predicted the decrease in the amount of nitrogen consumed by the tips decreases over the course of the experiments due to decreasing biomass.

2.4.2 Temperature

F. vesiculosus tips integrated the nitrogen solutions best when cultured at 5℃, followed by 10℃ and then 15℃ (Figure 2.2). Given that Graiff et al. (2015) found the optimum temperature range for growth of Baltic Sea *F. vesiculosus* is between 10℃ and 20℃, this suggests that high rates of nitrogen uptake (associated with higher temperatures (Pedersen et al. 2004; Young et al. 2007)) are not necessarily linked with effective integration of nitrogen isotopes. These results could support the idea that fractionation is more likely to occur when macroalgal tips are experiencing optimal nitrogen uptake (Dudley et al. 2010; Raimonet et al. 2013; Swart et al. 2014; Gröcke et al. 2017; Howarth et al. 2020). However, there didn't appear to be a difference between the amount of nitrogen consumed between the tips cultured at different temperatures (Figure 2.2) which doesn't support this theory. These results contrast with those reported by Umezawa et al. (2007) who reported that the brown macroalga *Padina australis* had δ^{15}N values more reflective of the solution δ^{15}N value at higher temperatures. This could be explained by interspecies differences in nitrogen uptake, demonstrating the need to fully examine the effects of environmental factors on nitrogen isotope uptake for different species to be used in biomonitoring. A recent study on the red macroalga, *Chondrus Crispis*, however, found very similar results to this study (Howarth et al. 2020). In this study the macroalgal tissue better integrated the nitrogen isotopic signal at a temperature lower than the optimum reported for growth (Howarth et al. 2020).

The *F. vesiculosus* tips collected for this study, were growing in an intertidal zone of the North Sea, which experiences a range of sea surface temperatures, ranging between 5℃ and 15℃, hence the temperatures chosen for this study (McQueen & Marshall, 2017). Therefore, it is not assumed that the tips have adapted to a small range of temperatures as it has been observed that macroalgae can adapt to varying salinities (Nygard & Ekelund 2006; Nygard & Dring 2008; Barboza et al. 2019).

2.4.2 Salinity

Salinity was found to have varying effects on the uptake of the isotopically-labelled nitrogen solutions according to temperature (Figure 2.2). Tips cultured in solutions with 35 mg/L salinity integrated the nitrogen solutions most effectively, followed by tips in solutions with salinities of 20 and 10 mg/L respectively, but only in experiments conducted at 5℃ and 10 ℃ (figures 2.1 and 2.2). For tips cultured at 15℃, salinity appeared to have no effect on the uptake of nitrogen isotopes (Figure 2.2). There was a significant difference between tips cultured in 10 mg/L and 35 mg/L salinity, as determined by post-hoc Tukey tests (Table 2.5). These results indicate that the δ^{15}N values of *F. vesiculosus* tips are most reflective of δ^{15}N-DIN values when in typical marine salinity values, of 35 mg/L, at least at the lower temperatures of 5℃ and 10℃ (Figure 2.2). This is not a surprising result given that these macroalga tips were collected from an intertidal area where salinity values will be close to 35 mg/L, and hence they could have adapted to this salinity (Barboza et al. 2019). This, however, does not support the hypothesis that fractionation between the water column and macroalgal tissue is greatest when optimum conditions for nitrogen uptake are occurring, if it is assumed that tips cultured at 35 mg/L are experiencing optimum nitrogen uptake. Again, there was not observed to be an increased amount of nitrogen consumed by these tips cultured at the higher salinity value (Figure 2.3).

However, these results have implications for the use of *F. vesiculosus* as a biomonitor, suggesting that macroalgae subject to lower salinities than they have experienced do not integrate δ^{15}N values in the environment as effectively. Resultingly, transplanting macroalgae in estuaries that are characterised by extremely variable salinity values, both daily and annually, may not be the most effective way to biomonitor for sources of nitrogen pollution. There could be estuarine macroalgae that have adapted to the highly variable salinity values, and therefore could be appropriate to transplant into the estuaries, but further work here is needed to examine whether varying salinities

will have an effect on the ability of the tips to effectively integrate nitrogen isotopes that reflect the δ^{15}N values in the environment. Nygard & Dring (2008) reported that high nutrient concentrations provided tolerance to lower salinities for *F. vesiculosus*, this finding is not supported by this study, at least in terms of tolerance to the integration of nitrogen solutions.

It is generally expected that macroalgae such as *Fucus* and *Ulva* can tolerate salinities between 10 and 100 mg/L (Hurd et al. 2014) but this study demonstrates it is not necessarily the values of optimum or tolerable environmental conditions that describe how reflective the δ^{15}N values of macroalgal tips will be of δ^{15}N-DIN values. It was observed in the experiments that tips cultured in 10 mg/L salinity, and to a lesser extent 20 mg/L salinity, that there was release of pigments from cell walls, likely due to hypo-osmotic stress. This could explain the fact that tips cultured in these solutions did not have δ^{15}N values that were as reflective of δ^{15}N-DIN as tips in the 35 mg/L salinity solution. This is again, reflective of the fact these tips were not adapted to such low salinities.

It is unclear why the Δ^{15}N values of the tips cultured at 15℃ were not affected by salinity values (figures 2.1 and 2.2). One explanation could be that the temperature was too high for the *F. vesiculosus* tips and hence integration of the nitrogen solutions was not effective and so the salinity of the solution made no difference to uptake. No other variables were changed between this experiment and the other experiments conducted at 5 and 10, so this is likely the only explanation. Temperature and salinity were not found to have an interaction effect on Δ^{15}N values in the *F. vesiculosus* tips (Table 2.3). In further studies, the biomass of the macroalga tips should be recorded to determine growth rates between different variables. Unfortunately, due to time constraints, this measure was not able to be carried out in this study.

Macroalgae may be more sensitive to changes in salinity rather than temperature, given that it is believed fractionation is occurring at optimum conditions for nitrogen uptake (higher temperatures), but this does not appear to be the case with optimum salinity conditions.

2.4.3 Limitations

The author is aware that due to both financial and timing constraints, there are several limitations that affect this study. For example, having the macroalgal tips completely submerged in solution is

not reflective of the actual environmental conditions of intertidal and estuarine macroalgae in particular. Future studies should seek to engineer a study design that will allow for alternating emersion and immersion of the macroalgal tips. In addition to this, time was a limiting factor and as such the experiments could only run for 14 days each, when it has been previously been reported that *F. vesiculosus* tips equilibrate with δ^{15}N-DIN values after 2-3 weeks (Umezawa et al. 2007); hence, the tips could've required more time to integrate the δ^{15}N signal. Furthermore, there were financial constraints that meant that the nitrogen concentrations could not be measured fully and only a selection of concentrations were taken, therefore there were not enough data to interpret relationships and have statistical power. Once outliers were excluded, the relative standard deviation (RSD) of the stock nitrogen solutions were on average 7% for each experiment. In further work, the nitrogen concentrations should be measured at more regular time intervals and with more repeats, and this should be combined with biomass weighing to understand the kinetics of nitrogen uptake. Despite this, as far as the author is aware, this is the first study to characterise how salinity can affect the uptake of isotopically-labelled nitrogen solutions by *F. vesiculosus* tips. Given this, this study provides interesting insights on the effects temperature and salinity can have on the integration of δ^{15}N values by *F. vesiculosus*.

2.5 Conclusions

Findings from this study demonstrate that both temperature and salinity can independently have a significant effect on how closely macroalgal δ^{15}N values reflect those of their environment. Further, there was a greater than 1 ‰ deviation between macroalgal δ^{15}N values and δ^{15}N-DIN values at all temperatures and salinities by the end of the 14 days culturing. This is an important finding as it affects the use of macroalgae, particularly *F. Vesiculosus* as a biomonitor; in the author's opinion it would be desirable to conduct trials using macroalgal tips as biomonitors in locations that have minimal nutrient loading, for at least a year or two to establish any variations that may occur due to annual changes in temperature, or indeed other environmental factors. Shorter trials could be done in the environment to establish whether daily cycles of temperature and salinity change have any effects on the uptake of nitrogen isotopes on macroalgal tips, although this would be unexpected for perennial species such as *F. vesiculosus* given they take 2-3 to assimilate δ^{15}N values in the environment (Bailes and Gröcke 2020).

Chapter 3: Nitrogen pollution in North East England: assessing sources of nitrogen using *Fucus vesiculosus* as a biomonitor over spatial and temporal scales

3.1 Introduction

In order to test *F. vesiculosus* as a biomonitor for determining sources of nitrogen pollution in estuaries and coastal areas, native macroalgae can be collected to evaluate the variation in $\delta^{15}N$ values between areas and over time (Costanzo et al. 2001; Gartner et al. 2002; Orlandi et al. 2014; Signa et al. 2020).

For a review of biomonitoring studies using macroalgae, see Garcia-Seoane et al. (2018). It is highlighted in this review that transplanted macroalgae is thought to be better at reflecting $\delta^{15}N$-DIN values after a period of equilibration. However, there are significant costs associated with transplanting macroalgae, as opposed to collecting native macroalgae, and given the scope of this project it was decided to collect native macroalgae.

3.2 Methodology

3.2.1 Study area background

Approximately 2.5 million people live in the North-East of the UK, with the majority of people living in the areas of Tyne and Wear, including Newcastle, Gateshead and Sunderland, and the Tees Valley which includes Middlesbrough (Environment Agency & DEFRA 2009). Approximately 67% of the river basin in the North-East is used for agriculture or forestry, and the main industries are chemical, food, drink, petrochemicals, metal sectors and transport equipment (Environment Agency & DEFRA 2009). This study will focus on the coastline between North Tyneside and Hartlepool, including the estuaries of the Tyne and Wear, and downstream stretches of the Tyne and Wear (Figure 3.1).

The North and South Tyne rise in the Cheviot and Penine hills respectively, before converging at Warden. The Tyne from here then flows eastwards to the North Sea through the large urban area of Tyneside (Environment Agency and DEFRA 2009). The Tyne catchment has several areas of recognised national importance for nature conservation including river shingle sites and upland peat bogs (Environment Agency and DEFRA 2009). Furthermore, the catchment is of high ecological

value, with salmon, brown trout and coarse fish populations all being supported by rivers in the catchment (Environment Agency and DEFRA 2009). The Port of Tyne is a major handling facility on the river and is one of the largest in the UK (Environment Agency and DEFRA 2009). Water quality has improved in recent years, thanks to the decline in heavy industries such as coal mining, however there are still issues that can have negative impacts on water quality in the catchment such as old industrial sites, contaminated land and road run off (Environment Agency and DEFRA 2009). In 2009 50 % of the river and lake water bodies in the catchment were classed as having good chemical and ecological status overall (Environment Agency and DEFRA 2009).

The River Wear rises at Kilhope Burn and Burnhope Burn at Wearhead in the northern Pennines and flows west along a stretch of 107 km to the North Sea at Sunderland (Kelly, 2002). The Wear basin has an area of approximately 1044 km^2 and has an average flow of 18 $m^3 s^{-1}$ (Neal, et al., 2000). The middle reaches of the Wear are dominated by arable farmland; however, this area has a history of coal mining, sand/aggregate and shale extraction, causing problems with minewater discharge (Neal, et al., 2000). This part of the catchment forms part of the North Pennines Area of Outstanding Natural Beauty and as such, has many features of high conservation value (Environment Agency & DEFRA, 2009). The coastal areas of the catchment were also dominated by coal mining, there were several deep mines that extended far out under the seabed, but these mines have since closed (Environment Agency & DEFRA, 2009). Although the water quality in the catchment has improved more recently, there is still work to be done; in 2009 only 24% of river and lake water bodies in the Wear catchments were assessed as having good status overall (chemical and ecological) (Environment Agency & DEFRA, 2009). There are multiple sewage treatments works that discharge to the River Wear, with a few of them having tertiary treatment to remove nitrogen from discharges (DEFRA, 2002). Despite this, point source releases from sewage works and combined sewage outfalls are major reasons for failures in the Wear catchment (Environment Agency & DEFRA, 2009).

The estuaries of the Tyne and Wear face pressure of pollution from industrial discharges, excess nutrient loading from run-off and sewage (Environment Agency & DEFRA 2009). According to draft classifications, these estuaries are not reaching good status and have only achieved moderate status. In 2009 only 14% of estuaries had good status overall in the North-East region, but 86% of coastal waters had this status (Environment Agency & DEFRA 2009). One of the key actions proposed by the River Basin Management plan for the Northumbria River Basin District is identifying diffuse pollution from urban, agricultural and mining sources (Environment Agency & DEFRA, 2009).

This study will seek to evaluate diffuse sources of nitrogen pollution coming from urban and agricultural activities.

3.2.2 Sampling design

Vegetative tips of *F. vesiculosus* were collected from the River Wear in October and December 2020, and then March, May and July 2021. Tips were collected from the North-East coastline and the Tyne in February and June 2021. Tips were selected at random for harvesting, but effort was made to select tips that were most likely to have been submerged for the longest periods of time, i.e., those closest to the water edge, whilst following safety precautions. During the different collection periods, the accessibility of each site was determined, and sites were excluded from collection if it was deemed unsafe to collect the macroalga tips. The tips were then stored in a freezer (<-18°C) before being placed into a drying oven set at 60°C for at least 24 hours until completely dry. Following this, the tips were subsampled for stable isotope analysis by cutting two small fragments from the tip apex to generate replicate samples. Macroalgal samples were then weighed between 1.5 and 2.5 mg using a microbalance, into 6 mm x 4 mm tin capsules. Replicate analyses were performed for the majority of samples.

There is the possibility that not all tips collected were strictly the species *F. vesiculosus*, due to the cross-breeding with other *Fucus* species, including *Fucus spiralis* and *Fucus serratus*. This is expected to have minimal impact on the study conducted because these species have very similar life and ecological strategies.

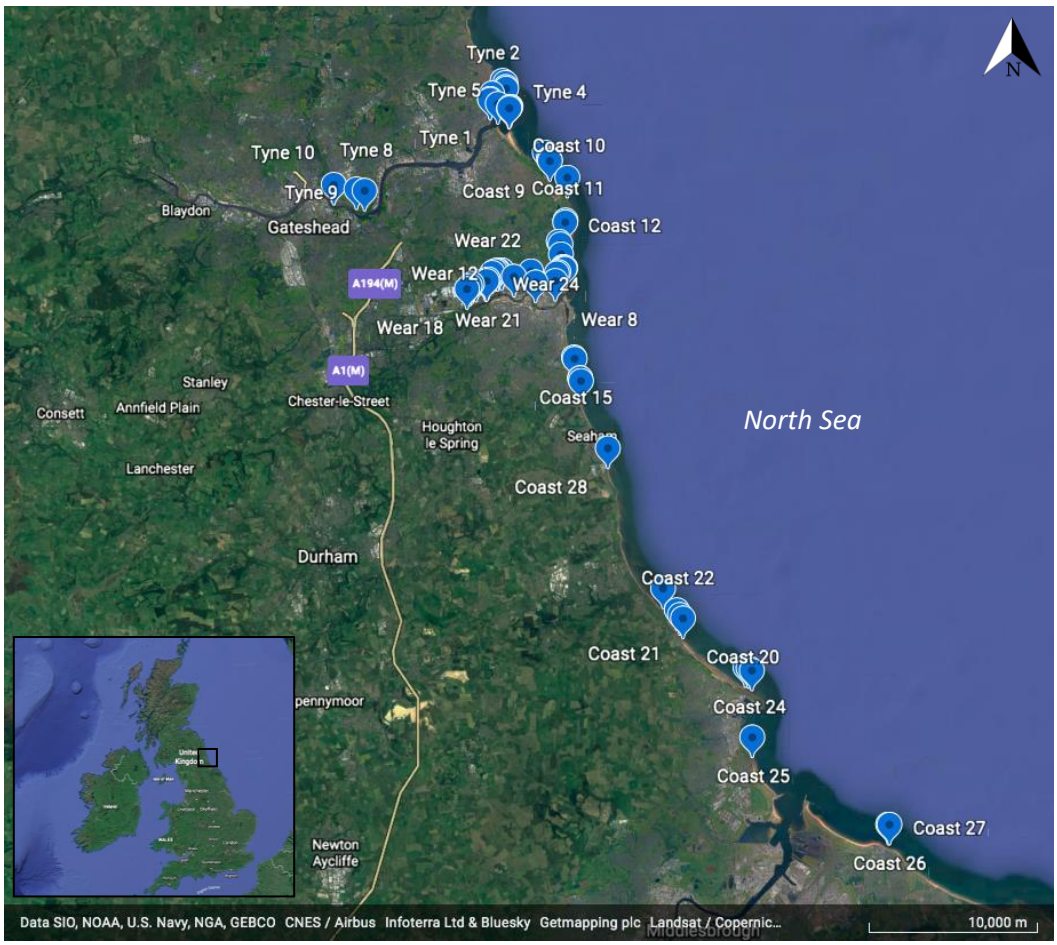


Figure 3.1: Study area in the North East of England, UK and sampling locations.

3.2.3 Isotopic analysis

Isotopic analysis was performed using a ECS 4010 elemental analyser (Costech, Valencia, CA, USA) connected to a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer. The mass spectrometer was calibrated using internal reference samples (e.g., glutamic acid, α-cellulose, IVA urea) and international reference standards (e.g., IAEA-600, IAEA-CH-3, IAEA-CH-6, IAEA-N-1, IAEA-N-2, NBS 19, USGS40, USGS24). All these samples were analysed in duplicate with every run. The standard deviation of the standards was better than ±0.1 ‰ (1σ) for carbon and nitrogen isotope ratios.

3.2.4 Data analysis

The data were analysed using Microsoft Excel, where average $\delta^{15}N$ values were generated by taking the mean $\delta^{15}N$ values of the five tips taken from each sampling location for each time. SPSS software was used to produce statistical data, using several one-ways ANOVAs to determine if there is a

significant difference between sampling locations for each collection period. Assumptions were tested for and met, except for the homogeneity of variances and as such the Welch F statistic was used to examine differences, and appropriate Games-Howell post-hoc tests conducted.

3.3 Results

3.3.1 River Wear

3.3.1.1 October 2020

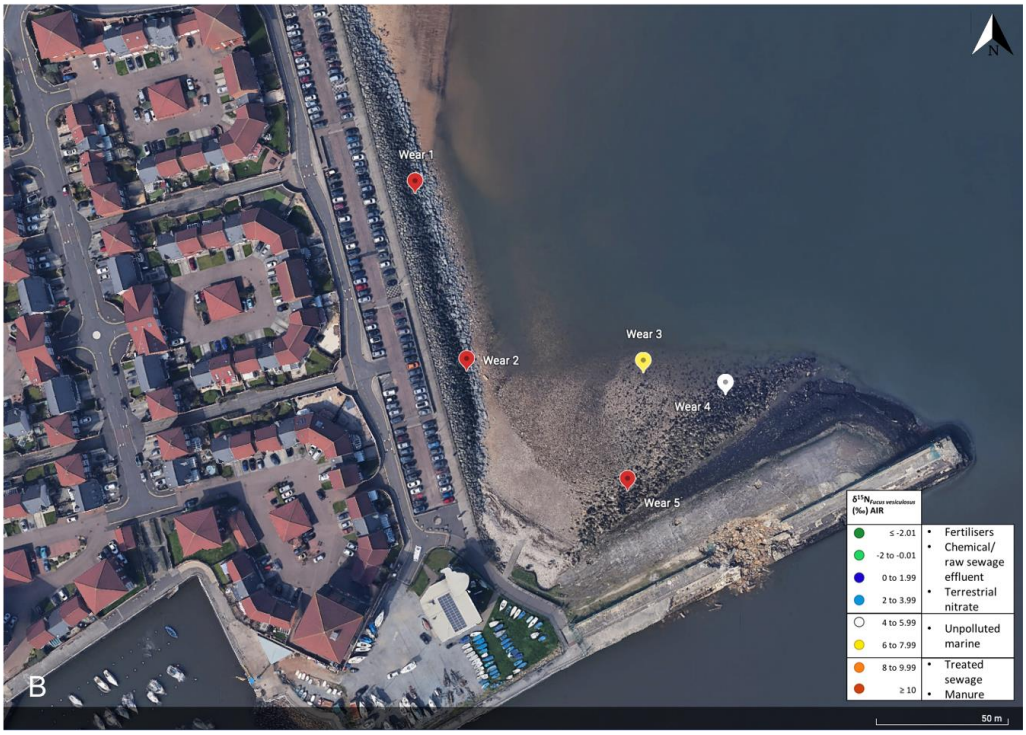


Figure 3.2: Mean δ^{15}N values for *F. vesiculosus* samples collected along the banks of the River Wear (A) and Roker Beach (B) in October 2020 (n=5).

F. vesiculosus tips collected in October 2020 were found to have elevated δ^{15}N values, with an average value of 8.95 ‰ ± 0.71 ‰ (Figure 3.2). Only three sampling sites (*Wear 3*, *4*, and *19*) recorded tips with δ^{15}N values in the unpolluted marine range (4-8 ‰). The majority of other sites (76% of sites) recorded high δ^{15}N values that suggest these locations were affected by nitrogen pollution originating from either treated sewage or manure in the watercourse. *F. vesiculosus* tips sampled from the site *Wear 10* have drastically lower δ^{15}N values than other sites, with an average of 0.54 ‰ ± 0.65 ‰. Interestingly, this low δ^{15}N signal is not seen further downstream, with the closest downstream site *Wear 9* recording high δ^{15}N values.

F. vesiculosus tips collected from Roker Beach (Figure 3.2B) show a range of δ^{15}N values despite being within close proximity (within 50-100 m). Sites *Wear 1*, *2*, *5* record high values that suggest inputs of sewage or manure. In contrast sites *Wear 3* and *4*, which are in closer proximity to the sea, and therefore will be submerged for longer periods of time, show average δ^{15}N values in the unpolluted marine range.

3.3.1.2 December 2020

Figure 3.3: Mean δ^{15}N values for *F. vesiculosus* samples collected along the banks of the River Wear in December 2020 (n=5).

F. vesiculosus tips collected in December 2020 had an average δ^{15}N value of 7.5 ‰ ± 0.92 ‰ (Figure 3.3), which is within the unpolluted marine range and a decrease from the average value recorded in October 2020. Despite this decrease in average δ^{15}N values, just over half of all sites (55 %) still record δ^{15}N values outside of the unpolluted marine range, although there has been an increase in the number of sites that record δ^{15}N values in this range from the previous collection. *F. vesiculosus* tips sampled from site *Wear 17* recorded low average δ^{15}N values with a high standard deviation at 3.39 ‰ ± 1.99 ‰. There could be some evidence of this low δ^{15}N signal being captured by the δ^{15}N values of the tips collected from site *Wear 19*, for which the site has an average value of 5.10 ‰ ± 1.36 ‰. Interestingly, the site *Wear 10* which recorded a low average δ^{15}N value in October 2020, shows a high average value of 8.33‰ ± 0.85‰ in December 2020, an increase in δ^{15}N value of 7.79 ‰.

Similar to the *F. vesiculosus* tips collected in October 2020, the tips collected in December 2020 from Roker Beach (Figure 3.3B), show a range of δ^{15}N values, although they are lower on average than in the previous collection. Site *Wear 3* records the lowest average value of 5.13‰ ± 1.16‰ which is in closest proximity to the sea. Sites *Wear 1* and *2* record high δ^{15}N values that suggest inputs of sewage or manure, and sites *Wear 4* and *5* record δ^{15}N values in the unpolluted marine range.

3.3.1.3 March 2021

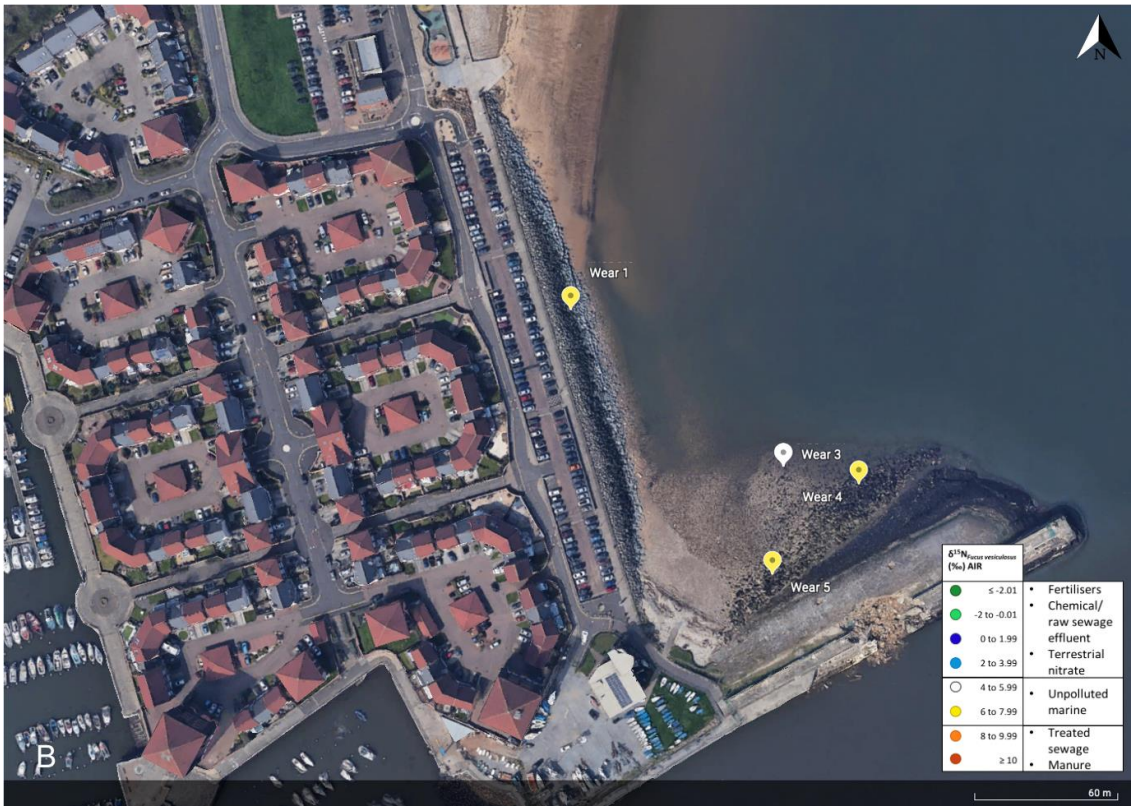


Figure 3.4: Mean δ^{15}N values for *F. vesiculosus* samples collected along the banks of the River Wear in March 2021 (n=5).

The average δ^{15}N value of *F. vesiculosus* tips collected in March 2021 decreased again from previous collections to 6.01 ‰ ± 0.87 ‰ (Figure 3.4). The majority of sites (87 %) recorded δ^{15}N values in the unpolluted marine range. This is a complete contrast to the collection in October 2020 where only three sites (14 %) had average δ^{15}N values in the unpolluted marine range. Sites *Wear 17* and *19* recorded low average δ^{15}N values whilst site *Wear 24* recorded an elevated δ^{15}N value. Site *Wear 17* had also previously recorded a low average δ^{15}N value in the December 2020 collection. There could be some evidence here that the low δ^{15}N signal recorded at sites *Wear 17* and *19* has been also assimilated further downstream at site *Wear 20* which has an average δ^{15}N value of 4.45 ‰ ± 1.49 ‰.

All *F. vesiculosus* tips collected in March 2021 at Roker Beach had δ^{15}N values in the unpolluted marine range, and do not show as much of a range in values as previous collections. Site *Wear 3* again records the lowest average δ^{15}N value with a value of 5.53 ‰ ± 0.48 ‰.

3.3.1.4 May 2021

Figure 3.5: Mean $\delta^{15}N$ values for *F. vesiculosus* samples collected along the banks of the River Wear in May 2021 (n=5).

In May 2021 the average $\delta^{15}N$ value of the *Fucus* tips collected was 7.48 ‰ ± 0.99 ‰ (Figure 3.5) which is an increase from average values in March 2021 but within the unpolluted marine range of values. Around half of the sites (53 %) in this collection recorded $\delta^{15}N$ values outside the unpolluted marine range, which is a decrease from the previous collection. Site *Wear 17* again records a low $\delta^{15}N$ average value of 3.50 ‰ ± 1.69 ‰. There doesn't appear to be a continued low $\delta^{15}N$ signal downstream, of this site as potentially seen previously with the collection in December 2020 and March 2021 collections.

All sites at Roker Beach collected in May 2021 record *F. vesiculosus* tips with high average $\delta^{15}N$ values (Figure 3.5B) and all sites have similar values, there is not a large range. The average values are higher than those recorded by *Fucus* tips collected in March 2021. Site *Wear 3* does not have the lowest $\delta^{15}N$ average value as with previous collections.

3.3.1.5 July 2021

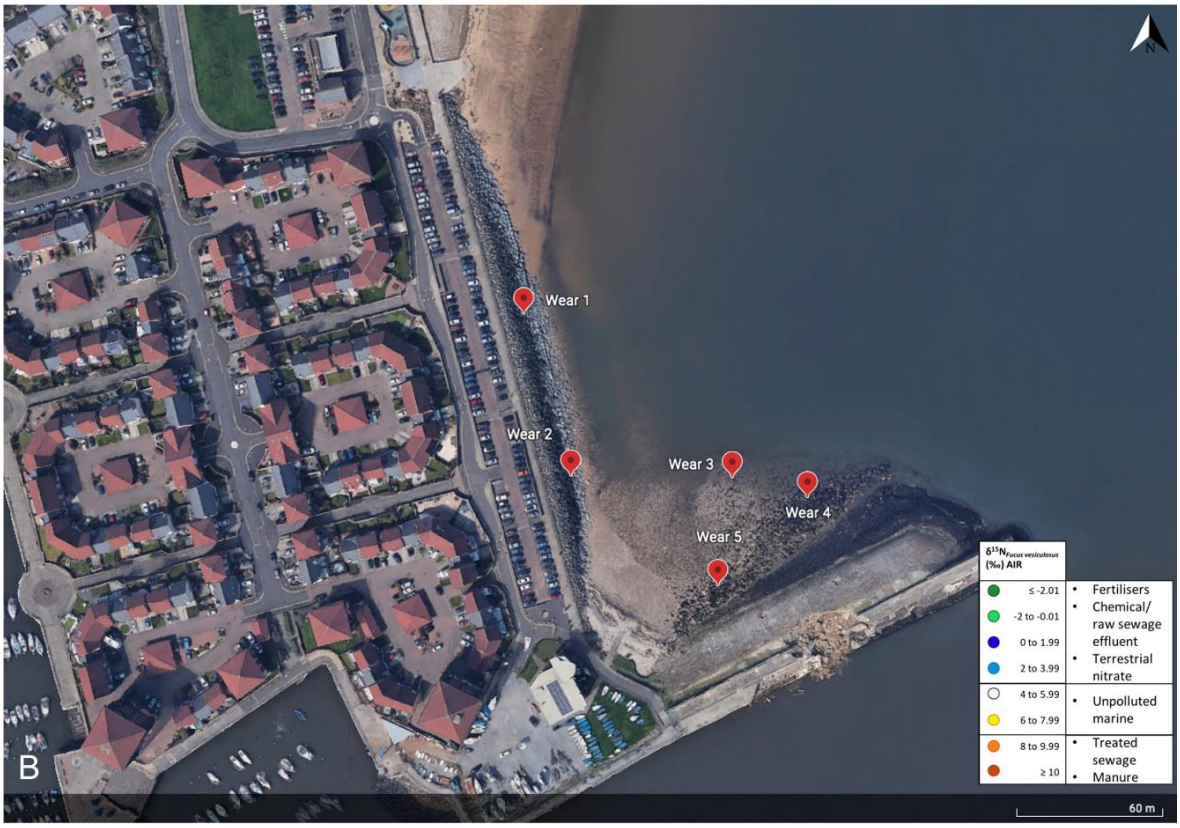


Figure 3.6: Mean δ^{15}N values for *F. vesiculosus* samples collected along the banks of the River Wear in July 2021 (n=5).

The average δ^{15}N of *F. vesiculosus* tips collected from the River Wear in July 2021 was 10.59 ‰ ± 0.71 ‰, which is an increase on the previous collection in May 2021. This average value is elevated and hence is outside of the unpolluted marine range. Only one site, *Wear 23*, has an average δ^{15}N value within the unpolluted marine range, meaning 95 % of all sites have δ^{15}N values outside of this range. The majority of sites record average δ^{15}N values over 10 ‰ (Figure 3.6). Sites that had previously recorded very low δ^{15}N values, *Wear 10* and *17*, record elevated average δ^{15}N values.

Sites at Roker Beach record very high average δ^{15}N values in July 2021, all greater than 10‰. These values are again an increase from the previous collection, with the highest average value at site *Wear 3*, which previously had lowest δ^{15}N values, with a value of 13.07 ‰ ± 0.48 ‰. Standard deviations are low apart from at site *Wear 2* with a value of 11.57 ‰ ± 2.34 ‰.

3.3.1.6 General trends

	Month of collection	Welch F statistic	Df1	Df2	P
δ^{15}N (‰) of F. vesiculosus tips collected from the Wear	October 2020	95.891	20	51.699	<0.001
	December 2020	13.853	19	45.465	<0.001
	March 2021	20.682	22	52.992	<0.001
	May 2021	31.486	18	44.781	<0.001
	July 2021	33.375	19	46.098	<0.001

Table 3.1: Results from repeated one-way ANOVA analyses carried out using SPSS software on the δ^{15}N values of *F. vesiculosus* tips collected from around the River Wear and its estuary. A significance level of 0.05 was used, meaning p values less than or equal to 0.05 represent a statistically significant result, i.e., there is less than a 5% probability the null hypothesis is correct. Significant values are highlighted in bold.

There was a significant difference between the δ^{15}N values of *F. vesiculosus* tips collected from different locations around the River Wear and its estuary for every collection period (Table 3.1).

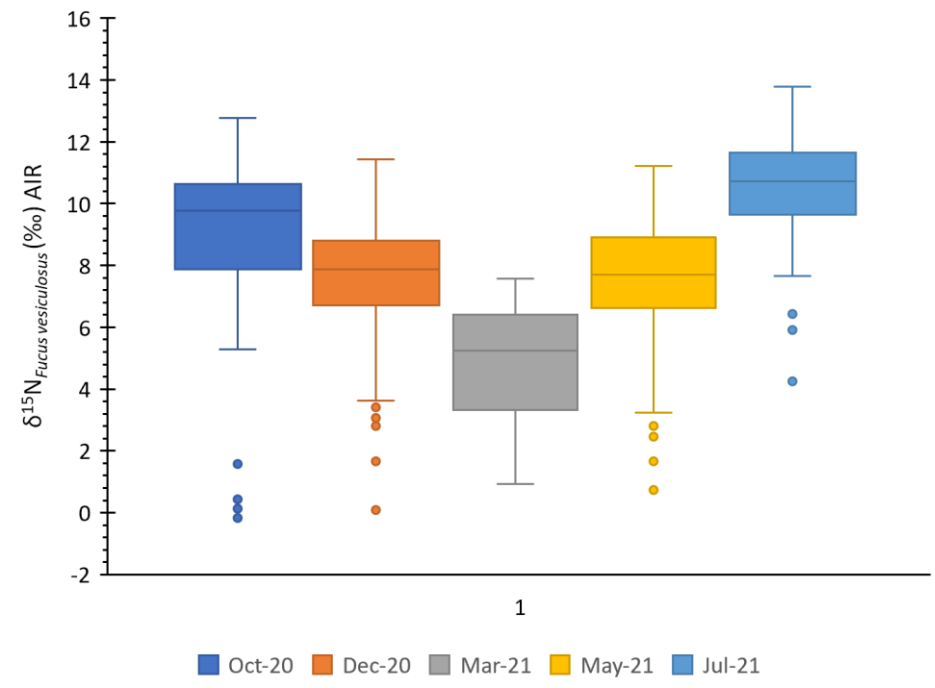


Figure 3.7: δ^{15}N values of *F. vesiculosus* tips collected from around the River Wear and its estuary in October and December 2020, and March and May 2021.

δ^{15}N values of *F. vesiculosus* tips collected from the River Wear were on average high in October 2020, displaying a wide interquartile range (Figure 3.7). There were also some *F. vesiculosus* tips that had outlier δ^{15}N values, recording low values between approximately 0 and 2 ‰ (Figure 3.7). Following this, there was a decrease in the median δ^{15}N values of *F. vesiculosus* tips collected in December 2020 and the interquartile range decreased in size. There were still outlier δ^{15}N values in the *F. vesiculosus* tips collected in December, but these had a wider range than in October, between 0 and 4 ‰. In March 2021, there was another decrease in the median δ^{15}N value of *F. vesiculosus* tips, although the interquartile range was broader than both October and December 2020 (Figure 3.7). Tips from this time of collection did not have outlier δ^{15}N values, however this could be as a result of the values being lower overall, the range extends to approximately 1 %, and therefore values that were outliers in previous collections are included within the range. Following this collection, the median δ^{15}N value increases for tips collected in May 2021, with a median of 7.70 ‰, this collection displays a distribution in values extremely similar to the values of those tips collected in December 2020 (Figure 3.7). Then, there was an increase in the median value for δ^{15}N values of tips collected in July 2021, reaching the highest value of all collections previously. The interquartile range is relatively narrow, similar to the distribution of δ^{15}N values from December 2020 and May 2021. There were outliers from the collection in July 2021, although they had higher values than the previous outliers, with a range of approximately 4 to 6.5 ‰. Overall, there is a cyclical trend in δ^{15}N values of *F. vesiculosus* tips collected from the River Wear and its estuary between October 2020 and July 2021. The δ^{15}N values began with a high median value of 9.77 ‰ in the polluted range (treated sewage/manure), before then decreasing to a low in March 2021 with a median value of 5.24 ‰ in the unpolluted marine range, and then increasing until the high in July 2021 of 10.71 ‰ which is again in the polluted range (Figure 3.7). There were outliers associated with all collections apart from those tips collected in March 2021.

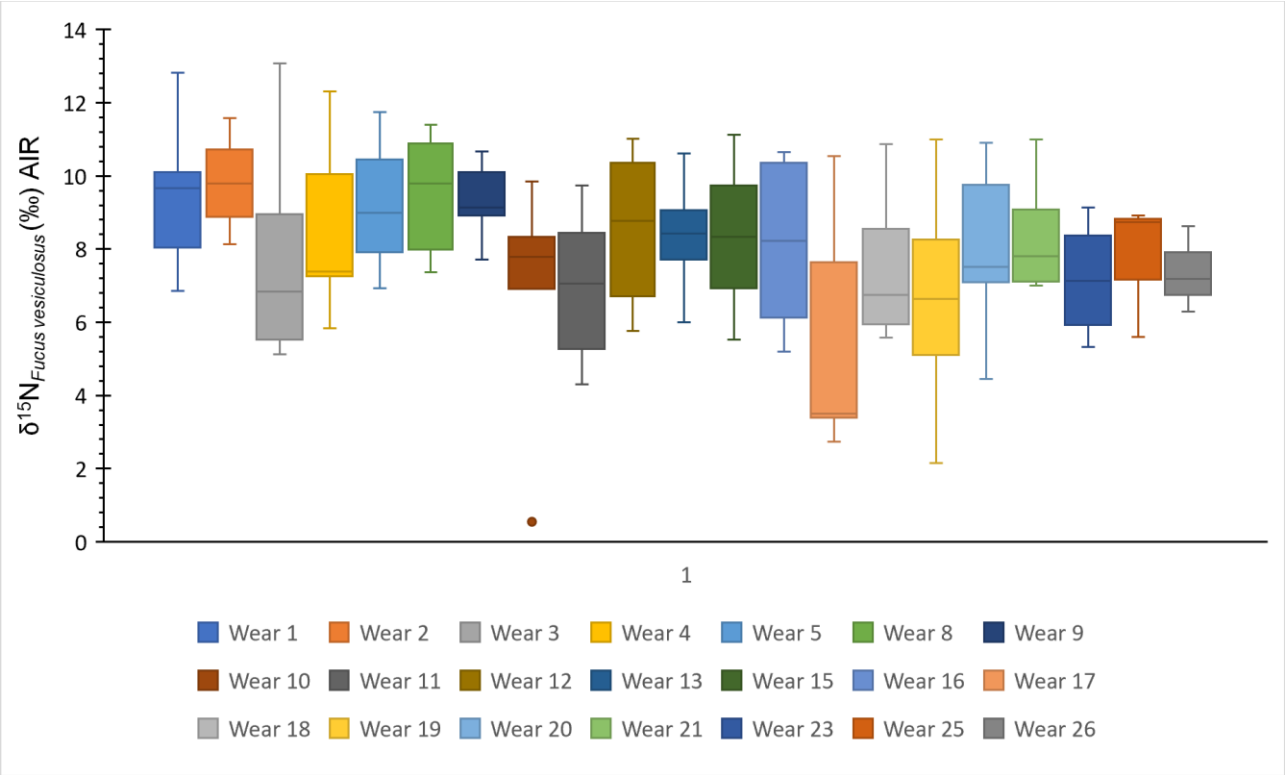


Figure 3.8: Average δ^{15}N values of *F. vesiculosus* tips collected from each site along the River Wear over five collection periods (October 2020, December 2020, March 2021, May 2021, July 2021). n=5.

Macroalgal δ^{15}N values varied temporally at each site along the River Wear (Figure 3.8). The majority of sites had a variation of more than or equal to 4 ‰ (i.e., the range of δ^{15}N values for unpolluted marine nitrogen) when comparing the range of average δ^{15}N values for each site. Those sites that did not vary to this extent include sites *Wear 2*, *9*, *23*, *25* and *26*. Site *Wear 10*, whilst not having a large interquartile range, does have an outlier with a low δ^{15}N value. For the majority of sites, the median δ^{15}N value was in the polluted range, above 8 ‰, indicating pollution from treated sewage or manure (Figure 3.8).

3.3.2 North East coastline and Tyne

3.3.2.1 February/March 2021

Figure 3.9: Mean $\delta^{15}N$ values for *F. vesiculosus* sampled collected along the coastline of North East England, UK in February and March 2021 (n=5).

F. vesiculosus tips collected from the mouth of the River Tyne (sites *Tyne 1*, *2*, *3*, *4*, *5* and *11*) in February 2021 generally had low δ^{15}N values (Figure 3.9). These sites all generally had high standard deviations of δ^{15}N values, demonstrating a spread of δ^{15}N values within the *F. vesiculosus* tips. These sites are all located within the estuary of the River Tyne, enclosed by piers on both the north and south edges, which isolates the sites from the North Sea to some extent. The site with the lowest average δ^{15}N value was site *Tyne 2* with a value of -3.75 ‰ ± 2.55 ‰. Site *Tyne 11* tips recorded slightly higher average δ^{15}N values than these other sites at 0.17 ‰ ± 1.08 ‰ and is the only site of the estuary sites located on the southern side of the Tyne. Sites *Tyne 6* and *7*, *Coast 1*, *2*, *3* and *4* record *F. vesiculosus* tips with average δ^{15}N values in the unpolluted marine range and have relatively low standard deviations of δ^{15}N values. These sites are considered to be coastal sites, given they are located on the seaward sides of the piers. *F. vesiculosus* tips collected further upstream in the River Tyne, sites *Tyne 9* and *10* record tips with average δ^{15}N values in the unpolluted marine range. Site *Tyne 8* records *Fucus* tips with an average δ^{15}N value of -1.7 ‰ ± 1.87 ‰, which is a low value in the range that could be impacted by raw sewage, fertilisers or terrestrial nitrate. *Tyne 8* is downstream of sites *Tyne 9* and *10*. Sites located at Marsden Beach (*Coast 6*, *7*, *8* and *9*) record *F. vesiculosus* tips with average δ^{15}N values in the unpolluted marine range. The range of average δ^{15}N values is small. Site *Coast 10* located at Souter Lighthouse also records an average δ^{15}N value in the unpolluted marine range (Figure 3.9). *F. vesiculosus* tips collected from Whitburn Beach (sites *Coast 11* and *12*) and Seaburn (*Coast 13*) record δ^{15}N values in the unpolluted marine range. Figure 3.3 shows slightly higher average δ^{15}N values for *F. vesiculosus tips* at three of the sites, *Coast 14* at Ryhope Beach and *Coast 16* and *17* at Seaham Beach. *Coast 15* and *Coast 18* record average δ^{15}N values in the unpolluted marine range. Despite sites *Coast 17* and *18* being only ~20m away from one another, there is a difference of 1.55 ‰ in average δ^{15}N values. *F. vesiculosus* tips collected from Blackhall Rocks (*Coast 19*) and Crimdon Beach (*Coast 20* and *21*) record high average δ^{15}N values outside of the unpolluted marine range with low standard deviations (Figure 3.9). Site *Coast 22* at Crimdon Beach records an average δ^{15}N value within the unpolluted marine range. Further south on the coastline, sites *Coast 23*, *24* and *25* all record average δ^{15}N values within the unpolluted marine range (Figure 3.3). Site *Coast 26* at Seaton Carew Beach records Fucus tips with average δ^{15}N values in the unpolluted marine range, as well as sites *Coast 27* and *28* at Redcar (Figure 3.3). All sites had low standard deviation values of δ^{15}N.

In general, the average δ^{15}N value of *F. vesiculosus* tips collected from the North East coastline in February/March 2021 was 6.68 ‰ ± 0.52 ‰ and the average value for River Tyne *F. vesiculosus tips*

was 0.70‰ ± 1.60‰. When comparing the River Tyne and the River Wear in this same time period, there is a large difference in average δ^{15}N values, of 5.31 ‰. The River Tyne *F. vesiculosus tips* and North East coastline *F. vesiculosus* have a difference of 5.98 ‰ in average δ^{15}N values. The River Wear and North East coastline *Fucus* average δ^{15}N values are comparable, with only a small difference of 0.67‰.

3.3.2.2 June 2021

Figure 3.10: Mean $\delta^{15}N$ values for *F. vesiculosus* sampled collected along the coastline of North East England, UK in June 2021 (n=5).

F. vesiculosus tips collected in June 2021 from the mouth of the River Tyne (sites *Tyne 1*, *2*, *3* and *11*) had low $\delta^{15}N$ values, but not quite as ^{15}N-depleted as those tips collected in February 2021 (figures 3.8 and 3.9). Site *Tyne 4*, however, had an average $\delta^{15}N$ value in the unpolluted marine range, which is in contrast to the average value of the tips collected in February 2021 which had a much lower average $\delta^{15}N$ value of -2.97‰ ± 1.51‰. Site *Tyne 11* again records slightly higher average $\delta^{15}N$ values than the sites *Tyne 1*, *2*, and *3*, with an average $\delta^{15}N$ value of 3.53 ‰ ±0.42 ‰, which is an increase from the previous collection. The standard deviations of $\delta^{15}N$ values for these sites are generally high, demonstrating the variation between individual *F. vesiculosus* tips, but did not show the same large spread as those tips collected in February 2021. Site *Coast 2* records an average $\delta^{15}N$ value in the unpolluted marine range, with sites Coast 1 and 3, and *Tyne 6* and *7*, recording average $\delta^{15}N$ values in the elevated range of values, indicating treated sewage or manure pollution (Figure 3.10). These coastal sites maintained their relatively low $\delta^{15}N$ standard deviation values. *F. vesiculosus* tips collected from the riverine sites *Tyne 9* and *10*, again record average $\delta^{15}N$ values in the unpolluted marine range, although the values are slightly higher than those collected in February 2021 (Figure 3.10). Site *Tyne 8* records an average $\delta^{15}N$ value of -6.32 ‰ ±1.09 ‰ which is substantially lower than in the previous collection (Figure 3.9). Sites on Marsden Beach (*Coast 7*,

8, and *9*) record *F. vesiculosus* tips with average δ^{15}N values in the unpolluted marine range for June 2021. These values have remained fairly consistent with *F. vesiculosus* tips from the previous collection (figures 3.9 and 3.10). *vesiculosus* tips from *Coast 10* record elevated δ^{15}N values in June 2021, with an average value of 9.09 ‰ ± 1.18 ‰, an increase from February tips that had an average of 7.76 ‰ ± 0.29 ‰. Sites on Whitburn and Seaburn beaches (*Coast 11* and *13*, respectively) record *F. vesiculosus* tips with average δ^{15}N values in the elevated range, indicating a potential input of treated sewage or manure (Figure 3.10). *Coast 11* in particular has a very high average δ^{15}N value of 12.37‰ ± 1.06‰. Sites at Ryhope and Seaham beaches (Coast 14, 15, 16, 17 and 18) all record *F. vesiculosus* tips with elevated average δ^{15}N values of greater than 10‰. All sites have similar average δ^{15}N values with low standard deviations. This is in contrast to the previous collection where some sites recorded *Fucus* tips with δ^{15}N values in the unpolluted marine range and some with elevated values in close proximity (Figure 3.10). Sites at Crimdon Beach (*Coast 20*, *21* and *22*) record *F. vesiculosus* tips with average δ^{15}N values greater than 10‰ (Figure 3.10). These sites had shown elevated δ^{15}N values in the previous collection (excluding *Coast 22*) but the values increased for June 2021. In March 2021, sites at Hartlepool Beach recorded average δ^{15}N values in the unpolluted marine range; these values all increased in June 2021, recording elevated average δ^{15}N values, indicating a potential input of treated sewage or manure into the area (Figure 3.10).

Overall, the average δ^{15}N value of *Fucus* tips collected from along the North East coastline increased in June 2021 from the previous collection in February/March 2021, with an average of 9.49 ‰ ± 0.61 ‰. The average δ^{15}N value for *Fucus* collected from River Tyne sites also increased to 3.48 ‰ ± 0.75 ‰. Comparing River Tyne *F. vesiculosus tips* collected in June and River Wear *F. vesiculosus* collected in July, there is a difference in average δ^{15}N values of 7.11 ‰, which is a larger difference than in the February/March collection. River Tyne tips and North East coastline tips have a difference in average δ^{15}N values of 6.01 ‰, which is comparable to the difference in February/March 2021. For *F. vesiculosus* collected from the River Wear in July and North East coastline *F. vesiculosus* collected in June, there is a difference in average δ^{15}N values of 1.10 ‰, which is an increase from the February/March 2021 collection.

3.3.2.3 General trends

Month of collection	Welch F statistic	Df1	Df2	P

$\delta^{15}N$ (‰) of F. vesiculosus tips collected from the Tyne	February 2021	50.474	27	64.566	**<0.001**
	June 2021	53.171	20	48.532	**<0.001**

Table 3.2: Results from repeated one-way ANOVA analyses carried out using SPSS software on the $\delta^{15}N$ values of *F. vesiculosus* tips collected from around the River Tyne and its estuary. A significance level of 0.05 was used, meaning p values less than or equal to 0.05 represent a statistically significant result, i.e., there is less than a 5% probability the null hypothesis is correct. Significant values are highlighted in bold.

There was a significant difference between the $\delta^{15}N$ values of *F. vesiculosus* tips collected from different locations around the River Tyne and its estuary for every collection period (Table 3.2).

	Month of collection	**Welch F statistic**	**Df1**	**Df2**	**P**
$\delta^{15}N$ (‰) of F. vesiculosus tips collected from the North East coastline	February 2021	50.474	27	64.566	**<0.001**
	June 2021	53.171	20	48.532	**<0.001**

Table 3.3: Results from repeated one-way ANOVA analyses carried out using SPSS software on the $\delta^{15}N$ values of *F. vesiculosus* tips collected from around the North East coastline. A significance level of 0.05 was used, meaning p values less than or equal to 0.05 represent a statistically significant result, i.e., there is less than a 5% probability the null hypothesis is correct. Significant values are highlighted in bold.

There was a significant difference between the $\delta^{15}N$ values of *F. vesiculosus* tips collected from different locations around the North East coastline for every collection period (Table 3.3).

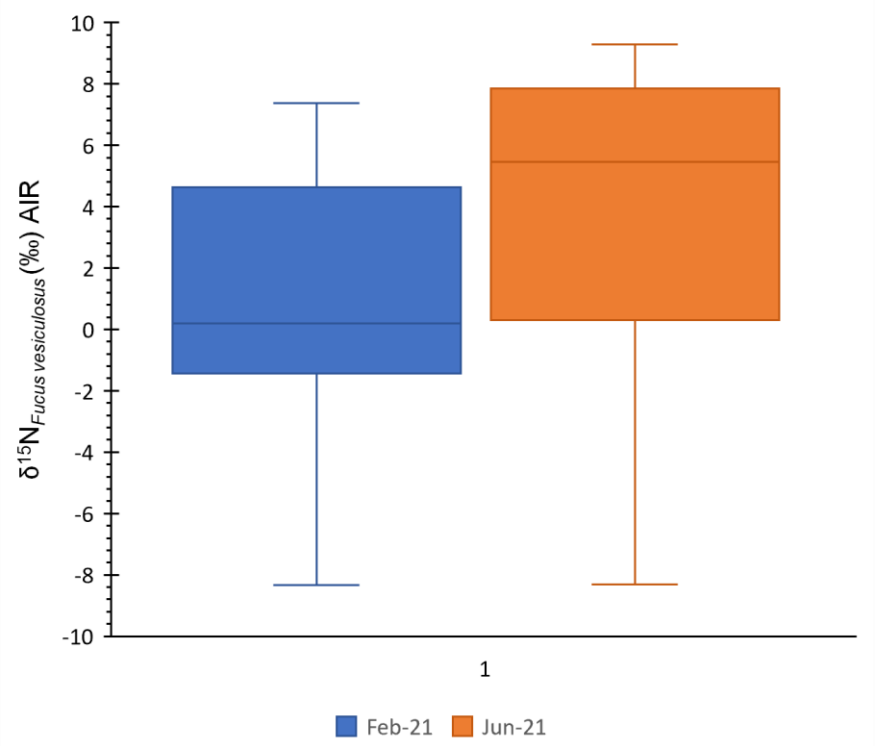


Figure 3.11: $\delta^{15}N$ values of *F. vesiculosus* tips collected from around the River Tyne and its estuary in February and June 2021.

$\delta^{15}N$ values of *F. vesiculosus* tips collected from the River Tyne in February 2021 were low on average (0.19 ‰), displaying a very wide interquartile range and with an overall range between ~ -8 ‰ and 8 ‰ (Figure 3.11). Following this, *F. vesiculosus* tips collected from the River Tyne in June 2021 had a higher median value compared to the previous collection, of 5.46 ‰, again with a very wide interquartile range between ~ 0 and 8‰ (Figure 3.10). Despite this higher average, the range of $\delta^{15}N$ values was still very wide this collection, with values between ~ -8 ‰ and 9 ‰ (Figure 3.10).

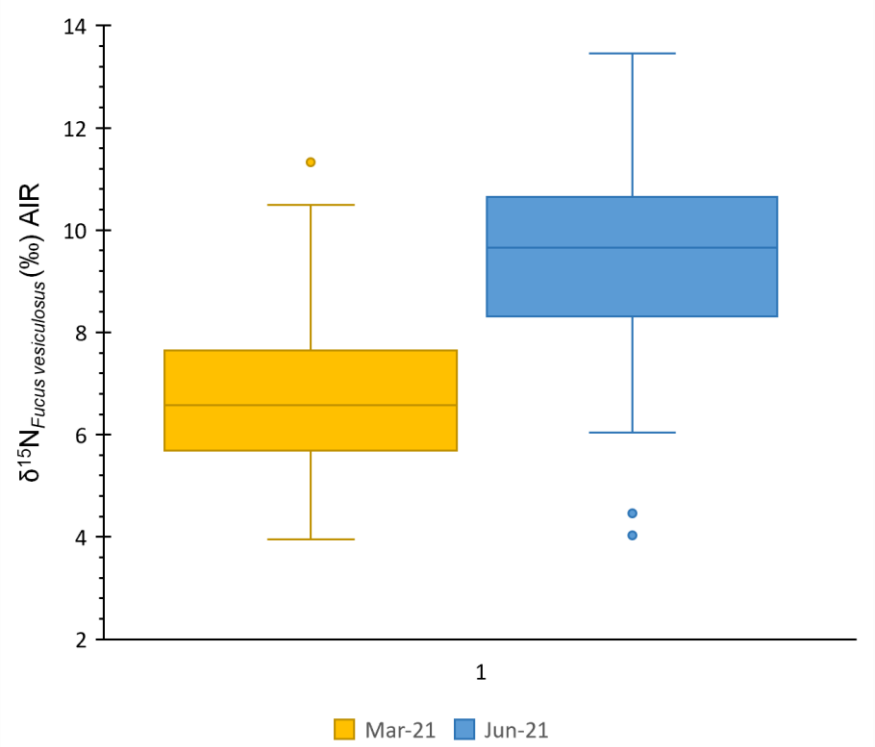


Figure 3.12: δ^{15}N values of *F. vesiculosus* tips collected from around the North East coastline in March and June 2021.

δ^{15}N values of *F. vesiculosus* tips collected along the North East coastline in March 2021 had a median value of 6.58 ‰, with a slightly narrow interquartile range (Figure 3.12). Tips from this collection had δ^{15}N values ranging from 4 to 10.5 ‰, with an outlier at higher values (Figure 3.12). The median δ^{15}N value then increased in tips collected in June 2021, with a value of 9.65 ‰, and a wider interquartile range (Figure 3.11). δ^{15}N values ranged from ~ 6 to 13.5 ‰ in this collection with a couple of outliers with lower δ^{15}N values around 4 ‰ (Figure 3.12).

3.3.3 Individual site temporal comparisons

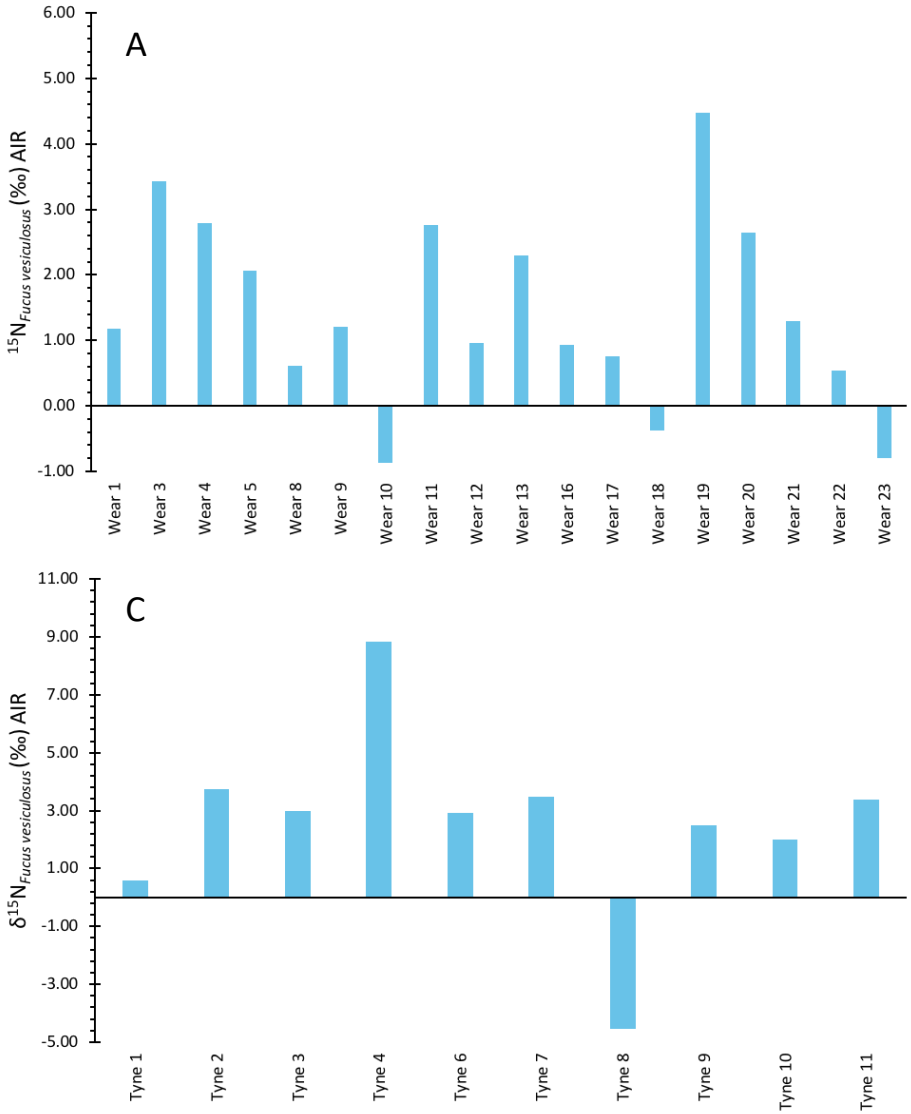


Figure 3.13: Average difference in $\delta^{15}N$ values of *F. vesiculosus* tips collected from each site along the River Wear (A), North East coastline (B) and River Tyne (C) over two collection periods (average June 2021 (May for Wear) $\delta^{15}N$ values minus average March 2021 $\delta^{15}N$ values). n=5.

Given that there were two collection periods for the North East coastline and the River Tyne, similar collection periods were chosen to compare to the River Wear for changes in macroalgal δ^{15}N values at different sites. Average δ^{15}N values varied temporally at all sites, between March and May/June 2021 (Figure 3.13). Several sites showed a large amount of variation between collection periods, in particular those sites with more than or equal to 4 ‰ variation (i.e., the range of δ^{15}N values for unpolluted marine nitrate) include sites *Wear 19*, *Coast 11* and *13*, and *Tyne 4* and *8* (Figure 3.13). These sites were highlighted when mapped spatially as those with polluted δ^{15}N values at some collections, deviating greatly from the unpolluted nitrogen range (figures 3.2-3.6, 3.9, 3.10). Those sites with a low amount of variation (δ^{15}N values less than or equal to 1 ‰) include sites *Wear 8*, *10*, *12*, *16*, *17*, *18*, *22* and *23*, *Coast 7*, *8*, *9*, and *19*, and *Tyne 1*. For the majority of sites, δ^{15}N values increased from March to May/June 2021, excluding sites *Wear 10*, *18* and *23*, and *Tyne 8*. These results indicate the δ^{15}N signal in the environment is not stable throughout the year, or macroalgal metabolism changes throughout the year.

3.4 Discussion

3.4.1 Temporal trends

When considering trends of δ^{15}N values in native macroalgae, there are two overarching factors that could influence these values: the type and potential mix of nitrogen sources that have been taken up by the macroalgae, and macroalgal physiology that could change the δ^{15}N values of these sources. To add to this, both nitrogen sources and macroalgal physiology can change interannually, and as such it can be difficult to explain changing δ^{15}N values of native macroalgae. Therefore, results from Chapter Two of this thesis are paramount to be able to explain the trends seen in the results in this chapter. It assumed in the literature that δ^{15}N values of macroalgal tips are generally representative of δ^{15}N-DIN values (Gartner et al. 2002; Dudley et al. 2010; García-Seoane et al. 2018 and references therein), although there are studies that report results stating macroalgal δ^{15}N values aren't representative of δ^{15}-DIN (Swart et al. 2014). This thesis has found that temperature and salinity can have a significant effect on δ^{15}N uptake by *F. vesiculosus* non-fertile tips (Chapter Two). Therefore, this must be considered, alongside findings from other workers that have documented an approximate 1 ‰ deviation between macroalgal δ^{15}N values and δ^{15}N-DIN (Umezawa et al. 2007; Teichberg et al. 2007; 2008; Barr et al. 2007; Cornelisen et al. 2007).

F. vesiculosus tips collected from the River Wear showed a cyclical trend in this study, with median values decreasing from October 2020 to December 2020, before then increasing to a high in July 2021 (Figure 3.7). *F. vesiculosus* tips collected from the River Tyne and North-East coastline also recorded higher $\delta^{15}N$ values in the summer month collection (June 2021) compared to the winter month of collection (March 2021) (Figure 3.7). Average $\delta^{15}N$ values are not stable at each site, showing significant variations between collection periods (Figure 3.8). Considering biogeochemical cycles, there are a number of explanations for this trend. Higher primary production rates in the summer months could explain higher $\delta^{15}N$ values seen in *F. vesiculosus* tips, due to the fact that organisms in general prefer to take up lighter nitrogen, and hence leaving heavier nitrogen in the system that the macroalgal tips then take up (Ahad et al. 2006). This is on the assumption that macroalgal tips also do not have a preference for lighter nitrate (Deutsch & Voss, 2006). Furthermore, during the summer months microbial processes are more active (Ahad et al. 2006), for example denitrification increases with temperature increases, which produces a heavier pool of remaining nitrate (Baeta et al. 2009; Bergamino & Richoux 2014). This could be reflected in the higher $\delta^{15}N$ values recorded by the *F. vesiculosus* tips in the summer. On the other hand, in the winter months microbial activity is generally low meaning $\delta^{15}N$ values in the environment will be less impacted by biogeochemical transformations, and hence $\delta^{15}N$ values in theory are more reflective of source values. Also, in the winter months the river discharges were higher (Figure 3.14), therefore the $\delta^{15}N$ values can be more representative of terrestrial nitrogen sources (Ahad et al. 2006). Given this, the low $\delta^{15}N$ values recorded by the River Wear *F. vesiculosus* tips in March 2021, and Tyne and North East coastline tips in February 2021, could reflect a greater proportion of terrestrial-derived nitrate (figures 3.7, 3.11, 3.12 and 3.14).

It is also important to consider that the different nitrogen sources could've impacted the tips at different stages of the year, for example, the timing of fertiliser application could've affected the $\delta^{15}N$ values seen in the macroalgal tips. Low $\delta^{15}N$ values seen in the winter months could be explained by a higher proportion of fertiliser-derived nitrogen being present in the environment (Heaton 1986). Higher discharge rates could mobilise nitrate from soils that have been treated with fertiliser and hence increase nitrate concentrations, whilst simultaneously decreasing $\delta^{15}N$ values due to the greater proportion of ^{15}N-depleted nitrate being present. However, manure is often used as an agricultural fertiliser and this has characteristically high $\delta^{15}N$ values (Xue et al. 2009), and so it becomes more difficult to entangle the agricultural signal unless it is known which type of fertiliser is being applied to fields in a given catchment. Unfortunately investigating the types of fertiliser

applied to fields in the catchment is beyond the scope of this study. Given that much of the study is in a nitrate vulnerable zone (Environment Agency, 2021), this limits the application of nitrate at certain times of the year and is much stricter than for areas that have to follow the general legislation (Environment Agency & DEFRA, 2018). However, it is generally acknowledged that farmers don't strictly follow these rules and as such, timing of fertiliser application could vary throughout the year. In the summer months, the increase in δ^{15}N values in *F. vesiculosus* tips at all locations on average could be explained by an increasing proportion of heavy-nitrogen sources being present in the environment, i.e., an increased amount of treated sewage or manure (Xue et al. 2009; Dailer et al. 2010). If farmers were applying manure to their fields in the summer, this could lead to an increase in the proportion of heavy nitrate in the environment, however given that discharge is much lower in the summer months (Figure 3.14), the runoff from manure-treated fields may not reach the aquatic environment in significant enough quantities to influence the δ^{15}N signals. An increased amount of treated sewage in the environment could also be responsible for higher δ^{15}N values being reported in *F. vesiculosus* in the summer months. This effect has been reported by Signa et al. (2020) in Cyprus due to the influence of the summer tourist season increasing the amount of processed sewage being released. However, the North East generally doesn't receive a large amount of tourism in the summer months and therefore, given this, and the fact there are no other explanations for an increase in sewage at different times of the year this is unlikely to be the cause of variation in δ^{15}N values in the summer months. The higher δ^{15}N values could be as a result of the decreased amount of discharge in rivers (Figure 3.14), and hence the amount of terrestrial-derived nitrate is lower, meaning that the proportion of nitrogen sources changes, and therefore increases the δ^{15}N signal due to decreasing the amount of light nitrogen in the system.

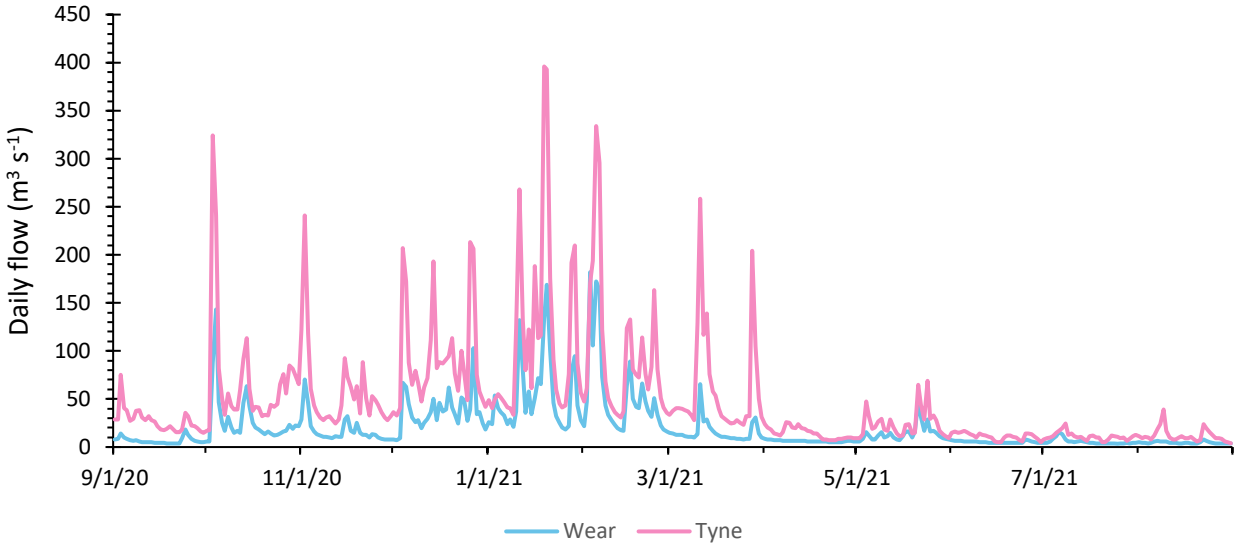


Figure 3.14: Daily mean flow from the River Wear (site: Chester-Le-Street, grid reference: NZ2830451226) and the River Tyne (site: Bywell, grid reference: NZ0391361684). Flow data from 01/09/2020 to 31/08/2021, sourced from the Hydrology Data Explorer produced by Defra (DEFRA, 2021).

3.4.1.1 Macroalgal physiology

As discussed previously it is essential to consider the fact that macroalgal physiology could cause fractionation of the δ^{15}N-DIN signal in the environment. Results from Chapter Two of this thesis show that temperature and salinity can have a significant effect on the extent to which *F. vesiculosus* δ^{15}N values are reflective of δ^{15}N-DIN values. Other workers have identified a 1 ‰ deviation between macroalgal δ^{15}N and δ^{15}N-DIN values. Results from this study also report a more than a 1 ‰ deviation between macroalgal δ^{15}N and δ^{15}N-DIN values (Chapter Two).

As such, the δ^{15}N values in collected native *F. vesiculosus* could have been affected by these environmental factors, and this therefore makes it difficult to interpret the sources of nitrogen pollution in the environment, using their signature δ^{15}N values. Chapter Two reported similar results to Howarth et al. (2020) in that macroalgal tips did not have δ^{15}N value as reflective of δ^{15}N-DIN when cultured at higher temperatures. If it is assumed that baseline δ^{15}N values for *F. vesiculosus* tips are around 4-8 ‰, the unpolluted marine signal, and also that *F. vesiculosus* tips do not integrate δ^{15}N values as effectively at higher temperatures (Howarth et al. 2020; Chapter Two), then it can be assumed that the high δ^{15}N values recorded by the tips in the summer months do indicate

a δ^{15}N signal with a high δ^{15}N value, but the true δ^{15}N signal in the environment is higher than recorded by the *F. vesiculosus* tips. As such, it can be said that *F. vesiculosus* tips can be used as an indicator of δ^{15}N values, in that an approximation of the δ^{15}N signal in the environment can be deciphered, but it must be taken into consideration that the δ^{15}N values are not truly reflective of the δ^{15}N signal in the environment, at least over a period of 14 days (Chapter Two). At lower temperatures in the winter months, tips are more reflective of the δ^{15}N signal in the environment, however after two weeks incubation, the tips still did not reach 100% equilibrium with δ^{15}N-DIN values (Chapter Two). Therefore, although tips may be more reflective of the δ^{15}N signal in the environment in the winter months, it can be said that even so *F. vesiculosus* tips should be used as an indicator of potential sources of nitrogen pollution in the environment. Actions to reduce nitrogen pollution can then target suspected sources of nitrogen pollution, and by continuously monitoring the δ^{15}N values of *F. vesiculosus*, regulators can assess whether these actions have returned the δ^{15}N values to unpolluted levels.

Chapter Two reports that *F. vesiculosus* tips incubated at lower than normal (35 ‰) salinity integrate δ^{15}N signals less effectively, which has implications for estuarine macroalgae that experience shifts in salinity daily due to tidal action and annually due to changes in precipitation. Typically, salinity levels in estuaries decline in the latter winter and spring months when discharge and precipitation are greater due to dilution, and in the summer months salinity values are greater due to an increase in temperatures and evaporation (NOAA, 2022). Therefore, according to results from Chapter Two, *F. vesiculosus* tips collected in the summer months may be more reflective of the δ^{15}N signal in the environment, and less reflective at lower salinities in the winter/spring months, based on salinity as a factor alone. This is the opposite effect of temperature, according to this study and Howarth et al. (2020), and so there could be an offset between the effects of temperature and salinity on macroalgal δ^{15}N values at different points of the year, that could mean the overall δ^{15}N value is actually reflective of the δ^{15}N signal in the environment. However, the extent to which each factor affects the uptake of nitrogen by *F. vesiculosus* tips is not known, and therefore further studies should look to address accounting for each factor. Furthermore, there are daily fluctuations of salinity in estuarine environments due to tidal cycles, and therefore estuarine *F. vesiculosus* tips may not be appropriate for monitoring nitrogen pollution using δ^{15}N values but may give a more representative idea of the types of nitrogen pollution that may be present in an area.

3.4.2 Spatial trends

3.4.2.1 River Wear

δ^{15}N values recorded by *F. vesiculosus* tips collected from the River Wear are generally high in October 2020 and July 2021, with the majority of locations recording tips with δ^{15}N values in the polluted range (figures 3.2, 3.6, 3.7). Tips collected in December 2020 and May 2021 had an overall average of ~ 7.5 ‰, with an approximately even split between tips recording δ^{15}N values in the polluted range and unpolluted marine range (figures 3.3, 3.5, 3.7). Tips collected in March 2020 record on average values in the unpolluted marine range (figures 3.4 and 3.7). Spatially, there is a loose trend towards higher δ^{15}N values down-river, approaching the mouth of the estuary, however this isn't a consistent trend over time (Figures 3.2-3.6). This could be explained by the fact that input of treated sewage will increase downstream due to several sewage inputs (DEFRA, 2002), which will increase the δ^{15}N signal in the environment due to increasing the proportion of this source.

3.4.2.2 River Tyne

Tips collected from the River Tyne record low δ^{15}N values in March 2021 and June 2021, although the average values are higher in June, but still in the polluted range (figures 3.8A, 3.9A, 3.10). These values are indicative of nitrogen sourced from either fertilisers, chemical/raw sewage effluent, or terrestrial nitrate, or a mixture of these sources (Xue et al. 2009). A previous study identified high rates of nitrification in the Tyne due to ammonium originating from sewage discharges (Ahad et al. 2006). This process results in isotopically light nitrate being present in the water column, due to the nitrification step having an enrichment factor between -19 ‰ to -35 ‰ (Mariotti et al. 1981). Therefore, the low δ^{15}N signals seen in the River Tyne at both timepoints could be explained by the nitrification of sewage inputs into the river. Alternatively, the low δ^{15}N signals could originate from agricultural fertilisers or chemical effluent (Xue et al. 2009). Given that the Tyne catchment is no longer subject to discharges from heavy industry like it once was (Tyne Catchment Partnership, 2022), this is unlikely to be the source of low δ^{15}N values in this area. Agriculture is present in upper reaches of the Tyne catchment, and so this could be responsible for the low δ^{15}N values present as a diffuse pollutant.

3.4.2.3 North East coastline

On average, *F. vesiculosus* tips collected in March 2021 recorded δ^{15}N values in the unpolluted range, whereas tips collected in June 2021 recorded a high average δ^{15}N value in the polluted range (treated sewage and manure) (figures 3.9, 3.10, 3.12). There does not appear to a spatial trend in δ^{15}N values of *F. vesiculosus* tips collected from the North East coastline (figures 3.8 and 3.9). Although, in March 2021 only a few sites recorded δ^{15}N values outside of the unpolluted marine range (figures 3.8 and 3.11), suggesting a potential input of treated sewage of diffuse manure runoff in these areas: Ryhope, Seaham and Crimdon beaches. In June 2021 these same sites record δ^{15}N values of >10 ‰ which is relatively high compared to other sites in this collection, despite a general increase at all sites (Figure 3.9).

Overall, it appears that given the difference between δ^{15}N values recorded by *F. vesiculosus* tips between different locations, sites along the North East coastline are diluted by the δ^{15}N signal of unpolluted marine nitrate (Riera 1998; Riera et al., 2000; Savage & Elmgren 2004; Orlandi et al. 2014), despite varying inputs from the rivers. δ^{15}N values in *F. vesiculosus* tips collected in the summer months are higher than the winter values, suggesting that denitrification as a result of higher temperatures is responsible for increasing these values despite no apparent change in nitrogen sources.

3.4.2.4 Sewage outfalls

Nitrification of ammonium sourced from sewage inputs has been previously reported by several studies (Hashimoto et al. 1999; de Wilde and de Bie 2000). This process produces lighter nitrate than the δ^{15}N value of the ammonium, and therefore sewage inputs in temperate estuaries could be responsible for lower δ^{15}N signals (Ahad et al. 2006). This process could offer explanation for the low δ^{15}N values observed in both the River Wear and the River Tyne. Sites on the River Wear that experienced low δ^{15}N values were found to be in close proximity to CSOs (The Rivers Trust, 2022), and sites on the Tyne could've been influenced by several CSOs, as well as from treated sewage outfalls (The Rivers Trust 2022). Particularly on the River Wear, these outlier low δ^{15}N values at certain sites aren't temporally stable, suggesting the *F. vesiculosus* tips can detect when untreated sewage has been released from CSOs. *F. vesiculosus* tips are thought to have an integration time of 2-3 weeks for δ^{15}N-DIN (Umezawa et al. 2007), however results from this study find that two weeks may not be enough time to reach equilibrium (Chapter Two). Furthermore, those *F. vesiculosus* tips that are located in close proximity to sewage outfalls, where salinity will be lower due to the influx

of low salinity wastewater, might not reflect the $\delta^{15}N$ signal of the influence of sewage in the environment accurately (Chapter Two). Despite this, outlier low $\delta^{15}N$ values could suggest that untreated sewage is being released into rivers, even if the macroalgal $\delta^{15}N$ value recorded is not equal to the $\delta^{15}N$-DIN value from the sewage, the indication of a general trend can help to resolve the source as untreated sewage.

3.4.2.5 Emersion/immersion

One factor that was not accounted for was the effect of emersion on $\delta^{15}N$ values. Collected *F. vesiculosus* tips were always collected around low tide, and tips closest to the water's edge were selected if this was able to be done safely, therefore the tips collected should represent tips that are immersed for the longest period, to allow for time to equilibrate with $\delta^{15}N$-DIN values. Kim et al. (2013) found evidence for the release of nitrogen from red macroalgae during emersion which therefore causes fractionation of macroalgal $\delta^{15}N$ values from $\delta^{15}N$-DIN values. Further studies should seek to investigate this effect further and determine whether this would have a significant effect on the potential for *F. vesiculosus* to be used as a biomonitor, perhaps with *in vitro* studies.

3.4.3 Nitrogen loading in the North East

Taking into account the effects of biogeochemical cycling and macroalgal physiology that could affect $\delta^{15}N$ values, the average $\delta^{15}N$ values from each site at each sampling time point can help to identify sources of nitrogen pollution in the region. It can be observed that despite outliers and annual fluctuations, the general (diffuse) pollution source for the River Wear appears to be from that of heavy $\delta^{15}N$ source, i.e., treated sewage or manure (Xue et al. 2009) (Figure 3.15). Given that the upper reaches of the Wear are dominated by agricultural lands (Neal et al. 2000), applications of manure could be polluting the Wear, however given that when discharges increase in spring, the $\delta^{15}N$ values decrease, this is unlikely as any $\delta^{15}N$ signal from manure should in theory be shown as it is washed from fields. Therefore, treated sewage is most likely to be the dominant diffuse pollution source for the River Wear. For point source pollution, untreated sewage from CSO's appears to be the most likely source of pollution (The Rivers Trust, 2022). For the River Tyne, the dominant diffuse pollution source is isotopically lighter, with low $\delta^{15}N$ values, suggesting an input of synthetic fertilisers or chemical/raw sewage effluent, or terrestrial nitrate into the river (Figure 3.15). Given that the Tyne is also dominated by agricultural lands upstream (Environment Agency

and DEFRA, 2009), synthetic fertilisers could be the reason behind the low $\delta^{15}N$ values. However, the same signal is not seen in the River Wear, which also drains agricultural lands, and so this explanation is hard to resolve, unless the proportion of fertiliser derived nitrogen is much greater in the Tyne or farmers used organic sources of fertiliser to a greater extent in the Wear catchment (Xue et al. 2009). The dominant $\delta^{15}N$ signal in the Tyne could originate from point source pollution from untreated sewage being nitrified if the proportion of this pollution is greater than the diffuse pollutants impacting the river (Mariotti et al. 1981, Ahad et al. 2006). Alternatively, the river could be displaying the signal of terrestrial nitrate (Mayer et al. 2002) but given that the river serves a metropolitan area (Newcastle, Gateshead and South Shields) this is unlikely due to the fact there will be anthropogenic inputs into the river. Higher $\delta^{15}N$ values in the summer months could be as a result of a higher input of treated sewage or manure into the Tyne, or a falling proportion of untreated sewage. It is unclear from the data how this trend could be explained and as such, I believe increases in denitrification in the summer is responsible for altering the $\delta^{15}N$ signal in the environment (Baeta et al. 2009). The North East coastline appears to be affected by heavy $\delta^{15}N$ values in the summer months, in a similar manner by which the River Wear and Tyne macroalgal $\delta^{15}N$ values also increased in the summer (Figure 3.15). This suggests that the $\delta^{15}N$ values in coastal macroalgae are coupled to the $\delta^{15}N$ values of estuarine/riverine macroalgae, despite the baseline values differing, the temporal trend is very similar. Overall, the North East coastline could be impacted by treated sewage or manure, by which the signal is diluted by increased discharge from the Wear and Tyne (Figure 3.14) containing more terrestrially derived ^{14}N-enriched nitrogen in the winter. However, given that the Tyne is impacted by low $\delta^{15}N$ pollution, the coastal sites around the Tyne do not reflect this low $\delta^{15}N$ signal, suggesting the coastal signal could be regulated by marine unpolluted nitrogen. This would support the idea that denitrification is at least partially responsible for increasing $\delta^{15}N$ values in summer (Baeta et al. 2009). Overall, the North East is affected by nitrogen pollution from both diffuse and point sources; these sources vary between rivers and potentially temporally, while the coastline appears to be impacted by heavy $\delta^{15}N$ nitrogen sources but follows a similar temporal trend to the estuarine/riverine macroalgae.

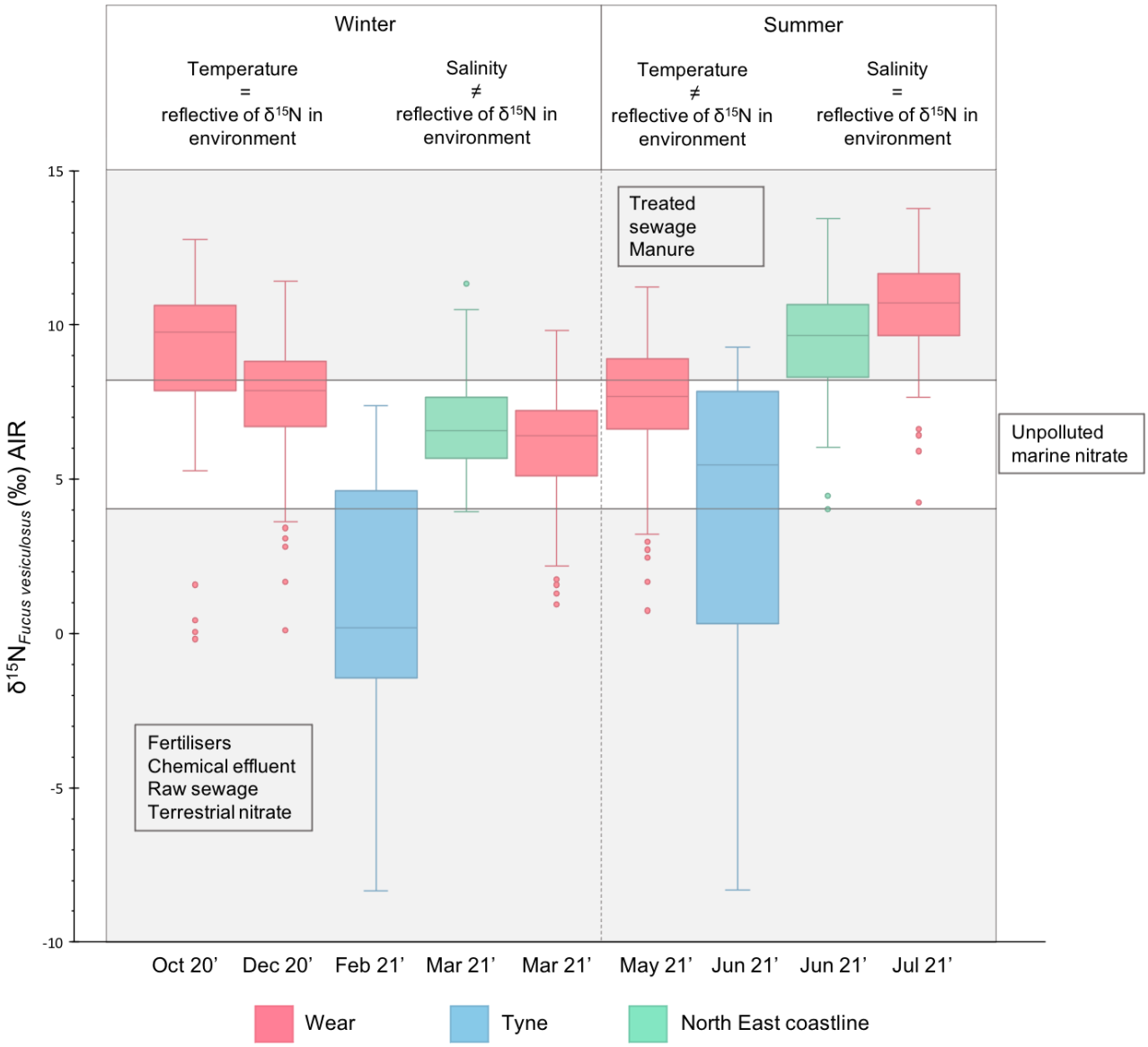


Figure 3.15: δ^{15}N values of F. vesiculosus tips collected from around the River Wear, River Tyne and North East coastline between October 2020 and July 2021. Grey boxes indicate the range of δ^{15}N values in the environment expected from various sources of nitrogen pollution. Also shown is the expected effects of temperature and salinity on δ^{15}N values in macroalgal tips as a function of whether they're reflective of δ^{15}N-DIN on a broad winter vs summer basis, as detailed from the results of Chapter Two.

3.4.4 Limitations

There are of course many other factors that can affect the uptake of δ^{15}N values by macroalgal tips such as light availability and intensity, concentration of DIN, bacterial processes at the surface of the frond, uptake of DON, and emersion relative to immersion. As such, the results from Chapter Two of this thesis cannot fully offer explanation for trends seen in native macroalgae temporally,

but these data do define the effects of temperature and salinity on the uptake of nitrogen isotopes by *F. vesiculosus* tips. Furthermore, due to restrictions on time and resources, then complicated by restrictions due to the Covid-19 pandemic, the North East coastline and River Tyne macroalgae was not able to be sampled as regularly as the River Wear macroalgae. This would've provided better opportunity to compare nitrogen loading in the North East as a whole. To add to this, measurements of water DIN and δ^{15}N-DIN could've helped to ground truth some of the interpretations of the macroalgal δ^{15}N values. Unfortunately, again financial and time restrictions in this study meant this was not possible.

3.5 Conclusions

Overall, the δ^{15}N values of native *F. vesiculosus* tips collected from the rivers Wear and Tyne, and the North East coastline are likely as a result of a combination of factors discussed. There is a significant difference in macroalgal δ^{15}N values between sampling locations for each collection period in both the River Wear and Tyne and also along the North East coastline. δ^{15}N values also vary throughout the year. Due to the effects of macroalgal physiology and biogeochemical factors, δ^{15}N values in *F. vesiculosus* can give an indication of the type and proportion of nitrogen pollution that is affecting an area, but these values cannot be used to accurately predict δ^{15}N-DIN values. Therefore, I recommend the need for long-term monitoring studies using *F. vesiculosus* tips to establish baseline values, over a spatially large area, in order to understand how the sources of nitrogen pollution are changing through space and time.

Conclusions

A comprehensive literature review was undertaken to identify factors that could affect the fractionation of nitrogen isotopes between macroalgal tissue and δ^{15}N-DIN. This review summarised a range of both environmental and biological factors that could cause such fractionation including: nitrogen uptake by macroalgae, nitrogen concentration, nitrogen speciation, other nutrients i.e., phosphate and DON, temperature, biogeochemical cycling, irradiance, salinity and bacterial processes at macroalgal frond. Not all of these potential sources of fractionation have been quantified, only changes temperature and irradiance have been suggested to cause an approximate 1 ‰ difference between macroalgal δ^{15}N and δ^{15}N-DIN (Umezawa et al. 2007; Howarth et al. 2020; Dudley et al. 2010).

Experiments were conducted to assess whether temperature and salinity can have an effect on the uptake of nitrogen isotopes by *F. vesiculosus* and therefore whether fractionation is likely between macroalgal δ^{15}N and δ^{15}N-DIN values. The data suggest that both temperature and salinity have a significant effect on how reflective macroalgal δ^{15}N values are of δ^{15}N-DIN, but there is not an interaction effect between them. After 14 days of incubation in artificial seawater containing isotopically-labelled nitrate, there was a greater than 1 ‰ difference between δ^{15}N and δ^{15}N-DIN. Therefore, it can be assumed that changes in temperature and salinity are likely to cause fractionation between macroalgal δ^{15}N and δ^{15}N-DIN values (given previously documented equilibrium time of two-three weeks) (Bailes and Gröcke, 2020).

A study of δ^{15}N values in *F. vesiculosus* collected periodically around North East England revealed a significant difference in macroalgal δ^{15}N values between sampling locations for each collection period. As such, macroalgal δ^{15}N values were found to vary spatially and temporally. In the River Wear, the dominant source of diffuse pollution was found to be wastewater, i.e., treated sewage or manure, with point source pollution originating from untreated sewage discharging from CSO's. The low δ^{15}N values recorded by Tyne *F. vesiculosus* suggest an input of untreated sewage, synthetic fertilisers or terrestrial nitrate to the river. It is more difficult to resolve this source, but given contextual arguments, the point source inputs of untreated sewage could be greater proportionally than any diffuse pollution and so dominate the δ^{15}N signal recorded. The North East coastline appears to be impacted by heavy δ^{15}N nitrogen sources such as treated sewage or manure in the

summer months but looks to be in the unpolluted marine range over winter. This could be as a result of increased terrestrial nitrogen signals with lower δ^{15}N values diluting the δ^{15}N signal as discharge from rivers increased in winter. However, the extremely low δ^{15}N values recorded by River Tyne macroalgae do not appear to impact coastal macroalgae close to the Tyne. Therefore, the coastal signal may be regulated by the unpolluted marine nitrogen δ^{15}N values, whilst following the same overall trend as the rivers of increasing δ^{15}N values in summer. Overall, using δ^{15}N values in *F. vesiculosus*, this thesis has found that nitrogen pollution in North East England originates from different sources in the Wear and Tyne, affected by treated sewage and untreated sewage/fertilisers, respectively, and the coastline is regulated by unpolluted marine nitrogen. Furthermore, macroalgal δ^{15}N values at the vast majority of sites were higher in the summer collection period than in the winter collection suggesting an impact from biogeochemical cycling, i.e., denitrification. δ^{15}N values in *F. vesiculosus* tips can be used as an indicator of sources of nitrogen loading in the environment, but more work needs to establish potential fractionation between water column and macroalgal δ^{15}N values.

Recommendations for future research

Areas for future research have been highlighted throughout this thesis, however the following areas have been deemed a priority by the author:

- As identified in Chapter One of this thesis, there remains large gaps in the literature regarding the fractionation between macroalgal δ^{15}N and δ^{15}N-DIN and the factors that can cause this fractionation. Future research must seek to address these gaps by conducting experiments to quantify fractionation caused by environmental and biological factors.
- To improve experimental work, δ^{15}N values should be measured in the culture media at frequent points during the course of incubation as well as nitrogen concentrations. Furthermore, conditions should mimic that of the environment, i.e., emersion and immersion should be simulated in the lab to understand the effects on δ^{15}N fractionation. Also, perennial species such as *F. vesiculosus* should be cultured for longer time periods to allow for equilibration.
- To trial *F. vesiculosus* as a biomonitor, longer term (i.e., 1-3 years) studies should be conducted in 'pristine' areas that are known not to be polluted with excess nitrogen, in order

to establish any variations in $\delta^{15}N$ that are as a result of biogeochemical cycling and environmental factors such as temperature and salinity.

- If fractionation was quantified in experimental studies, this could then be applied as an offset to studies conducted in the environment, given that environmental conditions are known and measured.
- Collections of native macroalgae must be sampled at regular intervals throughout the year to ensure proper interpretations of nitrogen loading in an area.
- Sampling water DIN concentrations and $\delta^{15}N$-DIN values could be a helpful measure to help confirm the use of *F. vesiculosus* as a biomonitor, however given the fact that these values can vary greatly in rivers, estuaries and in coastal areas, averaging these values over time would be useful. As such, sampling design and data analysis would need to be carefully considered (DEFRA, 2021).

Supporting Information

A	10 mg/L salinity + $NaNO_3$						
Day of experiment	Jar 1 (‰)	Jar 2 (‰)	Jar 3 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
1	6.15	7.30	6.99	6.82	0.49	0.36	5.87
5	6.21	6.72	7.56	6.83	0.56	0.34	5.58
6	6.28	5.27	7.29	6.28	0.83	0.90	14.61
7	6.59	6.85	6.77	6.74	0.11	0.44	7.14
8	6.69	6.87	6.51	6.69	0.15	0.49	7.87
11	5.24	4.75	5.10	5.03	0.21	2.15	34.82
12	6.37	5.89	4.97	5.74	0.58	1.44	23.26
13	6.58	6.19	3.61	5.46	1.32	1.72	27.83
14	5.50	2.67	5.06	4.41	1.24	2.77	44.77

B	20 mg/L salinity + $NaNO_3$						
Day of experiment	Jar 4 (‰)	Jar 5 (‰)	Jar 6 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
1	8.12	6.84	8.32	7.76	0.66	-0.58	-9.38
5	6.57	5.63	5.95	6.05	0.39	1.13	18.26
6	6.46	7.13	5.41	6.34	0.71	0.84	13.63
7	5.81	7.19	6.08	6.36	0.60	0.82	13.30
8	6.55	5.36	4.37	5.43	0.89	1.75	28.35
11	4.65	4.07	4.15	4.29	0.26	2.89	46.71
12	3.70	5.60	5.93	5.08	0.98	2.10	34.03
13	4.03	5.17	3.05	4.08	0.87	3.09	50.08

14	2.61	4.84	4.82	4.09	1.05	3.09	49.95

C	35 mg/L salinity + $NaNO_3$						
Day of experiment	Jar 7 (‰)	Jar 8 (‰)	Jar 9 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
1	4.92	7.37	5.80	6.03	1.01	1.15	18.57
5	7.05	6.40	7.00	6.82	0.30	0.36	5.82
6	6.79	5.30	5.87	5.99	0.62	1.19	19.25
7	4.30	5.45	4.35	4.70	0.53	2.48	40.09
8	6.03	5.43	4.75	5.40	0.52	1.78	28.78
11	5.22	4.17	4.49	4.63	0.44	2.55	41.30
12	4.85	4.14	2.42	3.80	1.02	3.38	54.64
13	3.61	3.76	2.58	3.32	0.52	3.86	62.46
14	4.44	4.78	5.58	4.94	0.48	2.24	36.30

D	35 mg/L salinity						
Day of experiment	Jar 10 (‰)	Jar 11 (‰)	Jar 12 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
1	7.40	6.86	6.67	6.98	0.31	0.20	3.23
5	8.24	8.43	7.92	8.19	0.21	-1.02	-16.45
6	8.27	6.77	7.20	7.42	0.63	-0.24	-3.84
7	7.48	5.46	6.21	6.38	0.83	0.80	12.89
8	6.15	7.04	6.58	6.59	0.37	0.59	9.55
11	8.97	6.73	8.35	8.02	0.94	-0.84	-13.59
12	8.17	7.08		**7.62**	**0.55**	**-0.45**	**-7.23**
13	6.05	5.02	5.94	5.67	0.46	1.51	24.42
14	5.68	5.59	5.73	5.66	0.06	1.51	24.50

Table S1: Raw, average and standard deviations of $\delta^{15}N$ values of *F. vesiculosus* tips cultured at 10°C, in solutions of 10 mg/L salinity + $NaNO_3$ (A), 20 mg/L salinity + $NaNO_3$ (B), 35 mg/L salinity + $NaNO_3$ (C), and 35 mg/L salinity (D), over a period of 14 days. Also presented are the calculated $\Delta^{15}N$ (‰) and % equilibrium (methods for calculation in thesis). Values in bold indicate data are missing from the calculated values. Missing values were as a result of errors during the running of the mass spectrometer.

A	10 mg/L salinity + $NaNO_3$						
Day of experiment	Jar 1 (‰)	Jar 2 (‰)	Jar 3 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
1	6.77	6.50	6.77	6.68	0.13	0.60	9.57
4	5.44	5.96	5.79	5.73	0.22	1.55	24.69
5	5.90	5.65	6.07	5.87	0.17	1.41	22.48
6	7.01	6.02	7.13	6.72	0.50	0.56	8.93
7	5.63	5.21	5.24	5.36	0.19	1.92	30.62
11	5.12	4.79	5.30	5.07	0.21	2.21	35.19
12	6.17	4.89	5.22	5.43	0.54	1.85	29.52
13	4.17	3.47	3.43	3.69	0.34	3.59	57.15
14	5.97	4.23	4.40	4.87	0.78	2.41	38.41

B	20 mg/L salinity + NaNO3						
Day of experiment	Jar 4 (‰)	Jar 5 (‰)	Jar 6 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
1	7.39	7.01	6.87	7.09	0.22	0.19	3.07
4	6.36	6.45	6.26	6.36	0.07	0.93	14.73
5	6.64	6.60	6.88	6.71	0.12	0.57	9.15
6	4.49	5.75	7.71	5.98	1.33	1.30	20.68
7	4.48	6.57	5.72	5.59	0.86	1.69	26.95
11	5.24	1.95	3.05	3.41	1.37	3.87	61.60
12	2.87	4.32		**3.60**	**0.73**	**3.69**	**58.67**
13	2.98	4.95	4.99	4.31	0.94	2.98	47.38

14	5.51	4.01	5.64	5.06	0.74	2.23	35.45

C	35 mg/L salinity + NaNO3						
Day of experiment	Jar 7 (‰)	Jar 8 (‰)	Jar 9 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
1	5.87	6.86	5.75	6.16	0.50	1.12	17.90
4	7.88	6.01	6.38	6.76	0.81	0.53	8.36
5	4.31	5.21	8.18	5.90	1.65	1.38	21.98
6	5.50	4.39	4.27	4.72	0.55	2.56	40.78
7		4.52	4.68	**4.60**	**0.08**	**2.68**	**42.65**
11	3.94	4.31	4.23	4.16	0.16	3.12	49.72
12	2.72	4.65	4.37	3.91	0.85	3.37	53.61
13	2.66	3.01	2.83	2.83	0.14	4.45	70.83
14	4.01	4.07	4.05	4.05	0.02	3.24	51.51

D	35 mg/L salinity						
Day of experiment	Jar 10 (‰)	Jar 11 (‰)	Jar 12 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
1	7.30	6.57	7.22	7.03	0.33	0.25	4.00
4	8.64	7.15	8.74	8.18	0.73	-0.89	-14.22
5	7.41	7.32	7.63	7.45	0.13	-0.17	-2.72
6	8.22	6.33	7.93	7.49	0.83	-0.21	-3.32
7	7.48		7.33	**7.40**	**0.08**	**-0.12**	**-1.93**
11	7.36	8.39	8.43	8.06	0.49	-0.78	-12.39
12	8.16	7.45	7.95	7.85	0.30	-0.57	-9.08
13	7.58	8.46	7.41	7.82	0.46	-0.53	-8.48
14	7.14	8.80	7.62	7.85	0.70	-0.57	-9.07

Table S2: Raw, average and standard deviations of $\delta^{15}N$ values of Fucus vesiculosus (F. vesiculosus) tips cultured at 5°C, in solutions of 10 mg/L salinity + $NaNO_3$ (A), 20 mg/L salinity + $NaNO_3$ (B), 35 mg/L salinity + $NaNO_3$ (C), and 35 mg/L salinity (D), over a period of 14 days. Also presented are the calculated $\Delta^{15}N$ (‰) and % equilibrium (methods for calculation in thesis). Values in bold indicate data are missing from the calculated values. Missing values were as a result of errors during the running of the mass spectrometer.

A	10 mg/L salinity + $NaNO_3$						
Day of experiment	Jar 1 (‰)	Jar 2 (‰)	Jar 3 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
3	8.49	9.38	9.76	9.21	0.53	0.28	3.28
4	9.09	9.24	6.69	8.34	1.17	1.15	13.55
5	8.74	9.93	9.78	9.48	0.53	0.00	0.04
6	10.16	8.53	8.94	9.21	0.69	0.28	3.25
7	8.46	8.01	8.70	8.39	0.29	1.10	12.96
11	8.58	6.84	7.65	7.69	0.71	1.80	21.16
12	8.36	7.90	8.21	8.16	0.19	1.33	15.66
13	7.87	7.95	7.53	7.78	0.18	1.70	20.05
14	8.84	9.15	9.24	9.08	0.17	0.41	4.84

B	20 mg/L salinity + $NaNO_3$						
Day of experiment	Jar 4 (‰)	Jar 5 (‰)	Jar 6 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
3	9.69	9.11	10.34	9.71	0.50	-0.22	-2.64
4	9.92	8.33	9.45	9.23	0.67	0.25	2.97
5	9.78	10.06	9.58	9.80	0.20	-0.32	-3.74
6	7.99	8.81	8.01	8.27	0.38	1.22	14.34
7	9.00	9.07	8.52	8.86	0.25	0.62	7.33
11	8.42	8.05	9.01	8.49	0.40	0.99	11.69
12	7.68	7.70	7.82	7.73	0.06	1.75	20.66
13	7.55	9.36	10.70	9.20	1.29	0.28	3.33

14	7.40	6.78	9.06	7.75	0.96	1.74	20.51

C	35 mg/L salinity + NaNO3						
Day of experiment	Jar 7 (‰)	Jar 8 (‰)	Jar 9 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
3	9.02	9.78	8.79	9.20	0.42	0.29	3.42
4	9.71	8.73	8.92	9.12	0.43	0.37	4.31
5	7.29	8.11	8.23	7.88	0.42	1.61	18.97
6	8.87	7.90		**8.39**	**0.49**	**1.10**	**12.97**
7	9.34	7.98	8.80	8.71	0.56	0.78	9.19
11	8.43	8.22	6.36	7.67	0.93	1.82	21.41
12	8.49	8.13	8.77	8.47	0.27	1.02	12.03
13	7.73	8.37	9.16	8.42	0.58	1.07	12.59
14	8.66	6.85	8.41	7.97	0.80	1.52	17.88

D	35 mg/L salinity						
Day of experiment	Jar 10 (‰)	Jar 11 (‰)	Jar 12 (‰)	Average $\delta^{15}N$ (‰)	Std. deviation (‰)	$\Delta^{15}N$ (‰)	% equilibrium
3	11.85	10.36	10.28	10.83	0.72	-1.34	-15.81
4	9.82	9.82	10.87	10.17	0.50	-0.68	-8.07
5	9.73	11.10	10.41	10.42	0.56	-0.93	-10.94
6	9.28	9.44	9.89	9.54	0.26	-0.05	-0.59
7	8.49	9.35	10.20	9.34	0.70	0.14	1.68
11	8.72	9.34	10.10	9.39	0.57	0.10	1.17
12	9.64	9.64	10.47	9.92	0.39	-0.43	-5.08
13	10.11	10.02	9.16	9.76	0.43	-0.28	-3.27
14	10.38	9.89	9.65	9.97	0.31	-0.49	-5.74

Table S3: Raw, average and standard deviations of $\delta^{15}N$ values of Fucus vesiculosus (F. vesiculosus) tips cultured at 15℃, in solutions of 10 mg/L salinity + $NaNO_3$ (A), 20 mg/L salinity + $NaNO_3$ (B), 35 mg/L salinity + $NaNO_3$ (C), and 35 mg/L salinity (D), over a period of 14 days. Also presented are the calculated $\Delta^{15}N$ (‰) and % equilibrium (methods for calculation in thesis). Values in bold indicate data are missing from the calculated values. Missing values were as a result of errors during the running of the mass spectrometer.

A	10℃							
Day of experiment	Jar 1 (mg/L)	Jar 4 (mg/L)	Jar 7 (mg/L)	Jar 10 (mg/L)	10 mg/L salinity + $NaNO_3$ (mg/L)	20 mg/L salinity + $NaNO_3$ (mg/L)	35 mg/L salinity + $NaNO_3$ (mg/L)	35 mg/L salinity (mg/L)
0					11.46	11.62	12.07	0.039
5	3.95	3.05	3.06	<0.002	11.86	11.41	11.28	0.037
8	9.491	8.892	8.626	0.005	10.93	10.2	10.36	0.036
12	9.921	8.819	8.481	<0.002	10.581	2.956	3.444	0.038
14	10.503	2.708	2.842	<0.002				

B	5℃							
Day of experiment	Jar 1 (mg/L)	Jar 4 (mg/L)	Jar 7 (mg/L)	Jar 10 (mg/L)	10 mg/L salinity + $NaNO_3$ (mg/L)	20 mg/L salinity + $NaNO_3$ (mg/L)	35 mg/L salinity + $NaNO_3$ (mg/L)	35 mg/L salinity (mg/L)
0					12.31	11.78	11.78	0.04
4	5.13	4.05	4.96	<0.002	11.54	11.35	10.9	0.037
7	8.26	8.24	8.62	<0.002	12.36	11.68	11.48	0.025
11	10.112	8.752	8.949	<0.002	12.880		11.984	0.030
14	12.047	11.82	11.73	0.037				

C	15℃

Day of experiment	Jar 1 (mg/L)	Jar 4 (mg/L)	Jar 7 (mg/L)	Jar 10 (mg/L)	10 mg/L salinity + $NaNO_3$ (mg/L)	20 mg/L salinity + $NaNO_3$ (mg/L)	35 mg/L salinity + $NaNO_3$ (mg/L)	35 mg/L salinity (mg/L)
0					11.72	10.22	10.81	0.003
4	5.13	4.05	4.96	<0.002	11.63	13.06	13.24	0.032
7	8.91	10.98	11.15	<0.002	11.9	12.02	11.66	0.033
11	8.57	10.82	10.51	<0.002	12.1	11.86	11.74	0.036
14	9.38	11.84	11.56	<0.002				

Table S4: Nitrate concentrations of water samples taken on given days of each experiment at 10℃, 5℃ and 15℃ (A, B and C respectively). Concentrations are given in mg/L. Water samples taken from jars were from after culturing macroalgal tips, whereas solution samples were taken as soon as each solution was prepared. All water samples were stored at 4℃ before analysis.

	Experiment		
	10℃	5℃	15℃
$\delta^{15}N$ (‰)	6.55	8.01	8.25
	5.54	7.00	8.83
	8.04	6.34	9.46
	7.74	7.58	8.95
	6.49	7.60	9.92
	7.41	8.26	10.52
	7.24	7.19	10.22
	7.98	6.32	10.80
	7.43	7.36	8.24
	7.36	7.15	9.67
Average $\delta^{15}N$ (‰)	7.18	7.28	9.49

Std. deviation (‰)	0.73	0.60	0.86
Date of collection	27/05/2021	11/06/2021	25/06/2021

Table S5: $\delta^{15}N$ values of *Fucus vesiculosus* tips collected from site Coast 2 before tips were cultured in various solutions. Average initial collection values were used to calculate $\Delta^{15}N$ values. The date of collection is given for each round of macroalgae collected.

Dependent Variable: D15N

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	21.783[a]	8	2.723	4.020	0.008	0.654
Intercept	125.370	1	125.370	185.092	0.000	0.916
Temperature	10.884	2	5.442	8.034	0.003	0.486
Salinity	6.034	2	3.017	4.454	0.028	0.344
Temperature * Salinity	6.008	4	1.502	2.218	0.110	0.343
Error	11.515	17	0.677			
Total	154.301	26				
Corrected Total	33.298	25				

a. R Squared = .654 (Adjusted R Squared = .491)

Table S6 : ANOVA test comparing the $\Delta^{15}N$ values of *F. vesiculosus* tips cultured in isotopically-labelled artificial seawater solutions containing $NaNO_3$ with varying temperature and salinity. A significance level of 0.05 was used, meaning p values less than or equal to 0.05 represent a statistically significant result, i.e., there is less than a 5% probability the null hypothesis is correct. Generated using SPSS software.

Dependent Variable: D15N

Tukey HSD

(I) Temperature	(J) Temperature	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5℃	10℃	0.5756	0.39991	0.344	-0.4504	1.6015
	15℃	1.5122*	0.39991	0.004	0.4863	2.5381
10℃	5℃	-0.5756	0.39991	0.344	-1.6015	0.4504
	15℃	0.9367	0.38797	0.067	-0.0586	1.9319
15℃	5℃	-1.5122*	0.39991	0.004	-2.5381	-0.4863
	10℃	-0.9367	0.38797	0.067	-1.9319	0.0586

Based on observed means.
The error term is Mean Square(Error) = .677.

*. The mean difference is significant at the .05 level.

Table S7: Post-hoc Tukey test for the variable Temperature on $\Delta^{15}N$ values, generated using a two-way ANOVA in SPSS.

Dependent Variable: D15N

Tukey HSD

(I) Salinity	(J) Salinity	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
10 mg/L	20 mg/L	-0.8275	0.39991	0.126	-1.8534	0.1984
	35 mg/L	-1.0478*	0.38797	0.038	-2.0431	-0.0525
20 mg/L	10 mg/L	0.8275	0.39991	0.126	-0.1984	1.8534
	35 mg/L	-0.2203	0.39991	0.847	-1.2462	0.8056
35 mg/L	10 mg/L	1.0478*	0.38797	0.038	0.0525	2.0431
	20 mg/L	0.2203	0.39991	0.847	-0.8056	1.2462

Based on observed means.
The error term is Mean Square(Error) = .677.

*. The mean difference is significant at the .05 level.

Table S8: Post-hoc Tukey test for the variable Salinity on $\Delta^{15}N$ values, generated using a two-way ANOVA in SPSS.

Site name	Latitude	Longitude
Wear 1	**54°55'10.8"N**	**1°21'47.7"W**
Wear 2	**54°55'08.7"N**	**1°21'46.7"W**
Wear 3	**54°55'08.6"N**	**1°21'43.0"W**
Wear 4	**54°55'08.3"N**	**1°21'41.0"W**
Wear 5	**54°55'06.8"N**	**1°21'43.5"W**
Wear 6	**54°55'00.7"N**	**1°22'04.4"W**
Wear 7	**54°54'58.4"N**	**1°22'09.1"W**
Wear 8	**54°54'43.7"N**	**1°22'14.6"W**
Wear 9	**54°54'40.8"N**	**1°23'21.1"W**
Wear 10	**54°55'02.3"N**	**1°23'36.7"W**
Wear 11	**54°54'51.6"N**	**1°24'33.7"W**
Wear 12	**54°55'04.2"N**	**1°25'15.6"W**

Wear 13	**54°55'03.6"N**	**1°25'28.0"W**
Wear 14	**54°55'03.0"N**	**1°25'30.4"W**
Wear 15	**54°55'02.5"N**	**1°25'37.9"W**
Wear 16	**54°54'59.1"N**	**1°25'46.8"W**
Wear 17	**54°54'33.5"N**	**1°27'03.1"W**
Wear 18	**54°54'27.7"N**	**1°27'12.3"W**
Wear 19	**54°54'37.2"N**	**1°26'55.8"W**
Wear 20	**54°54'41.6"N**	**1°26'41.6"W**
Wear 21	**54°54'42.4"N**	**1°26'08.8"W**
Wear 22	**54°54'42.8"N**	**1°26'07.1"W**
Wear 23	**54°54'46.8"N**	**1°26'01.7"W**
Wear 24	**54°54'43.4"N**	**1°26'05.3"W**

Table S9: Latitude and longitude values for each site location around the River Wear. Google Maps was used to log the locations of sites, and hence values are an approximation of the exact location of sampling.

Site name	Latitude	Longitude
Coast 1	**55°00'17.4"N**	**1°24'51.6"W**

Coast 2	**55°00'18.4"N**	**1°24'50.6"W**
Coast 3	**55°00'20.4"N**	**1°24'48.5"W**
Coast 4	**55°00'21.7"N**	**1°24'46.2"W**
Coast 6	**54°58'41.1"N**	**1°22'36.5"W**
Coast 7	**54°58'38.0"N**	**1°22'33.4"W**
Coast 8	**54°58'36.4"N**	**1°22'32.8"W**
Coast 9	**54°58'52.7"N**	**1°22'50.3"W**
Coast 10	**54°58'03.9"N**	**1°21'34.1"W**
Coast 11	**54°56'40.7"N**	**1°21'40.4"W**
Coast 12	**54°56'37.2"N**	**1°21'41.0"W**
Coast 13	**54°55'54.5"N**	**1°21'57.0"W**
Coast 14	**54°52'15.4"N**	**1°21'11.3"W**
Coast 15	**54°52'12.5"N**	**1°21'09.1"W**
Coast 16	**54°51'29.0"N**	**1°20'48.4"W**
Coast 17	**54°51'34.4"N**	**1°20'49.7"W**
Coast 18	**54°51'34.7"N**	**1°20'51.2"W**
Coast 19	**54°44'47.2"N**	**1°16'12.1"W**
Coast 20	**54°43'49.1"N**	**1°15'05.5"W**
Coast 21	**54°43'55.8"N**	**1°15'15.3"W**
Coast 22	**54°44'04.9"N**	**1°15'26.4"W**
Coast 23	**54°42'14.1"N**	**1°11'36.0"W**
Coast 24	**54°42'11.1"N**	**1°11'25.0"W**
Coast 25	**54°42'07.1"N**	**1°11'14.6"W**
Coast 27	**54°37'08.6"N**	**1°03'29.8"W**
Coast 28	**54°37'07.8"N**	**1°03'21.4"W**

Table S10: Latitude and longitude values for each site location along the North East coastline. Google Maps was used to log the locations of sites, and hence values are an approximation of the exact location of sampling.

Site name	Latitude	Longitude
Tyne 1	**55°00'36.9"N**	**1°25'55.4"W**
Tyne 2	**55°00'47.2"N**	**1°25'48.7"W**
Tyne 3	**55°00'35.1"N**	**1°25'55.2"W**
Tyne 4	**55°00'57.8"N**	**1°25'01.9"W**
Tyne 5	**55°00'59.8"N**	**1°25'01.4"W**
Tyne 6	**55°01'11.8"N**	**1°25'12.6"W**
Tyne 7	**55°01'07.9"N**	**1°25'03.2"W**
Tyne 8	**54°57'37.9"N**	**1°32'58.0"W**
Tyne 9	**54°57'40.4"N**	**1°33'25.3"W**
Tyne 10	**54°57'49.5"N**	**1°34'42.6"W**
Tyne 11	**55°00'27.1"N**	**1°25'29.0"W**

Table S11: Latitude and longitude values for each site location around the River Tyne. Google Maps was used to log the locations of sites, and hence values are an approximation of the exact location of sampling.

Oct-20		**Dec-20**		**Mar-21**		**May-21**		**Jul-21**	
Sample ID	**$\delta^{15}N$ (‰)**	**Sample ID**	**$\delta^{15}N$ (‰)**	**Sample ID**	**$\delta^{15}N$ (‰)**	**Sample ID**	**$\delta^{15}N$ (‰)**	**Sample ID**	**$\delta^{15}N$ (‰)**
WEAR 1-1	10.49	WEAR 1-1	9.19	WEAR 1-1	7.64	WEAR 1-1	8.54	WEAR 1-1	12.57
WEAR 1-2	7.38	WEAR 1-2	10.01	WEAR 1-2	7.07	WEAR 1-2	7.61	WEAR 1-1 R	12.59
WEAR 1-2 R	9.15	WE-1-2 R	9.43	WEAR 1-2 R	6.81	WEAR 1-2 R	8.19	WEAR 1-2	12.40

WEAR 1-3	11.16	WEAR 1-3	10.29	WEAR 1-3	7.32	WEAR 1-3	7.37	WEAR 1-3	13.66
WEAR 1-4	10.99	WEAR 1-4	9.42	WEAR 1-4	5.75	WEAR 1-4	7.58	WEAR 1-3 R	13.78
WEAR 1-4 R	10.87	WEAR 1-4 R	9.59	WEAR 1-4 R	5.99	WEAR 1-4 R	8.08	WEAR 1-4	12.59
WEAR 1-5	11.12	WEAR 2-1	8.74	WEAR 1-5	7.48	WEAR 1-5	8.94	WEAR 1-5	12.63
WEAR 1-6	10.11	WEAR 2-2	9.42	WEAR 3-1	4.90	WEAR 2-1	8.90	WEAR 1-5 R	12.41
WEAR 1-6 R	9.70	WEAR 2-2 R	8.42	WEAR 3-1 R	4.93	WEAR 2-1 R	9.03	WEAR 2-1	5.91
WEAR 2-1	11.28	WEAR 2-3	7.46	WEAR 3-2	5.54	WEAR 2-2	9.31	WEAR 2-2	13.17
WEAR 2-2	10.00	WEAR 2-4	7.24	WEAR 3-3	5.47	WEAR 2-2 R	9.23	WEAR 2-2 R	12.69
WEAR 2-2 R	10.48	WEAR 2-4 R	2.19	WEAR 3-3 R	5.94	WEAR 2-3	9.39	WEAR 2-3	12.18
WEAR 2-3	10.75	WEAR 2-5	7.52	WEAR 3-4	5.59	WEAR 2-4	9.32	WEAR 2-4	12.23
WEAR 2-4	9.98	WEAR 3-1	5.44	WEAR 3-5	5.41	WEAR 2-5	8.83	WEAR 2-4 R	12.71
WEAR 2-4 R	9.24	WEAR 3-1 R	4.62	WEAR 3-5 R	6.46	WEAR 2-5 R	9.10	WEAR 2-5	12.11
WEAR 2-5	11.39	WEAR 3-2	7.24	WEAR 4-1	7.67	WEAR 3-1	8.61	WEAR 3-5	13.16
WEAR 3-1	6.74	WEAR 3-3	5.05	WEAR 4-2	7.50	WEAR 3-1 R	8.92	WEAR 3-5 R	12.50
WEAR 3-1 R	6.76	WEAR 3-3 R	4.02	WEAR 4-2 R	7.52	WEAR 3-2	8.36	WEAR 3-1	13.49
WEAR 3-2	6.33	WEAR 3-4	6.54	WEAR 4-3	6.93	WEAR 3-3	9.85	WEAR 3-2	12.76
WEAR 3-3	6.80	WEAR 3-5	3.62	WEAR 4-4	7.58	WEAR 3-3 R	10.06	WEAR 3-2 R	12.32

WEAR 3-3 R	6.74	WEAR 3-5 R	4.48	WEAR 4-4 R	6.76	WEAR 3-4	8.96	WEAR 3-3	13.62
WEAR 3-4	5.89	WEAR 4-1	7.85	WEAR 4-5	6.80	WEAR 3-5	7.98	WEAR 3-4	13.69
WEAR 3-5	7.83	WEAR 4-2	4.65	WEAR 5-1	6.81	WEAR 4-1	9.54	WEAR 3-4 R	13.01
WEAR 3-5 R	7.52	WEAR 4-3	8.44	WEAR 5-1 R	8.10	WEAR 4-1 R	9.92	WEAR 4-1	12.17
WEAR 4-1	6.62	WEAR 4-3 R	7.59	WEAR 5-2	7.00	WEAR 4-2	9.86	WEAR 4-2	12.55
WEAR 4-2	5.80	WEAR 4-4	7.06	WEAR 5-3	6.64	WEAR 4-3	10.17	WEAR 4-2 R	12.30
WEAR 4-2 R	6.17	WEAR 4-5	8.32	WEAR 5-3 R	7.69	WEAR 4-3 R	11.22	WEAR 4-3	11.62
WEAR 4-3	5.44	WEAR 4-5 R	7.83	WEAR 5-4	6.36	WEAR 4-4	9.48	WEAR 4-4	12.73
WEAR 4-4	5.28	WEAR 5-1	8.90	WEAR 5-5	6.21	WEAR 4-5	9.86	WEAR 4-4 R	12.51
WEAR 4-4 R	5.67	WEAR 5-2	7.15	WEAR 5-5 R	6.65	WEAR 4-5 R	10.33	WEAR 4-5	12.29
WEAR 5-1	10.15	WEAR 5-2 R	7.70	WEAR 8-1	7.55	WEAR 5-1	9.68	WEAR 5-1	12.92
WEAR 5-2	9.88	WEAR 5-3	8.64	WEAR 8-2	7.55	WEAR 5-2	8.24	WEAR 5-1 R	12.91
WEAR 5-2 R	9.77	WEAR 5-4	7.33	WEAR 8-2 R	7.97	WEAR 5-2 R	8.37	WEAR 5-2	11.89
WEAR 5-3	12.78	WEAR 5-4 R	6.42	WEAR 8-3	6.83	WEAR 5-3	8.57	WEAR 5-3	11.42
WEAR 5-4	11.23	WEAR 5-5 A	9.30	WEAR 8-4	7.51	WEAR 5-3 R	8.90	WEAR 5-3 R	12.05
WEAR 5-4 R	10.95	WEAR 5-5 B	7.81	WEAR 8-4 R	6.70	WEAR 5-4	9.65	WEAR 5-4	10.84
WEAR 5-5	10.05	WEAR 8-1	8.57	WEAR 8-5	7.42	WEAR 5-5	9.52	WEAR 5-5	11.01

WEAR 5-6	9.84	WEAR 8-2	8.81	WEAR 9-1	7.10	WEAR 8-1	8.61	WEAR 5-5 R	10.95
WEAR 5-6 R	9.38	WEAR 8-3	8.81	WEAR 9-1 R	7.08	WEAR 8-2	8.31	WEAR 8-1	10.12
WEAR 6-1	10.42	WEAR 8-3 R	9.14	WEAR 9-2	9.24	WEAR 8-2 R	9.28	WEAR 8-1 R	10.55
WEAR 6-2	9.95	WEAR 8-4	10.77	WEAR 9-3	7.49	WEAR 8-3	7.92	WEAR 8-2	10.97
WEAR 6-2 R	10.44	WEAR 8-5	11.06	WEAR 9-3 R	8.12	WEAR 8-4	7.37	WEAR 8-3	12.98
WEAR 6-3	10.65	WEAR 8-5 R	11.43	WEAR 9-4	7.14	WEAR 8-4 R	7.47	WEAR 8-3 R	13.22
WEAR 6-3 R	10.49	WEAR 9-1	8.55	WEAR 9-5	7.77	WEAR 8-5	6.89	WEAR 8-4	10.66
WEAR 6-4	9.77	WEAR 9-2	9.08	WEAR 9-5 R	7.74	WEAR 9-1	8.56	WEAR 8-5	11.32
WEAR 6-5	10.75	WEAR 9-2 R	9.59	WEAR 10-1	8.53	WEAR 9-2	9.74	WEAR 9-1	9.75
WEAR 6-5 R	10.40	WEAR 9-3	10.18	WEAR 10-2	7.01	WEAR 9-2 R	9.45	WEAR 9-1 R	9.36
WEAR 6-6	9.86	WEAR 9-4	8.82	WEAR 10-2 R	7.05	WEAR 9-3	8.01	WEAR 9-2	9.81
WEAR 7-1	8.96	WEAR 9-4 R	9.66	WEAR 10-3	7.81	WEAR 9-4	8.17	WEAR 9-3	9.16
WEAR 7-1 R	8.66	WEAR 9-5	8.12	WEAR 10-4	9.04	WEAR 9-4 R	8.59	WEAR 9-3 R	9.70
WEAR 7-2	7.40	WEAR 10-1	8.10	WEAR 10-4 R	8.76	WEAR 9-5	9.40	WEAR 9-4	10.82
WEAR 7-3	8.51	WEAR 10-1 R	8.89	WEAR 10-5	6.26	WEAR 9-5 R	9.47	WEAR 9-5	10.82
WEAR 7-3 R	8.38	WEAR 10-2	8.68	WEAR 11-1	3.90	WEAR 10-1	7.36	WEAR 9-5 R	11.38
WEAR 7-4	7.95	WEAR 10-3	7.27	WEAR 11-1 R	3.86	WEAR 10-2	7.40	WEAR 10-1	10.59

WEAR 7-5	8.26	WEAR 10-3 R	8.36	WEAR 11-2	6.60	WEAR 10-2 R	8.28	WEAR 10-2	8.88
WEAR 7-5 R	8.10	WEAR 10-4	6.80	WEAR 11-3	2.69	WEAR 10-3	8.49	WEAR 10-2 R	8.10
WEAR 8-1	10.06	WEAR 10-5	9.28	WEAR 11-3 R	2.94	WEAR 10-3 R	8.55	WEAR 10-3	10.41
WEAR 8-2	11.33	WEAR 10-5 R	9.26	WEAR 11-4	6.01	WEAR 10-4	3.57	WEAR 10-4	10.53
WEAR 8-2 R	11.42	WEAR 11-1	4.71	WEAR 11-5	4.12	WEAR 10-5	4.74	WEAR 10-4 R	10.38
WEAR 8-3	10.50	WEAR 11-2	4.29	WEAR 12-1	3.71	WEAR 11-1	7.71	WEAR 10-5	10.00
WEAR 8-4	10.95	WEAR 11-2 R	4.52	WEAR 12-1 R	3.89	WEAR 11-1 R	7.98	WEAR 11-1	9.04
WE-8-4 R	10.87	WEAR 11-3	3.08	WEAR 12-2	5.99	WEAR 11-2	8.84	WEAR 11-1 R	8.42
WEAR 8-5	11.13	WEAR 11-4	8.07	WEAR 12-3	6.41	WEAR 11-3	6.28	WEAR 11-2	9.63
WEAR 8-6	10.51	WEAR 11-4 R	7.75	WEAR 12-3 R	6.63	WEAR 11-3 R	5.36	WEAR 11-3	10.05
WEAR 8-6 R	10.96	WEAR 11-5	4.48	WEAR 12-4	6.43	WEAR 11-4	6.72	WEAR 11-3 R	9.65
WEAR 8-7	11.07	WEAR 12-1	8.67	WEAR 12-5	7.25	WEAR 11-5	6.62	WEAR 11-4	9.65
WEAR 9-1	10.92	WEAR 12-1 R	8.38	WEAR 13-1	7.06	WEAR 11-5 R	6.95	WEAR 11-5	11.24
WEAR 9-1 R	11.01	WEAR 12-2	8.19	WEAR 13-1 R	6.61	WEAR 12-1	7.13	WEAR 11-5 R	10.16
WEAR 9-2	10.95	WEAR 12-3	8.65	WEAR 13-2	4.15	WEAR 12-2	7.57	WEAR 12-1	9.94
WEAR 9-3	11.33	WEAR 12-3 R	8.57	WEAR 13-3	6.61	WEAR 12-2 R	7.51	WEAR 12-2	11.58
WEAR 9-3 R	11.01	WEAR 12-4	8.38	WEAR 13-3 R	6.97	WEAR 12-3	8.02	WEAR 12-2 R	11.26

WEAR 9-4	10.60	WEAR 12-5	9.60	WEAR 13-4	6.20	WEAR 12-4	4.40	WEAR 12-3	11.85
WEAR 9-5	10.09	WEAR 12-5 R	9.68	WEAR 13-5	5.31	WEAR 12-4 R	4.75	WEAR 12-4	10.80
WEAR 9-5 R	9.67	WEAR 13-1	9.20	WEAR 13-5 R	5.02	WEAR 12-5	7.64	WEAR 12-4 R	10.69
WEAR 9-6	10.46	WEAR 13-2	7.96	WEAR 14-1	6.91	WEAR 13-1	9.32	WEAR 12-5	11.01
WEAR 10-1	-0.18	WEAR 13-3	8.82	WEAR 14-2	2.98	WEAR 13-1 R	9.75	WEAR 13-1	10.99
WEAR 10-1 R	0.44	WEAR 13-3 R	9.09	WEAR 14-2 R	2.99	WEAR 13-2	9.90	WEAR 13-1 R	11.44
WEAR 10-2	0.05	WEAR 13-4	7.39	WEAR 14-3	7.35	WEAR 13-3	7.47	WEAR 13-2	10.84
WEAR 10-3	1.58	WEAR 13-5	8.75	WEAR 14-4	6.00	WEAR 13-3 R	8.51	WEAR 13-3	10.56
WEAR 10-3 R	1.64	WEAR 13-5 R	8.65	WEAR 14-4 R	6.37	WEAR 13-4	7.80	WEAR 13-3 R	9.64
WEAR 10-4	0.18	WEAR 14-1	6.72	WEAR 14-5	5.13	WEAR 13-5	6.51	WEAR 13-4	10.38
WEAR 10-5	0.14	WEAR 14-2	7.21	WEAR 15-1	5.01	WEAR 13-5 R	7.00	WEAR 13-5	10.40
WEAR 10-5 R	0.52	WEAR 14-2 R	7.08	WEAR 15-1 R	4.65	WEAR 16-1	6.18	WEAR 13-5 R	10.69
WEAR 11-1	7.69	WEAR 14-3	5.93	WEAR 15-2	5.24	WEAR 16-2	2.97	WEAR 16-1	10.54
WEAR 11-2	8.11	WEAR 14-4	5.28	WEAR 15-3	4.57	WEAR 16-2 R	2.81	WEAR 16-2	10.70
WEAR 11-2 R	7.57	WEAR 14-4 R	4.94	WEAR 15-3 R	4.71	WEAR 16-3	7.51	WEAR 16-2 R	10.79
WEAR 11-3	6.79	WEAR 14-5	8.15	WEAR 15-4	5.46	WEAR 16-4	7.68	WEAR 16-3	10.46
WEAR 11-4	9.76	WEAR 15-1	8.79	WEAR 15-5	6.98	WEAR 16-4 R	8.04	WEAR 16-4	10.72

WEAR 11-4 R	9.69	WEAR 15-2	8.51	WEAR 15-5 R	7.57	WEAR 16-5	7.76	WEAR 16-4 R	10.61
WEAR 11-5	9.51	WEAR 15-2 R	8.45	WEAR 16-1	5.69	WEAR 17-1	3.23	WEAR 16-5	10.71
WEAR 12-1	10.64	WEAR 15-3	8.22	WEAR 16-2	4.98	WEAR 17-1 R	3.59	WEAR 17-1	10.89
WEAR 12-1 R	9.76	WEAR 15-4	8.61	WEAR 16-2 R	5.01	WEAR 17-2	1.67	WEAR 17-1 R	10.97
WEAR 12-2	10.39	WEAR 15-4 R	7.96	WEAR 16-3	2.18	WEAR 17-3	4.51	WEAR 17-2	9.52
WEAR 12-2 R	11.13	WEAR 15-5	7.86	WEAR 16-4	6.28	WEAR 17-3 R	0.74	WEAR 17-3	11.04
WEAR 12-3	10.77	WEAR 16-1	7.33	WEAR 16-4 R	6.10	WEAR 17-4	2.72	WEAR 17-3 R	10.85
WEAR 12-4	10.50	WEAR 16-1 R	7.28	WEAR 16-5	6.18	WEAR 17-5	5.76	WEAR 17-4	9.91
WEAR 12-4 R	9.51	WEAR 16-2	7.33	WEAR 17-1	1.58	WEAR 17-5 R	5.77	WEAR 17-5	10.82
WEAR 12-5	10.76	WEAR 16-3	8.48	WEAR 17-1 R	1.77	WEAR 18-1	4.53	WEAR 17-5 R	10.37
WEAR 12-6	10.53	WEAR 16-3 R	8.57	WEAR 17-2	2.78	WEAR 18-2	5.37	WEAR 18-1	10.85
WEAR 12-6 R	9.47	WEAR 16-4	7.92	WEAR 17-2 R	3.17	WEAR 18-2 R	5.95	WEAR 18-2	11.06
WEAR 15-1	12.02	WEAR 16-5	9.56	WEAR 17-3	2.24	WEAR 18-3	5.81	WEAR 18-2 R	11.37
WEAR 15-2	10.97	WEAR 16-5 R	9.27	WEAR 17-4	3.29	WEAR 18-4	5.70	WEAR 18-3	10.60
WEAR 15-2 R	11.00	WEAR 17-1	4.18	WEAR 17-4 R	3.54	WEAR 18-4 R	5.92	WEAR 18-4	11.08
WEAR 15-3	10.86	WEAR 17-2	3.11	WEAR 17-5	3.55	WEAR 18-5	5.78	WEAR 18-4 R	9.92
WEAR 15-4	11.40	WEAR 17-2 R	2.81	WEAR 18-1	6.41	WEAR 19-1	6.83	WEAR 18-5	11.24

WEAR 15-4 R	11.05	WEAR 17-3	0.10	WEAR 18-1 R	6.48	WEAR 19-1 R	7.57	WEAR 19-1	10.33
WEAR 15-5	10.55	WEAR 17-4	5.99	WEAR 18-2	5.72	WEAR 19-2	7.12	WEAR 19-1 R	10.47
WEAR 16-1	11.03	WEAR 17-4 R	5.89	WEAR 18-3	5.89	WEAR 19-3	4.82	WEAR 19-2	11.22
WEAR 16-1 R	10.91	WEAR 17-5	1.67	WEAR 18-3 R	6.16	WEAR 19-3 R	6.02	WEAR 19-3	11.74
WEAR 16-2	11.24	WEAR 18-1	6.96	WEAR 18-4	5.21	WEAR 19-4	6.62	WEAR 19-3 R	10.62
WEAR 16-3	9.39	WEAR 18-1 R	7.46	WEAR 18-5	5.67	WEAR 19-5	6.84	WEAR 19-4	11.76
WEAR 16-3 R	9.11	WEAR 18-2	7.86	WEAR 18-5 R	6.05	WEAR 19-5 R	7.22	WEAR 19-5	11.17
WEAR 16-4	10.46	WEAR 18-3	5.53	WEAR 19-1	0.94	WEAR 20-1	6.73	WEAR 19-5 R	10.65
WEAR 17-1	7.85	WEAR 18-4	5.03	WEAR 19-2	1.82	WEAR 20-2	6.84	WEAR 20-1	10.91
WEAR 17-1 R	7.91	WEAR 18-4 R	6.50	WEAR 19-3	1.30	WEAR 20-2 R	7.26	WEAR 20-2	11.37
WEAR 17-2	8.83	WEAR 18-5	7.89	WEAR 19-3 R	1.77	WEAR 20-3	7.41	WEAR 20-2 R	9.93
WEAR 17-3	7.54	WEAR 19-1	3.73	WEAR 19-4	3.27	WEAR 20-4	6.83	WEAR 20-3	11.38
WEAR 17-3 R	7.44	WEAR 19-1 R	3.42	WEAR 19-5	2.64	WEAR 20-4 R	7.35	WEAR 20-4	10.90
WEAR 17-4	5.92	WEAR 19-2	5.57	WEAR 19-5 R	3.32	WEAR 20-5	7.20	WEAR 20-4 R	10.61
WEAR 17-5	8.10	WEAR 19-3	6.65	WEAR 20-1	2.76	WEAR 21-1	8.76	WEAR 20-5	11.23
WEAR 17-5 R	7.63	WEAR 19-3 R	6.49	WEAR 20-2	2.95	WEAR 21-1 R	8.57	WEAR 21-1	4.24
WEAR 18-1	8.82	WEAR 19-4	3.07	WEAR 20-2 R	2.84	WEAR 21-2	6.50	WEAR 21-2	6.42

WEAR 18-2	8.30	WEAR 19-5	6.03	WEAR 20-3	6.87	WEAR 21-3	8.08	WEAR 21-2 R	6.63
WEAR 18-2 R	8.56	WEAR 19-5 R	5.88	WEAR 20-4	5.25	WEAR 21-3 R	8.82	WEAR 21-3	7.66
WEAR 18-3	8.57	WEAR 20-1	6.73	WEAR 20-4 R	5.36	WEAR 21-4	8.32	WEAR 21-4	8.28
WEAR 18-4	8.71	WEAR 20-2	8.95	WEAR 20-5	5.12	WEAR 21-5	8.93	WEAR 21-4 R	8.79
WEAR 18-4 R	9.03	WEAR 20-2 R	8.59	WEAR 21-1	7.51	WEAR 21-5 R	9.55	WEAR 23-1	8.66
WEAR 18-5	7.94	WEAR 20-3	7.03	WEAR 21-1 R	7.26	WEAR 22-1	11.23	WEAR 23-2	8.76
WEAR 19-1	6.73	WEAR 20-4		WEAR 21-2	7.45	WEAR 22-2	6.74	WEAR 23-2 R	9.33
WEAR 19-1 R	7.16	WEAR 20-4 R	6.85	WEAR 21-3	6.78	WEAR 22-2 R	6.94	WEAR 23-3	8.87
WEAR 19-2	7.29	WEAR 20-5	6.94	WEAR 21-3 R	6.61	WEAR 22-3	9.52	WEAR 23-4	8.89
WEAR 19-3	7.65	WEAR 25-1	9.06	WEAR 21-4	8.50	WEAR 22-4	8.22	WEAR 23-4 R	8.93
WEAR 19-3 R	7.77	WEAR 25-1 R	8.18	WEAR 21-5	6.39	WEAR 22-4 R	6.76	WEAR 23-5	10.57
WEAR 19-4	9.31	WEAR 25-2	8.56	WEAR 21-5 R	6.74	WEAR 22-5	9.32	WEAR 25-1	8.27
WEAR 19-5	9.96	WEAR 25-3	8.85	WEAR 22-1	8.52	WEAR 23-1	2.47	WEAR 25-2	8.66
WEAR 19-5 R	9.84	WEAR 25-3 R	9.06	WEAR 22-2	7.97	WEAR 23-1 R	3.29	WEAR 25-2 R	8.41
WEAR 19-6	6.68	WEAR 25-4	8.82	WEAR 22-2 R	8.47	WEAR 23-2	4.24	WEAR 25-3	8.45
WEAR 19-7	10.22	WEAR 25-5	9.32	WEAR 22-3	8.17	WEAR 23-3	6.51	WEAR 25-4	9.33
WEAR 20-1	10.56	WEAR 25-5 R	9.56	WEAR 22-4	7.00	WEAR 23-3 R	6.57	WEAR 25-5	9.22

WEAR 20-1 R	10.56	WEAR 26-1	6.74	WEAR 22-4 R	6.50	WEAR 23-4	6.23	WEAR 25-5 R	8.78
WEAR 20-2	10.28	WEAR 26-2	7.59	WEAR 22-5	8.38	WEAR 23-5	6.54	WEAR 26-1	8.83
WEAR 20-3	9.25	WEAR 26-2 R	7.67	WEAR 23-1	7.49	WEAR 23-5 R	6.80	WEAR 26-1 R	8.68
WEAR 20-3 R	9.29	WEAR 26-3	7.02	WEAR 23-1 R	7.26			WEAR 26-2	8.43
WEAR 20-4	7.67	WEAR 26-4	7.63	WEAR 23-2	5.09			WEAR 26-3	7.95
WEAR 20-5	10.12	WEAR 26-4 R	6.45	WEAR 23-3	6.05			WEAR 26-3 R	8.13
WEAR 20-5 R	10.26	WEAR 26-5	7.22	WEAR 23-3 R	6.29			WEAR 26-4	8.11
WEAR 21-1	11.09			WEAR 23-4	4.57			WEAR 26-5	9.52
WEAR 21-2	11.24			WEAR 23-5	6.12			WEAR 26-5 R	9.42
WEAR 21-2 R	11.60			WEAR 23-5 R	6.11				
WEAR 21-3	10.20			WEAR 24-1	7.08				
WEAR 21-4	10.82			WEAR 24-2	8.20				
WEAR 21-4 R	10.86			WEAR 24-3	7.95				
WEAR 21-5	11.21			WEAR 24-3 R	8.18				
WEAR 23-1	7.63			WEAR 24-4	9.83				
WEAR 23-2	6.52			WEAR 24-5	8.51				
WEAR 23-2 R	5.85			WEAR 24-5 R	7.82				

WEAR 23-3	8.20			WEAR 25-1	6.20				
WEAR 23-4	9.74			WEAR 25-2	6.46				
WEAR 23-4 R	9.45			WEAR 25-2 R	5.86				
WEAR 23-5	9.45			WEAR 25-3	4.68				
WEAR 24-1	10.57			WEAR 25-4	5.95				
WEAR 24-1 R	10.36			WEAR 25-4 R	5.38				
WEAR 24-2	9.87			WEAR 25-5	4.64				
WEAR 24-3	9.39			WEAR 26-1	6.00				
WEAR 24-3 R	9.43			WEAR 26-2	6.46				
WEAR 24-4	9.11			WEAR 26-2 R	6.57				
WEAR 24-5	10.50			WEAR 26-3	4.36				
				WEAR 26-4	6.68				
				WEAR 26-4 R	6.89				
				WEAR 26-5	7.09				

Table S12: δ^{15}N values of individual *F. vesiculosus* tips collected from each site around the River Wear at each collection time. Tips are labelled by their location, the first number is the site, the number after the dash is the number tip at that site, and R dictates the tip is a repeat. For example, WEAR 4-5 R represents a tip collected from site WEAR 4, it is the fifth tip collected from that site and is a repeat of that tip.

Feb/Mar-21		Jun-21	
Sample ID	**$\delta^{15}N$ (‰)**	**Sample ID**	**$\delta^{15}N$ (‰)**
COAST 1-1	5.21	COAST 1-1	7.06
COAST 1-1 R	5.39	COAST 1-1 R	6.04
COAST 1-2	5.62	COAST 1-2	6.94
COAST 1-3	5.59	COAST 1-3 R	6.84
COAST 1-3 R	5.89	COAST 1-4	6.93
COAST 1-4	6.22	COAST 1-5	7.35
COAST 1-5	6.59	COAST 1-5 R	7.12
COAST 1-5 R	6.61	COAST 2-1	7.75
COAST 2-1	6.69	COAST 2-2	8.30
COAST 2-2	7.00	COAST 2-2 R	8.58
COAST 2-2 R	6.35	COAST 2-3	8.14
COAST 2-3	5.15	COAST 2-4	8.82
COAST 2-4	6.58	COAST 2-4 R	8.56
COAST 2-4 R	6.77	COAST 2-5	8.76
COAST 2-5	5.22	COAST 3-1	8.00
COAST 3-1	5.04	COAST 3-1 R	7.90

COAST 3-1 R	4.81	COAST 3-2	8.40
COAST 3-2	5.66	COAST 3-3	8.12
COAST 3-3	4.73	COAST 3-3 R	8.72
COAST 3-3 R	5.14	COAST 3-4	8.49
COAST 3-4	5.66	COAST 3-5	9.65
COAST 3-5	5.69	COAST 3-5 R	9.32
COAST 3-5 R	5.78	COAST 7-1	8.03
COAST 4-1	5.98	COAST 7-1 R	8.13
COAST 4-2	6.12	COAST 7-2	7.55
COAST 4-2 R	5.05	COAST 7-3	4.03
COAST 4-3	5.93	COAST 7-3 R	4.46
COAST 4-4	4.08	COAST 7-4	6.64
COAST 4-4 R	4.27	COAST 7-5	7.47
COAST 4-5	6.41	COAST 7-5 R	8.17
COAST 4-6	5.91	COAST 8-1	7.67
COAST 4-6 R	6.27	COAST 8-2	7.84
COAST 5-1	5.66	COAST 8-2 R	6.97

COAST 5-2	4.76	COAST 8-3	7.29
COAST 5-2 R	4.69	COAST 8-4	6.35
COAST 5-3	4.58	COAST 8-4 R	6.36
COAST 5-4	4.46	COAST 8-5	8.02
COAST 5-4 R	4.58	COAST 9-1	6.52
COAST 5-5	3.96	COAST 9-2	6.42
COAST 6-1	5.90	COAST 9-2 R	6.76
COAST 6-1 R	6.04	COAST 9-3	7.44
COAST 6-2	5.41	COAST 9-4	8.33
COAST 6-3	5.15	COAST 9-4 R	8.74
COAST 6-3 R	4.74	COAST 9-5	6.36
COAST 6-4	5.03	COAST 10-1	7.62
COAST 6-5	6.09	COAST 10-1 R	7.53
COAST 6-5 R	6.44	COAST 10-2	7.84
COAST 7-1	6.90	COAST 10-3	9.21
COAST 7-2	7.04	COAST 10-3 R	9.50
COAST 7-2 R	6.23	COAST 10-4	10.40

COAST 7-3	5.44	COAST 10-5	10.32
COAST 7-4	6.63	COAST 10-5 R	10.33
COAST 7-4 R	5.87	COAST 11-1	13.35
COAST 7-5	6.40	COAST 11-2	13.46
COAST 8-1	6.59	COAST 11-2 R	10.47
COAST 8-1 R	6.72	COAST 11-3	11.33
COAST 8-2	6.76	COAST 11-4	12.64
COAST 8-3	6.02	COAST 11-4 R	13.32
COAST 8-3 R	7.38	COAST 11-5	12.03
COAST 8-4	6.83	COAST 13-1	10.94
COAST 8-5	7.19	COAST 13-1 R	9.82
COAST 8-5 R	6.98	COAST 13-2	8.86
COAST 9-1	6.83	COAST 13-3	9.80
COAST 9-2	6.96	COAST 13-3 R	9.82
COAST 9-2 R	6.81	COAST 13-4	9.14
COAST 9-3	8.03	COAST 13-5	10.00
COAST 9-4	6.55	COAST 13-5 R	8.53

COAST 9-4 R	7.25	COAST 14-1	10.00
COAST 9-5	7.08	COAST 14-2	11.19
COAST 10-1	7.46	COAST 14-2 R	11.09
COAST 10-2	7.75	COAST 14-3	11.09
COAST 10-3	7.55	COAST 14-4	10.11
COAST 10-3 R	7.45	COAST 14-4 R	10.02
COAST 10-4	7.78	COAST 14-5	9.94
COAST 10-5	8.07	COAST 15-1	11.18
COAST 10-5 R	8.27	COAST 15-1 R	11.30
COAST 11-1	9.11	COAST 15-2	10.70
COAST 11-2	6.85	COAST 15-3	10.50
COAST 11-2 R	7.13	COAST 15-3 R	10.38
COAST 11-3	5.77	COAST 15-4	11.45
COAST 11-4	7.89	COAST 15-5	11.40
COAST 11-4 R	7.62	COAST 15-5 R	10.71
COAST 11-5	7.07	COAST 16-1	10.15
COAST 12-1	6.31	COAST 16-2	10.41

COAST 12-1 R	6.61	COAST 16-3	10.67
COAST 12-2	6.25	COAST 16-3 R	10.11
COAST 12-3	5.30	COAST 16-4	10.58
COAST 12-3 R	6.17	COAST 16-5	11.86
COAST 12-4	5.55	COAST 16-5 R	11.54
COAST 12-5	5.15	COAST 17-1	10.33
COAST 12-5 R	5.56	COAST 17-2	11.32
COAST 13-1	6.28	COAST 17-2 R	11.47
COAST 13-2	5.55	COAST 17-3	11.28
COAST 13-2 R	5.79	COAST 17-4	10.24
COAST 13-3	5.32	COAST 17-4 R	10.20
COAST 13-3 R	5.55	COAST 17-5	10.29
COAST 13-4	4.83	COAST 18-1	10.36
COAST 13-5	5.88	COAST 18-1 R	10.29
COAST 13-5 R	5.70	COAST 18-2	10.21
COAST 14-1	8.28	COAST 18-3	10.63
COAST 14-1 R	8.31	COAST 18-3 R	10.50

COAST 14-2	7.32	COAST 18-4	10.54
COAST 14-3	7.85	COAST 18-5	10.74
COAST 14-3 R	7.78	COAST 18-5 R	10.46
COAST 14-4	8.25	COAST 19-1	10.07
COAST 14-5	8.39	COAST 19-2	9.12
COAST 14-5 R	9.50	COAST 19-2 R	8.88
COAST 15-1	7.94	COAST 19-3	8.37
COAST 15-2	6.81	COAST 19-4	9.32
COAST 15-2 R	6.34	COAST 19-4 R	9.16
COAST 15-3	7.72	COAST 19-5	8.71
COAST 15-4	7.83	COAST 20-1	10.75
COAST 15-4 R	6.51	COAST 20-2	12.15
COAST 15-5	7.61	COAST 20-2 R	12.36
COAST 16-1	9.17	COAST 20-3	12.51
COAST 16-1 R	8.86	COAST 20-4	12.34
COAST 16-2	8.75	COAST 20-4 R	12.27
COAST 16-3	7.75	COAST 20-5	12.60

COAST 16-3 R	8.04	COAST 21-1	10.89
COAST 16-4	9.09	COAST 21-1 R	10.92
COAST 16-5	8.86	COAST 21-2	10.87
COAST 16-5 R	8.57	COAST 21-3	11.48
COAST 17-1	6.42	COAST 21-3 R	11.08
COAST 17-2	9.29	COAST 21-4	11.07
COAST 17-2 R	8.90	COAST 21-5	11.08
COAST 17-3	7.81	COAST 22-1	9.17
COAST 17-4	8.44	COAST 22-1 R	9.32
COAST 17-4 R	8.83	COAST 22-2	9.65
COAST 17-5	7.65	COAST 22-3	10.42
COAST 18-1	6.44	COAST 22-3 R	11.04
COAST 18-1 R	6.29	COAST 22-4	9.65
COAST 18-2	6.19	COAST 22-5	10.75
COAST 18-3	7.64	COAST 22-5 R	10.60
COAST 18-3 R	7.56	COAST 23-1	9.69
COAST 18-4	5.69	COAST 23-2	9.92

COAST 18-5	6.47	COAST 23-2 R	9.91
COAST 18-5 R	6.87	COAST 23-3	10.36
COAST 19-1	8.64	COAST 23-4	10.37
COAST 19-2	9.16	COAST 23-4 R	10.20
COAST 19-2 R	9.15	COAST 23-5	8.91
COAST 19-3	8.26	COAST 24-1	9.31
COAST 19-4	7.68	COAST 24-1 R	9.38
COAST 19-4 R	8.58	COAST 24-2	9.43
COAST 19-5	7.49	COAST 24-3	9.38
COAST 20-1	9.33	COAST 24-3 R	9.33
COAST 20-1 R	8.07	COAST 24-4	8.79
COAST 20-2	11.33	COAST 24-5	8.70
COAST 20-3	9.73	COAST 24-5 R	8.91
COAST 20-3 R	9.95	COAST 25-1	7.46
COAST 20-4	9.48	COAST 25-2	8.73
COAST 20-4 R	9.41	COAST 25-2 R	8.94
COAST 20-5	10.49	COAST 25-3	7.97

COAST 21-1	8.89	COAST 25-4	8.41
COAST 21-1 R	8.83	COAST 25-4 R	8.50
COAST 21-2	7.69	COAST 25-5	8.25
COAST 21-3	7.82		
COAST 21-3 R	7.91		
COAST 21-4	6.80		
COAST 21-5	8.24		
COAST 21-5 R	8.61		
COAST 22-1	7.45		
COAST 22-2	7.73		
COAST 22-2 R	7.47		
COAST 22-3	6.70		
COAST 22-4	7.45		
COAST 22-4 R	7.33		
COAST 22-5	7.39		
COAST 23-1	7.61		
COAST 23-1 R	7.18		

COAST 23-2	7.26		
COAST 23-3	6.82		
COAST 23-3 R	5.98		
COAST 23-4	5.85		
COAST 23-5	7.02		
COAST 23-5 R	6.95		
COAST 24-1	5.76		
COAST 24-2	5.39		
COAST 24-2 R	5.38		
COAST 24-3	4.65		
COAST 24-4	5.71		
COAST 24-4 R	5.68		
COAST 24-5	4.44		
COAST 25-1	6.50		
COAST 25-1 R	6.08		
COAST 25-2	6.47		
COAST 25-3	7.08		

COAST 25-3 R	7.32		
COAST 25-4	7.32		
COAST 25-5	6.40		
COAST 25-5 R	6.36		
COAST 26-1	5.92		
COAST 26-2	5.91		
COAST 26-2 R	6.30		
COAST 26-3	6.98		
COAST 26-4	6.52		
COAST 26-4 R	6.45		
COAST 26-5	6.06		
COAST 27-1	3.95		
COAST 27-2	4.30		
COAST 27-2 R	4.45		
COAST 27-3	4.57		
COAST 27-4	4.23		
COAST 27-4 R	4.44		

COAST 27-5	4.47		
COAST 28-1	4.97		
COAST 28-3	4.52		
COAST 28-3 R	4.47		
COAST 28-4	4.11		
COAST 28-5	4.67		
COAST 28-5 R	5.12		

Table S13: $\delta^{15}N$ values of individual *F. vesiculosus* tips collected from each site along the North East coastline at each collection time. Tips are labelled by their location, the first number is the site, the number after the dash is the number tip at that site, and R dictates the tip is a repeat. For example, COAST 4-5 R represents a tip collected from site COAST 4, it is the fifth tip collected from that site and is a repeat of that tip.

Feb/Mar-21		**Jun-21**	
Sample ID	**$\delta^{15}N$ (‰)**	**Sample ID**	**$\delta^{15}N$ (‰)**
TYNE 1-1	-1.06	TYNE 1-1	-0.67
TYNE 1-1 R	-1.78	TYNE 1-2	1.37
TYNE 1-2	1.57	TYNE 1-2 R	1.33
TYNE 1-3	-0.19	TYNE 1-3	0.82
TYNE 1-3 R	-4.59	TYNE 1-4	-0.47

TYNE 1-4	1.23	TYNE 1-5	-1.59
TYNE 1-5	0.99	TYNE 2-1	0.02
TYNE 1-5 R	0.23	TYNE 2-1 R	0.00
TYNE 2-1	-0.61	TYNE 2-2	0.31
TYNE 2-2	-0.79	TYNE 2-3	1.11
TYNE 2-2 R	-1.42	TYNE 2-3 R	0.32
TYNE 2-3	-4.87	TYNE 2-4	0.85
TYNE 2-4	-5.72	TYNE 2-5	-1.17
TYNE 2-4 R	-5.39	TYNE 2-5 R	-1.38
TYNE 2-5	-7.44	TYNE 3-1	0.47
TYNE 3-1	-8.03	TYNE 3-2	-0.73
TYNE 3-1 R	-8.34	TYNE 3-2 R	-0.75
TYNE 3-2	0.00	TYNE 3-3	-1.40
TYNE 3-3	0.16	TYNE 3-4	1.71
TYNE 3-3 R	-5.56	TYNE 3-4 R	1.99
TYNE 3-4	0.13	TYNE 3-5	0.68
TYNE 3-5	-0.13	TYNE 4-1	5.28

TYNE 3-5 R	0.21	TYNE 4-1 R	5.65
TYNE 4-1	-5.26	TYNE 4-2	6.12
TYNE 4-2	-1.00	TYNE 4-3	5.64
TYNE 4-2 R	-0.75	TYNE 4-3 R	5.52
TYNE 4-3	-2.88	TYNE 4-4	6.07
TYNE 4-4	-3.91	TYNE 4-5	5.46
TYNE 4-4 R	-3.92	TYNE 4-5 R	7.23
TYNE 4-5	-3.08	TYNE 6-1	9.26
TYNE 5-1	-0.59	TYNE 6-2	8.77
TYNE 5-1 R	-1.11	TYNE 6-2 R	8.85
TYNE 5-2	-0.06	TYNE 6-3	9.28
TYNE 5-3	-0.47	TYNE 6-4	7.99
TYNE 5-3 R	-2.20	TYNE 6-4 R	8.12
TYNE 5-4	-0.39	TYNE 6-5	8.56
TYNE 5-5	-2.24	TYNE 7-1	8.39
TYNE 5-5 R	-0.38	TYNE 7-1 R	8.03
TYNE 6-1	6.43	TYNE 7-2	8.87

TYNE 6-2	4.66	TYNE 7-3	7.85
TYNE 6-2 R	4.94	TYNE 7-3 R	8.09
TYNE 6-3	4.22	TYNE 7-4	7.85
TYNE 6-4	7.38	TYNE 7-5	8.81
TYNE 6-4 R	7.10	TYNE 7-5 R	8.60
TYNE 6-5	5.56	TYNE 8-1	-5.60
TYNE 7-1	4.22	TYNE 8-2	-6.17
TYNE 7-1 R	4.68	TYNE 8-2 R	-6.48
TYNE 7-2	4.42	TYNE 8-3	-5.40
TYNE 7-3	4.39	TYNE 8-4	-7.34
TYNE 7-3 R	4.24	TYNE 8-4 R	-8.31
TYNE 7-4	5.97	TYNE 8-5	-4.92
TYNE 7-5	5.41	TYNE 9-1	7.12
TYNE 7-5 R	5.23	TYNE 9-1 R	7.20
TYNE 8-1	-2.92	TYNE 9-2	6.80
TYNE 8-2	0.59	TYNE 9-3	7.20
TYNE 8-2 R	0.65	TYNE 9-4	8.00

TYNE 8-3	-2.64	TYNE 9-5	6.23
TYNE 8-4	-1.76	TYNE 9-5 R	6.00
TYNE 8-4 R	-1.38	TYNE 10-1	8.24
TYNE 8-5	-5.04	TYNE 10-2	7.76
TYNE 9-1	5.48	TYNE 10-2 R	7.40
TYNE 9-1 R	5.82	TYNE 10-3	6.82
TYNE 9-2	2.95	TYNE 10-4	5.83
TYNE 9-3	4.76	TYNE 10-5 R	7.90
TYNE 9-3 R	4.07	TYNE 11-1	2.75
TYNE 9-4	5.00	TYNE 11-2 R	4.00
TYNE 9-5	3.58	TYNE 11-3	3.54
TYNE 9-5 R	3.64	TYNE 11-4	3.73
TYNE 9-6	4.68	TYNE 11-5	3.66
TYNE 10-1	6.78		
TYNE 10-1 R	6.80		
TYNE 10-2	4.62		
TYNE 10-3	5.31		

TYNE 10-3 R	5.16		
TYNE 10-4	2.86		
TYNE 10-5	5.63		
TYNE 10-5 R	5.47		
TYNE 11-1	-0.52		
TYNE 11-2	0.24		
TYNE 11-2 R	0.66		
TYNE 11-3	-1.46		
TYNE 11-4	-0.14		
TYNE 11-4 R	0.06		
TYNE 11-5	2.34		

Table S14: δ^{15}N values of individual *F. vesiculosus* tips collected from each site around the River Tyne at each collection time. Tips are labelled by their location, the first number is the site, the number after the dash is the number tip at that site, and R dictates the tip is a repeat. For example, TYNE 4-5 R represents a tip collected from site TYNE 4, it is the fifth tip collected from that site and is a repeat of that tip.

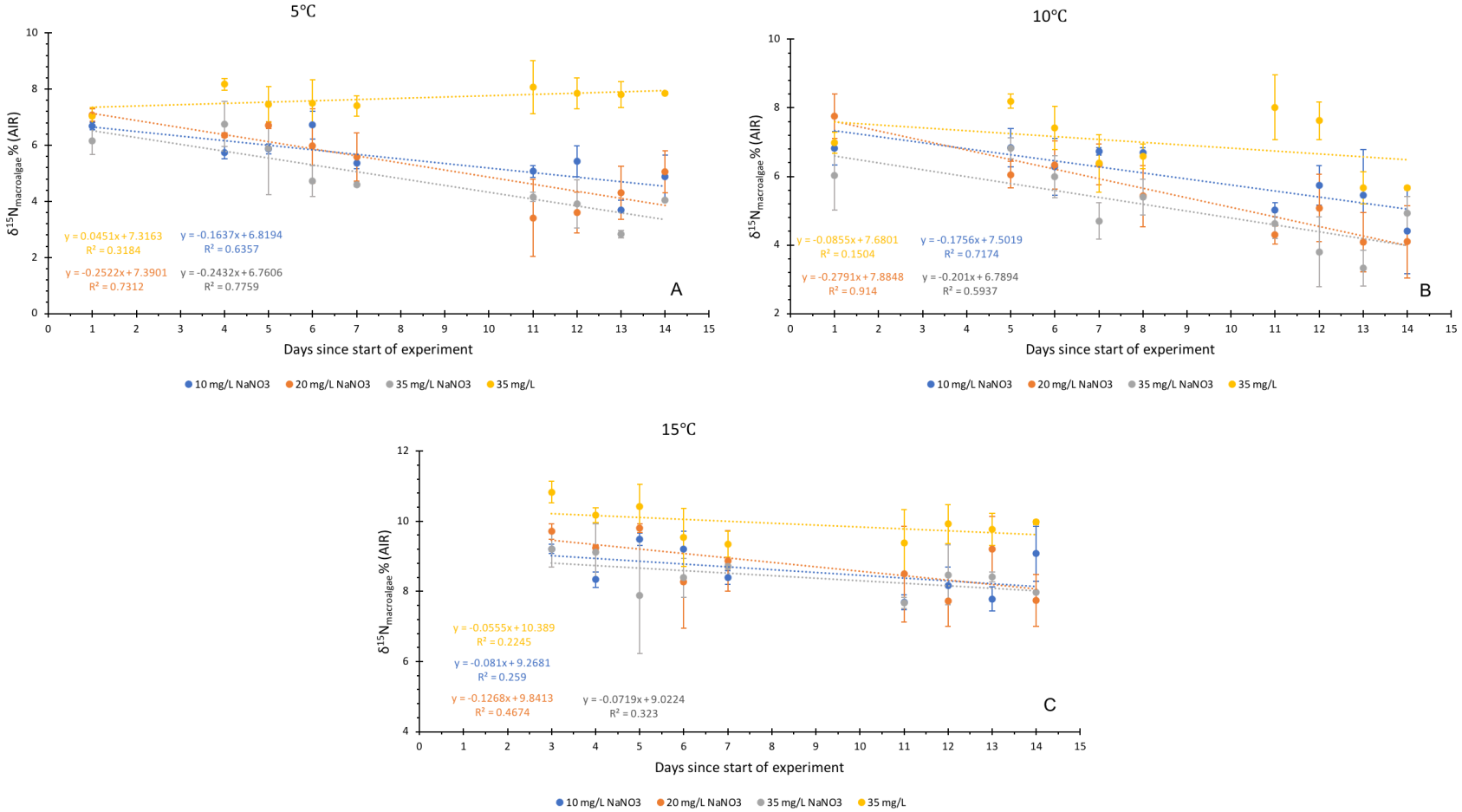


Figure S1: $\delta^{15}N$ values of *F. vesiculosus* tips cultured in solutions of varying salinity (S_A) containing $NaNO_3$ at a concentration of 11.3 mg NO_3^--N/L. One solution did not contain $NaNO_3$ to act as the control. Three experiments were conducted at 5°C, 10°C, and 15°C, representing graphs A, B, and C respectively. Means and standard deviations are representative of 3 sampled tips. Note the y-axis does not begin at zero.

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