Macroalgae Stable Isotope Analysis to Trace Sewage Nitrogen Pollution in Estuarine Environments

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Abstract

Sewage pollution is a widespread issue across the UK causing significant ecological damage to rivers, estuaries, and coastal environments. The issue has drawn widespread media attention over the high number of sewage spills and illegal discharges. Use of $\delta^{15}N$ values in macroalgae tissue can discriminate between pollutant sources and has been successfully used to identify sewage nitrogen inputs across the globe. This method could provide a cheap and easy method of tracing sewage inputs to UK waterbodies. High $\delta^{15}N$ values indicate an anthropogenic source (i.e., sewage) whereas lower values (~ 0 ‰) suggest an artificial source (i.e., fertiliser). Macroalgae is considered a reliable bio-indicator of nitrogen sources, although the technique is limited to only a handful of studies in the UK. A methodological approach was taken sampling *Fucus vesiculosus* and *Ulva* sp. at Staithes (North Yorkshire, UK) to understand $\delta^{15}N$ variation within a small bay. High $\delta^{15}N$ values were linked to a sewage treatment facility upstream. The harbour recorded relatively homogenous $\delta^{15}N$ values suggesting sites were representative of the whole harbour. Only one exception recorded a significantly different $\delta^{15}N$ value within the harbour; attributed to a WWTW Pump. *Fucus vesiculosus* and *Ulva* sp. were also used to record seasonal $\delta^{15}N$ variation for the highly populated Mersey Estuary in the UK. Consistently high $\delta^{15}N$ values (~ 14–18 ‰) revealed widespread sewage nitrogen loading across the entire estuary. *Ulva* sp. showed significant enrichment in summer, although values were high for all collection months and suggest sewage nitrogen is a year-round issue for the Mersey Estuary. Further work is required to produce a monthly data set and extend $\delta^{15}N$ to include samples upstream. The Mersey record was extended to include historical $\delta^{15}N$ data from herbaria macroalgae donated by the World Museums Liverpool. This is the first study using herbaria $\delta^{15}N$ analysis in the UK. A 200-year $\delta^{15}N$ record was produced for the estuary, revealing a shift from industrial and/or raw sewage nitrogen inputs in the early 1800s to a treated sewage nitrogen source into present day. Variation in $\delta^{15}N$ appeared to coincide with policy changes regarding water quality and sewage infrastructure although more data is required. Herbaria was shown to be a useful tool when tracing historical nitrogen pollutants and further analysis of other herbaria records could reveal similar trends for other

UK estuaries. *Fucus vesiculosus* and *Ulva* sp. are reliable bio-monitors, they are widespread making results comparable to other European studies; $\delta^{15}N$ analysis of these species should be considered as an alternative method for identification of sewage nitrogen across the UK.

Statement of Copyright

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Chapter 1: Introduction

1.1 Nitrogen and Water Pollution

Environmental and economic challenges due to high nitrogen (N) concentrations are well documented: negative impacts from eutrophication and hypoxia exist at local, regional, and national scales (Diaz & Rosenberg, 2008; Nixon, 1995; Smith & Schindler, 2009; Wurtsbaugh et al., 2019). Nixon (1995) defined eutrophication as "an increase in the rate of supply of organic matter to an ecosystem": this term now describes ecosystems where anthropogenic activity has elevated biologically available nutrient concentrations to a point where it is no longer a limiting factor on primary productivity.

Nitrogen is biologically unavailable as nitrogen gas (N_2), it first must be converted to biologically useful compounds. Prokaryotes naturally fix N_2 to biologically available ammonia (NH_3), the reaction is catalysed by the enzyme nitrogenase. Nitrogen fixing bacteria facilitate oxidation of NH_3 to nitrite (NO_2^-) and to nitrate (NO_3^-) through a series of chemical reactions listed in Appendix 1. Natural fixation processes contribute 203 Tg N to the environment a year (Fowler et al., 2013). However, anthropogenic activity is now the primary method of biological nitrogen addition to the environment: inputting 210 Tg N yr^{-1} (Fowler et al., 2013; Swaney et al., 2012; Wurtsbaugh et al., 2019). N_2 gas can be artificially fixed on reaction with H_2 gas under high pressure in the Haber-Bosch process: 80% of the resulting NH_3 is used in agricultural fertilisers worldwide (Fowler et al., 2013). The remaining NH_3 is used in the manufacture of chemicals and other industrial processes (Fowler et al., 2013). Wastewater (i.e., treated and/or raw sewage) inputs are also a significant anthropogenic nitrogen input to ecosystems (Glasgow & Burkholder, 2000; Herbert, 1999). Coastal waters receive > 6 Tg N from wastewater discharge a year, 63 % of which is treated sewage (Tuholske et al., 2021). The combination of industrial nitrogen fixation and sewage discharges has rapidly accelerated the rate at which N is becoming available to primary producers (Galloway et al., 2004; Glasgow & Burkholder, 2000; Herbert, 1999).

Nitrogen is a requirement of photosynthesis, incorporated into amino acids, nucleic acids, and adenosine triphosphate (ATP), for example Herbert (1999). Thus, when nutrient concentrations are no longer limiting and environmental conditions favourable, primary productivity can accelerate, leading to instances of algal blooms and elevated biological oxygen demands (BOD) (Camargo & Alonso, 2006; Howarth & Marino, 2006). Eutrophication is considered the primary threat to estuarine stability (Camargo & Alonso, 2006; Diaz & Rosenberg, 2008). Hypoxic “dead zones” are formed when oxygen is depleted through excessive consumption by primary consumers (Diaz & Rosenberg, 2008). Biologically available nitrogen is removed by an anaerobic process facilitated by denitrifying prokaryotes that convert NO_3^- to N_2 gas (Appendix 1). Rates of denitrification are far exceeded by the rate of nitrification due to human activity; consequently, biologically available nitrogen inputs have risen by 120 % since the 1970s (Galloway et al., 2008; Swaney et al., 2012; Valiela et al., 1997). Dinoflagellate (red tides) and cyanobacterial blooms may also occur during excessive nutrient supply, with the potential to release harmful toxins to the environment (Paerl & Otten, 2013; Wurtsbaugh et al., 2019). Fish kills and species diversity loss are commonly associated with eutrophic waters (Adams et al., 2020; Camargo & Alonso, 2006; Wurtsbaugh et al., 2019). Loss of established communities such as fish and seagrasses can restructure food webs and cause significant biodiversity loss whereby opportunistic algae *Ulva* sp. and/or *Cladophora* sp., for example, dominate ecosystems with high nutrient supply (Camargo & Alonso, 2006; Wurtsbaugh et al., 2019).

Sewage discharges are a primary source of excessive nutrient (or nitrogen) loading to aquatic environments (Valiela et al., 1997). Wastewater adds to the nutrient load of waterbodies but is also a major concern regarding public health. Bacteria such as *E. coli*, *Salmonella*, viral pathogens, and parasites are commonly found in sewage discharges; they can result in gastroenteritis, diarrhoea and, in extreme cases, death (Chahal et al., 2016). Chlorination and UV light are used to remove these pathogens; further treatment stages are in place to remove nitrogen, particulate solids, and larger debris (Chahal et al., 2016). UK wastewater treatment works (WWWTs) are permitted to discharge wastewater to rivers, estuaries, and coastal environments (DEFRA, 2012; Environmental Audit Committee, 2022). A 50 mg/l nitrate limit was set in 1980 by the World Health Organisation; all discharges in the UK are limited to this threshold (Drinking Water Inspectorate, 2023). WWTWs operating at a capacity >10,000 people and/or discharging to riverine or estuarine environments require biological denitrification of wastewater to remove dissolved organic nitrogen (DON), nitrates and ammonia (DEFRA, 2012). Of the 18,000 outflows in the UK approximately 15,000 of these are combined sewer overflows (CSOs) permitted to discharge raw sewage during periods of "exceptional rainfall" (Environmental Audit Committee, 2022). In these instances, unquantified concentrations of effluent are released into waterbodies with implications for excessive nitrogen loading, eutrophication, and poor ecological health. Limited information is available regarding CSO discharge for all UK Water Companies, there is a reliance on citizen science to monitor and track instances of raw sewage discharge to the environment (BBC, 2023a; Woodward, 2023). The UK Government is currently facing widespread public outcry over failings to effectively monitor wastewater discharges and prosecute water companies over sewage pollution (BBC, 2021, 2023b; Environment Agency, 2023; Quaranta et al., 2022; The Independent, 2022).

The UK has consistently failed to meet water quality targets: only 14% of UK rivers were of "Good Chemical Status" as of 2019, by 2027 this is expected to fall to 6% (Environment Agency, 2022; Environmental Audit Committee, 2022). Of the 86% of rivers that failed, sewage pollution was linked to more than a third of cases (Environmental Audit Committee, 2022). Across the UK there are numerous reports of sewage incidents, CSOs spilled for 1.75 million hours in 2022 alone (BBC, 2022a; The Rivers Trust, 2022). CSO discharges are not classed as a serious pollution incident by the Environment Agency despite recommendations to do so due to the ecological damage and adverse effects on human health (Environmental Audit Committee, 2022). The frequency of illegal discharges (where CSOs discharge at times of low flow) is now deemed unacceptable by the Environment Agency; 2022 saw three companies total 3,500 hours of dry spill (BBC, 2023b; Environment Agency, 2023). Effluent discharged at low flow to estuaries and/or rivers has a longer residence time, lower flows cannot as effectively flush away sewage inputs (Howarth & Marino, 2006). Estuaries are especially vulnerable ecosystems: the enclosed morphology and large tidal range can trap greater amounts of pollutants (e.g., nitrates) significantly prolonging the pollutant residence time (Adams et al., 2020; Howarth & Marino, 2006). Failings by privatised water companies in the UK has led to a situation where 25-30 % of nitrate in UK waterways is from sewage inputs and 93 % of estuarine environments exceed the Water Framework Directive nitrogen standard (Environment Agency, 2019). As such policy changes are required to monitor and reduce sewage nitrogen loads to aquatic environments in the UK (Environment Agency, 2018).

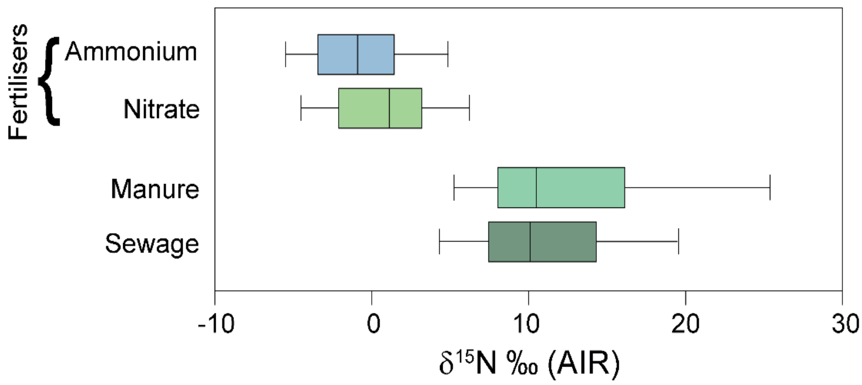
Restrictions of 50 mg/l for nitrate concentrations in discharge were put in place in 1980 in the UK. Water companies face fines imposed by the Environment Agency and Ofwat for breaching this limit. Prior to 2016 the UK was subject to EU Water Framework Directive regulation and since leaving the European Union the UK has retained much of the same legislation regarding water quality (House of Lords, 2023). In 2009, the UK was prosecuted by the EU regarding sewage pollution however since 2016 companies no longer face EU prosecution (The Guardian, 2009). In 2017 the Environment Agency was solely responsible for prosecuting Thames Water with fines of £20.3 million for spills in 2012 and 2014 (The Independent, 2017). Southern Water were fined a record £90 million for illegal discharges, however prosecutions can take several months, and public consensus reveals many do not think fines go far enough to reduce illegal discharges (House of Lords, 2023). Despite Event Duration Monitors currently in place on 91 % of all CSOs (increasing to 100 % by the end of 2023), budget cuts have reduced the EA’s capacity to monitor these CSOs so they cannot effectively respond to and prosecute companies over all incidents (DEFRA, 2022a; Environmental Audit Committee, 2022; House of Lords, 2023). In some locations citizen scientists have taken it upon themselves to report illegal discharges (BBC, 2022b, 2023a). Self-reporting is also expected of water companies: the Thames Water discharge map (Thames Water, 2023) is a promising start – however exact quantities of sewage effluent, nitrate and ammonia concentrations are not reported. Information is only provided for the Thames Water region. The Rivers Trust, (2022) map, whilst an excellent resource for identifying and publishing sewage spills across the UK provides information only for the number of hours sewage has spilled and not in real time. United Utilities, for example, has been found to have spilled sewage > 400,000 hours in 2022 which is higher than all other companies, but no further information is available (DEFRA, 2022b). Whilst the UK Government’s plan to prohibit illegal CSO discharges on all outflows by 2050 is good, more immediate action is required to monitor and reduce current discharges (DEFRA, 2022a;

House of Lords, 2023). Better transparency is needed by private water companies; United Utilities is regularly criticised over its unwillingness to share data, poor record on sewage spills and illegal discharges (DEFRA, 2022b; Woodward, 2023). Clearly more needs to be done to monitor these CSOs and understand where in our rivers, coasts and estuaries sewage is being discharged. A cost-effective and rapid method of identification is needed to better understand the extent of the UK's sewage pollution problem.

1.2 Nitrogen Isotopes and Sewage

Nitrogen has two stable isotopes: ^{14}N and ^{15}N. Heaton (1986) summarised the benefits of using stable isotopes ratios ($\delta^{15}N$ (‰) = (R_{sample} /($R_{standard}$ – 1))*10^3) as a method of discriminating between various nitrogen sources. Anthropogenic sewage and artificial (agricultural) are two sources of fixed nitrogen (i.e., biologically useful) that have a distinct isotopic signature (Fig. 1). Their distinct signatures are generated through bacterially mediated kinetic fractionation processes (Heaton, 1986; Peterson & Fry, 1987). Artificially fixed nitrogen (i.e., fertilisers) is sourced from N_2 (g) through the Haber-Bosch Process. N_2 (g) has a $\delta^{15}N$ value ~ 0 ‰. Minimal fractionation occurs during industrial fixation; the resulting NH_4^+ (i.e., fertiliser) is isotopically light (enriched in ^{14}N) due to the isotopically light source nitrogen (N_2) (Heaton, 1986).

Figure 1: Artificial and anthropogenic nitrogen sources isotope range (adapted from Bailes & Gröcke, 2020).



Sewage derived nitrogen, however, is more enriched in ^{15}N compared to ^{14}N (Heaton, 1986). Wastewater contains biologically available nitrogen as ammonia and urea ($CO(NH_2)_2$). Nitrification of NH_4^+ to nitrate is facilitated by nitrifying bacteria followed by denitrification of NH_3^- to N_2 (g) by bacteria under anaerobic conditions which removes nitrogen from the system (Wagner & Loy, 2002). Bacterially mediated processes have a higher ^{14}N turnover rate; more ^{14}N enriched N_2 (g) is produced (Dahnke & Thamdrup, 2013). The remaining nitrate and sewage sludge will be depleted in ^{14}N.

A secondary method is the hydrolysis of urea to NH_4^+ which causes a temporary rise in pH to basic (or alkaline) conditions, facilitating further ammonia production (Heaton, 1986; Peterson & Fry, 1987). Isotopically light ammonia will rapidly volatise, preferentially removing more ^{14}N from the system. This further enriches the remaining solution is in ^{15}N, as the pH decreases with volatilisation of ammonia, NH_4^+ is converted instead to ^{15}N enriched nitrate (Heaton, 1986). Ammonia volatilisation is widely cited, however findings from waste stabilisation ponds suggest biological removal (i.e., denitrification) as the dominant nitrogen removal mechanism (Camargo Valero et al., 2010; Carmargo Valero & Mara, 2007; Costanzo et al., 2001; Dailer et al., 2010; Heaton, 1986; Peterson & Fry, 1987).

When effluent and/or treated water is discharged it is no longer under anaerobic conditions and nitrogen removal will slow (Wagner & Loy, 2002). Water receiving WWTW discharges will reflect this elevated nitrogen isotope signature due to the presence of enriched nitrates and ammonia (Connolly et al., 2013; Dailer et al., 2010; Tucker et al., 1999). Since the mid-1980s the number of studies has grown exponentially detailing the benefits of nitrogen isotope analysis as a low-cost and rapid method of pollution identification (Costanzo et al., 2001; Gröcke et al., 2017; Heaton, 1986; MacKenzie et al., 2014; Peterson & Fry, 1987).

Due to differing treatment processes the $\delta^{15}N$ value for sewage derived nitrogen can vary between 8 ‰ to > 20 ‰ depending on the effluent source (Alldred et al., 2023; Samper-Villarreal, 2020; Savage & Elmgren, 2004; Xue et al., 2009). Treated (i.e., denitrified) sewage will be enriched in ^{15}N due to prolonged removal of DIN; some WWTW $\delta^{15}N$ values may be highly enriched > 18 ‰ (Dailer et al., 2010). Regions that rely on septic tanks for sewage treatment will produce more varied nitrogen isotope values due to an incomplete denitrification treatment process, such as sporadic leaks and condition of the septic tank (Alldred et al., 2023; Steffy & Kilham, 2004). Conversely untreated (or raw) sewage may be isotopically light yet high in DIN since it has not undergone a denitrification process (Barr et al., 2013). $\delta^{15}N$ values will typically reflect a human/animal effluent signature but may not be as enriched as treated sewage (Barr et al., 2013; Bird et al., 2022).

Typical oceanic $\delta^{15}N$ values are ~ 5 ‰, ranges indicative of "clean" coastal ecosystems can range between > 0 ‰ to ~ 8 ‰ (Dahnke & Thamdrup, 2013; Schubert et al., 2013; Xue et al., 2009). For example, a range between 6.6 ‰ and 8.8 ‰ was considered pristine for New Zealand (Barr et al., 2013) whereas 3 ‰ to 4 ‰ was considered "clean" for Scandinavia (Savage & Elmgren, 2004). Understanding the natural $\delta^{15}N$ baseline for a region is beneficial in determining other nitrogen inputs to an ecosystem (Barr et al., 2013; MacKenzie et al., 2014).

1.3 Tracing Sewage Using Nitrogen Isotopes

$\delta^{15}N$ analysis of living organisms (or bio-indicators) has been used for over 40-years to successfully trace nitrogen sources in aquatic environments (Samper-Villarreal, 2020). Studies require bio-indicators that are sessile, globally abundant species that accumulate contaminants and/or nutrients without being killed (Areco et al., 2021). Climate and biodiversity of the study area as well as the focus of the research often dictates the bio-indicator species used, common organisms include fish, macroalgae, seagrasses, oysters, and mussels (Elvines et al., 2023; Samper-Villarreal, 2020; Schubert et al., 2013).

Fish integrate $\delta^{15}N$ into muscle tissue over prolonged periods providing an average for $\delta^{15}N$ in the environment over time (Schlacher et al., 2005; Thompson et al., 2005). Schlacher et al. (2005) linked enriched values > 9.9 ‰ to wastewater pollution for an estuary; however, it was stated the need to kill each specimen as a limitation for use in environmental research. Jellyfish also present the same issues although were highly effective to trace nutrient dynamics through $\delta^{15}N$ values by MacKenzie et al. (2014) for the North Sea. More mobile species (e.g., fish) would not have been suitable (MacKenzie et al., 2014). In addition to spatial studies, $\delta^{15}N$ values can be used to trace effluent in food chains with multi-species studies. Enriched $\delta^{15}N$ values found in consumers such as grazing isopods indicate sewage nitrogen is transferred up the food chain (Dudley & Shima, 2010). Slower tissue turnover times in organism such as fish, crabs and isopods mean these bioindicators can be used to understand the average $\delta^{15}N$ ratio for that environment over a prolonged period (Dudley & Shima, 2010; Samper-Villarreal, 2020; Schlacher et al., 2005).

Sessile organisms such as molluscs are often used in nearshore and estuarine environments to trace anthropogenic nitrogen inputs (Hong et al., 2020; McKinney et al., 2001; Puccinelli et al., 2022). Studies have mapped spatial $\delta^{15}N$ changes to trace wastewater plumes; $\delta^{15}N$ values rapidly depleting away from sewage infrastructure (McKinney et al., 2001; Puccinelli et al., 2022). Isotopic analysis using fauna (i.e., molluscs, jellyfish, crustaceans) requires muscle tissue so the organism must be killed. This raises ethical questions regarding how environmentally friendly the use of such organisms is, especially when studying fragile ecosystems (MacKenzie et al., 2014; Puccinelli et al., 2022; Schlacher et al., 2005).

Marine plants and macroalgae could be considered a more ethical bioindicator: often only small parts of material need to be sampled and many species are globally abundant (Bunker et al., 2017). Plants and macroalgae also provide a more immediate $\delta^{15}N$ value of their environment, nitrogen uptake is faster in comparison to organisms such as fish or crustaceans (Gröcke et al., 2017; Samper-Villarreal, 2020). Macroalgae, in particular, is a widely used and popular $\delta^{15}N$ tracer of pollutants (García-Seoane et al., 2018): > 100 papers specifically cite macroalgae $\delta^{15}N$ as the primary method of nitrogen source identification (Samper-Villarreal, 2020).

1.4 Macroalgae and Isotope Research

1.4.1 Macroalgae Physiology and Nutrient Uptake

Macroalgae (or seaweeds) are classified into three taxonomic groups: Rhodophyta (red), Chlorophyta (green) and Phaeophyta (brown). All macroalgae are primary producers, they contain the pigment chlorophyll α which is used in photosynthesis to convert CO_2 and light into oxygen plus energy for growth (Bunker et al., 2017). They are found across all UK coastlines attached to rocks and artificial structures by a holdfast (Bunker et al., 2017). The entire seaweed body is termed the thallus and differentiated into a holdfast, stipe, meristem, and blades (Hurd et al., 1994). Often the stipe, meristem and blades are referred to as the frond (Bunker et al., 2017; Hurd et al., 1994).

Nutrient uptake (e.g., NO_3^- and NH_4^+) occurs across a diffusion boundary layer (DBL) at the growing tip of the thallus (i.e., blade) (Hurd et al., 1994; Roleda & Hurd, 2019; Viana et al., 2015). Nitrogen uptake rates are dictated primarily by the thallus surface area to volume (SA:V) ratio; the smaller the SA:V ratio and thicker the DBL, the slower the rate of diffusion (Roleda & Hurd, 2019; Rosenberg & Ramus, 1984). There are three pathways across the DBL: active transport, facilitated diffusion and/or passive transport (Hurd et al., 1994; Roleda & Hurd, 2019). Active transport requires use of carrier proteins and the molecule adenosine triphosphate (energy) to enable diffusion of nutrients into the cell. Macroalgae uses active transport when the concentration of nutrients is greater within the frond than the water column (Hurd et al., 1994; Roleda & Hurd, 2019). Facilitated diffusion requires an electrochemical gradient between the cell and water column to facilitate movement of nutrients through channel proteins and into the cell (Hurd et al., 1994). Passive diffusion does not require energy or channel proteins – nitrogen compounds passively diffuse across a lipid bilayer when the concentration of NO_3^- and/or NH_4^+ is greater in the water column compared to macroalgal cells (Hurd et al., 1994; Roleda & Hurd, 2019). Nutrient uptake rates are significantly different between opportunistic greens and often larger browns (Roleda & Hurd, 2019; Whitehouse & Lapointe, 2015). Macroalgae bio-monitoring studies often focus on widely distributed species common along most coastlines. *Fucus vesiculosus* (a brown macroalgae) and *Ulva* sp. (a green macroalgae) dominate the literature (Samper-Villarreal, 2020).

1.4.2 *Fucus vesiculosus* & *Ulva* sp.

Fucus vesiculosus (hereafter referred to as *Fucus*) is commonly used in European isotope studies (Bailes & Gröcke, 2020; Savage & Elmgren, 2004; Viana & Bode, 2013). It is widely distributed along sheltered coastlines often dominating the intertidal zone in temperate climates (Bothwell, 2023; Bunker et al., 2017). *Fucus* can also be found in some estuarine environments, it is therefore easily comparable between studies (Bunker et al., 2017; García-Seoane et al., 2018; Samper-Villarreal, 2020). The most recent growth (frond tips) is where nutrient uptake is fastest, with nitrogen assimilation taking between 14 to 16 days (Gröcke et al., 2017; Harrison & Hurd, 2001; Viana et al., 2015). Since *Fucus* growth is seasonal, NO_3^- is stored in cell vacuoles over winter (Harrison & Hurd, 2001; Roleda & Hurd, 2019). This NO_3^- reserve is then used in summer when growth conditions are more optimal (Harrison & Hurd, 2001): peak growth can reach ~ 5 cm/month (Raimonet et al., 2013).

Ulva sp. (hereafter referred to as *Ulva*) is an opportunistic green macroalgae genera exhibiting rapid growth in high nutrient environments (Brodie et al., 2007; Bunker et al., 2017). *Ulva* will bloom when high nutrient loads correspond with warm temperatures and optimal light conditions (Brodie et al., 2007; Lapointe et al., 2015; Whitehouse & Lapointe, 2015). It is globally distributed, and blooms are regularly used as an indicator of poor environmental health and/or wastewater pollution (Brodie et al., 2007; Teichberg et al., 2010). Isotopic studies often use *Ulva* to identify sewage derived nitrogen (García-Seoane et al., 2018). Species level identification is often impossible without further DNA or microscopy analysis (Bunker et al., 2017). *Ulva* species are morphologically similar; characterised by flat or tubular blades with high SA:V ratios. Blades and/or fronds are 1– 2 cells thick which enables rapid diffusion of nutrients into the seaweed. Nutrient assimilation is rapid (~ 48 hours) leading to rapid growth especially in summer months (Gröcke et al., 2017; Raimonet et al., 2013). *Ulva* uptakes both NO_3^- and NH_4^+, although it displays preferential uptake of NH_4^+ which peaks within the first three days of nutrient exposure (Flynn, 1991; Han et al., 2023; Naldi & Wheeler, 2002). Their wide distribution, rapid assimilation of nitrogen in the form of NO_3^-, NH_4^+ and minimal fractionation of nitrogen isotopes makes them a reliable alternative to traditional methods of identifying pollutant sources (Barr et al., 2013; Gröcke et al., 2017).

1.4.3 $\delta^{15}N$ and Macroalgae

The significance of isotopic fractionation in macroalgae at the nitrate reductase stage of nitrogen assimilation is debated regarding how accurately macroalgae will reflect the source $\delta^{15}N$ value (Cohen & Fong, 2005; Deutsh & Voss, 2006; Swart et al., 2014). Swart et al (2014) report $\delta^{15}N$ enrichment when NO_3^- concentration is limiting, and depletion of macroalgal $\delta^{15}N$ when concentrations are not limiting. However, Cohen & Fong (2005) argue fractionation during nutrient uptake is insignificant; marine concentrations of NO_3^- may be too low to cause any significant fractionation of the source N (Swart et al., 2014). Source $\delta^{15}N$-NO_3^- values are best reflected where there is minimal variation in the water column; in estuaries macroalgae has been found to accurately represent the water $\delta^{15}N$ (Deutsh & Voss, 2006). As such, fractionation of nitrogen by macroalgae species is negligible and they are considered a reliable indicator of the $\delta^{15}N$ ratio of the environment in which they live (Bailes & Gröcke, 2020; Lemesle et al., 2016; Viana et al., 2015).

Differing environmental parameters and research focuses means > 120 macroalgae species of species have been used in isotopic research (Dailer et al., 2010; Samper-Villarreal, 2020): N turnover rates, growth rates and ecological niche contributes to the vast array of species used. Often researchers use a multi-species approach to understand $\delta^{15}N$ values at better spatial and temporal resolution (Connolly et al., 2013; Samper-Villarreal, 2020). The variation in nitrogen turnover rates mean $\delta^{15}N$ trends can be interpreted on different time scales. Slower growing species can provide information on the baseline $\delta^{15}N$ signature of an ecosystem. *Ascophyllum nodosum*, for example, is a brown macroalgae which assimilates N over ~ 6 months so provides an average $\delta^{15}N$ value for half a year (Viana et al., 2015). However, opportunistic species (i.e., *Ulva*, *Cladophora* sp.) rapidly uptake and assimilate nitrogen, giving a more immediate $\delta^{15}N$ value and insight into the recent nitrogen loading regime (Gröcke et al., 2017). Studies that use species with different turnover and/or growth rates (e.g., Viana et al., 2015) can better identify average and recent nitrogen inputs to an environment. Challenges surrounding preferential uptake of NH_4^+ by opportunistic species (e.g., *Ulva*) can also be addressed by using another macroalgae genera (Cohen & Fong, 2005). Preferential uptake of one compound may produce a bias, masking the $\delta^{15}N$ value of an environment with both NO_3^- and NH_4^+ inputs from different sources (Cohen & Fong, 2005).

Ecological parameters lead to a dominance of one or two species in particular regions; for example, *Fucus* in European studies and *Ulva* for environments with very high nutrient loads (García-Seoane et al., 2018; Monteiro et al., 1997; Savage & Elmgren, 2004). Since most studies simply collect specimens growing in-situ species distribution is a large factor controlling study site location and macroalgae type (Carballeira et al., 2014; Savage, 2005). This method, when applied in Sweden, successfully traced sewage loading in *Fucus vesiculosus* (values > 10 ‰) over a 24 km profile from a sewage outflow (Savage, 2005). These studies are often limited by macroalgae distribution, alternative methods such as translocation experiments are used to deploy macroalgae in target locations where there is limited access or species distribution (Costanzo et al., 2001; Dailer et al., 2010; Gröcke et al., 2017). For example, the red macroalgae species *Catenella nipae* was transplanted to locations with minimal macroalgal distribution by (Costanzo et al., 2001), tissue recorded values between 7-9 ‰, supporting the existence of a sewage plume from the Brisbane River.

Use of macroalgae has expanded to include herbaria specimens to identify historical nitrogen regimes, extending the applications beyond modern day pollution analysis (Miller et al., 2020). Californian upwelling has been successfully modelled through stable isotope analysis of 200-year-old specimens by Miller et al. (2020). The use of macroalgal isotopes has declined over the past decade, but Miller et al. (2020) demonstrates a novel way in which macroalgae can be used to understand present and historical nitrogen sources (Samper-Villarreal, 2020).

1.5 Aims & Objectives

The Environment Agency's current monitoring strategy regarding effluent discharges has been labelled misleading, ineffective, and "a waste of money" (Environmental Audit Committee, 2022): evidently improved monitoring, research and better publication of data are required. Effluent volumes and concentrations from event duration monitoring is non-existent, and real time data currently available for only a handful of companies (Thames Water, 2023; Yorkshire Water, 2023). Macroalgal $\delta^{15}N$ analysis could be a cost-effective solution to identifying sewage nitrogen loading to UK estuaries and coastlines. Although current UK understanding is limited to a handful of studies, nitrogen isotopes have proved effective at identifying sewage nitrogen inputs (Alldred et al., 2023; Bailes & Gröcke, 2020). These were small scale studies in the UK; but macroalgae has been used successfully across mainland Europe to trace effluent pollution at a kilometre scale (Savage & Elmgren, 2004; Viana & Bode, 2013).

The objective of this research project is to use macroalgal $\delta^{15}N$ to identify sewage inputs to estuarine and coastal environments for the UK. A methodological study was also conducted to better understand how nitrogen isotopes vary within a system such as a small bay (Staithes, North Yorkshire). This research project is presented as three distinct papers that will be published in open access journals. The aims of the following chapters are presented as follows:

- Chapter 2 aims to produce an in-depth macroalgal record to understand inter-site $\delta^{15}N$ variation for a single bay (Staithes, Yorkshire) to better aid sampling methods and constrain nutrient mixing within single bays, harbours and/or estuaries.
- Chapter 3 focusses on the Mersey Estuary, a large estuary in the northwest of England. The aim of this chapter is to identify seasonal and spatial $\delta^{15}N$ variations for the Mersey Estuary. Is a sewage signal observed year-round or do pollutant sources fluctuate between industrial and anthropogenic in origin?
- Chapter 4 centres on the Mersey Estuary but expands the modern record to also include herbaria macroalgae with the objective of reconstructing historical nitrogen loading over the industrial period to present day for the Mersey Estuary. Chapter 4 was published on the 26th of April 2024 in Environmental Science: Advances.

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Nitrogen isotope variability of macroalgae from a small fishing village, Staithes Harbour, Yorkshire, UK

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Introduction

Anthropogenic activity has accelerated the rate of biologically available nitrogen that is added to the coastal environment, increasing the frequency of algal blooms, red tides, and eutrophication worldwide (Howarth, 2008; Howarth & Marino, 2006; Smith & Schindler, 2009; Wurtsbaugh et al., 2019). Sewage effluent is a significant cause of excess nutrients, specifically nitrogen. In the UK, Combined Sewer Overflows (CSOs) discharged for over 1.7 million hours in 2022 – a situation that has become the normal in the UK which has led to no waterbodies in England are of Good Overall Status according to the most recent report by the Rivers Trust (Environment Agency, 2023; The Rivers Trust, 2024). Over 90 % of UK estuaries fail to meet the 50 mg/l Water Framework Directive (WFD) nitrogen standard (Drinking Water Inspectorate, 2023), in 2018 16 English estuaries were designated as eutrophic or within a Nitrogen Vulnerable Zone (NVZ) (Environment Agency, 2018b, 2019a). Nutrient inputs (i.e., sewage pollution) have prolonged residence times in estuaries due to coastal morphology and tidal influence (Maier et al., 2009). Therefore, understanding the amount and types of nutrients (e.g., sewage, agricultural manure and/or fertilisers) into an estuary will help to evaluate and improve basin-wide management strategies.

Traditionally, sewage pollution is monitored through water analysis for *Escherichia coli* (*E. coli*), total nitrogen (TN) and biochemical oxygen demand (BOD) (Environment Agency, 2019b, 2022a). However, these types of analyses do not directly identify sewage, for example TN and BOD may also become elevated due to agricultural runoff (Crowther et al., 2002; Environment Agency, 2018b). Furthermore, sporadic testing of seawater for effluent pollution means some sites are only monitored 12 – 24 times a year and only during the summer (Environment Agency, 2019b). This data also only represents a snapshot in time and is costly to generate (Environment Agency, 2019b). Another method commonly used is nitrogen isotope analysis ($\delta^{15}N$) of water dissolved nitrate $\delta^{15}N_{NO_3^-}$ (Bronders et al., 2012; Ohte, 2013), which also has the same issues mentioned previously. However, nitrogen isotope analysis of photosynthetic organic matter (e.g., macrophytes, macroalgae, microalgae) can be used as an alternative method for determining nitrogen sources (Gröcke et al., 2017): it is inexpensive and records an average record of the nitrogen source during the growing period (Cohen & Fong, 2005; García-Seoane et al., 2018; Gröcke et al., 2017; Samper-Villarreal, 2020).

Nitrogen isotope analysis has been used to discriminate between anthropogenic and artificially derived nitrogen in aquatic environments for many decades (e.g., Heaton, 1986; Aravena et al., 1993; Savage, 2005; Lapointe et al., 2015). Anthropogenic sewage nitrogen sources have more elevated $\delta^{15}N$ values (Costanzo et al., 2001; Dailer et al., 2010; Heaton, 1986). Denitrification of nitrate (NO_3^-) favours the reduction of ^{14}N from nitrate to N_2 (g) compared to ^{15}N, leading to sewage effluent being enriched in ^{15}N (Dailer et al., 2010; Heaton, 1986; Risk et al., 2009). Other processes such as ammonia (NH_4^+) volatilisation will further elevate the $\delta^{15}N$ value of remaining wastewater (Heaton, 1986; Risk et al., 2009). Prolonged denitrification can elevate values to > +18 ‰ in specific environmental conditions (Barr et al., 2013; Dailer et al., 2010; Gartner et al., 2002; Riera et al., 2000). Untreated and/or raw sewage may be less elevated in $\delta^{15}N$ (~ +8 ‰) since it has not undergone denitrification (Barr et al., 2013; Risk et al., 2009). Industrially sourced nitrogen products (i.e., chemical fertiliser) have lower $\delta^{15}N$ values since atmospheric N_2 (g) is used in the making process (Heaton, 1986). Therefore, nitrogen isotopes can be used to distinguish between sewage sources versus chemical fertilizers (Bannon & Roman, 2008; Heaton, 1986; Peterson & Fry, 1987).

Macroalgae $\delta^{15}N$ has been used as a reliable tracer of nitrogen pollution in coastal environments (Brodie et al., 2007; Gröcke et al., 2017; Risk et al., 2009). For example, sewage effluent has been successfully traced using this method > 20 km from a point source with higher values usually associated with sewage infrastructure (Costanzo et al., 2001; Dailer et al., 2010; Savage & Elmgren, 2004). Baseline coastal $\delta^{15}N$ values or "natural" non-polluted environments have been proposed to be between +4 ‰ and +6 ‰ for the north-east Atlantic Ocean by Savage and Elmgren (2004). Very limited studies exist on using macroalgae $\delta^{15}N$ in UK (Alldred et al., 2023; Bailes & Gröcke, 2020; Gröcke et al., 2017). Alldred et al. (2023) produced a record for the Isles of Scilly; $\delta^{15}N$ values ~ +4 ‰ and +6 ‰ were recorded for areas least influenced by sewage infrastructure. Jones et al. (2018) used seagrass meadows across 11 UK sites, including the Isles of Scilly, and assumed $\delta^{15}N$ values < +6 ‰ to represent low anthropogenic influence.

Recent UK studies now include more industrialised areas of the UK such as County Durham (Bailes, 2022) and the Mersey Estuary (Alldred et al., 2024).

No standardised sampling technique exists for stable isotope macroalgae research and thus, a variety of approaches have been developed (e.g., García-Seoane et al., 2018). Barr et al. (2013) and Orlandi et al. (2017) focused on using a single species, whereas other studies have analysed the three dominant types – red (Rhodophyta), brown (Phaeophyceae), and green (Chlorophyta) (Lemesle et al., 2016). Most scientific studies on macroalgae $\delta^{15}N$ in Europe have dominantly used *Fucus* sp. and *Ulva* sp. (hereafter, *Fucus* and *Ulva*, respectively) due to their ubiquitous distribution and ease of identification (Bunker et al., 2017; García-Seoane et al., 2018; Samper-Villarreal, 2020). Nitrogen assimilation rates and nitrogen isotopic fractionation are well understood for these species (Bailes & Gröcke, 2020; Cohen & Fong, 2005; Swart et al., 2014). Assimilation rates can vary: for example, ~48 hours for *Ulva* (Budd & Pizzola, 2008; Lemesle et al., 2016), 14–30 days for *Fucus* and 1–7 months for *Ascophyllum nodosum* (Hill & White, 2008; Viana et al., 2015). Simple field collections of macroalgal growing *in situ* are common and restricted to the intertidal zone, < 2 m water depth (e.g. Thornber et al., 2008 & Titlyanov et al., 2011). Where macroalgae distribution and presence is limited translocation/deployment of isotopically labelled macroalgae can be used for assessing nitrogen pollution (Bailes & Gröcke, 2020; Costanzo et al., 2001). Translocated macroalgae also enables sampling of open oceans/deeper water as demonstrated by Howarth et al. (2019) to trace salmon farm effluent in Nova Scotia, Canada.

Macroalgae survey designs range from sampling a single coastal environment such as a lagoon or estuary to transects away from a point source (Savage, 2005; Thornber et al., 2008; Titlyanov et al., 2011; Viana & Bode, 2013). Many studies sample macroalgae across a large spatial area. For example, Viana and Bode, (2013) collected macroalgae from 10 sites > 80 km apart, whereas Savage and Elmgren, (2004) sampled 19 stations along a 36-km transect. Changes in coastal morphology is known to impact macroalgae $\delta^{15}N$ due to increased nutrient retention times in bays/lagoons (Raimonet et al., 2013; Titlyanov et al., 2011). Although large-scale studies over hundreds of kilometres may not capture subtle geospatial variation in $\delta^{15}N$ along the coast they will provide broad-scale changes.

Studies such as Titlyanov et al. (2011) where 9 sites from a 5 km^2 area were sampled may not record all environmental conditions affecting that area. Although estuarine studies are often incorporated into studies (e.g. Valiela et al., 1997; Riera et al., 2000; Raimonet et al., 2013), there are few that investigate in detail small bays and/or ports/harbours to determine the geospatial variation of nitrogen isotopes on a localised scale (e.g. Gartner et al., 2002; Dudley and Shima, 2010).

In this study, we selected a simple small fishing harbour in the north-east of England, Staithes, North Yorkshire, and collected seaweed geospatially over two periods of the year (May = Spring, September = Autumn). During each collection trip *Fucus* and *Ulva* were collected from 18 plots for nitrogen isotope analysis to understand the source and distribution of nitrogen in the harbour. This study indicates that a minimum number of 10 samples from each species is enough to characterise the $\delta^{15}N$ value of simple, small harbours: single river and single exit to the open ocean.

Study Site

Staithes is a small fishing harbour/village located on the north-east coast of North Yorkshire, England (Fig. 2.1A). The village has < 800 permanent residents, where most properties are second homes or holiday lets for the summer tourist season (Office for National Statistics, 2021). The Staithes Beck is a 25 km-long river draining a catchment of ~ 32 km^2 before discharging into the small harbour (0.03 km^2) and the North Sea (Environment Agency, 2022b). Beck is a Yorkshire word to denote a stream and/or small river; three small becks drain into the Staithes Beck including the Borrowby Dale Beck draining from the village of Hinderwell (Crowther et al., 2002). Wastewater treatment in the region is managed by the private water company Yorkshire Water. In 2001 the Hinderwell Sewage Treatment Works were constructed to redirect sewage from Hinderwell and Easington to the Staithes Long Sea Outfall (Environment Agency, 2022a). The Staithes Long Sea Outfall (Fig. 2.1B) discharges ~100 m north of the harbour into the North Sea (The Rivers Trust, 2022; Yorkshire Water, 2023b). Staithes has two Combined Sewer Overflows (CSOs), one discharges directly in the harbour (the Gun Gutter or Slipway) and the second directly to Staithes Beck (Fig. 2.1B) (Crowther et al., 2002;

The Rivers Trust, 2022; Yorkshire Water, 2023b). The Staithes Pumping Station is located on the western harbour wall (Fig. 1B) (The Rivers Trust, 2022).

Staithes Beck has a history of poor water quality: ecological status was rated Poor or Moderate until 2014 (Environment Agency, 2022b). Since 2015 the beck has been rated as Good ecological status but has consistently failed to reach Good Chemical Status (Environment Agency, 2022b). The Staithes Beck receives both anthropogenic and agricultural nitrogen pollution from surrounding farmland and sewerage infrastructure. However, there is limited data, for example, TN has not been reported since 2015 to present day (Environment Agency, 2021, 2022b). The Hinderwell Sewage Treatment Works was not fit for purpose upon completion in 2001, sewage leaks to the beck and Gun Gutter were a regular occurrence (Hinderwell Parish Council, Ms C. Barker, email pers. comm. 2023). In 2015 a major leak caused a reduction in dissolved oxygen, high ammonia concentrations and foul discoloured water into the beck and port, killing at least 100 fish in Staithes Beck (Environment Agency, 2018a). Yorkshire Water were fined £600,000 over the incident due to poor maintenance of rusted sewage tanks (Environment Agency, 2018a; Minting, 2017). Since then, public opinion over water quality has been low. An enquiry raised concerns over faecal pollution, poor water quality and dissatisfaction that Staithes Harbour was removed as a designated bathing site in 2016 (DEFRA, 2016). Since 2015, the Staithes Harbour has seen limited improvement, with over 1000 discharge hours of raw sewage from the Staithes Long Sea Outfall in 2020 (The Rivers Trust, 2022; Yorkshire Water, 2023b). Although CSOs discharge sewage into Staithes Harbour and Staithes Beck, however, real time information is unavailable as data is limited to 2019 – 2022 (The Rivers Trust, 2022; Yorkshire Water, 2023b).

Materials & Methodology

Macroalgae was collected from 18 areas (sites) that can be divided into three geographical zones: Staithes Beck (A – F), Staithes Harbour (G – L, P, Q, S, T) and North Sea (O, R) (Fig. 2.1B). Sites were chosen for accessibility, macroalgal cover and to produce good coverage of the harbour. Macroalgae was sampled on 26th September 2022 and 19th May 2023 to understand seasonal changes in $\delta^{15}N$

between Autumn and Spring. In each area at least 20 random macroalgae samples were collected, with a minimum of 10 *Fucus* samples. In September there was less *Ulva* present and so a minimum of 5 samples was set; this was increased to 10 samples in May due to abundant *Ulva* present. For *Fucus* the most recent growth was sampled, and fertile tips were ignored since they do not rapidly assimilate nitrogen (Viana et al., 2015). Sections of in-place *Ulva* between 3–5 cm^2 were sampled and squeezed to remove seawater. All samples were placed into individual lunch-money sized envelopes and subsequently dried in an oven set at 60˚C for between 48–72 hours. The tips of *Fucus* are assumed to record $\delta^{15}N$ values that represent the previous 2–4 weeks, whereas we have assumed that for *Ulva* it would represent ~2 days based on varying nitrogen assimilation rates (Gröcke et al., 2017; Viana et al., 2015).

All maps were produced using ArcGIS Pro 3.0, the size of each point represents the standard deviation in d15N for that site. The larger the point the greater the standard deviation. All graphs were drawn using Excel, Students T-Tests were used to test the significant difference between the Staithes Beck, Harbour, and North Sea Sites. A post-hoc Tukey Test was performed using R Studio to provide a comparison between all sites for both *Fucus* and *Ulva*.

Results

In September 2022, 305 individual macroalgae samples were collected. No *Ulva* samples were present at Sites L and S (Fig. 2.1B). *Fucus* produced an average of +9.7 ‰ ± 1.0 ‰ (n = 235) whilst *Ulva* was less positive at +8.8 ‰ ± 0.9 ‰ (n = 70). Staithes Beck averaged +10.4 ‰ ± 1.0 ‰ (n = 63) (*Fucus*) and +9.4 ‰ ± 0.7 ‰ (n = 26) (*Ulva*). Staithes Harbour recorded a lower average: +9.6 ‰ ± 0.9 ‰, n = 144 and +8.4 ‰ ± 0.8 ‰, n = 37, for *Fucus* and *Ulva* respectively. *Fucus* averaged +9.0 ‰ ± 0.5 ‰, n = 28 and *Ulva* averaged +8.3 ‰ ± 0.3 ‰, n = 7 for the North Sea. $\delta^{15}N$ values range between +7.2 ‰ (Site T) and +13.5 ‰ (Site A) for *Fucus* and between +6.8 ‰ (Site P) and +10.6 ‰ (Site B) for *Ulva*. Site B was the most elevated site for *Fucus* and *Ulva* with a $\delta^{15}N$ average of +11.1 ‰ ± 0.6 ‰ (n = 15) and +9.9 ‰ ± 0.5 ‰ (n = 5), respectively. The lowest average $\delta^{15}N$ was recorded at Site P for *Fucus* (+8.4 ‰ ± 0.9 ‰, n = 10) and at Site T for *Ulva* (+7.5 ‰ ± 0.6

‰, *n* = 5). Figure 2 illustrates the $\delta^{15}N$ range across all sites for September: Site F (*Fucus*) and H (*Ulva*) exhibited the largest $\delta^{15}N$ range whereas Site R had the smallest range for both species. All sites for Staithes Beck plot above the respective mean for both species. Staithes Harbour sites show some variation around the mean while Sites R and O plot below the Staithes Harbour mean (Fig. 2.2A, B). Figure 2.3 illustrates the range for each designated zone, all zones recorded significantly $\delta^{15}N$ values except between the Staithes Harbour and North Sea *Ulva* (p value > 0.05) (Table 2.1)

In May 2023, 351 macroalgae samples were collected: although no *Ulva* again was present and collected from Site L (Fig. 2.1B). May recorded a lower average $\delta^{15}N$ value than September 2022 in both *Fucus* (+8.4 ‰ ± 1.6 ‰, *n* = 184) and *Ulva* (+8.9 ‰ ± 1.1 ‰, *n* = 167) (Fig. 2.2A, B). Staithes Beck averaged +9.5 ‰ ± 1.3 ‰, *n* = 61 (*Fucus*) and +9.7 ± 0.9 ‰, *n* = 59 (*Ulva*). Staithes Harbour records a lower average: +8.3 ‰ ± 1.2 ‰ (*n* = 103) and +8.8 ‰ ± 0.9 ‰ (*n* = 88), for *Fucus* and *Ulva* respectively. *Fucus* averaged +5.9 ‰ ± 0.8 ‰ (*n* = 20) and *Ulva* averaged +7.5 ‰ ± 0.4 ‰ (*n* = 20) for the North Sea. $\delta^{15}N$ values range between +4.7 ‰ (Site R) to +12.5 ‰ (Site B) for *Fucus* and +5.3 ‰ (Site K) and +12.4 ‰ (Site A) for *Ulva* (Fig. 2.2A, B). In line with the results from September 2022, the most elevated $\delta^{15}N$ values are located in Staithes Beck with Sites D and G recording the highest average for *Fucus* (+9.8 ‰ ± 1.3 ‰, *n* = 10 and +9.8 ‰ ± 1.6 ‰, *n* = 10, respectively). Site A recorded the highest $\delta^{15}N$ average for *Ulva* (+10.6 ‰ ± 1.1 ‰, *n* = 10). Site O recorded the lowest average $\delta^{15}N$ for both *Fucus* and *Ulva*: +5.5 ‰ ± 0.4 ‰ (*n* = 10) and +7.3 ‰ ± 0.3 ‰ (*n* = 10), respectively (Fig. 2.2A, B).

Discussion

Staithes Harbour Site P

Of all the data generated in this study Site P produced anomalous results in September 2022 and May 2023 suggesting a difference in nitrogen pollution source or amounts compared to the other sites. September *Fucus* at Site P had significantly lower $\delta^{15}N$ averages compared to all other Staithes Harbour sites (Fig. 2.4A). *Ulva* $\delta^{15}N$ averages were generally lower but not significantly different from other Staithes Harbour sites (Fig. 4B) although in May 2023 there was significantly variability at this site. The $\delta^{15}N$ values from Site P were similar to the North Sea sites (Sites R and O) during September and May. Neighbouring sites S and Q are significantly different to Site P for *Fucus* in both collection periods.

During both sampling trips it was observed that water was trickling from under the rocks of the retaining wall opposite the Staithes Harbour Pumping Station (Fig. 2.1A, B). The pumping station transfers wastewater from local properties to the nearest wastewater treatment works, and has been managed and operated by Yorkshire Water since 2016 (Yorkshire Water, 2023a). There is limited information regarding the Staithes Harbour Pumping Station although significant leaks were reported in 2020 (The Rivers Trust, 2022; Yorkshire Water, 2023b). We hypothesise that the pumping station is still leaking and releasing raw sewage and/or household chemicals that are lowering the $\delta^{15}N$ in comparison to treated sewage (Barr et al., 2013; Risk et al., 2009): it requires further investigation by local authorities.

Seasonal $\delta^{15}N$ Variation

Fucus $\delta^{15}N$ is more positive across all three environmental zones in September 2022 compared to May 2023 (Table 2.2, Fig. 2.3A, B). On the other hand, *Ulva* shows no significant difference between September and May (Table 2.2), although more spatial variation is observed with more elevated $\delta^{15}N$ values in September (Fig. 2A). Lower $\delta^{15}N$ in May differs to seasonal trends observed by Raimonet et al. (2013) and Lemesle et al. (2016) where $\delta^{15}N$ was elevated in warmer months. The Staithes data more closely reflects findings by Bailes, (2022) from the County Durham coast (England) with more elevated $\delta^{15}N$ values in September compared to May. To generate a more thorough understanding of seasonal changes in $\delta^{15}N$ a study composed of monthly sampling would be required for all 17 sites. Although this would produce a very extensive dataset it would also come at a cost in terms of time and analyses (> 3,500 samples). This type of scientific approach would not, in our opinion, be warranted or show anything different to what is presented in this study.

The seasonal difference in $\delta^{15}N$ for *Fucus* and *Ulva* between September 2022 and May 2023 indicates that the nitrogen pollution source into Staithes Harbour is not significantly invariant between these seasons. Elevated $\delta^{15}N$ values (> +6 ‰) suggest an input from anthropogenic effluent (raw and/or treated) and/or animal manure, and thus limited input from agricultural fertilizers (Deutsh & Voss, 2006; Kroeger et al., 2006). Staithes Beck is known to receive sewage effluent from the Hinderwell Facility and from two holding tanks which regularly overflow after heavy rainfall (Hinderwell Parish Council, Ms C. Barker, email pers. comm. 2023). Organic fertiliser, such as animal manure, can also produce an elevated $\delta^{15}N$ values > +6 ‰ (Jones et al., 2018; Xue et al., 2009). In England the spreading of anthropogenic effluent/animal manure predominantly occurs in September in preparation for winter-sown crops (Kynetec, 2023). Since *Fucus* records a longer uptake of nitrogen pollution (i.e., slower assimilation rate) the more elevated $\delta^{15}N$ average compared to *Ulva* may represent this period of organic fertilisation whereas Ulva may only be recording the last few days of nitrogen input into Staithes Harbour (Gröcke et al., 2017; Kynetec, 2023). However, no information is publicly available on the amounts and type of fertiliser application in the surrounding farming area to support this interpretation.

Spatial $\delta^{15}N$ Variation

Macroalgal $\delta^{15}N$ varies spatially within the Staithes Harbour dataset. Figures 2.4 and 2.5 show elevated $\delta^{15}N$ values in Staithes Beck compared to the Staithes Harbour and North Sea. Sites A and B record the highest $\delta^{15}N$ average of > +11 ‰, suggesting denitrified sewage as a dominant source of the nitrogen pollution. Since it is reported that Staithes Beck receives treated sewage effluent from the Hinderwell Facility, this is supported by the macroalgae $\delta^{15}N$ data. Reduced mixing between freshwater and seawater may be a mechanism that concentrates the effluent upstream, allowing the macroalgae more time to incorporate the treated sewage effluent $\delta^{15}N$ signature (Barr et al., 2013): at least during periods of high tide. Macroalgae $\delta^{15}N$ values gradually decline from Staithes Beck into the Staithes Harbour (Figs. 2.4, 2.5). Macroalgae $\delta^{15}N$ values for Staithes Harbour (G – L, Q, S and T) exhibit a range of 5.1 ‰ in September 2022 compared to 6.4 ‰ in May 2023. There is a decrease in $\delta^{15}N$ on the order of ~3 ‰ in *Fucus* and *Ulva* from

Site A to the open ocean sites representing the North Sea (Figs. 2.4, 2.5). This trend is less apparent in September: *Fucus* ~2.5 ‰ and *Ulva* ~1.5 ‰ (Fig. 2.4). In May 2023 (Fig. 2.5B) Staithes Harbour recorded a higher $\delta^{15}N$ average compared to the North Sea: *Fucus* $\delta^{15}N$ average +5.9 ‰ ± 0.8 ‰ (*n* = 20) whereas *Ulva* is +7.5 ‰ ± 0.4 ‰ (*n* = 20). During the fieldtrip in May there was considerably more growth of *Ulva*, especially along the Staithes Beck and the outflow region in Staithes Harbour. Increased runoff of nutrients and warmer temperatures during May have promoted *Ulva* growth and nutrient uptake, that is potentially the cause behind this increased coverage. There is no significant difference for *Ulva* between these zones in September (p value = 0.79, Table 2.1) and it is interpreted to be caused by increased wave activity and mixing between Staithes Harbour and the North Sea, reduced growth rates and uptake of nutrients.

A Tukey pairwise comparison t-test (Tukey Test) was performed to statistically assess how similar each site was to one another in terms of mean isotope values, where for this test we have removed Site L from the analysis since it was a site that did not generate any *Ulva* results. Levene's test for homogeneity (Levene, 1960; Fox, 2015) provided evidence of heterogeneity of isotope values across groups, hence the Tukey test was based on constructing a generalised linear model for isotope value, with Site as regressor, for each isotope type-month pairing. The results of a Tukey test can be presented in the form of a compact number display, which attributes a shared group number between any two groups for which there is no evidence (at the 95% significance level) of a difference between their group means (Piepho, 2004). This grouping is graphically represented on the right of each subplot in Figure 2.6, with blue bars indicating the groups assigned to each site. We observed that 6 groups are required to categorise the sites in September (Fig. 2.6A), whereas 9 groups are required for May (Fig. 2.6B). Both months show considerable overlap between sites within Staithes Harbour: 10 for September (Group 3) and 6 for May (Group 3). The $\delta^{15}N$ data suggests that Staithes Harbour exhibits relatively low $\delta^{15}N$ variation between sites indicating that the harbour is relatively well-mixed between nitrogen pollution inputs (Staithes Beck) and the open ocean (North Sea). More Tukey test group overlaps between sites for September 2022 indicate that it is more homogenous in terms of nitrogen pollution input and mixing. There are more groups for May 2023, and in particular, fewer site pairs sharing any group number, which may represent differential uptake and assimilation in *Ulva* in comparison to *Fucus*: hence, the proliferation of *Ulva* in the mouth of the Staithes Beck and along its low tide course. During the winter months when *Fucus* is growing slowly it takes up nitrogen and stores it to subsequently use in the Spring (Lehvo et al., 2001; Young et al., 2007): this is an advantage since it does not then have to compete with opportunistic, faster assimilating macroalgae species such as *Ulva*. Therefore, the Tukey test suggests that to produce a representative $\delta^{15}N$ value for Staithes Harbour more sites may need sampling in Spring in comparison to Autumn.

Based on the dataset presented here, the Gun Gutter Outflow indicates minimal to no elevation in Site H from either sampling trip (Figs. 2.4, 2.5). The $\delta^{15}N$ average for both *Fucus* and *Ulva* at Site H falls within the range of $\delta^{15}N$ average values recorded from Staithes Harbour and Staithes Beck. This suggests that Site H was not influenced by the Gun Gutter Outflow at the time of collection. Or because of mixing in this part of the harbour any input from Site H is incorporated into the overall $\delta^{15}N$ signature of the harbour. A cautionary note is that during other periods of the year this outflow may have an impact of $\delta^{15}N$ in Site H.

This study shows that in a simple harbour environment, sampling in detail around the harbour is not required. In fact, 10 samples from each of the main source (i.e., river), the middle of the harbour (i.e., beach area) and the outer harbour (i.e., open sea) is enough to characterise the effect and distribution of nitrogen pollution when using $\delta^{15}N$. If a harbour has multiple sources, then we suggest that each source is sampled. Although seasonality may affect $\delta^{15}N$ values of the harbour, this is dependent on the whether the nitrogen pollution inputs change significantly (i.e., from sewage effluent to agriculture chemical fertiliser runoff). Although this study revealed a leaking pumping station (i.e., point source) this does not seem to have had any impact on the average $\delta^{15}N$ value of the harbour. Depending on the question/hypothesis to be investigated will depend on the sampling density, for example: (1) a broad geospatial coastal study will only require a minimum of 10 samples from within the central portion of the harbour to determine the average $\delta^{15}N$ value for that site; and (2) if you wanted to determine point sources and geospatial distribution in a harbour we would recommend a similar approach to that conducted in this study.

Conclusions

Spatial variation in macroalgae $\delta^{15}N$ was investigated from a small fishing harbour, Staithes, on the North Yorkshire coast of England. Over 300 macroalgae samples were collected in September 2022 (Autumn) and May 2023 (Spring). No significant difference was documented between these two sampling time intervals, although there is significant spatial variation in $\delta^{15}N$. In both time periods Staithes Beck was significantly different to the harbour. Sites within the harbour were statistically similar to one another, except for Site P, which recorded lower $\delta^{15}N$ values that were similar to the open marine sites. We propose this is due to a leaking pumping station near to Site P. A Tukey Test revealed a greater number of sites were statistically significantly distinct in May compared to September, indicating changes either in the mixing rate of the area and/or changes in species density preferentially taking up nitrogen pollution. This study indicates that large-scale macroalgae $\delta^{15}N$ surveys should avoid sampling from river inputs into a region, but instead sample more open parts of the harbour to obtain a representative average between the river (i.e., source of nitrogen pollutants) and the ocean (i.e., background marine value).

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Figure 2.1:

(A) Photograph of Staithes Harbour in 2022 (courtesy of Chris Riddell). (B) Sampling locations within Staithes Beck, Staithes Harbour and the North Sea. ArcGIS Pro 3.0 was used to produce this figure.

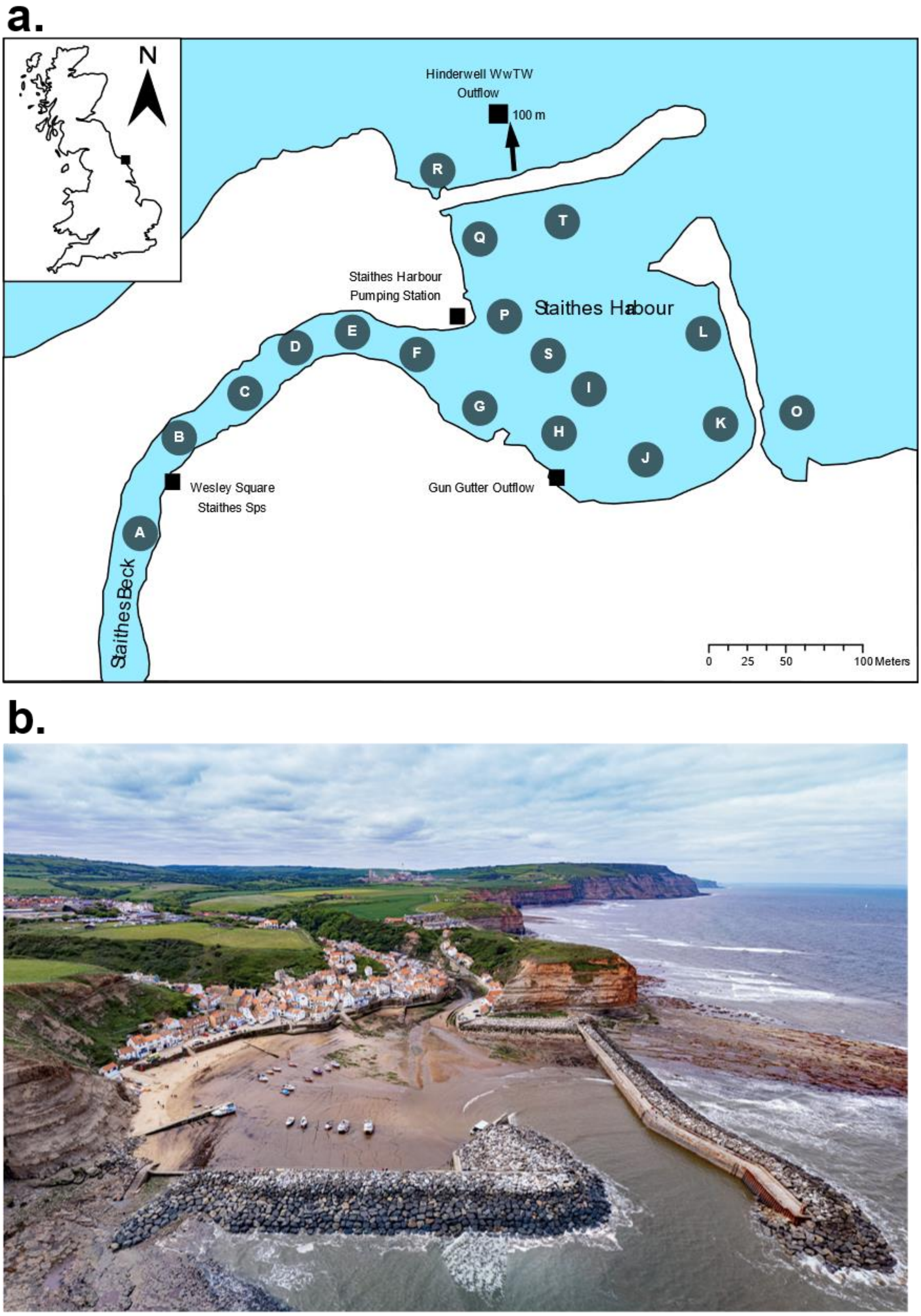


Figure 2.2:

$\delta^{15}N$ values recorded for *Fucus vesiculosus* (A) and *Ulva* sp. (B) shown for all sites in September and May. The red dash line represents the average for each species: (A) September +9.7 ‰ ± 1.0 ‰ (*n* = 235) and in May +8.4 ‰ ± 1.6 ‰ (*n* = 184), (B) September +8.8 ‰ ± 0.9 ‰ (*n* = 70) and in May +8.9 ‰ ± 1.1 ‰ (*n* = 197).

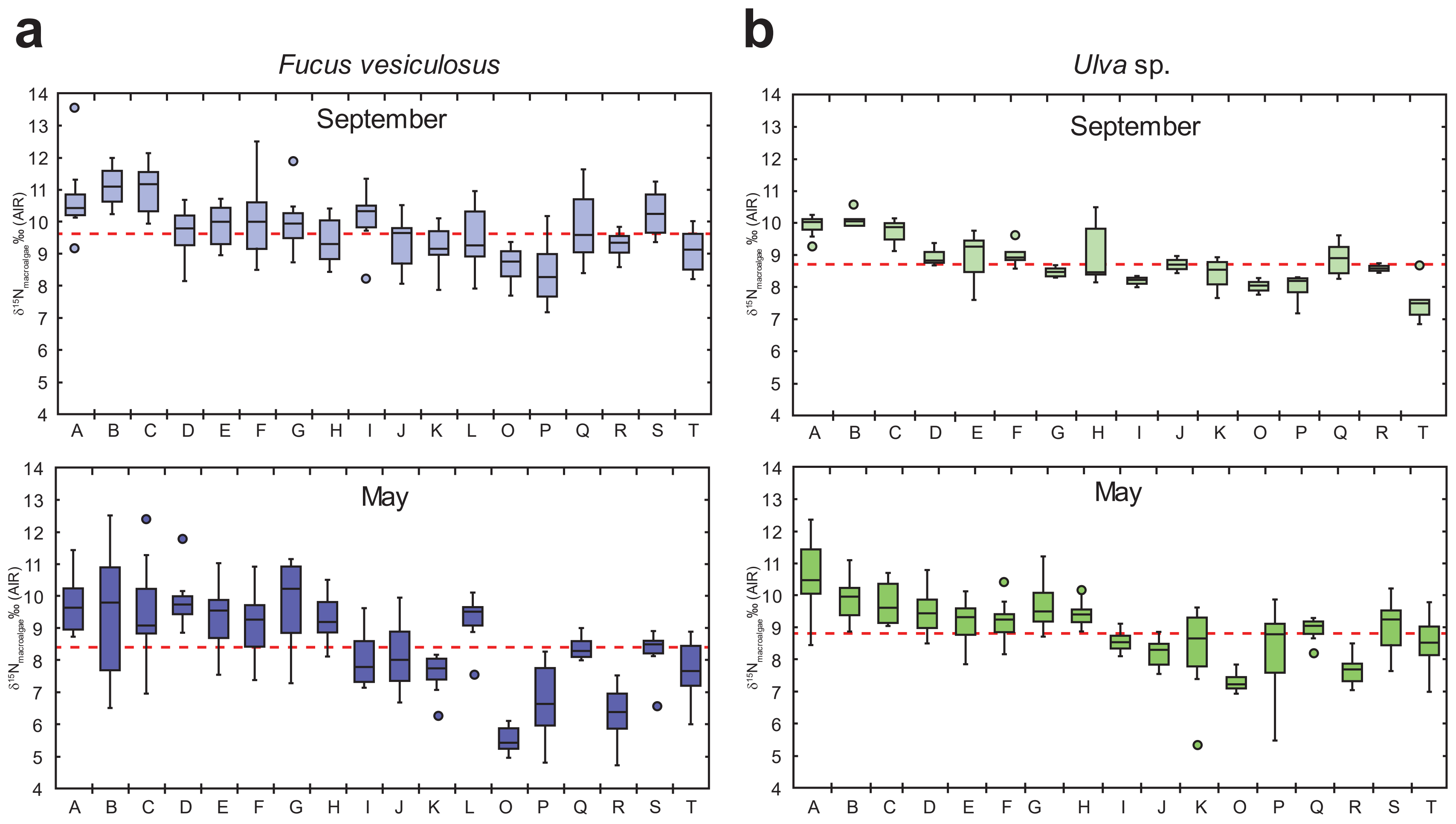


Figure 2.3:

$\delta^{15}N$ values recorded for September (A) and May (B) divided into Staithes Beck, Staithes Harbour and the North Sea

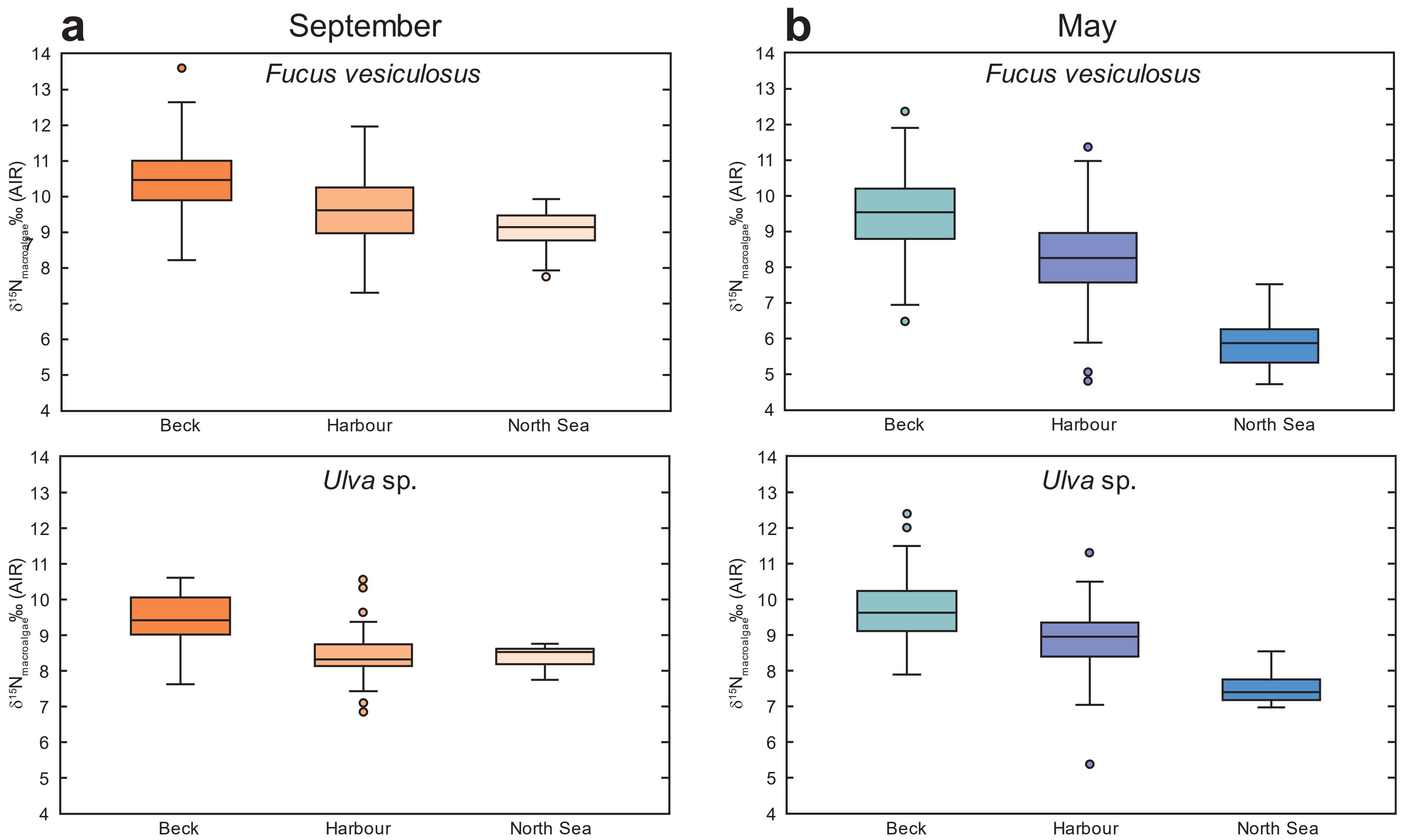


Figure 2.4:

Geospatial representation of $\delta^{15}N$ values for *Fucus vesiculosus* (A) and *Ulva* sp. (B) for September. Plots were produced using ArcGIS Pro 3.0

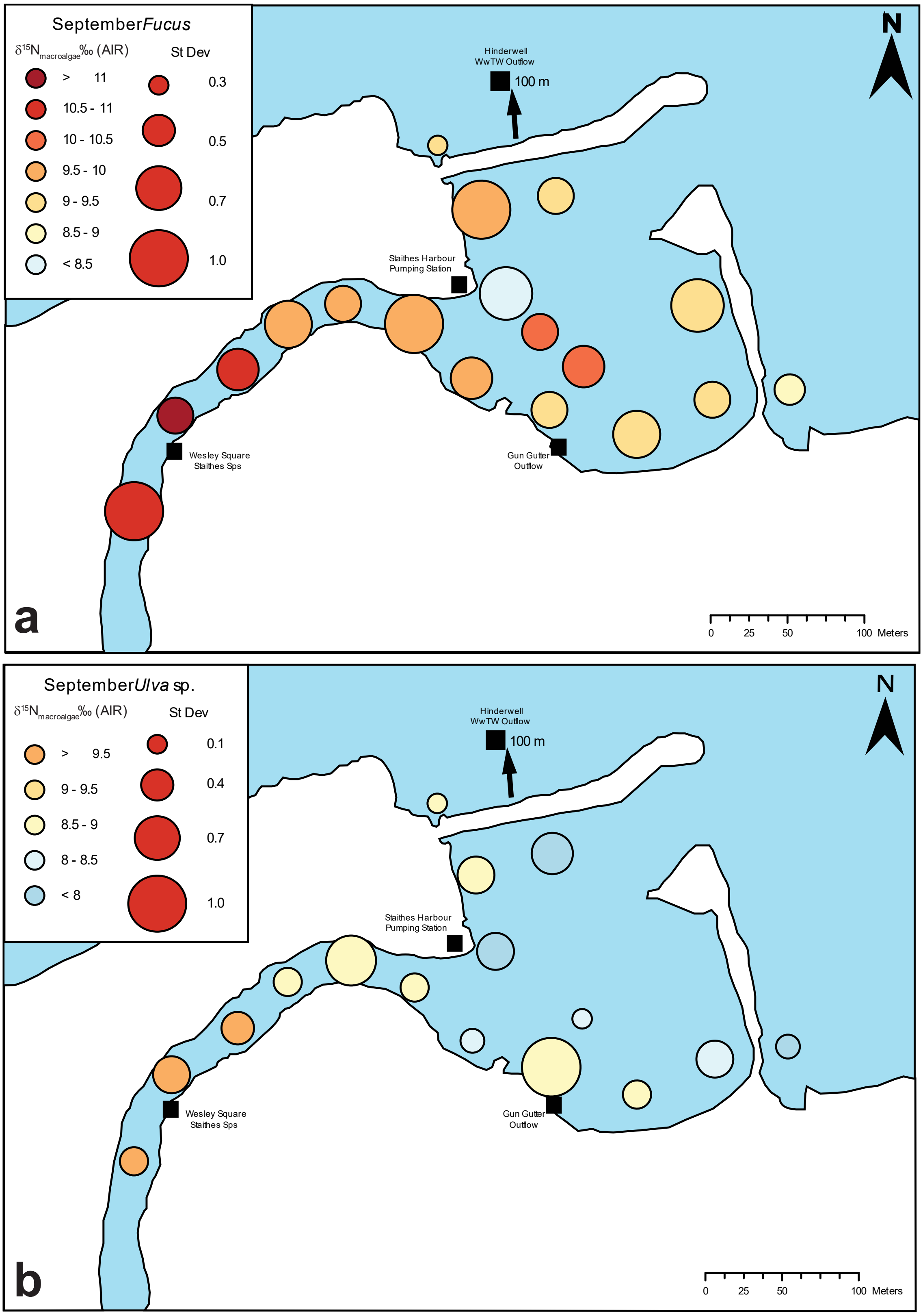


Figure 2.5:

Geospatial representation of δ^{15}N values for *Fucus vesiculosus* (A) and *Ulva* sp. (B) for May. Plots were produced using ArcGIS Pro 3.0

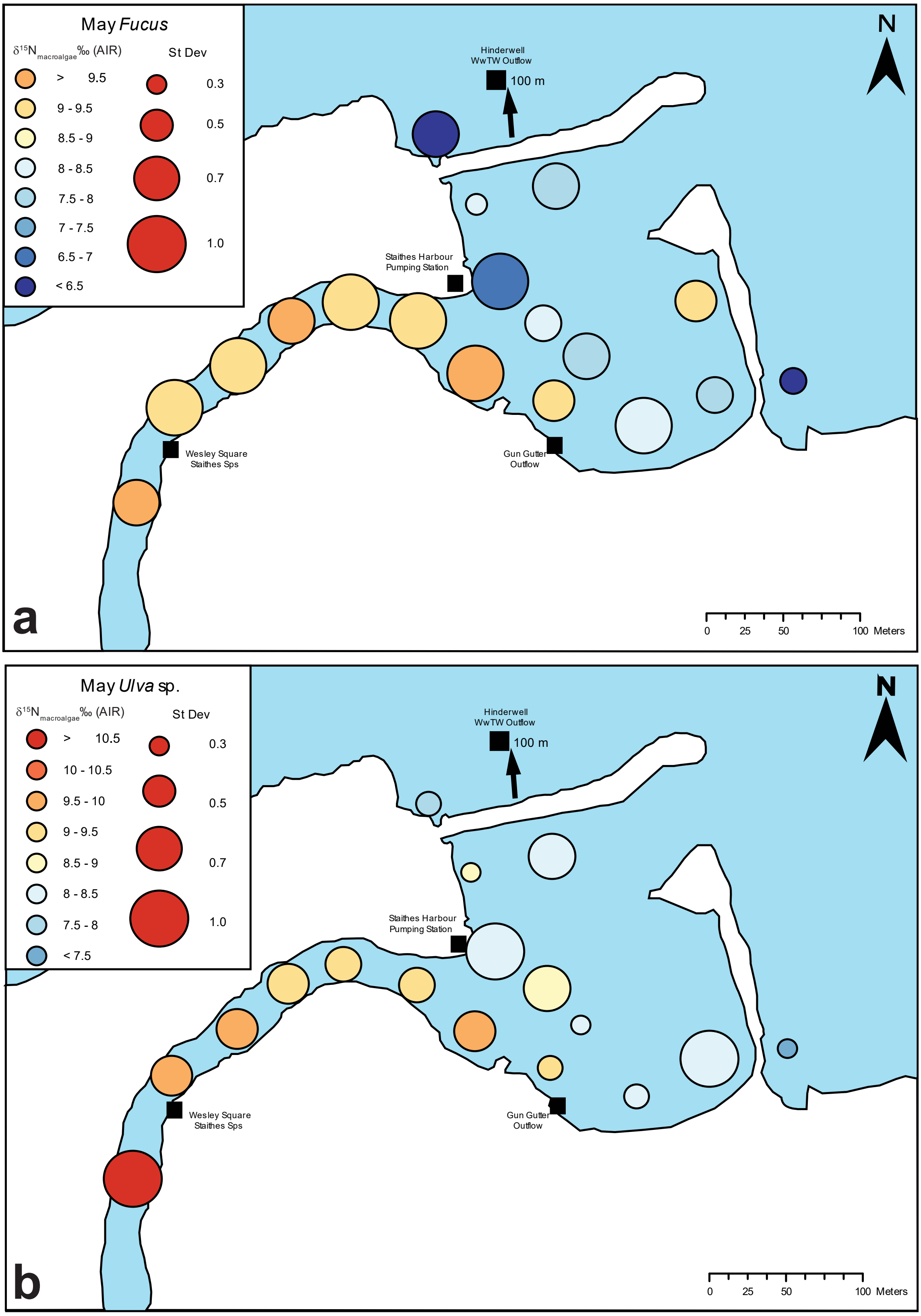
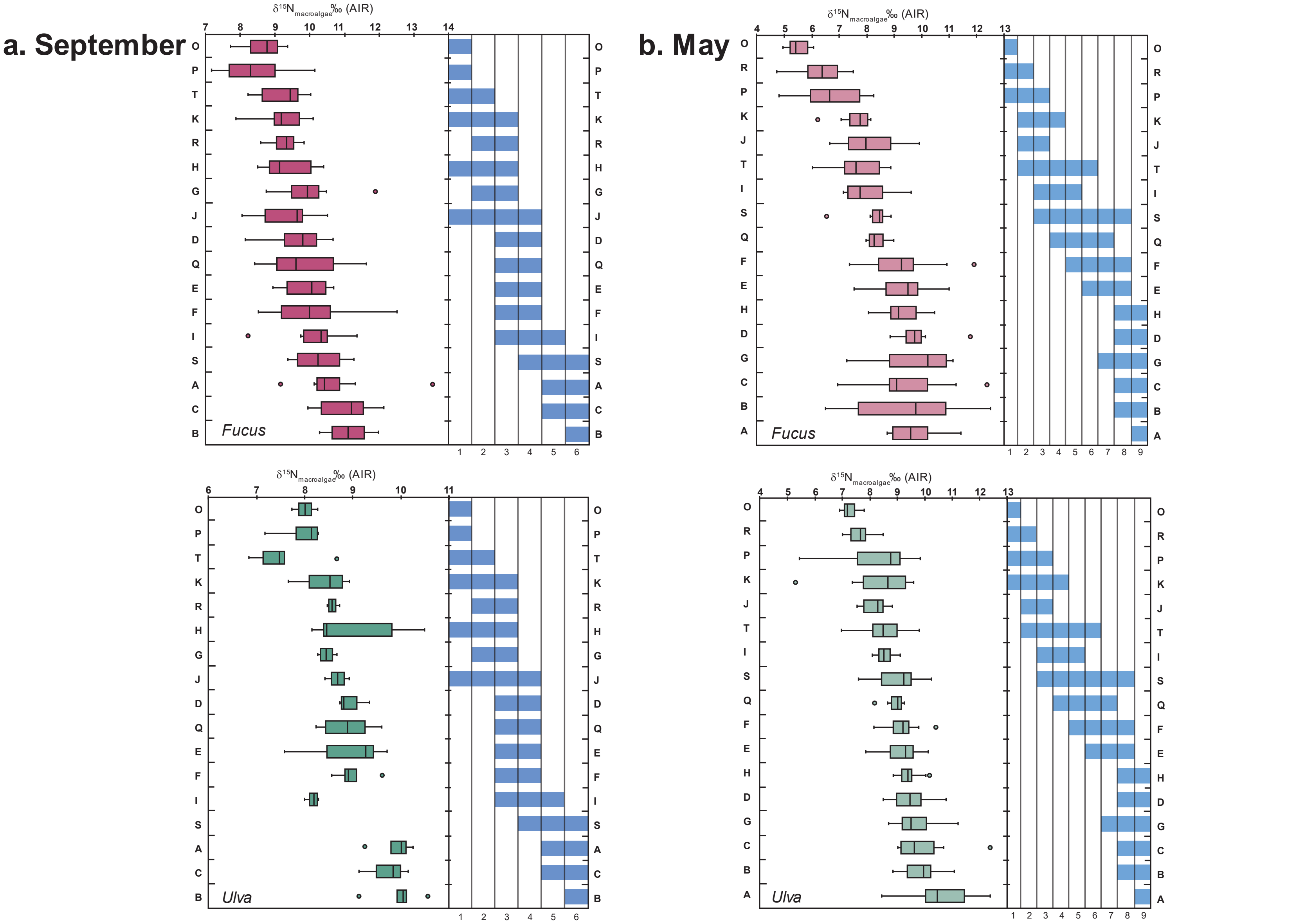


Figure 2.6:



Graphical representation of pairwise compact number display site groupings based on the Tukey Test results. If the blue bars for two sites overlap, thus sharing at least one group number, then there is no evidence of a difference between their group means. (A) Indicates that 6 groups were required to represent September sites. (B) Indicates that 9 groups were required for May. $\delta^{15}N$ values for each site are shown graphically as a boxplot.

Table 2.1:

Students T Test results for (1) comparison between regions, *Fucus* and *Ulva* combined, (2) comparison between regions *Fucus* and (3) comparison between regions for *Ulva*

T-Test Summary Table			
September	Staithes Beck	Staithes Harbour	North Sea
Staithes Beck		$1.73*10^{-8}$	$1.98*10^{-13}$
Staithes Harbour			$5.6*10^{-4}$
North Sea			
May	Staithes Beck	Staithes Harbour	North Sea
Staithes Beck		$1.34*10^{-12}$	$3.33*10^{-25}$
Staithes Harbour			$7.2*10^{-16}$
North Sea			

Fucus T-Test Summary Table			
September	Staithes Beck	Staithes Harbour	North Sea
Staithes Beck		$2.1*10^{-7}$	$8.7-10^{-13}$
Staithes Harbour			$5.5*10^{-5}$
North Sea			
May	Staithes Beck	Staithes Harbour	North Sea
Staithes Beck		$8.88*10^{-7}$	$3.39*10^{-21}$
Staithes Harbour			$7.72*10^{-15}$
North Sea			

Ulva T-Test Summary Table			
September	Staithes Beck	Staithes Harbour	North Sea
Staithes Beck		$6.5*10^{-7}$	$1.5*10^{-5}$
Staithes Harbour			0.79
North Sea			
May	Staithes Beck	Staithes Harbour	North Sea
Staithes Beck		$1.92*10^{-7}$	$1.65*10^{-23}$
Staithes Harbour			$1.65*10^{-12}$
North Sea			

Table 2.2:

Student T Test comparison between September & May for (1) *Fucus* vs *Ulva*, (2) for *Fucus*, and (3) *Ulva*

T-Test September vs May					
	May Fucus	May Ulva	Staithes Beck May	Staithes Harbour May	North Sea May
September Fucus	$1.33*10^{-18}$				
September Ulva		0.097			
Staithes Beck September			$86*10^{-4}$		
Staithes Harbour September				$6.55*10^{-12}$	
North Sea September					$7.38*10^{-18}$

Fucus T-Test September vs May			
	Staithes Beck May	Staithes Harbour May	North Sea May
Staithes Beck September	$1.65*10^{-5}$		
Staithes Harbour September		0.705	
North Sea September			$2.14*10^{-16}$

Ulva T-Test September vs May			
	Staithes Beck May	Staithes Harbour May	North Sea May
Staithes Beck September	0.132		
Staithes Harbour September		0.018	
North Sea September			$1.27*10^{-4}$

Chapter 3: Seasonal nitrogen isotope variation in macroalgae from the Mersey Estuary, UK

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Introduction

Discharges of both raw and treated sewage to UK waterbodies are becoming more frequent in recent years (Giakoumis & Voulvoulis, 2023; Gill et al., 2021). In addition to public health concerns over release of pathogens, such as *E. coli*, excessive nitrogen inputs have a detrimental cost on the environment and ecological health of rivers, estuaries, and coastlines (Florini et al., 2020; Metcalf et al., 2022; Nixon, 1995; Wurtsbaugh et al., 2019). As of 2019, no river was of "Good Chemical Status" and only 14 % of waterbodies were in "Good Ecological Heath" according to the Environment Agency (EA) (Bevan, 2020; Environmental Audit Committee, 2022). The UK's reliance on Combined Sewer Overflows (CSOs) has contributed significantly to this ongoing environmental crisis regarding water quality and ecological health of waterbodies (BBC, 2022a; Giakoumis & Voulvoulis, 2023). Sewage contains high concentrations of biologically available nitrate (NO_3^-) and ammonia (NH_4^+), at times of peak flow wastewater companies are permitted to discharge raw sewage directly to waterbodies through CSOs causing an influx of nutrients (NO_3^- & NH_4^+) to the environment. Usually, nitrogen is limiting in estuarine environments so when it is in excess primary productivity increases, commonly leading to biological blooms (Glibert, 2017; Nixon, 1995; Wurtsbaugh et al., 2019). Algal blooms can raise the biological oxygen demand (BOD), cause depletion of nutrients and oxygen resulting in eutrophication (Maier et al., 2009; Smith, 2003; Smith & Schindler, 2009).

In 2020 there were 400,000 sewage spills to UK rivers (BBC, 2021). In 2022, sewage overflows spilled for > 1.75 million hours, obtained from the 91 % of CSOs fitted with Event Duration Monitors (EDMs) (Environment Agency, 2023b). By the end of 2023 the EA hopes to have fitted EDMs to all CSOs, significantly improving the UKs ability to track sewage discharges (Environment Agency, 2023b). However, the UK is still failing to effectively reduce sewage discharges. Only 65 (or 0.5 %) of the 14,000 storm overflows have been upgraded, infrastructure is outdated and unable to cope with current population demands (Environment Agency, 2023b; House of Lords, 2023). There is a growing need to effectively monitor and reduce sewage nitrogen pollution or the UK will see instances of eutrophication rise significantly and irreversible damage to ecosystems.

Our current understanding of annual nitrogen inputs to UK estuaries is poorly documented since nitrogen concentration data is spatially and time constrained (The Rivers Trust, 2022; Woodward, 2023). An increase in productivity with temperature and light intensity causes nitrogen to become limited in summer months (Baeta et al., 2009; Ralston et al., 2014). Therefore, an influx of sewage nitrogen has greater potential for algal blooms in summer due to accelerated growth rates (Baeta et al., 2009; Barr et al., 2013; Teichberg et al., 2010). The growing number of illegal CSO discharges in the UK summer is particularly worrying; longer water residence times in estuaries and higher temperatures creates ideal growing conditions for macro- and micro-algal blooms (Philips et al., 2023; Raimonet et al., 2013). Thus, a greater understanding of annual nitrogen inputs is required to characterise any seasonal changes in nitrogen loading. Water samples used to generate nitrogen concentration data are highly influenced by short-term variations. *E. coli* samples are also thwarted with short-term variation, a lack of reproducibility in duplicate sampling, and typically only sampled in summer months in the UK (Devane et al., 2020; Environment Agency, 2023a). However, nitrogen isotopes from macroalgae provide a more ideal method for characterising nitrogen pollution inputs.

Nitrogen Isotopes in Macroalgae

Nitrogen isotope values ($\delta^{15}N$) in macroalgal tissue can discriminate industrial and anthropogenic (i.e., sewage) nitrogen sources (Dailer et al., 2010; Risk et al., 2009; Viana & Bode, 2013). High $\delta^{15}N$ values > 8 ‰ are indicative of sewage nitrogen whereby denitrification during wastewater treatment preferentially converts more ^{14}N to N_2 (g); ammonia volatilisation may also remove more ^{14}N from the system (Heaton, 1986; Savage, 2005). The remaining sewage sludge is therefore heavily enriched in ^{15}N (Heaton, 1986). Prolonged denitrification can produce very high $\delta^{15}N$ values, for example Dailer et al. (2010) provides information for multiple studies recording values > 18 ‰ associated with Wastewater Treatment Works (WWTW). Alternatively, nitrification of excess ammonia has been linked to elevated $\delta^{15}N$ values of residual ammonia in estuaries (Jacob et al., 2016; Middelburg & Nieuwenhuize, 2001). An isotopic preference for the conversion of 14N to NO_3^-

(i.e., nitrification) causes any remaining NH_4^+ to become highly enriched in 15N (Jacob et al., 2016; Middelburg & Nieuwenhuize, 2001). Since macroalgae uptakes both nitrate and ammonia it is possible that highly enriched $\delta^{15}N$ within macroalgal tissue may also come from NH_4^+ uptake as well as NO_3^- uptake (Roleda & Hurd, 2019). Conversely, industrial sources (i.e., agricultural fertilisers) have very low $\delta^{15}N$ values due to a nitrogen source from atmospheric N_2 (g) which has an $\delta^{15}N$ value ~ 0 ‰ (Heaton, 1986; Peterson & Fry, 1987).

This technique has been used globally to identify sewage nitrogen inputs to marine environments (Gröcke et al., 2017; Risk et al., 2009; Samper-Villarreal, 2020). It is a relatively cheap method of source discrimination, sampling is simple since macroalgae is widely distributed (Barr et al., 2013; Gröcke et al., 2017; Savage, 2005; Viana & Bode, 2013). Sampling methods are dependent upon the focus of the study. For example, transects are more effective when tracing effluent dilution from an outflow (Savage, 2005; Savage & Elmgren, 2004); whilst transplanting macroalgae can better trace sources of effluent in deeper water (e.g., salmon effluent) and/or where there is minimal macroalgae cover (Costanzo et al., 2001; Howarth et al., 2019).

Macroalgal $\delta^{15}N$ has been found to vary seasonally; warmer months produce higher $\delta^{15}N$ values (Lemesle et al., 2016; Raimonet et al., 2013). Species differences can also influence $\delta^{15}N$ variation – opportunistic species such as *Ulva* sp. (hereafter *Ulva*) uptake and assimilate nitrogen within ~48 hours (Gröcke et al., 2017; Thornber et al., 2008). By contrast, *Fucus vesiculosus* (hereafter *Fucus*) can take ~14 days to assimilate nitrogen (Bailes & Gröcke, 2020; Viana et al., 2015). Due to incorporation over different time scales the nitrogen loading regime can be assessed over an immediate, fortnightly, or sometimes 6-month period (Viana et al., 2015).

In well mixed environments macroalgae have consistent reproducibility (Alldred et al., Chapter 2), and their wide distribution allows for application in most coastal environments such as estuaries. Shifts in nitrogen inputs between anthropogenic, agricultural, and industrial have yet to be traced in a large, populated estuary in the UK. This chapter aims to produce an annual record of $\delta^{15}N$ for the Mersey Estuary in the northwest of England to understand how nutrient sources differ across this large estuary. A summer enrichment trend is expected, with both species recording elevated values in proximity to sewage infrastructure.

Study Site

The Mersey Estuary is a 26 km long estuary in the northwest of the UK, it is located at the mouth of the River Mersey where it discharges into Liverpool Bay, Irish Sea (Fig. 3.1) (Mersey Basin Campaign, n.d.). The Mersey Basin drains a catchment of ~ 5,000 km^2 with a population > 5 million people including Manchester, Stockport, and Liverpool (Hawkins et al., 2020; National Rivers Authority, 1995). Approximately 480,000 people reside in Liverpool located at the eastern bank of the estuary, this figure does not include the other large population centres such as Runcorn and Birkenhead on the west bank of the estuary (Fig. 3.1) (Burton, 2003; ONS, 2021). A large tidal range (> 10 m) causes strong currents across the entire estuary (Ritchie-Noakes, 1984). The inner estuary is characterised by large sand banks; this changes to a deep, fast-moving channel as the estuary narrows to 1.5 km (Fig. 3.1) (National Rivers Authority, 1995). The estuary is < 1 km at its narrowest point. Peak flow rates are observed in winter months between November and January for the Mersey averaging 22.5 m^3/s (data obtained from Ashton Weir over a 10-year period, see Supplementary Fig. 3.1) (National River Flow Archive, 2020). Low flow periods are seen between May and August averaging 9.5 m^3/s (National River Flow Archive, 2020). It is assumed that the estuary flow also peaks during winter and falls during the summer in line with the river flow.

Public opinion of water quality is low for the Mersey River and Mersey Estuary, due to over 200-years of industrial, agricultural and wastewater pollution entering the catchment (Burton, 2003; Hawkins et al., 2020). Between 1985 – 2010 the Mersey Basin Campaign was set up to improve environmental conditions and water quality (Burton, 2003; Meredith, 1992; Mersey Basin Campaign, n.d.; National Rivers Authority, 1995). Both metal contaminants and BOD were successfully improved (Hawkins et al., 2020). Improved BOD meant biodiversity recovered and now 37 fish species can be found in the estuary (Hawkins et al., 2020; The Mersey Rivers Trust, 2023). However, efforts to reduce nutrient concentrations have been less successful (Hawkins et al., 2020). In 2019, the estuary exceeded the transitional

Water Framework Directive (WFD) Dissolved Inorganic Nitrogen threshold (Greenwood et al., 2019). Sewage discharges have become increasingly frequent along the Mersey, CSOs spilled for 212,000 hours into the catchment in 2021 alone (The Rivers Trust, 2022). In 2022, a total of 977 sewage discharges into the Mersey River were recorded (BBC, 2023). High levels of nitrate have long been associated with point source sewage outflows along the Mersey and a growing number of reports suggest year-round sewage inputs to the Mersey are occurring, including times of low flow (BBC, 2022a, 2022b; Hawkins et al., 2020).

Materials and Methods

Macroalgae samples were collected from 38 sites: 25 from the River Mersey, 10 from the Docks and three from outflows (Fig. 3.1). Sites were sampled over a 3-day window in January (Winter), April (Spring), and July (Summer) 2023 to assess the seasonal differences in macroalgal $\delta^{15}N$. Approximately 5 – 15 macroalgae specimens were collected for each site, primarily *Fucus* and *Ulva*. The most recent non-fertile tips were collected for *Fucus* (taken from the longest frond), ~ 4 cm sections were collected for green and red species which were squeezed to remove seawater. All macroalgae samples were rinsed with seawater at the site of collection. In January a total of 178 *Fucus* and 57 *Ulva* samples were collected from the River Mersey, and 39 samples were collected from the Docks. Dock samples consisted of 6 *Ulva*, 21 *Cladophora* sp., 2 *Fucus* and 10 *Callithamnium corymbosum* (a red seaweed). In April a total of 170 *Fucus* and 95 *Ulva* samples were collected from the River Mersey, and a further 34 samples were collected from the docks (20 *Ulva*, 3 *Cladophora* sp., 9 *Fucus*, and 2 red seaweeds). In July, 132 *Fucus* and 116 *Ulva* were sampled from the River Mersey. 35 *Ulva* samples were collected from the docks, no *Cladophora* sp. was sampled.

Prior to stable isotope analysis, macroalgae samples were dried in an oven between 45–60°C for three days. For *Fucus*, the most recent growth of the thallus was analysed and assumed to represent the $\delta^{15}N$ value of its environment over ~ 14 days (Gröcke et al., 2017; Viana et al., 2015). Sub-samples of the other macroalgae, *Ulva*, *Cladophora* sp. and red seaweeds were analysed. All samples weighed between 1–1.5 mg. Stable isotope analysis was carried out at the Stable

Isotope Biogeochemistry Laboratory (SIBL) at Durham University following protocols outlined in Bailes and Gröcke (2020) and Gröcke et al. (2017). ArcGIS Pro (3.0) was used to generate a series of isoplots to visually show $\delta^{15}N$ values across the Mersey Estuary for each season. Isoplots display the average $\delta^{15}N$ value for each site, the point size varies by standard deviation for that site. Excel was used to generate all graphical figures (e.g., boxplots). Students T-Tests were also used to test the significant difference between each sample month for the Mersey Estuary and Docks,

In addition, 14 water samples from January, April and July were tested for NH_4-N, Total Organic Nitrogen (TON), NO_2-N, NO_3-N and NO_3 concentrations. 50 ml centrifuge tubes were filled with water collected directly from outflow pipes, dock water or the River Mersey and stored in a refrigerator until analysis. All water analyses were carried out on 25 ml samples at the James Hutton Institute, Scotland. The method is described as follows: for Total Oxidisable Nitrogen determination, the sample is pH buffered using an Imidazole based buffer and transferred to a copper cadmium coil where Nitrate is chemically reduced to Nitrite. The sample is then mixed with the sulphanilamide-NEDD colour reagent. The Sulphanilamide reacts with both the chemically reduced nitrite, plus any existing nitrite, forming a diazonium compound. This couples with N-(1-napthyl)-ethylenediamine dihydrochloride to form an azo dye. The resulting complex can be measured photometrically at 520 nm. A separate aliquot of the sample is analysed for Nitrite by omitting the cadmium reduction step so that the sulphanilamide-NEDD colour reagent can only react with any existing Nitrite. The Nitrate concentration can be determined by subtracting the Nitrite result from the Total Oxidisable Nitrogen result (SEAL Analytical, 2021).

Results

Seasonal Dock Record:

In January, dock $\delta^{15}N$ values ranged between -2.2 ‰ and +23.3 ‰ (*Ulva*, Site 30, *Callithamnium corybosum*, Site 16, respectively) (Fig. 3.2). The South Docks recorded an average $\delta^{15}N$ of 14.3 ‰ ± 4.6 ‰ (n = 37). The red species *Callithamnium* $\delta^{15}N$ average was enriched (18.1 ‰ ± 3.4 ‰, n = 10) in comparison to the green species *Cladophora* sp. (14.7 ‰ ± 2.6 ‰, n = 21) and *Ulva* (6.6 ‰ ± 1.6 ‰, n = 6). Alfred Dock (Site 30) recorded a lower $\delta^{15}N$ average in comparison to the South Docks (1.3 ‰ ± 2.7 ‰, n = 4); inclusion of Site 30 produces a slightly lower dock value of 13.0 ‰ ± 5.9 ‰ (n = 41) for January.

In April, $\delta^{15}N$ values range between 7.5 ‰ and 15.6 ‰ (Site 23 and Site 21 respectively). The South Docks produced a lower $\delta^{15}N$ average than winter (12.5 ‰ ± 2.5 ‰, n = 25) (Fig. 3.2). *Ulva* recorded the most elevated $\delta^{15}N$ average of 13.0 ‰ ± 2.2 ‰ (n = 20) in April, in contrast to January. Alfred Dock (Site 30) produced an average of 10.2 ‰ ± 0.7 ‰, n = 9 (*Fucus*) & 10.9 ‰ ± 0.3 ‰, n = 3 (*Ulva*): no significant difference is observed in the dock average on inclusion of Site 30 (12.3 ‰ ± 2.4 ‰, n = 28).

In July, only *Ulva* was sampled for the docks which recorded an average $\delta^{15}N$ of 11.3 ‰ ± 3.8 ‰ (n = 28). No significant difference is observed on inclusion of Alfred Dock (11.0 ‰ ± 3.8 ‰, n = 35). $\delta^{15}N$ ranges from 0.2 ‰ (Site 23) – 17.7 ‰ (Site 18). Figure 3.2 indicates there is no difference between dock macroalgal $\delta^{15}N$ across all collection months. January records the highest average; but a Students T -Test shows there is no statistical difference between April (p-value = 0.24) and July (p-value = 0.08). April and July also record no statistical difference (p-value = 0.14).

<u>Seasonal River Mersey Estuary Record:</u>

The Mersey Estuary $\delta^{15}N$ record uses data from Sites 0 to 34 (Fig. 3.1). Sites 35 and 36 have been omitted due to their location in Liverpool Bay as well as the outflows at Sites 1, 5 and 9 (Fig. 3.1). In January, $\delta^{15}N$ values range between 3.4 ‰ and 15.3 ‰ (Site 12 and Site 6, respectively) for *Fucus* and 7.2 ‰ and 13.9 ‰ (Site 8 and Site 25, respectively) for *Ulva* (Fig. 3.3). *Fucus* produced an average $\delta^{15}N$ value of 12.1 ‰ ± 2.2 ‰ (n = 160) for the Mersey Estuary, whereas it was lower for *Ulva* (11.9 ‰ ± 1.8 ‰, n = 51). No significant difference is evident between

January *Fucus* and *Ulva* (p value < 0.05). The lowest average $\delta^{15}N$ value for *Ulva* was at Site 9B (9.2 ‰ ± 0.7 ‰, *n* = 2) and at Site 11 for *Fucus* (8.7 ‰ ± 1.7 ‰, *n* = 10). Site 10 for *Ulva* and Site 34 for *Fucus* produced the highest $\delta^{15}N$ averages (16.8 ‰, n = 1 and 14.4 ‰ ± 0.4 ‰, *n* = 10, respectively).

In April, the Mersey Estuary recorded a $\delta^{15}N$ range between 8.4 ‰ and 14.7 ‰ for *Fucus* (Sites 36 and 8, respectively) (Fig. 3.3a). *Ulva* produced a range between 9.6 ‰ and 19.7 ‰ (Sites 9B and 11, respectively) (Fig. 3.3b). *Fucus* averaged 12.2 ‰ ± 1.4 ‰ (*n* = 155); no significant different was recorded with the winter *Fucus* collection (p value > 0.3). April *Ulva* produced a significant higher $\delta^{15}N$ average to *Fucus*: 14.8 ‰ ± 2.2 ‰, *n* = 80 (p value > 0.05). Site 36 produced the lowest average for both *Fucus* and *Ulva* (9.4 ‰ ± 0.7 ‰, *n* = 8 and 11.1 ‰ ± 0.7 ‰, *n* = 6 respectively). The highest average $\delta^{15}N$ for *Fucus* was at Site 6 (13.5 ‰ ± 1.0 ‰, *n* = 9) and Site 10 for *Ulva* (17.8 ‰ ± 1.3 ‰, *n* = 6).

For July, the $\delta^{15}N$ average was higher for the Mersey Estuary than January and April: 13.7 ‰ ± 3.3 ‰, *n* = 132 (*Fucus*) and 16.1 ‰ ± 2.9 ‰, *n* = 166 (*Ulva*) (Fig. 3.3). *Ulva* is significantly different to all other seasons (p value < 0.05). *Fucus* ranged between 2.8 ‰ (Site 12) and 22.6 ‰ (Site 7), whilst *Ulva* ranged between 4.0 ‰ (Site 14) and 21.1 ‰ (Site 7). Site 7 was significantly enriched, averaging > 19.0 ‰ for both species. Site 12 recorded the lowest $\delta^{15}N$ average for *Fucus* (5.7 ‰ ± 1.7 ‰, *n* = 7), whilst Site 36 produced the lowest $\delta^{15}N$ average for *Ulva* (10.0 ‰ ± 0.8 ‰, *n* = 8). Figure 3.3 suggests an enrichment trend from January to July for *Ulva*; but this is less pronounced in *Fucus* (Fig. 3.3).

<u>River Mersey Outflow Record:</u>

In January, outflows were relatively depleted in ^{15}N compared to the estuary. Sites 5 & 9 recorded the lowest $\delta^{15}N$ averages (0.7 ‰ ± 0.9 ‰ *n* = 3 & 2.5 ‰ *n* = 1, respectively). Site 9A was higher at 8.4 ‰ ± 1.4 ‰ (*n* = 4). For April, outflows were slightly higher than January: Site 5 recorded 8.9 ‰ ± 0.5 ‰ (*n* = 5). The July data set recorded lower $\delta^{15}N$ values for outflows. Sites 1 & 5 are very low (2.7 ‰ ± 0.3 ‰ *n* = 3 & 4.2 ‰ 2.9 ‰ ± *n* = 7, respectively). Site 9 shows no significant change to April (7.0 ‰ ± 1.3 ‰, *n* = 5).

<u>Water Analyses:</u>

For January, sites ranged from 0.2 – 216.8 mg/l in NO_3 concentration (Sites 9A & 5, respectively). Two water samples were collected from the CSO at Site 5 and averaged 212.5 mg/l ± 4.2 mg/l NO_3, four times the legal limit of 50 mg/l NO_3 in the UK(Drinking Water Inspectorate, 2023). A creek sampled at Site 8 also recorded NO_3 concentration above the UK limit at 56.4 mg/l, this was sampled from a freshwater creek draining into the Mersey. All other samples were below 50 mg/l, Site 1 had the next highest at 19.6 mg/l NO_3. The South Docks recorded lower NO_3 concentrations averaging 2.3 mg/l from samples collected from Sites 19, 21 & 24 (Fig. 1). Ammonia (ionised) also recorded high concentrations with Site 9A measuring 3.3 mg/l NH_4^+: this is above the recommended limit of 0.2 mg/l advised by the WFD(Environment Agency, 2007). In total, nine sites recorded concentrations above this limit- these are provided in Table 3.1.

In April Nitrate recorded a narrower concentration range compared to January: 0.01 to 176.1 mg/l (Sites 9A & 5). Site 5 (the WWTW outlet) recorded the highest NO_3^- concentration at 176.1 ± 2.4 mg/l (n = 2). Like January, Site 8 recorded the second highest nitrate concentration (47.4 mg/l): this is high but falls within the WFD limit. Docks recorded low NO_3^- concentrations (Table. 3.1). For July, nitrate concentrations ranged between 0.01 mg/l (Sites 12, 19 & 21) and 98.5 mg/l (Site 5). Sites 5 & 8 (62.4 mg/l NO_3^-) were again the two highest sites. Dock values were lower compared to in January and April (Table. 3.1).

Discussion

Seasonal $\delta^{15}N$ in the Liverpool Docks:

Liverpool Dock water levels are replenished through a pumping system, limited mixing is possible through opening of gates at Canning and Brunswick Docks resulting in a replenishment time of 6–12 months (Allen et al., 1995; Hawkins et al., 2020; Wilkinson et al., 1996). Therefore, the dock $\delta^{15}N$ record would be expected to show minimal change between months. January recorded the highest $\delta^{15}N$ average (14.3 ‰ ± 4.6 ‰, n = 37) and the widest $\delta^{15}N$ range (Fig. 3.2). No significant difference was recorded between each month: dock $\delta^{15}N$ remains consistently high across all three months (Fig. 3.2). $\delta^{15}N$ values are > 11.0 ‰, indicating a sewage nitrogen source exists for the docks. Only January records no significant difference between the docks and Mersey River (*Ulva* record) (p value > 0.09). Higher flow rates on average in winter (Supplementary Fig. 3.1) may increase mixing between the docks and Mersey; the dock macroalgae would reflect a similar value to the river as a result. April and July recorded significantly lower δ^{15}N values for the docks in comparison to the Mersey (p values < 0.05). Flow rates are lower for these months (Appendix 1): reduced mixing with the docks may be causing this offset in δ^{15}N. Lower dock δ^{15}N values is likely to be due to increased industrial and/or chemical runoff from drains. Potentially there is also a bio-filtration effect by *Mytilus edulis* (blue mussel) colonies (Hawkins et al., 2020; Wilkinson et al., 1996). Natural filtration is estimated at ~1.5 months (Alldred et al., Chapter 4) and may reduce δ^{15}N values; as well as masking any seasonal enrichment trends producing a relatively constant δ^{15}N across each month. This is discussed in greater detail in Chapter 4 in a historical context.

<u>Seasonal δ^{15}N in the River Mersey:</u>

The River Mersey recorded elevated δ^{15}N values in both *Fucus* and *Ulva* across all collection months, suggesting that the source of nitrogen is from sewage treatment works (Fig. 3.3) (Barr et al., 2013; Deutsh & Voss, 2006; Raimonet et al., 2013). *Fucus* δ^{15}N recorded no significant difference between January and April (p value > 0.3) with both seasons enriched > 12 ‰. *Fucus* became significantly more enriched in July (13.7 ‰ ± 3.3 ‰, *n* = 132), and had the widest variation in δ^{15}N across the Mersey Estuary (Fig. 3.3a). *Ulva* δ^{15}N produced a clearer seasonal trend increasing by ~2 ‰ between each month (Fig. 3.3b). The July *Ulva* collection was significantly enriched above the *Fucus* collection (p value < 0.05) (Fig. 3.3). Alldred et al (Chapter 4) discusses a September 2022 macroalgae collection which shows elevated δ^{15}N values in both *Fucus* and *Ulva*: these are comparable to July 2023 (Fig. 3.3). An isoplot for September 2022 has not been generated as it is much smaller in sample size and different locations were sampled compared to the other collection trips (e.g., only on the northern margin of the River Mersey).

Isotopic values are elevated above the “clean” isotopic range across the annual season (Fig. 3.3): consistent enrichment of *Fucus* reveals year-round sewage nitrogen loading (Barr et al., 2013; Savage & Elmgren, 2004). *Fucus* actively uptakes nitrogen during winter, clear enrichment in January supports a sewage source that persists into the summer months when *Fucus* nitrogen uptake is at a minimum (García-Seoane et al., 2018; Viana et al., 2015). *Ulva* also records an annual sewage nitrogen source; but significantly higher $\delta^{15}N$ values were recorded in summer (Fig. 3.3b). Similar trends for *Ulva* off the coast of France were reported by Lemesle et al. (2016) and Raimonet et al. (2013). This > 2.5 ‰ increase for *Ulva* in summer may relate to its opportunistic growth strategy and rapid incorporation of nitrogen (Gröcke et al., 2017; Thornber et al., 2008). The response observed in summer may relate to *Fucus* using nitrogen taken up in the winter and summer, whereas *Ulva* is potentially providing a more immediate nitrogen signature (Gröcke et al., 2017; Thornber et al., 2008). Hence, the species record an increase during summer months, both recording a source of effluent and as well as a biological system response (Thornber et al., 2008; Van Beusekom & De Jonge, 1998).

River Mersey macroalgae $\delta^{15}N$ is much more positive in comparison to other UK and European studies (Jones et al., 2018; Raimonet et al., 2013; Riera et al., 2000; Savage, 2005; Viana & Bode, 2013). Denitrification processes in the treatment of sewage is a very plausible mechanism generating positive $\delta^{15}N$ values (Costanzo et al., 2001; Heaton, 1986; Savage, 2005); however, this process typically produces $\delta^{15}N$ values between 10 – 14 ‰ (Dailer et al., 2010). Another environmental issue regarding WWTWs is the significant release of ammonia (NH_4^+). Concentrations for January were well above the permitted NH_4^+ limit (Table. 3.1) (Environment Agency, 2007). When ammonia is in excess $\delta^{15}N$ values of residual NH_4^+ can become very elevated (Jacob et al., 2016; Middelburg & Nieuwenhuize, 2001): $\delta^{15}N$ of ammonium has been recorded between 14 – 43 ‰ for the Thames Estuary (Middelburg & Nieuwenhuize, 2001). Macroalgal values > 18 ‰ resulting from nitrification of NH_4^+ is therefore not unreasonable to assume for the Mersey Estuary. A WWTW source of ammonia may also explain the higher $\delta^{15}N$ recorded in *Ulva* caused by preferential uptake of NH_4^+ (Gröcke et al., 2017; Roleda & Hurd, 2019). Higher $\delta^{15}N$ values in the upper estuary for *Ulva* (Fig. 3.4) correspond to proximity with a wastewater treatment plant plus higher ammonia concentrations (Table. 3.1). Ammonia concentration may be playing an important role in producing such high isotope values in addition to high volumes of treated sewage. Further investigation is required into $\delta^{15}N$ values of WWTW discharge;

plus, additional NH_4^+ concentration data for April and July to understand if this phenomenon exists into summer months when very high $\delta^{15}N$ values are recorded (Fig. 3.3b).

Geospatial $\delta^{15}N$ variation for the Mersey Estuary:

$\delta^{15}N$ values were found to vary spatially across the Mersey Estuary: higher values were recorded by both species in the upper estuary (Figs. 3.4 & 3.5). Isotopic values at outflows were very depleted in comparison to the river. The CSO (Site 5) recorded very low $\delta^{15}N$ values: < 2 ‰ (Fig. 3.4); chemically sourced ammonia discharged during the treatment process may be resulting in this industrial nitrogen signature (Dailer et al., 2010; Peterson & Fry, 1987). This also explains high ammonia concentrations recorded for January (Table 3.1). Interestingly, the river macroalgae still records a sewage nitrogen signal near outfall locations (Sites 1, 5 & 9, Fig. 3.4). This would suggest that either, (1) *Ulva* is recording a snapshot of the past ~48 hours and industrially sources ammonia was only discharged in this period or, (2) that sewage nitrogen is coming from further upstream in the Mersey and is the dominant source in the estuary. Routine monitoring would be required to more fully understand when and where the estuary outflows are discharging sewage.

Clearly a sewage nitrogen signal persists across the estuary; Figures 3.4 & 3.5 show values remain high regardless of season or species. Macroalgae does record a slight decline in $\delta^{15}N$ values from the upper estuary towards the mouth, although values remain between 10 – 14 ‰ (Figs. 3.4 & 3.5). *Ulva* sampled in July recorded the greatest difference (~8 ‰) pointing towards high sewage nitrogen loading furthest upstream (Fig. 3.4c). Extensive tidal flats and reduced turbidity may be concentrating sewage nitrogen in the upper estuary; before values drop to ~ 10 ‰ towards the mouth (Figs 3.4 & 3.5) (Barr et al., 2013; Raimonet et al., 2013). Previous studies have reported of a pollutant plume the Mersey Estuary impacts the Liverpool Bay area (Hawkins et al., 2020; P. G. W. Jones & Haq, 1963). Unpublished data analysed by SIBL also suggests a pollutant plume originating from the Mersey can be traced as far as the Cumbria coastline. Extending the study site further upstream in the Mersey may help ascertain where sewage nitrogen is originating from, multiple WWTW along the whole river are likely to be all contributing to this year-round sewage loading problem.

Conclusions

Macroalgae records a slight seasonal shift to higher $\delta^{15}N$ values in summer months for the Mersey Estuary. Although a strong seasonal cycle is masked by high year-round isotope values across the entire estuary. $\delta^{15}N$ is consistently > 12 ‰ for much of the estuary indicating significant and widespread sewage nitrogen loading. Treated sewage from multiple WWTWs is likely to be the dominant source. It also appears a cumulative effect is causing year-round sewage nitrogen loading. Pollutants from population centres such as Manchester and Stockport upstream drain into the Mersey Estuary and likely to be a major contributor of sewage nitrogen loading for this environment. More data is needed regarding monthly $\delta^{15}N$ information as well as further upstream for the Mersey to identify other CSO inputs. However, macroalgal $\delta^{15}N$ has been shown to be an effective tracer of sewage nitrogen loading within estuaries. The Mersey is clearly subject to high sewage loads suggesting current infrastructure is inefficient in removing sewage nitrogen from wastewater and the problem can extend far downstream of the outfall. Macroalgal $\delta^{15}N$ is a simple tool for identification of nitrogen sources for UK estuaries and one that should be applied more often.

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Figure 3.1

The Mersey Estuary and Liverpool Docks site locations. ArcGIS Pro (3.0) was used to generate all maps.

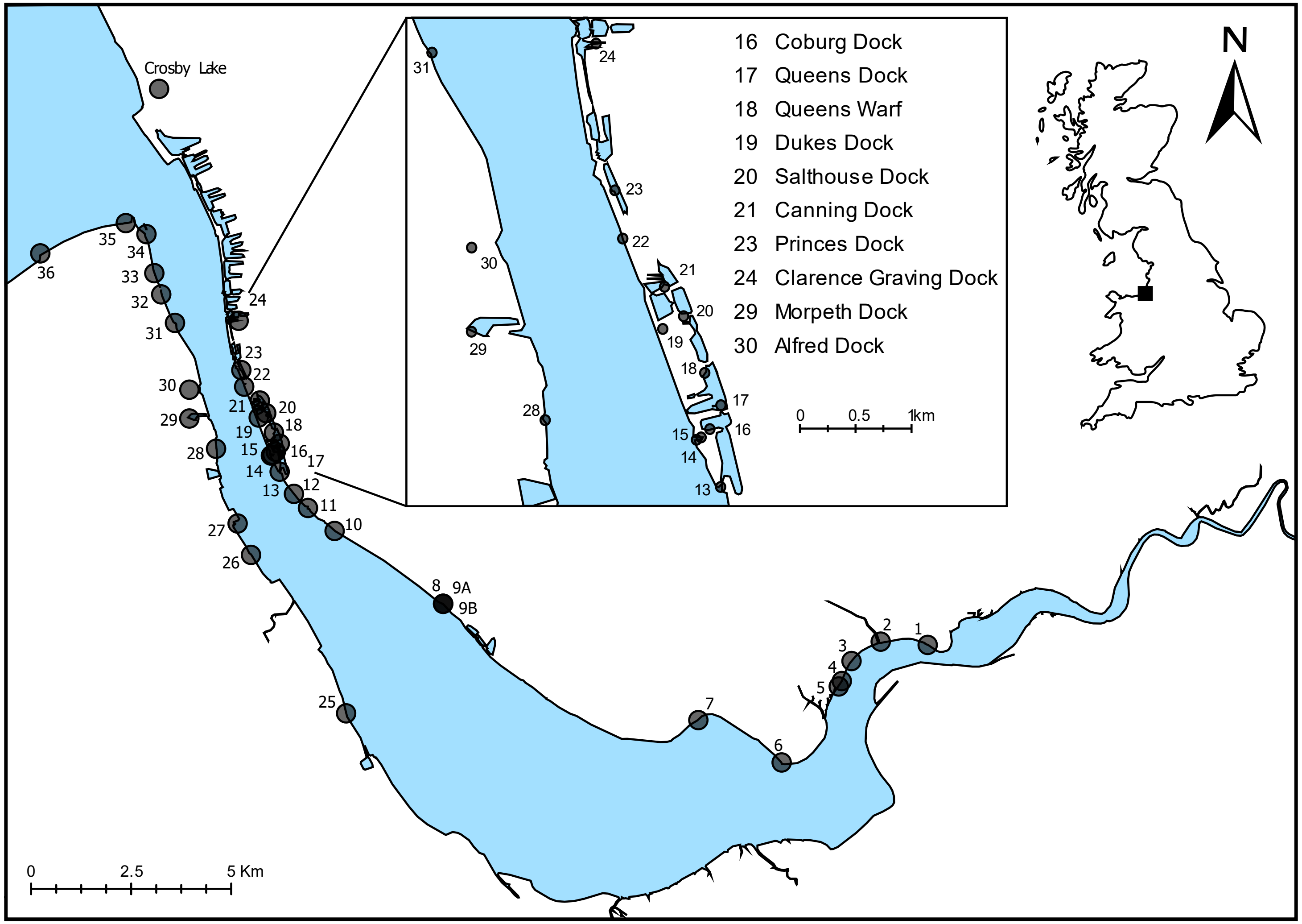

Figure 3.2

Seasonal $\delta^{15}N$ values for the Liverpool Docks. January *n* = 39, April *n* = 34 and in July *n* = 35

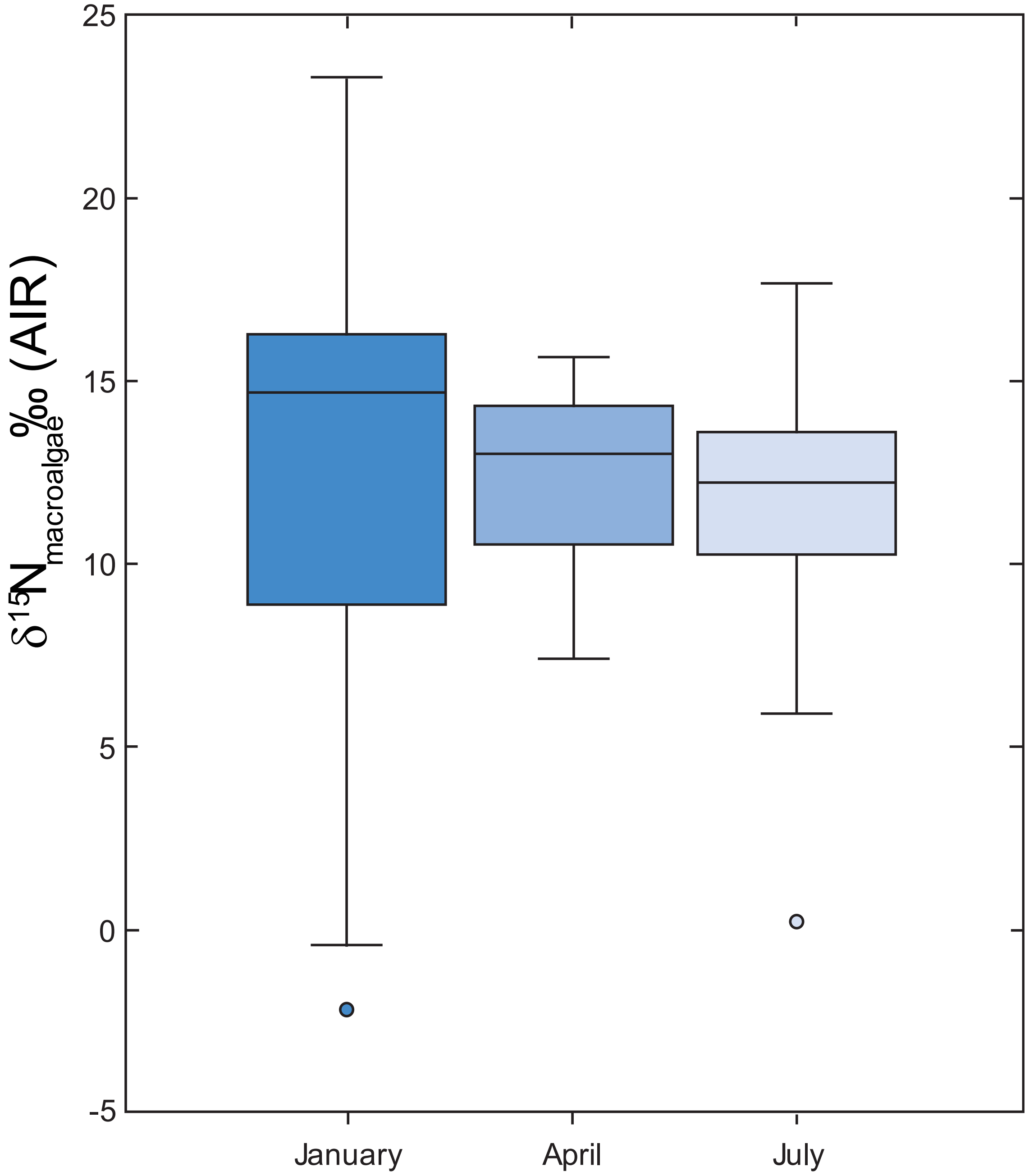

Figure 3.3

Seasonal $\delta^{15}N$ record for the Mersey Estuary, **3a** shows $\delta^{15}N$ values for *Fucus vesiculosus*: January n = 178, April n = 170 and in July n = 132. **3b** shows data for *Ulva* sp: January n = 57, April n = 95 and in July n = 116

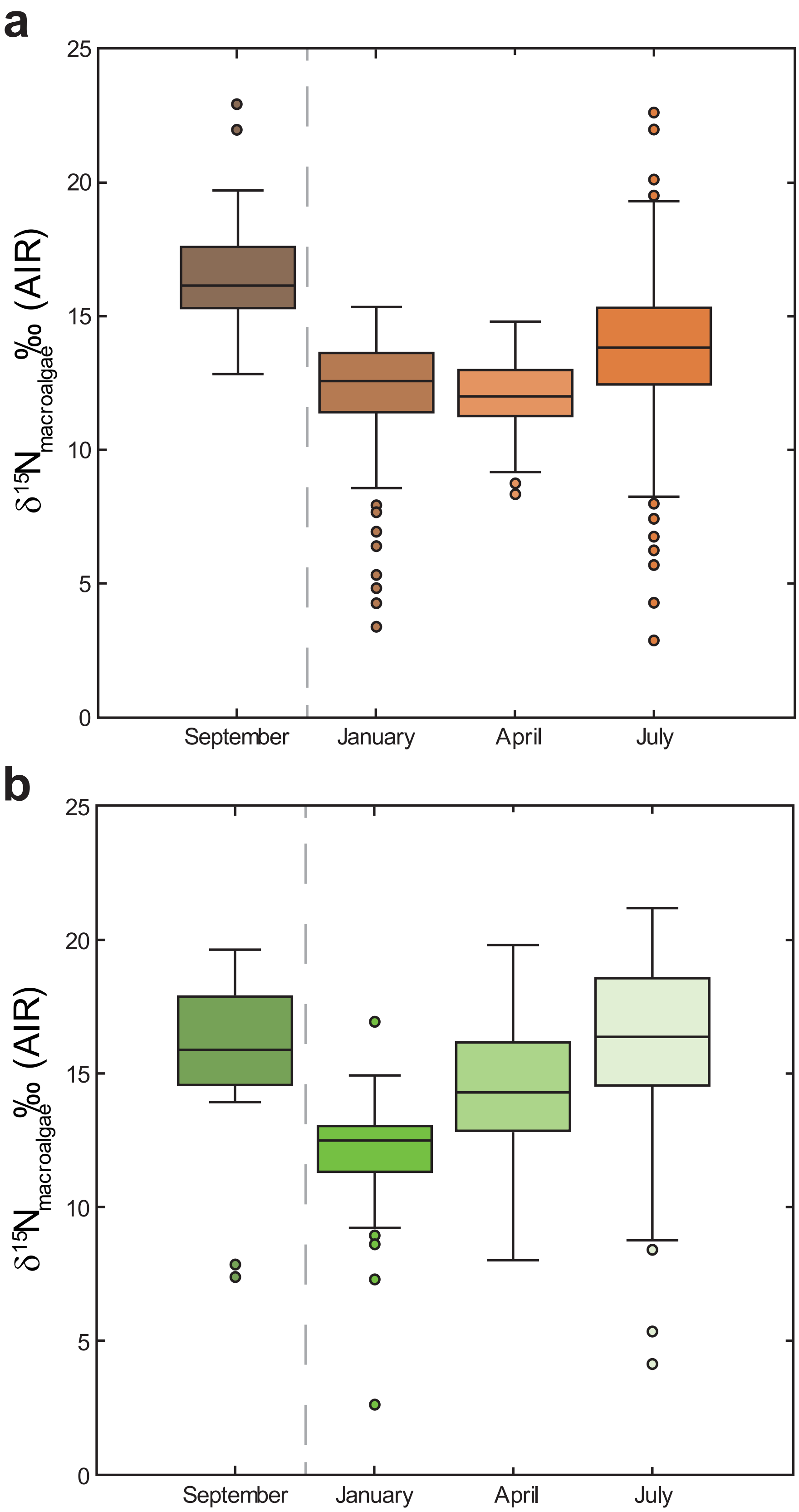

Figure 3.4

Ulva sp. isoplot for the Mersey Estuary and Liverpool Docks. **4a** shows $\delta^{15}N$ averages and standard deviations for each site for January, **4b** shows data for April and **4c** shows data for July. Numbers in bold refer to the nitrate concentration data. All plots were produced using ArcGIS Pro 3.0.

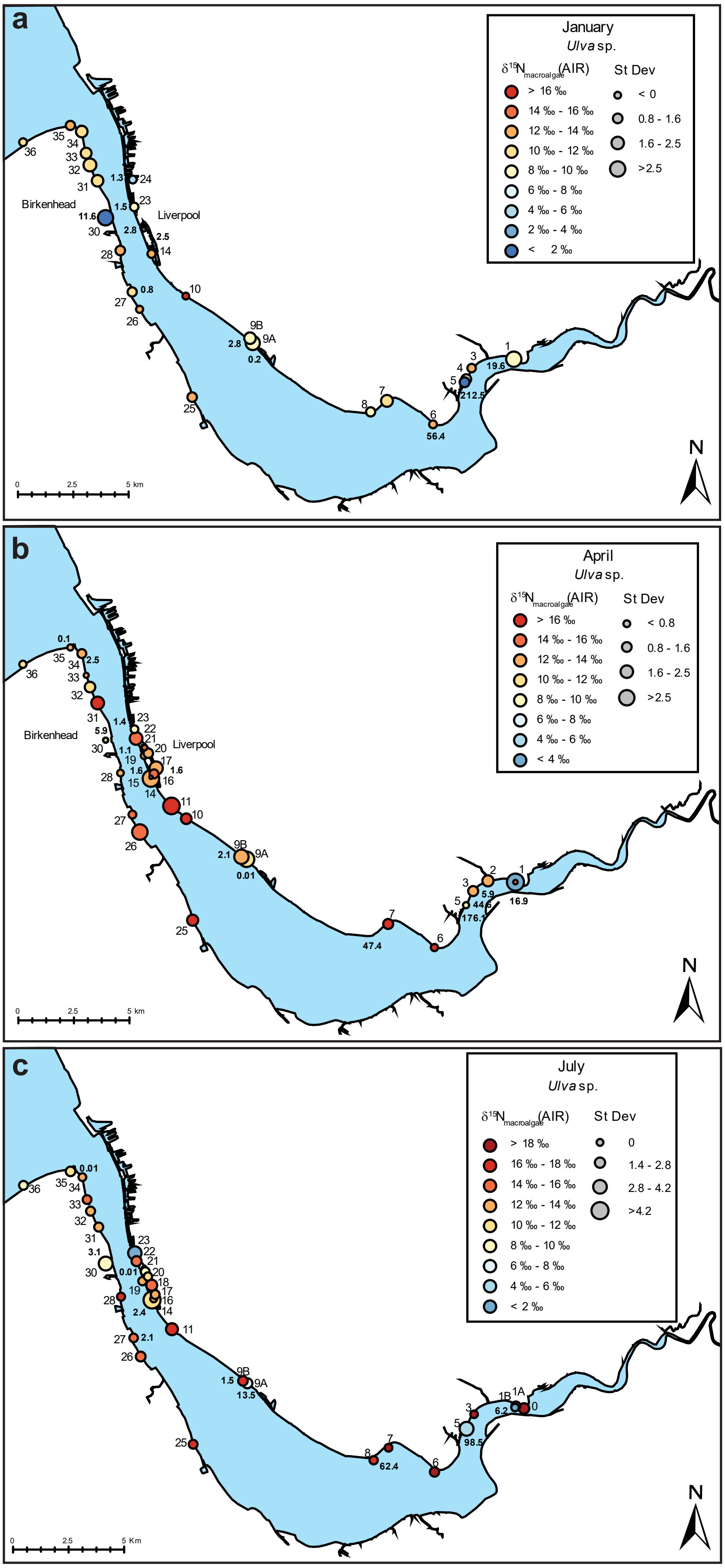

Figure 3.5

Fucus vesiculus isoplot for the Mersey Estuary and Liverpool Docks. **5a** shows $\delta^{15}N$ averages and standard deviations for each site for January, **5b** shows data for April and **5c** shows data for July. Numbers in bold refer to the nitrate concentration data. All plots were produced using ArcGIS Pro 3.0

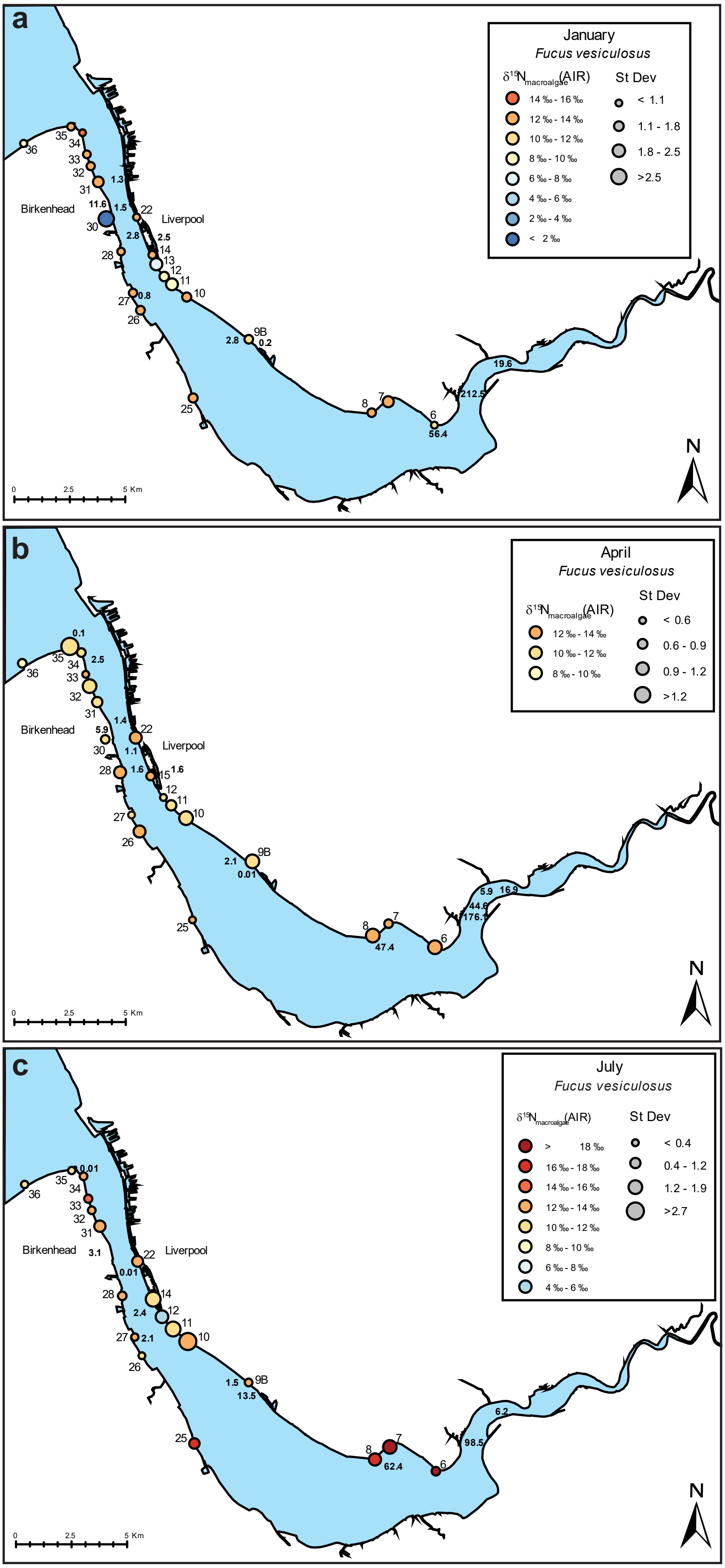

Table 3.1

Nitrate & Ammonia concentration data (mg/l) for the Mersey Estuary and Liverpool Docks. Concentrations highlighted in red indicate where concentrations breach regulations according to the Drinking Water Inspectorate & Environment Agency. Concentration analyses were provided by the James Hutton Institute, Scotland.

Site Number	January NO_3 mg/l	January NH_4^+ mg/l	April NO_3 mg/l	July NO_3 mg/l
Site 9A	0.2	3.34	0.01	13.5
Site 35			0.1	0.01
Site 19	2.5	0.41	1.1	0.01
Site 23	1.5	0.02	1.4	
Site 17			1.6	
Site 21	2.8	0.27	1.6	0.01
Site 9B	2.8	0.29	2.1	1.5
Site 34		0.4	2.5	0.01
Site 3			5.9	
Site 30	11.6	1.32	5.9	3.1
Site 1	19.6	3.19	16.9	6.2
Site 4			44.6	
Site 8	56.4	0.05	47.4	62.4
Site 5	212.5	0.02	176.1	98.5
Site 14				2.4
Site 24	1.3	0.37		
Site 27	0.8	0.35		2.1
Site 31				
Site 32				
Crosby Lake	0.1	0.2		

Nitrogen Isotopes in Herbaria Document Historical Nitrogen Pollution in the River Mersey, England

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Introduction:

Wastewater discharge in UK rivers and coastal environments are becoming more frequent leading to a decline in water quality. Currently, there are no rivers in England that have Good Overall Health Status and only 15 % of rivers are of Good Ecological Health Status (Bevan, 2020; Environment Agency, 2022; Environmental Audit Committee, 2022). This is in stark contrast to Scotland which has ~ 57% of rivers with Good (or Better) Overall Health Status (The Rivers Trust, 2024). Policy changes to permit Combined Sewer Overflows (CSOs) to discharge raw sewage at times of peak flow has been detrimental to the ecological health of UK rivers (Gill et al., 2021). Many wastewater treatment facilities are inadequate to cope with current population levels and have had minimal investment for decades, causing the number and frequency of CSOs to increase (Gill et al., 2021): this environmental crisis was graphically highlighted in the BBC Two documentary, *Our Troubled Rivers* (BBC & Whitehouse, 2023). Discharge during periods of low flow in rivers increases the residence time of the pollutants, enhancing the detrimental impact it has on the environment. Excessive nutrient loads are responsible for phytoplankton, algal and macrophyte blooms which subsequently reduce oxygen contents causing eutrophication of water bodies (Bermejo et al., 2022; Glibert, 2017; Smith, 2003). The current environmental issues in England rivers are exacerbated by budget cuts in the Environment Agency, which has hindered their ability to monitor, designate and prosecute water companies over illegal discharges of sewage (Gill et al., 2021; House of Commons, 2023; The Independent, 2022). It is also hampered by an unwillingness from privatised water companies to openly share data on discharge amounts and dates, resulting in the public taking action to report and monitor wastewater discharges (BBC, 2022; Woodward, 2023). There is widespread concern nationally over the effects that wastewater (i.e., raw and treated sewage) release has had on riverine and coastal environments.

Sewage effluent that reaches the coastal ecosystem can be identified through nitrogen isotope ($\delta^{15}N$) analysis of organisms living in that environment (e.g., macroalgae, mussels and fish) (Costanzo et al., 2001; Samper-Villarreal, 2020; Savage & Elmgren, 2004; Tucker et al., 1999). Macroalgae is less-frequently used as a bio-monitor to trace nitrogen sources, especially in the UK (García-Seoane et al., 2018; Samper-Villarreal, 2020). Macroalgae takes up nitrogen (e.g., ammonium, nitrate) with minimal nitrogen isotope fractionation and therefore can be used to discriminate the nitrogen source in the marine environment (Cohen & Fong, 2005). Different nitrogen sources (e.g., fertilisers, raw and treated sewage) have distinct isotopic averages. Treated sewage effluent is often identified through $\delta^{15}N$ values greater than +7 ‰ in macroalgae (Bailes & Gröcke, 2020; Samper-Villarreal, 2020; Xue et al., 2009). On the other hand, nitrogen pollution derived from industrial chemical processes (e.g., artificial fertilisers) produce $\delta^{15}N$ values near to atmospheric nitrogen, 0 ‰ (Bateman & Kelly, 2007; Heaton, 1986; Orlandi et al., 2017). Although there are numerous studies that have used macroalgae $\delta^{15}N$ to identify sewage pollution, there are only a handful of studies that exist for the UK despite the ongoing sewage pollution crisis (Alldred et al., 2023; Bailes & Gröcke, 2020; Gröcke et al., 2017; Samper-Villarreal, 2020).

Museum herbarium collections contain a vast amount of ecological and environmental information that is often underutilised in the study of recent anthropogenic change (Lister et al., 2011; Pyke & Ehrlich, 2010). Only recently have researchers considered herbaria as a tool to track biogeographical, environmental, and climatological changes (Lavoie, 2013; Lister et al., 2011; Pie et al., 2022). Although herbaria relate to all forms of dried material, such as vascular plants, macroalgae, bryophytes, lichens, and fungi, 82 % of current research has focussed on accessing vascular plant collections (Lavoie, 2013). Herbaria studies have dominantly been used to record population densities, distribution, and organism morphology to infer environmental conditions, but more recently this has extended to include DNA sequencing and chemical analyses (Davis, 2022; Lavoie, 2013; Pyke & Ehrlich, 2010). Macroalgae herbaria are starting to be used to investigate the marine environment although it remains under-utilised on samples prior to the 20th century (Alfonso et al., 2022; Lavoie, 2013; Miller et al., 2020). Recently, Miller et al. (2020) used macroalgae herbaria and nitrogen isotopes to reconstruct past upwelling trends along the Californian coast – this approach was adopted since that is the dominant environmental mechanism affecting nitrogen isotopes in that region.

In this study, we used macroalgae specimens collected and stored in the herbarium at the World Museum, National Museums Liverpool to reconstruct historical nitrogen pollution in the River Mersey and Liverpool Docks. Herbaria specimens from the River Mersey were assessed for their suitability for destructive sampling. Only those where there was adequate material available to have a portion removed without damaging the scientific integrity of the specimen for future research were used in this study. Very delicate small specimens, for example, were avoided and for pre-1900 specimens only one sample per year was permitted for sampling: again, to preserve the herbarium collection for future research. The collection has been added to relatively consistently for the same region between 1821–1860 and from the 1960s to present day. Sampling gaps occur during World War I and II, which may be reflected in many herbarium records around Europe during this time interval. Although this is unfortunate from a scientific perspective, the herbaria record available will allow us to reconstruct major changes in nitrogen between the industrial and wastewater treatment eras. The River Mersey has witnessed significant anthropogenic changes over the past 200 years, and thus is an ideal natural setting to assess the use of macroalgae $\delta^{15}N$ from herbaria as a proxy for reconstructing historical nitrogen pollution.

The River Mersey

The River Mersey has a catchment area of ~1800 mi^2 and includes Manchester, Lancashire and Cheshire in the north-west of England (Hawkins et al., 2020). It flows for 69 miles before widening into the Mersey Estuary that stretches for almost 16 miles (Figure 4.1) (Mersey Basin Campaign, n.d.). The large metropolitan city of Liverpool is located at the mouth of the River Mersey (Figure 4.1), where large tidal ranges (> 10 m) cause strong currents and large sand banks (Ritchie-Noakes, 1984). The Mersey Basin has grown in population size from ~500,000 (1821) to >5 million (2021); this estimate includes other nearby population centres such as Manchester, Liverpool, and Salford (Burton, 2003; Burton et al., 2003; ONS, 2021). This region is serviced by the private water company, United Utilities Group PLC. The River Mersey and Mersey Estuary have a long history of pollution and poor water quality since the early 1800s (Burton, 2003; Ritchie-Noakes, 1984). Nitrate plumes originating in the River Mersey have seriously impacted Liverpool Bay since the 1960s (Hawkins et al., 2020; Jones & Haq, 1963). Public outcry in the 1980s incentivised the launch of the Mersey Basin Campaign (Burton, 2003;

Meredith, 1992). The aim was to clean up the polluted River Mersey after it was described as an “affront to the standards a civilised society should demand” by the then Secretary of State for the Environment, Lord Heseltine (BBC, 2021). Unfortunately, pollution issues still exist in the River Mersey. For example, data extracted from The Rivers Trust Sewage Map (The Rivers Trust, 2022) shows that in 2021 the River Mersey catchment area experienced over 212,000 hrs of effluent discharge prior to processing through 12–24 hrs (for reference, a year = 8,766 hrs).

The River Mersey and Mersey Estuary macroalgae herbaria $\delta^{15}N$ record spans 197 years consisting of 69 macroalgae specimens collected between 1821 (*Enteromorpha compressa*, Liverpool) and 2018 (*Polysiphonia stricta*, Queens Dock): including the time gap previously mentioned. Many different macroalgae species have been used to generate this $\delta^{15}N$ record, because a range of specimen types are collected for herbariums: these are often selected based on casual observation, ecological monitoring, identification and taxonomy, or more frequently because of their fragility, rarity, and beauty on herbarium paper. A brown seaweed, such as *Fucus vesiculosus* (bladder wrack), is not as eye-catching as a delicate red seaweed and because it is very abundant around the UK still exist coastline. The same macroalgae species are often not routinely collected for herbariums and hence, generating a long-term species-specific $\delta^{15}N$ record will not be possible: we suspect this will be an inherent issue with many herbarium collections around the world.

Irrespective of the use of different macroalgae species the $\delta^{15}N$ record produced in this study reveal consistent and significant changes over the past 200 years that can be related to societal changes and a major neoliberalism event in 1989 in the UK (Jessop, 2018): the privatisation of water companies.

F. vesiculosus collected from Eastham in 1978 recorded the most elevated $\delta^{15}N$ value of +30.6 ‰, whereas the lowest $\delta^{15}N$ value was –4.1 ‰ collected from Otterspool in 1968 (also *F. vesiculosus*). $\delta^{15}N$ values of macroalgae above +20 ‰ are very rare in the literature and suggest extreme environmental conditions (Dailer et al., 2010): in this case, we interpret these elevated $\delta^{15}N$ values as a result of continued, voluminous release of sewage into the River Mersey.

To place the herbaria $\delta^{15}N$ record in the context of the present-day situation, a suite of macroalgae from the River Mersey and South Docks were collected in September 2022. Many different macroalgae species were collected from the South Docks and produced an average $\delta^{15}N$ value of +10.6 ‰ ± 3.2 ‰ (n = 27) (Figure 4.2). However, macroalgae $\delta^{15}N$ from the River Mersey average +16.5 ‰ ± 2.0 ‰ (n = 50) and +15.5 ‰ ± 2.7 ‰ (n = 22) for *Fucus* sp. and *Ulva* sp., respectively (Figure 4.3). The herbaria $\delta^{15}N$ data from the River Mersey and the Mersey Docks will be discussed separately. Due to the limited sample size, we have grouped the herbaria $\delta^{15}N$ data into age ranges and discussed results for each age range.

The South Docks Herbaria Record

The South Docks in Liverpool are an interconnected system (Allen et al., 1995), and therefore the herbaria macroalgae $\delta^{15}N$ record is being treated as a single composite record. Figure 4.2 shows the herbaria South Docks $\delta^{15}N$ record between 1981 and 2018. An 1846 herbaria sample collected from Princes Dock is the only specimen collected during the 1800s and so this data has been omitted from further discussion (*F. vesiculosus*, $\delta^{15}N$ = +8.2 ‰). $\delta^{15}N$ values show no clear trend in the South Docks over the 40-year period (Figure 4.2). The South Docks herbaria record has an average $\delta^{15}N$ value of +13.7 ‰ ± 3.8 ‰ (n = 23), which is significantly more elevated (p value > 0.003) than the 2022 dock average (+10.6 ‰ ± 3.1 ‰, n = 27).

Allen et al. (Allen et al., 1995) report low public opinion of dock water quality in the 1990s. Efforts to limit mixing between the river and docks were obtained through the installation of a pump in 1992 to replenish water levels, thus reducing turnover rates of the docks seawater to be between 6–12 months (Allen et al., 1995; Hawkins et al., 2020; Wilkinson et al., 1996). A reduced replenishment rate may be the reason behind dock herbaria not reflecting River Mersey macroalgae $\delta^{15}N$ values. The lower $\delta^{15}N$ average of the dock herbaria and modern macroalgae compared to the River Mersey suggests two potential mechanisms: (a) increased industry-sourced nitrogen pollution entering the docks (e.g., drains, road runoff);

and/or (b) water filtering (i.e., cleansing) by the presence of the bivalve, *Mytilus edulis* (blue mussel) (Wilkinson et al., 1996). Since the mid-1980s there have been several reports of blue mussel colonies thriving in the docks; they were introduced to Graving Dock as part of a bio-filtration experiment and naturally settled in Albert Dock and subsequently throughout the South Dock system (Hawkins et al., 2020; Wilkinson et al., 1996). The duration it would take for blue mussels to filter the volume of water in Albert dock (170,000 m^3) is calculated to every four days. Scaled up to include the entire South Dock complex (1.28 million m^3) the duration to filter all that water would be ~30 days assuming blue mussel density is consistent throughout the docks (Connolly et al., 2013; Wilkinson et al., 1996). Nitrogen is consumed by microalgae and phytoplankton in the docks and they are consumed by filtering blue mussels resulting in lower macroalgae $\delta^{15}N$ values. This is consistent with a blue mussel experiment showing that water nitrate and blue mussel tissue are more depleted in $\delta^{15}N$ than the nitrogen spike in the water when nitrate concentrations in the water are high (Pruell et al., 2020). Dock water samples analysed in this study indicate low to moderately high nitrate concentration ranges from 0.01 and 11.6 mg/l (*n* = 13, January–July 2023). Therefore, blue mussels, or other filtering organisms, should be considered as a natural bio-remediator in ports/harbours/docks, but they should not be considered as a solution without rectifying the cause.

Whilst the dock herbaria and modern macroalgae $\delta^{15}N$ records show less elevated values compared to the River Mersey, the docks still record elevated signatures (+13.7 ‰ and +10.6 ‰, respectively): such elevated macroalgae $\delta^{15}N$ would suggest that the dock water still contains anthropogenic nitrogen sourced from raw and/or treated sewage. Since the River Mersey is the primary source of seawater for the docks, a sewage nitrogen $\delta^{15}N$ signature is unavoidable. The modern and herbaria macroalgae $\delta^{15}N$ record (Figure 4.2) indicates that dock water quality has remained relatively constant since its redevelopment in the 1970s and introduction of the blue mussel ecosystem in the 1980’s.

River Mersey, 1821–1863: Victorians and Raw Sewage

Between 1821 and 1863 herbaria $\delta^{15}N$ values range between +4.7 ‰ (*Ectocarpus granulosus* 1863 and *Enteromorpha compressa* 1853) and +21.5 ‰ (*Enteromorpha intestinalis* 1849) (Figure 3). The majority of the herbaria specimens from this period have $\delta^{15}N$ values that fall between +4.7 ‰ and +8.8 ‰ (n = 14) with four other results greater than +11 ‰. A single specimen (*E. intestinalis*) from Bootle in 1849 recorded a $\delta^{15}N$ value of +21.5 ‰. This time interval records an average $\delta^{15}N$ value of +8.3 ‰ ± 4.1 ‰ (n = 18) or with exclusion of the 1849 Bootle sample, +7.5 ‰ ± 2.6 ‰ (n = 17). Figure 4.3 also shows the global average deep-water $\delta^{15}N$ value of +4.8 ‰ and the expected range (+4 ‰ to +6 ‰) for macroalgae in the North Atlantic (Savage & Elmgren, 2004; Viana & Bode, 2013).

Although the 1821–1863 River Mersey herbaria record has a limited amount of $\delta^{15}N$ data (n = 18) and information regarding their precise sampling location and precise date, the $\delta^{15}N$ range is significantly different to the 1990–2013 (p value < 0.02, n = 13) and 2022 (p-value = 1.6×10^{-7}, n = 69) datasets: it is not significantly different to 1949–1983 (p value > 0.1, n = 11). During the 1800's the Mersey Basin and Mersey Estuary were dominantly influenced by industrial activities: cotton, alkali and hydrogen-chloride production, ship building and transport of coal (Burton, 2003; Ritchie-Noakes, 1984). However, industry of this kind preceded the discovery and explosion of the use of nitrogen gas in industrial processes. For example, agricultural fertilisers made from nitrogen gas ($\delta^{15}N \approx 0$ ‰) were not generated until the discovery of the Haber–Bosch process and large-scale processing in 1913 (Cao et al., 2018; Galloway et al., 2017). Therefore, industrial processes would not be the cause behind lower $\delta^{15}N$ values compared to the other time intervals in this study.

River pollution in England was rife during the Victorian Era (Rosenthal, 2014). The main route of sewage/wastewater disposal in the 19th century was to cast it into rivers and/or cesspits, with the latter infiltrating into the hydrological system (Hughes, 2013). Between 1847–1858 an 80-mile-long sewer network with 48 outflows discharging into the River Mersey was constructed, which would have concentrated sewage waste in the river (Burton, 2003; Porter, 1973). Sewage waste reached such a level that it caused several national cholera outbreaks

(Davenport et al., 2019); the largest of these outbreaks occurring in 1849. It is interesting to observe that this cholera outbreak coincides with the most elevated $\delta^{15}N$ value (Bootle, +21.5 ‰) in our herbaria record and with the highest cholera mortality rate in large towns reported for England (Liverpool, 11.3 deaths per 1000 people) (Davenport et al., 2019). Even after the cholera outbreaks there are reports of massive sewage issues: for example, the Great Stink of London in 1858 when the River Thames was so heavily contaminated it sparked the conception of modern-day sewerage systems (Halliday, 1999).

Principally, we postulate that the input of raw sewage (e.g., not treated, and hence not denitrified (Lee & Liao, 2021) was the cause behind the 1821–1863 $\delta^{15}N$ values recorded for the River Mersey. Raw human sewage would have a similar $\delta^{15}N$ value to the diet they were consuming (Reid et al., 2023) and based on a modern-day equivalent dietary value this would represent a range between +4 ‰ and +8 ‰ (Bird et al., 2022). Although raw sewage can explain the $\delta^{15}N$ record, agricultural practices cannot be excluded. In addition, leaching and weathering of soil-derived nitrogen from fields using organic fertilisers (i.e., manure) may also be contributing to the $\delta^{15}N$ signature (Heaton, 1986). To our knowledge no soil-nitrogen isotope studies exist for Merseyside. In addition, no water quality data (i.e., nitrate concentration) exists this far back in time for this region, unlike the River Thames, London (Burton et al., 2003; Howden et al., 2010; Whelan et al., 2022). Although the precise nitrogen source is unknown the macroalgae $\delta^{15}N$ record from 1821–1863 is most likely caused by raw sewage (e.g., human and animal husbandry), it is evident that it was different to the modern River Mersey record (1990–present).

The ~100-year gap from 1863 is unfortunate since it would have been interesting to understand whether the macroalgae $\delta^{15}N$ record would follow an increasing trend or suddenly shift. There is some information pertaining to water quality from historical records during this time interval. No “waterweeds” (i.e., macroalgae) were present in the River Mersey between the 1870s and ~1900s which can be attributed to water pollution (Burton et al., 2003). Furthermore, from the 1900s unregulated sewage and industrial discharges persisted until the 1950s when wastewater discharge permits were first introduced (Jones, 2000; OFWAT, 2006).

River Mersey 1949–1989: Divergent Nitrogen Pollution Sources

The herbaria $\delta^{15}N$ record for the 1970s is more elevated in comparison to the $\delta^{15}N$ record for the 1800s. The River Mersey macroalgae herbaria record from 1949 to 1983 shows the greatest range in $\delta^{15}N$ (34.7 ‰) with an average value of +13.0 ‰ ± 9.2 ‰ (n = 11). The lowest $\delta^{15}N$ value of -4.1 ‰ recorded in *F. vesiculosus* occurred in 1968 at Otterspool, and the most elevated $\delta^{15}N$ value at +30.6 ‰ (also by *F. vesiculosus*) occurred a year later in Eastham. It is difficult to accurately determine or constrain the cause behind the large variation in $\delta^{15}N$ between 1949–1983. It records one of the most elevated macroalgae $\delta^{15}N$ values (i.e., sewage/denitrification) ever recorded to date, but is immediately followed by a negative $\delta^{15}N$ value indicative of industrial pollution. Due to this large range in $\delta^{15}N$ it is not significant different from any of the age range groups we have assigned to this data set (see Figure 4.3).

Since the majority of the $\delta^{15}N$ values in this time interval are elevated, when removing the lowest two $\delta^{15}N$ values, the average value becomes +16.2 ‰ ± 6.9 ‰. Standard processes in wastewater treatment plants cause the sewage effluent to become enriched in ^{15}N due to denitrification (Rogers, 2003). This time interval has very elevated $\delta^{15}N$ values in comparison to background "natural" macroalgae (Savage & Elmgren, 2004), thus implying that it is heavily influenced by nitrogen pollution created by wastewater treatment plants.

Decades of historical release of sewage and industrial pollution has led to the serious decline in the health status of UK water bodies. Massive cuts in funding and spending during the 1970s and 1980s exacerbated specifically the impact of sewage pollution on the environment making it a national problem that needed addressing (Meredith, 1992). The Control of Pollution Act (1974, 1974) introduced the requirement that local stakeholders had to apply for permits to discharge sewage effluent and industrial waste or face prosecution. This was heralded as a major legal improvement although it was slow to implement nationally (Howarth, 1989). In the 1970s the Mersey Estuary was considered the most polluted estuary in the UK receiving significant wastewater inputs that subsequently led to high nitrate plumes in Liverpool Bay (Burton, 2003; Hawkins et al., 2020; Jones, 2000; Meredith, 1992; Porter, 1973). High nutrient fluxes as a result of sewage wastewater discharges caused Biochemical Oxygen Demand (BOD) in the late 1960s to average 20 mg/l; the enriched $\delta^{15}N$ values observed in herbaria collected for this interval corroborate the input of denitrified sewage wastewater (Figure 4.3) (Hawkins et al., 2020). Growing public demand for improved water quality at the peak of the Mersey pollution crisis in the mid to late 1980s saw the establishment of the Mersey Basin Campaign (Kidd & Shaw, 2000; Meredith, 1992). At the same time public concern over environmental quality was growing across the nation (Meredith, 1992). The Conservative Government and the then Prime Minister, Margaret Thatcher, actioned the privatisation of water companies in England and Wales through the Water Act 1989 (The Water Act, 1989). Even then, as it is now, privatisation of the water companies was primarily focused on macro-economic policies – to inject much-needed cash into an infrastructure that had received little investment for decades (Meredith, 1992; Schaefer, 2009). With a failing regulatory system between Defra (Department for Environment, Food and Rural Affairs), the Environment Agency and Ofwat (Water Services Regulation Authority) there has been a continued lack of investment on being sustainable and to protect the environment (Schaefer, 2009).

Mersey Estuary, 1990–2013: The Start of the Sewage Era

The herbaria $\delta^{15}N$ record depicted in Figure 4.3 indicates that the privatisation of water companies had an impact on the macroalgae nitrogen isotope signature in the River Mersey. It resulted in a more stability in herbaria $\delta^{15}N$ values (average = +12.3 ‰ ± 2.5 ‰, n = 13) excluding one outlier, +4.9 ‰ (*F. vesiculosus*, 1997, Grassendale). Although the record is more stable, the $\delta^{15}N$ values are elevated in comparison to a background “natural” macroalgae range (+4 ‰ to +6 ‰). The herbaria $\delta^{15}N$ values are significantly more elevated than the 1821–1863 period (p value < 0.02, n = 13), which is interpreted as a result of wastewater treatment processes that elevate the ^{15}N content of sewage effluent. It is proposed that the stability in $\delta^{15}N$ in this time interval was a result of stricter controls on discharges, monitoring and increased chemical processing (i.e., ammonia) in wastewater treatment plants (Bakker, 2005; DEFRA, 2002).

During this time interval, stricter regulations were implemented by the European Union on the UK Government to address water quality issues. This legally enforced that the level of nitrate released into freshwater and marine environments was set to a maximum limit of 50 mg/l of NO_3^- (or 11.3 mg/l of nitrate N) (OFWAT, 2006). Environmental action, regulations and investment around the Mersey Basin led to the reduction of BOD from >300 t/y to ~ 50 t/y in the River Mersey from the 1970s to the early 2000s; however the Mersey Estuary was still classified as eutrophic in 2001 (Hawkins et al., 2020).

Mersey Estuary, 2022: A Peak in Nitrogen Sewage Pollution?

The $\delta^{15}N$ macroalgae data from September 2022 is very elevated (+16.5 ‰ ± 1.9 ‰) and significantly different from all previous time intervals, except for 1949–1983. $\delta^{15}N$ values are consistently more elevated with the lowest value of +12.8 ‰ and the highest +22.8 ‰. Elevated $\delta^{15}N$ values > +15 ‰ are rarely recorded in macroalgae studies, and only a handful of studies have recorded values > 20 ‰, and none previously in the UK (Alldred et al., 2023; Bailes & Gröcke, 2020; Dailer et al., 2010; Van Wynsberge et al., 2024; Grocke et al., unpub. data). Although BOD assessments indicate that the Mersey Estuary has improved, the Environment Agency reports it is “not achieving good status” due to industry, with no indication of water detriment coming from the water industry (Environment Agency, 2019; Howden et al., 2010). The Environment Agency also give “Low Confidence” that targets to reach “Good” nitrogen levels will be achieved by 2027 (Aertebjerg et al., 2001; Environment Agency, 2019). The herbaria $\delta^{15}N$ data indicates that the dominant ‘nitrogen’ pollution signal in the Mersey Estuary is caused by wastewater (i.e., sewage) and not chemical pollutants. Recent herbaria and modern macroalgae $\delta^{15}N$ dataset suggests that denitrified sewage input into the waters is extensive, and well above the “natural” range expected for a coastal and estuarine environment in the North Atlantic.

Although there is a time-gap between 1990–2013 and 2022 $\delta^{15}N$ datasets there is a clear increase. Since 2012 the Environment Agency has been aware of illegal sewage discharges by United Utilities and have been accused of “knowingly permitting” such discharges to occur (House of Commons, 2023). DEFRA state that CSOs are only permitted to discharge during periods of heavy, continuous rainfall, and will be tightened to permit discharges only where “there is no adverse ecological impact” by 2050 (DEFRA, 2022). Although there is abundant Social Media evidence that water company regulations on discharging are not being adhered to (BBC, 2023); additional evidence was presented in a recent BBC Panorama report documenting how pollution incidents are being covered up (BBC Panorama, 2023).

The Liverpool sewerage infrastructure is one of the oldest sewerage systems in the UK (National Rivers Authority, 1995), and discharges predominantly through CSOs (i.e., 84% of outlets to the River Mersey). We interpret the elevated macroalgae $\delta^{15}N$ values in the River Mersey as directly sourced from these CSOs which routinely discharge sewage effluent directly into the river. For example, in 2022 the River Mersey received a total of 3,346 hours of sewage dumping (i.e., 4.6 months) [43,80,81]dominantly occurring around Manchester and Warrington (Liverpool Echo, 2023; The Rivers Trust, 2022; Top of the Poops, 2024; United Utilities, 2022). Although nitrate assimilation can lead to elevated residual nitrate $\delta^{15}N$ values its impact is relatively minor, on the order of 5 ‰ to 8 ‰ (Fripiat et al., 2019). Thus, denitrification processes must be a primary cause for generating elevated $\delta^{15}N$ recorded in macroalgae. There are four potential mechanisms and sources:

(1) denitrified sewage effluent from wastewater treatment plants. It is well documented that wastewater treatment processes that use anaerobic conditions can elevate effluent $\delta^{15}N$ values above +10 ‰ (Onodera et al., 2021);

(2) increase nitrogen productivity and deposition of nitrate as a consequence of elevated wastewater effluent discharges. Nitrogen consumption in the water profile via surface water productivity (i.e., phytoplankton) will preferentially remove ^{14}N, resulting in more elevated nitrate $\delta^{15}N$ values in the water column. Burial of the nitrate and nitrogen-bound organic matter will also remove ^{14}N in preference to ^{15}N.

(3) denitrified groundwater nitrate. A 1999 study on groundwater nitrate indicated that sewage effluent was leaking into the aquifer beneath Liverpool (Whitehead et al. 1999) – this was based on nitrate $\delta^{15}N$ and microbiological analyses. However, the sample with elevated *E. coli* and

faecal streptococci contents was not analysed for nitrate $\delta^{15}N$. The other samples in that study ranged from –11.9 ‰ to +13.2 ‰ (average +6.8 ‰ ± 8.7 ‰), and therefore, aquifer discharge is not a cause for elevated $\delta^{15}N$ in the Mersey Estuary; and

(4) denitrification in estuarine sediment. The tidal range for the River Mersey is large (4 to 10 m). Due to the volume of water being replenished daily it is reasonable to state that the water column would be well-oxygenated, and hence, increase the depth of the suboxic layer in the sediment. Nitrification of wastewater ammonia would occur in the water column, and nitrification in the sediment profile will produce elevated $\delta^{15}N$ pore-water ammonia values as nitrate is depleted in concentration (Alkhatib et al., 2012; Lehmann et al., 2007). Subsequent denitrification in the sediment profile is therefore a function of oxygen supply and penetration, as well as nitrate replenishment from the water column into the sediment profile. Although the Mersey Estuary has the largest total inorganic nitrogen load (3959 $\times 10^6$ moles y^{-1}) in an estuary in the UK (Nedwell et al., 2002), further research on nitrification and denitrification in Mersey Estuary sediment is required.

Of the four options above, the most preferred explanation as a cause for the elevated $\delta^{15}N$ in macroalgae in the Mersey Estuary is due to (1) and (2) — increased denitrified and raw wastewater sewage effluent entering the River Mersey and Mersey Estuary. At present, water companies are undeterred by fines imposed by regulatory bodies and [1]have no incentive to change current operational practices (i.e., releasing effluent during dry periods). Funding cuts to the Environment Agency have also limited their ability to properly monitor, respond, assess and impose fines to water companies in breach of environmental standards (Environment Agency, 2022; House of Commons, 2023; The Independent, 2022). The decline in river water quality (and subsequently, estuaries and coastal settings) is exacerbated by a continued, significant lack of investment from water companies to improve and repair their infrastructure to accompany increases nationally in population and corresponding changes in present/future climate; illegal discharging from CSOs has become more frequent as population increases (Giakoumis & Voulvoulis, 2023). Although England water companies have until 2050 to achieve infrastructure upgrades and compliance, by that time the environmental damage to rivers, estuaries and coastal settings may be irreversible (DEFRA, 2022). Continued nitrogen isotopic monitoring of macroalgae will show whether improvements are made in the future or whether the Mersey Estuary will remain a an environment impacted by sewage pollution.

Conclusion:

The Mersey Estuary has been identified as a heavily polluted environment since the early 1800s. Museum herbaria provide an excellent source of material to reconstruct historical pollution. Despite the number of available samples for this study, it has been possible to depict broad scale nitrogen isotope changes and trends in the Mersey Estuary since 1821.

A macroalgae herbaria $\delta^{15}N$ dataset from 1813 to 2013 reveals three major nitrogen pollution episodes: (1) 1821–1863 is dominated by raw sewage; (2) 1949–1983 is influenced by industrial, agricultural and treated sewage processes; and (3) 1990–2013 is recording treated and raw sewage pollution. Although BOD has decreased from very elevated levels in the 1960s, the River Mersey still contains a significant proportion of nitrogen sewage pollution as interpreted by $\delta^{15}N$ macroalgae values. Privatisation of water companies in England and Wales in 1989 was driven by economics and not environmental sustainability. Its impact on herbaria $\delta^{15}N$ values was to stabilise the variability (and hence, the influence of varying nitrogen pollution sources). The modern macroalgae $\delta^{15}N$ record reflects increasing sewage nitrogen pollution into the River Mersey – through denitrified wastewater effluent and release of raw sewage. The very elevated $\delta^{15}N$ values are interpreted to be a consequence of limited regulation, underinvestment, and legislation changes.

Poor water quality is an incessant problem in the UK – the elevated macroalgae $\delta^{15}N$ values recorded in both herbaria and modern samples from the Mersey Estuary highlight an ongoing failure to reduce nitrogen pollution in our rivers. This study demonstrates the usefulness of macroalgae $\delta^{15}N$ in determining the nitrogen pollution source into the Mersey Estuary over the past 200 years and hence, indicative of a whole catchment source problem. The Mersey Estuary $\delta^{15}N$ record showcases how the nitrogen cycle has been influenced by human processes that have had a lasting impact on our riverine/estuarine environments. Large-scale nitrogen pollution from wastewater treatment plants is transforming the nitrogen cycle in UK rivers, which necessitates a critical shift in increased investment into wastewater treatment infrastructure and a re-evaluation of environmental monitoring methods and policy.

Materials and Methods:

A total of 72 herbaria macroalgae specimens were sampled at the World Museum, Liverpool, providing a sample record from 1821 to 2018. The macroalgae specimens are from multiple locations and different macroalgae species for the Mersey Estuary and Liverpool South Docks (see Figure 4.1): specific samples details are provided in the supplementary data file. A scalpel was used to remove the longest macroalgae thallus tips, which represent the last growth of the sample for all macroalgae specimens. The sample was then transferred into microcentrifuge tubes until processing for stable isotope analysis. It has been reported that nitrogen isotopic ratios are not impacted by paper type and so herbaria are assumed to be comparable to specimens pressed on modern acid-free paper (Miller et al., 2020). Even knowing this we still inspected every herbarium sample to prove that no sample had herbarium paper attached. Herbarium specimens were compared to a modern geospatial sample set from the River Mersey collected in September 2022: this sample set was collected for comparison with the herbaria samples – most of which were also collected in the summer season. Fifty *F. vesiculosus* and 22 *Ulva* sp. modern specimens were sampled from the River Mersey in addition to 27 specimens collected from the South Docks (Figure 4.1). Dock samples include 22 *Ulva* sp., nine *Cladophora* sp. and two C*allithamnion corymbosum*. Modern specimens were dried in an oven between 45–60°C – this drying method has no impact on the bulk signature of macroalgae stable isotope ratios. Once dried, sub-samples of macroalgae and the most recent growing tip of *Fucus* sp. were weighed between 1.1 – 1.5 mg into tin capsules for stable isotope analysis. All nitrogen isotope analyses were carried out in the Stable Isotope Biogeochemistry Laboratory (SIBL) at Durham University.

Nitrogen isotopes are reported against atmospheric nitrogen (AIR) and accuracy is continuously monitored both in-house and international standards: in-house standards are calibrated against international standards (e.g., IAEA-600, IAEA-N1, IAEA-N2). Analytical uncertainty is typically ± 0.1 ‰ (1 sd) for replicate analyses of our standards. Most herbaria specimens only had enough material for one analysis due to strict museum limitations on sample destruction. For further details on analytical methods see Bailes and Gröcke (2020) and Gröcke et al. (2017).

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Figure 4.1:

Study area of the Mersey Estuary with modern sample sites (red) and herbarium sites (grey). ArcGIS Pro 3.0 was used to produce this figure.

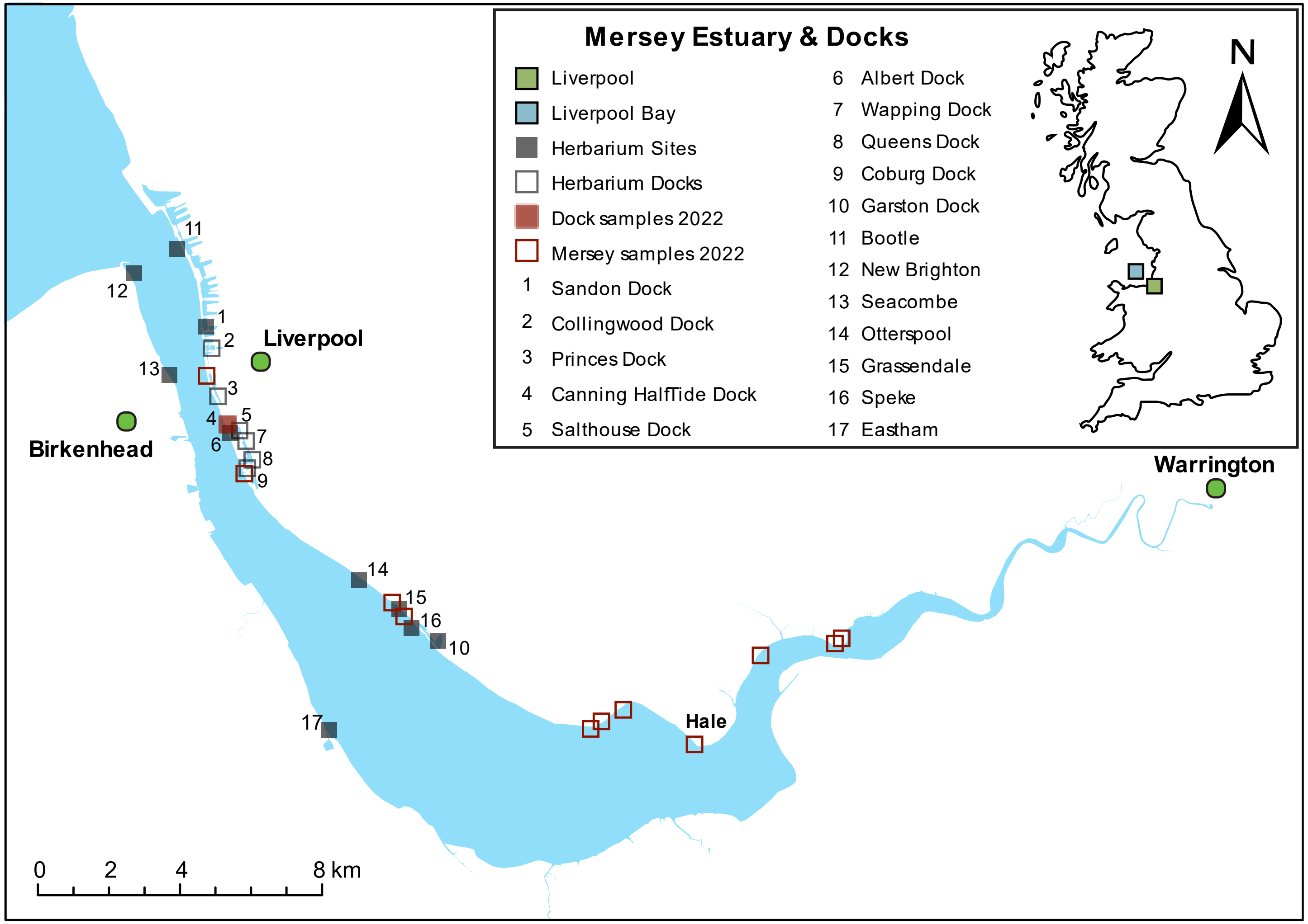


Figure 4.2:

Herbaria and modern nitrogen isotope record for the South Docks of Liverpool. Herbarium data points are shown in blue (*n* = 23); the modern nitrogen isotope range is provided as a boxplot collected in September 2022 (*n* = 27). The grey box represents the "natural" isotopic range (+4 ‰ to +6 ‰) for the North Atlantic (Alldred et al., 2023) and the dashed line the global nitrate $\delta^{15}N$ value (Sigman & Casciotti, 2001).

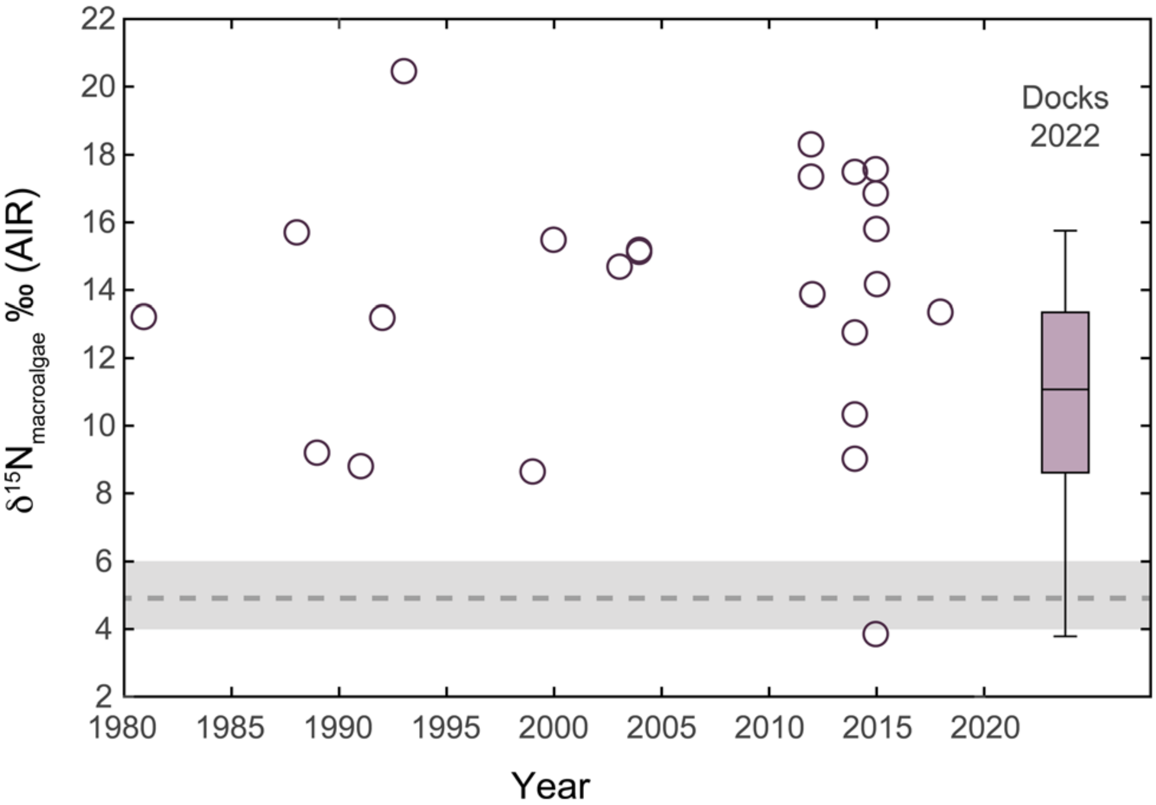
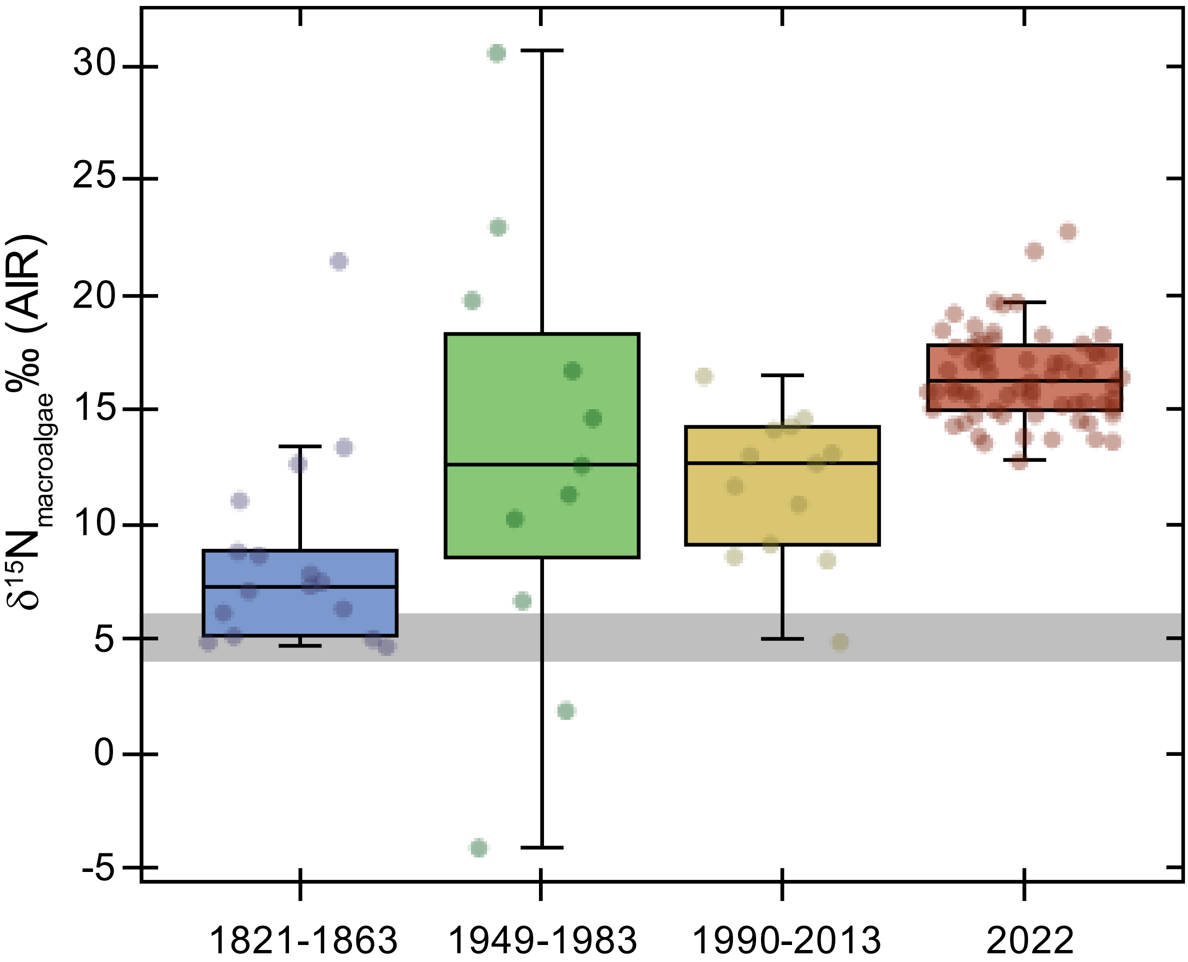


Figure 4.3:

Herbaria and modern nitrogen isotope record for the Mersey Estuary between 1821 and 2013 (n = 42) and 2022 (n = 69). There is significant statistical difference between 1821–1863 and 1990–2013 and 2022. There is no statistical difference between 1949–1983 due to the large range in $\delta^{15}N$ for this time interval, spanning the entire range of the whole dataset.



Chapter 5: Conclusions

5.1. Concluding Remarks

Alternative methods for monitoring sewage pollution utilising bio-monitors (i.e., macroalgae) should be considered in the UK alongside traditional methods. Three datasets were collected and analysed to investigate the use of macroalgal $\delta^{15}N$ in the context of spatial, temporal, and historic variation for the UK. *Fucus vesiculosus* and *Ulva* sp. were considered ideal species for bio-monitoring in the UK; they were common in both study locations and across all seasons. Winter sampling proved more successful for *Fucus* collection, whilst warmer months favoured *Ulva*.

The methodological approach taken at Staithes, Yorkshire found significant spatial variation in macroalgal $\delta^{15}N$ between environments (i.e., rivers, harbours, and open sea). Macroalgae recorded no difference between sampling months. Macroalgal $\delta^{15}N$ is limited in that they record a point in time, therefore sampling of both *Fucus* and *Ulva* can provide insights to nutrient inputs for two time periods but is still limited. Monthly sampling at Staithes could provide insights into seasonal nitrogen trends, although this was outside of the scope of this project. Staithes revealed significant differences between the Staithes Beck, Harbour, and North Sea for both seasons. The Beck recorded significantly higher $\delta^{15}N$ values (> 9.5 ‰) for both species. The Hinderwell Treatment Facility was identified as a likely source of leaking effluent to the Beck. A Tukey Test revealed the harbour was relatively homogeneous, as well as a potentially more well mixed environment in September. Macroalgal d15N also revealed effluent discharge from a Pumping Station for Site P, although this had minimal impact on the rest of the harbour. Clearly, nitrogen isotopes are beneficial in identifying where sewage effluent is concentrating and may provide an additional monitoring tool to identify where clean up efforts should be concentrated. Macroalgae provides a good indication of nutrient inputs to an environment when sampling well mixed environments such as harbours and/or estuaries. The Staithes dataset revealed large-scale surveys should carefully consider their study design to ensure samples are representative of the average for that environment. Combining the Staithes dataset with additional data such as more nitrate water concentration data, *E. coli* and other parameters would be very beneficially to building a more detailed picture of the Staithes Beck and Harbour.

A seasonal study was undertaken for the Mersey Estuary to understand how nitrogen sources may fluctuate naturally and to also to identify instances of industrial and anthropogenic nitrogen pollution. Two seasonal records were produced, one for the estuary and one for the docks. No clear trend was observed for the docks between January, April, or July, $\delta^{15}N$ values remaining consistently high (~ 10–15 ‰). Macroalgae $\delta^{15}N$ for the estuary also remained high throughout all seasons. *Ulva* showed an enrichment trend between January to July, from ~ 12 ‰ to ~ 18 ‰. *Fucus* did not produce as clear of a trend, although July was significantly higher than January at ~ 14 ‰. Both the estuary and the docks suggest a sewage nitrogen source persists across all seasons. Macroalgae $\delta^{15}N$ was highest in the upper estuary, suggesting sewage may be concentrating here and/or there is a dilution effect. Nitrogen isotopes were also successful in identifying point sources along the estuary. The limited macroalgal cover and/or access along stretches of the estuary were unfortunate and meant further sampling upstream was not possible. As with Staithes, the record is limited in that nitrogen inputs can only be known on a ~48 hour to 2-week time scale depending on species. Such high values across all seasons and locations for the Mersey Estuary suggest this environment is affected by a persistent treated sewage source across the entire estuary. Extending the record to include species such as *Ascophyllum nodosum* may provide further insights into the average nitrogen source on a time scale of ~6 months. The data set would also benefit from additional nitrate $\delta^{15}N$ data on water analyses as well as comparison to more traditional methods. But, macroalgal $\delta^{15}N$ clearly shows a persistent sewage nitrogen problem across the entire estuary.

Herbaria macroalgae was used successfully to trace historical nitrogen inputs to the Mersey Estuary and South Docks over 200-years. The docks produced a sporadic $\delta^{15}N$ record; the data set is very limited and comprised of single samples for a handful of docks. The estuary record is much more useful. Gaps in the record were unavoidable; but broad trends can still be identified from the record. Most notably the shift from lower $\delta^{15}N$ to higher $\delta^{15}N$ values from the early 1800s into present day. This indicates the dominant nitrogen source to Mersey Estuary has changed from an industrial and/or raw sewage source to an anthropogenic sewage source. The modern 2022 collection also supported a sewage nitrogen source and followed the rising $\delta^{15}N$ trend of the 2010s. The Mersey Estuary record also highlights the effect of policy changes on pollutant sources. Increases in $\delta^{15}N$ appear to coincide with political events such as privatisation and Brexit. This technique has not yet been applied in the UK, and never for tracing historical pollutant sources. This study should be considered a promising start of this novel isotope technique that can be applied to other collections across the UK. Herbaria macroalgae may be a very powerful tool for reconstructing past environmental pollutants where other proxies are not available.

5.2 Future Recommendations and Work

Macroalgal $\delta^{15}N$ could be an effective bio-monitoring tool across much of the UK; *Fucus* and *Ulva* are very common, and samples can be collected very quickly. However, as with all methods, limitations do exist if macroalgal $\delta^{15}N$ was to be used as an alternative to traditional methods. Instead, it would be highly effective as an additional method to current nitrate $\delta^{15}N$ analysis, *E. coli* monitoring and standard concentration analysis. It is important to draw attention to several considerations that would benefit this and future projects of a similar nature.

- Monthly sampling of Staithes and the Mersey Estuary may have provided greater insight into seasonal nitrogen trends and potentially identified when nitrogen sources to these environments fluctuates. Sampling over a longer timeframe or additional species with varying nitrogen turnover rates may be beneficial for future studies.
- Due to permits and difficulties working with Private Water Companies no data could be obtained for the exact $\delta^{15}N$ value of WWTW effluent. This would constrain speculation around the effect of prolonged denitrification on the $\delta^{15}N$ ratio and the reasons for such elevated values in the Mersey – are these a cumulative effect of such high sewage loads or produced during the denitrification process and an indication of highly treated sewage? Pairing macroalgal $\delta^{15}N$ with $\delta^{15}N$ analyses of water and nitrate concentration data would help to reinforce conclusions. But ultimately more transparency is needed from Water Companies relating to effluent discharges.

- The seasonal Mersey record whilst useful, does not address the issue surrounding where such high $\delta^{15}N$ values are coming from since it does not appear to be the outflows to the estuary. Extending sampling to include macrophytes may identify pollutant plumes from outflows further upstream.
- The UK has many herbaria records such as those housed at the Natural History Museum and the Edinburgh Royal Botanic Garden. There is potential for a more complete herbaria record, therefore historical nitrogen inputs could be assessed for multiple UK ports, estuaries, and coastlines. It would be interesting to see if similar trends are observed elsewhere in the UK.
- At present the Stable Isotope Biogeochemistry Lab has macroalgae samples from Cardiff to Inverness. Compiling this large data set would produce the first large scale macroalgal $\delta^{15}N$ study for the UK. This would aid in identification of sewage nitrogen loading for much of the UK coastline. Future projects should consider sampling the rest of the UK coastline to complete this record. It would be the first macroalgal $\delta^{15}N$ record for an entire country with benefits of identifying where sewage nitrogen loading is most prevalent.

Chapter 6: Appendix

Appendix 1: The Nitrogen Cycle

Nitrogen Fixation:

$$N_2 + 8H^+ + 6e^- \rightarrow 2NH_4^+$$

Nitrogen + Hydrogen → Ammonium + Hydrogen

Nitrification:

$$NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$$

Ammonia + Oxygen → Nitrite + Hydrogen

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$$

Nitrite + Water → Nitrate + Hydrogen

Denitrification:

Nitrate reduction to Nitrite

$$2NO_3^- + 4H^+ + 4e^- \rightarrow 2NO_2^- + 2H_2O$$

Nitrite reduction to Nitric Oxide

$$2NO_2^- + 4H^+ + 2e^- \rightarrow 2NO + 2H_2O$$

Nitric Oxide reduction to Nitrous Oxide

$$2NO + 2H^+ + 2e^- \rightarrow N_2O + H_2O$$

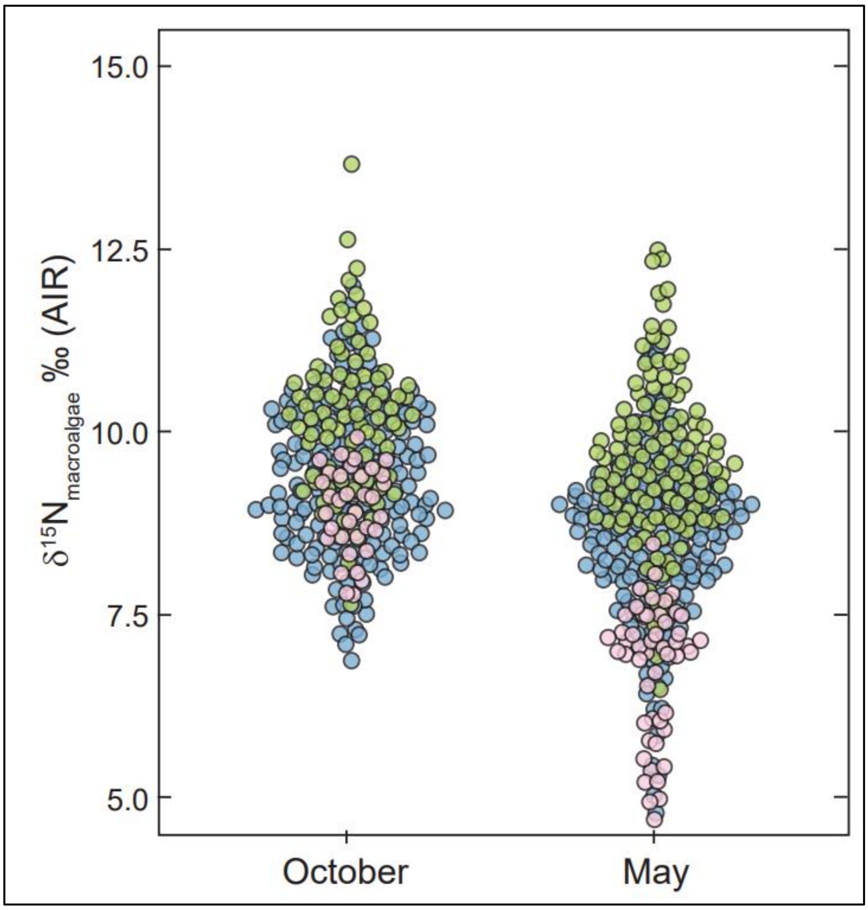
Nitrous Oxide reduction to Nitrogen (g)

$$N_2O + 2H^+ + 2e^- \rightarrow N_2 + H_2O$$

Simplified to:

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O$$

Appendix 2: Chapter 2 Data Set & Additional Figures



Appendix Figure 2.1. Violin Plot for *Fucus vesiculosus* $\delta^{15}N$ values for Staithes showing slightly lower $\delta^{15}N$ values in May compared to October and an offset between the River (green), Harbour (blue) and North Sea (pink) sites.

October *Fucus*		**October *Ulva***		**May *Fucus***		**May *Ulva***	
Site	**$\delta^{15}N$**	**Site**	**$\delta^{15}N$**	**Site**	**$\delta^{15}N$**	**Site**	**$\delta^{15}N$**
A	10.44	A	10.10	A	11.44	A	12.35
A	10.98	A	9.26	A	10.62	A	11.96
A	10.40	A	10.01	A	10.27	A	11.46
A	11.31	A	9.79	A	10.06	A	11.32
A	10.45	A	10.24	A	9.66	A	10.52
A	9.16	B	10.55	A	9.53	A	10.37
A	10.16	B	10.11	A	9.38	A	10.13
A	10.13	B	9.12	A	8.81	A	9.99
A	13.54	B	10.04	A	8.79	A	9.93
A	10.39	B	9.92	A	8.74	A	8.42
B	10.73	C	9.84	B	12.50	B	11.05
B	11.72	C	10.15	B	11.19	B	10.97
B	11.97	C	9.14	B	10.95	B	10.21
B	11.50	D	8.81	B	10.80	B	10.08
B	10.39	D	8.72	B	10.65	B	9.94

B	11.07	D	9.34	B	9.78	B	9.59
B	11.59	E	8.47	B	8.06	B	9.36
B	11.14	E	7.59	B	7.72	B	9.31
B	10.27	E	9.71	B	7.67	B	8.84
B	10.62	E	9.44	B	7.04	C	10.68
C	10.18	E	9.26	B	6.49	C	10.58
C	9.95	F	9.08	C	12.38	C	10.32
C	11.48	F	8.84	C	11.25	C	10.30
C	10.80	F	9.61	C	10.54	C	9.62
C	10.01	F	8.56	C	9.89	C	9.27
C	12.13	F	8.92	C	9.77	C	9.12
C	11.40	G	8.28	C	9.08	C	9.05
C	11.57	G	8.35	C	8.96	C	9.02
C	10.98	G	8.66	C	8.85	D	10.76
C	11.78	G	8.55	C	8.79	D	10.12
D	10.68	H	10.49	C	8.09	D	9.88
D	9.77	H	10.25	C	6.94	D	9.82
D	8.15	H	8.50	D	11.76	D	9.65
D	8.74	H	8.41	D	10.13	D	9.45
D	10.30	H	8.14	D	9.98	D	9.32
D	9.24	H	8.38	D	9.78	D	9.11
D	9.44	I	8.31	D	9.74	D	8.83
D	9.85	I	8.26	D	9.51	D	8.57
D	10.64	I	7.99	D	9.43	D	8.48
D	9.89	I	8.13	D	9.14	E	10.11
E	9.36	J	8.44	D	8.85	E	9.73
E	10.61	J	8.95	E	10.99	E	9.58
E	10.43	K	8.93	E	10.14	E	9.52
E	10.33	K	8.53	E	9.92	E	9.49
E	10.58	K	7.65	E	9.68	E	9.10
E	10.70	K	8.09	E	9.60	E	8.79
E	9.97	K	8.77	E	9.41	E	8.73
E	8.95	O	8.27	E	8.81	E	8.72
E	10.16	O	8.02	E	8.66	E	7.84
E	9.06	O	7.74	E	8.24	F	10.39
E	9.33	P	7.18	E	7.53	F	9.79
E	9.98	P	8.03	F	11.91	F	9.44
E	9.02	P	8.26	F	10.91	F	9.28
F	10.66	P	8.29	F	9.73	F	9.22
F	10.87	Q	8.24	F	9.60	F	9.21
F	10.40	Q	8.29	F	9.33	F	8.94
F	10.40	Q	8.85	F	9.20	F	8.82
F	8.51	Q	8.91	F	8.85	F	8.56
F	9.61	Q	9.36	F	8.28	F	8.14
F	9.15	Q	9.61	F	8.14	G	11.20
F	9.32	R	8.50	F	7.37	G	10.42
F	8.97	R	8.71	G	11.14	G	10.11
F	12.52	R	8.48	G	11.06	G	9.84
G	9.51	R	8.62	G	11.00	G	9.61
G	10.43	T	7.58	G	10.57	G	9.37

G	10.28	T	8.66	G	10.23	G	9.29
G	9.73	T	6.83	G	10.21	G	9.13
G	10.02	T	7.05	G	10.01	G	8.85
G	9.48	T	7.57	G	8.45	G	8.69
G	10.15	T	7.40	G	7.96	H	10.14
G	9.16			G	7.26	H	10.03
G	9.48			H	10.47	H	9.54
G	9.61			H	10.43	H	9.45
G	9.93			H	10.15	H	9.43
G	10.48			H	9.44	H	9.33
G	10.26			H	9.16	H	9.22
G	11.89			H	9.16	H	9.12
G	8.75			H	8.97	H	9.01
H	10.25			H	8.91	H	8.85
H	10.22			H	8.83	I	9.11
H	10.03			H	8.74	I	9.03
H	8.92			H	8.06	I	8.77
H	10.41			I	9.61	I	8.58
H	8.82			I	8.93	I	8.57
H	9.12			I	8.63	I	8.42
H	8.81			I	8.45	I	8.42
H	8.88			I	7.87	I	8.30
H	8.84			I	7.65	I	8.09
H	9.12			I	7.51	I	8.09
H	8.49			I	7.25	J	8.82
H	9.15			I	7.25	J	8.56
H	10.02			I	7.13	J	8.47
I	11.35			J	9.92	J	8.40
I	10.58			J	9.73	J	8.28
I	10.30			J	8.91	J	7.97
I	10.34			J	8.84	J	7.77
I	8.22			J	8.53	J	7.57
I	9.73			J	7.97	J	7.53
I	10.36			J	7.57	K	9.58
I	10.67			J	7.50	K	9.56
I	10.50			J	7.20	K	9.29
I	10.50			J	6.85	K	8.98
I	9.75			J	6.64	K	8.65
I	9.77			K	8.15	K	8.62
I	10.07			K	8.15	K	7.77
I	10.02			K	8.04	K	7.36
J	9.91			K	8.01	K	5.31
J	9.74			K	7.78	O	7.80
J	9.66			K	7.72	O	7.55
J	9.60			K	7.56	O	7.50
J	8.07			K	7.33	O	7.24
J	8.75			K	7.06	O	7.20
J	8.60			K	6.22	O	7.16
J	10.52			L	10.09	O	7.14
K	8.55			L	9.64	O	7.06

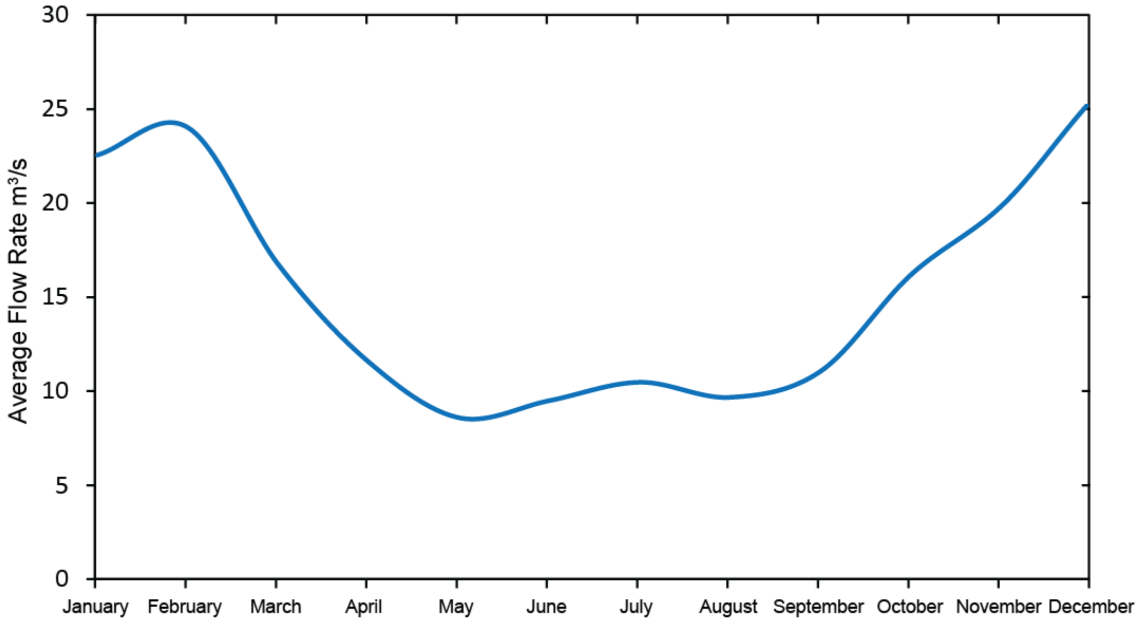
K	9.76	L	9.63	O	6.97
K	8.97	L	9.62	O	6.90
K	9.23	L	9.52	P	9.84
K	8.98	L	9.46	P	9.18
K	10.06	L	9.37	P	9.13
K	9.24	L	8.97	P	8.97
K	9.12	L	8.87	P	8.86
K	7.89	L	7.53	P	8.64
K	8.98	O	6.09	P	7.98
K	9.50	O	6.03	P	7.40
K	8.55	O	5.94	P	7.07
K	9.93	O	5.54	P	5.45
K	10.11	O	5.43	Q	9.26
L	10.96	O	5.38	Q	9.23
L	8.74	O	5.23	Q	9.17
L	7.96	O	5.22	Q	9.09
L	9.09	O	4.99	Q	9.02
L	7.88	O	4.95	Q	9.02
L	8.97	P	8.25	Q	8.93
L	9.20	P	7.99	Q	8.75
L	8.93	P	7.75	Q	8.65
L	10.57	P	7.69	Q	8.17
L	9.51	P	6.70	R	8.48
L	8.91	P	6.57	R	8.07
L	10.26	P	6.23	R	7.87
L	10.47	P	5.86	R	7.74
L	8.93	P	5.04	R	7.70
L	10.71	P	4.80	R	7.62
L	10.06	Q	8.98	R	7.50
L	10.84	Q	8.64	R	7.25
L	8.43	Q	8.59	R	7.24
L	9.36	Q	8.58	R	7.01
L	10.07	Q	8.36	S	10.23
O	7.91	Q	8.17	S	9.84
O	9.05	Q	8.14	S	9.57
O	9.22	Q	8.08	S	9.31
O	8.50	Q	8.03	S	9.27
O	7.72	Q	7.98	S	9.21
O	8.31	R	7.51	S	8.83
O	8.01	R	7.14	S	8.30
O	8.77	R	6.99	S	8.21
O	9.32	R	6.72	S	7.59
O	9.07	R	6.54	T	9.80
O	9.04	R	6.17	T	9.36
O	8.63	R	6.06	T	8.99
O	9.37	R	5.79	T	8.97
P	7.46	R	5.75	T	8.50
P	7.79	R	4.71	T	8.46
P	7.25	S	8.88	T	8.38
P	7.19	S	8.69	T	8.02

P	8.88		S	8.58	T	7.60
P	8.29		S	8.57	T	6.97
P	8.86		S	8.54		
P	9.12		S	8.39		
P	8.15		S	8.26		
P	9.66		S	8.19		
P	8.04		S	8.13		
P	10.17		S	6.53		
P	7.56		T	8.88		
P	8.70		T	8.76		
P	9.37		T	8.72		
Q	9.59		T	8.16		
Q	9.37		T	7.79		
Q	9.19		T	7.61		
Q	9.59		T	7.34		
Q	10.23		T	7.30		
Q	8.81		T	7.10		
Q	10.36		T	6.95		
Q	9.02		T	6.00		
Q	8.94					
Q	8.41					
Q	10.80					
Q	11.19					
Q	11.06					
Q	11.63					
R	9.85					
R	9.46					
R	9.56					
R	8.83					
R	9.34					
R	9.33					
R	9.00					
R	9.54					
R	8.59					
R	9.62					
R	8.82					
R	9.43					
R	9.24					
R	9.08					
R	9.54					
S	10.31					
S	10.86					
S	11.12					
S	11.19					
S	11.27					
S	10.74					
S	11.18					
S	10.39					
S	9.54					
S	10.23					

S	9.66			
S	9.36			
S	10.06			
S	10.21			
S	9.55			
S	9.54			
S	9.79			
S	10.17			
T	8.54			
T	9.43			
T	8.73			
T	8.50			
T	8.53			
T	8.87			
T	9.91			
T	9.45			
T	10.03			
T	9.72			
T	9.58			
T	8.22			

Appendix 3:

Appendix Figure 3.1. 10-year average monthly flow data for the River Mersey, recorded at Ashton Weird (SJ7723993563). UK National River Flow Archive.



Appendix Figure 3.2. Students T-Test Results for the Mersey Estuary and Docks for d15N for each month of collection. All calculations were done using Excel.

	$\delta^{15}N$ Docks (January)	$\delta^{15}N$ Docks (April)	$\delta^{15}N$ Docks (July)	Fucus $\delta^{15}N$ Mersey (January)	Fucus $\delta^{15}N$ Mersey (April)	Fucus $\delta^{15}N$ Mersey (July)	Ulva $\delta^{15}N$ Mersey (January)	Ulva $\delta^{15}N$ Mersey (April)	Ulva $\delta^{15}N$ Mersey (July)
$\delta^{15}N$ Docks (January)		0.24	0.08				0.09		
$\delta^{15}N$ Docks (April)			0.14					$5.89*10^{-4}$	
$\delta^{15}N$ Docks (July)									$2.49*10^{-7}$
Fucus $\delta^{15}N$ Mersey (January)					0.34	$3.57*10^{-7}$	0.16		
Fucus $\delta^{15}N$ Mersey (April)						$1.76*10^{-8}$		$1.34*10^{-11}$	
Fucus $\delta^{15}N$ Mersey (July)									$7.95*10^{-9}$
Ulva $\delta^{15}N$ Mersey (January)									$1.97*10^{-21}$
Ulva $\delta^{15}N$ Mersey (April)									$3.21*10^{-7}$
Ulva $\delta^{15}N$ Mersey (July)									

Chapter 3 Data Set

January:

Lab Revised Site Number	Latitude	Longitude	Original January Site Number	Fucus Average	Fucus std	Ulva average	ulva std	Cladophora Average	Cladophora std	CC Average	CC Std	PC Average	PC Std	Porphyra Average	Porphyra Std	Ascophyllum Avergae	Ascophyllum std
1	53.349354	-2.740408	1			9.8	5.2										
2	53.349745	-2.759004															
3	53.345553	-2.768927	2			12.3	0.5										
4	53.341037	-2.772488	4			9.5	0.9										
5	53.339815	-2.773616	3			0.7	0.9										
6	53.32259	-2.794688	5	11.3	0.4	12.7	0.3										
7	53.331891	-2.826113	6	12.8	1.5	11.7	1.5										
8	53.357313	-2.922425	7	11.2	0.9	9.4	2.2										
10	53.373333	-2.963514	9	12.5	1.0	16.8	0.0										
11	53.378414	-2.973629	10	8.7	1.7												
12	53.381541	-2.978967	11	9.7	1.1											8.2	0.0
13	53.386476	-2.984437	12	6.0	1.7												
14	53.390267	-2.987184	23	12.8	0.7	12.8	0.4										
15	53.390076	-2.987815															
16	53.390915	-2.986006	13					16.1	1.2	16.6	0.3						
17	53.392764	-2.984547	14					15.6	2.1	16.4	0.0						
18	53.39523	-2.986803	15					17.0	1.7	17.4	1.5						
19	53.398561	-2.992598	16					14.9	0.5	22.8	0.5						
20	53.399563	-2.989783	17					13.7	1.3								
21	53.401805	-2.992434	18					12.9	2.0	14.1	1.4						
22	53.405463	-2.998214	22	13.2	0.5												
23	53.409164	-2.999366	19			8.2	0.5	6.8	0.0								
24	53.420233	-3.000632	20			5.1	0.3										
25	53.332415	-2.958273	24	13.0	1.0	13.4	0.8										
26	53.367699	-2.994765	25	12.8	0.9	12.6	0.1										
27	53.374709	-2.999964	26	13.1	0.8	11.0	0.7										
28	53.391416	-3.008456	27	13.0	0.6	12.7	0.8										
29	53.398129	-3.018651	28														
30	53.404597	-3.018792	29	0.7	2.5	1.3	2.7										
31	53.419542	-3.024553	30	13.2	1.4	11.4	1.4							13.3	0.6		
32	53.425909	-3.029881	31	13.2	0.8	11.6	1.8							13.0	0.4		
33	53.430653	-3.03259	32	13.4	0.7	10.9	1.2							12.6	0.0		
34	53.439389	-3.035767	33	14.4	0.4	11.6	1.4							11.9	0.1		
35	53.4417902	-3.0435904	34	12.6	0.6	12.9	0.7					9.1	1.0	11.4	0.7		
36	53.434718	-3.075567	35	10.0	0.5	11.9	0.3					8.5	0.2				
9A	53.357299	-2.922355	8A			8.4	1.4										
9B	53.357313	-2.922425	8B	12.3	0.8	9.2	0.7										
Crosby Lake	53.472039	-3.031803	21														

<u>April:</u>

Site Name	Lab Revised Site Number	Latitude	Longitude	Original April Site Number	Fucus Average	Fucus std	Ulva average	ulva std	cladophora average	cladophora std	porphyra average	porphyra std	cc average	cc std	pc average	pc std
Westbank Dockland P	1	53.349354	-2.740408	1			15.6	0.2								
Westbank Dockland P	1	53.349354	-2.740408	1			2.7	4.3								
Ditton Brook	2	53.349745	-2.759004	4			12.8	1.4								
Halebank	3	53.345553	-2.768927	3			12.6	1.2								
Nature Reserve	4	53.341037	-2.772488													
Sewage Outflow	5	53.339815	-2.773616	5			8.9	0.5								
Hale Lighthouse	6	53.32259	-2.794688	2	13.5	1.0	17.0	0.7								
Dungeon Lane	7	53.331891	-2.826113	6	12.9	0.7	16.5	1.2								
Airport	8	53.357313	-2.922425	7	13.1	1.0										
Britannia Inn	10	53.373333	-2.963514	9	11.2	1.0	17.8	1.3								
Columbus Quay	11	53.378414	-2.973629	10	11.8	0.8	16.6	2.4								
Harrison Way	12	53.381541	-2.978967	11	11.9	0.6										
Dock Gates	13	53.386476	-2.984437													
Coburg Dock (Mersey	14	53.390267	-2.987184	13			12.4	0.0	12.2	0.2						
Coburg Dock (Mersey	15	53.390076	-2.987815	12	13.1	0.7	13.0	2.4			11.2	1.2	11.4	0.0		
Coburg Dock	16	53.390915	-2.986006	15			14.6	1.0								
Queens Dock (Waters	17	53.392764	-2.984547	14			13.1	1.8								
Queens Warf (Dock)	18	53.39523	-2.986803													
Dukes Dock	19	53.398561	-2.992598	18			13.9	0.6	14.3	0.0						
Salthouse Dock	20	53.399563	-2.989783	17			12.2	1.1								
Canning Dock	21	53.401805	-2.992434	16			15.2	0.4	10.5	0.0			8.9	0.4		
Mersey Ferry	22	53.405463	-2.998214	19	12.9	0.9	14.2	1.7			12.6	0.4				
Princes Dock	23	53.409164	-2.999366	20			8.3	0.8	8.2	0.0						
Clarence Graving Doc	24	53.420233	-3.000632													
Eastham Country Park	25	53.332415	-2.958273	21	12.1	0.6	16.1	1.4								
New Ferry	26	53.367699	-2.994765	22	12.1	0.9	15.6	2.2								
Rock Ferry Pier	27	53.374709	-2.999964	23	11.6	0.4	15.6	0.8								
Alabama Way	28	53.391416	-3.008456	24	12.7	0.9	13.5	0.6	11.7	0.0			16.4	0.0		
Morpeth Dock	29	53.398129	-3.018651													
Alfred Dock	30	53.404597	-3.018792	25	10.2	0.7	10.9	0.3								
Egremont Promenade	31	53.419542	-3.024553	26	11.6	0.8	16.6	1.7			13.7	0.1			10.3	0.1
Egremont Promenade	32	53.425909	-3.029881	27	11.5	1.0	12.0	1.3			12.8	0.0				
Holland Road	33	53.430653	-3.03259	28	12.5	0.6	14.0	0.3			13.6	0.5				
New Brighton (Tower)	34	53.439389	-3.035767	29	11.0	0.7	13.9	1.0	10.6	0.0	12.9	0.5				
New Brighton (Beach)	35	53.4417902	-3.0435904	30	11.0	1.2	13.2	0.5			11.1	0.6			10.6	1.0
Wirral Way	36	53.434718	-3.075567	31	9.4	0.7	11.5	0.7			12.0	0.5				
Grassendale Outflow	9A	53.357299	-2.922355	8A			10.2	2.2								
Grassendale	9B	53.357313	-2.922425	8B	11.8	1.0	13.4	2.0								
Crosby Lake	Crosby Lake	53.472039	-3.031803													

July

Original July Site Number	Lab Revised Number	Latitude	Longitude	Fucus Average	Fucus std	Fucus Count	Ulva Avergae	Ulva std	Ulva Count
1	Site 0	53.34768	-2.735702				18.8	1.5	6
2a	Site 1A	53.349336	-2.740634				19.0	0.9	4
2b	Site 1B	53.349336	-2.740634				2.7	0.3	3
3	Site 3	53.345785	-2.768527				19.3	0.3	3
4	Site 5	53.339889	-2.773527				4.2	2.9	7
5	Site 6	53.32232	-2.794847	18.1	0.8	7	19.0	1.0	9
6	Site 7	53.331902	-2.82612	19.3	1.9	9	19.7	0.4	8
7	Site 8	53.326782	-2.83607	17.5	1.6	8	17.3	0.6	6
8a	Site 9A	53.357234	-2.922067				7.0	1.3	5
8b	Site 9B	53.357234	-2.922067	13.4	0.6	6	17.3	1.1	6
9	Site 10	53.373416	-2.963501	12.1	2.7	8			
10	Site 11	53.378627	-2.973552	12.1	2.5	8	18.0	2.1	4
11	Site 12	53.383263	-2.981207	5.7	1.7	7			
12	Site 14	53.390314	-2.98727	10.4	2.2	8	10.8	4.2	9
13	Site 16	53.390714	-2.986214				14.0	0.0	2
14	Site 17	53.392521	-2.985217				12.4	0.8	6
16	Site 18	53.396264	-2.987668				14.8	1.7	5
17	Site 19	53.398399	-2.992747				13.1	0.6	4
18	Site 21	53.401709	-2.992161				10.0	0.8	5
19	Site 20	53.399753	-2.990291				10.7	0.6	3
20	Site 22	53.405463	-2.998214	12.1	1.3	8	14.5	1.3	4
21	Site 23	53.409164	-2.999366				2.2	2.7	3
22	Site 25	53.332304	-2.958169	16.5	1.2	7	16.6	0.8	8
23	Site 26	53.367409	-2.994431	12.0	0.4	5	15.8	1.2	8
24	Site 27	53.37491	-2.999395	13.8	0.5	8	15.1	0.7	7
25	Site 28	53.391453	-3.008305	13.8	0.8	10	16.9	0.5	6
26	Site 30	53.404751	-3.018929				9.6	2.9	7
27	Site 31	53.41943	-3.024087	13.9	1.4	10	13.5	0.9	6
28	Site 32	53.425799	-3.029752	13.2	0.6	8	13.2	0.9	7
29	Site 33	53.430447	-3.032244	14.6	0.7	8	15.2	0.8	6
30	Site 34	53.43948	-3.035652	13.8	0.6	8	13.9	0.4	8
31	Site 35	53.441729	-3043724	11.6	0.5	9	11.6	1.1	6
32	Site 36	53.435823	-3.075441	11.0	0.5	8	10.0	0.8	8

January *Fucus*		**January *Ulva***		**January (Other)**		**April *Fucus***		**April *Ulva***		**April (Other)**		**July *Fucus***		**July *Ulva***	
SIBL ID	$\delta^{15}N$	SIBL ID	$\delta^{15}N$	SIBL ID	$\delta^{15}N$	SIBL ID	$\delta^{15}N$	SIBL ID	$\delta^{15}N$	SIBL ID	$\delta^{15}N$	SIBL ID	$\delta^{15}N$	SIBL ID	$\delta^{15}N$
01-2023 F 5-1	10.7	01-2023 U 1-1	13.3	01-2023 C 13-2	14.9	F 2-2	13.3	U-1-1	15.4	CC-12-16	11.4	F-5-10	17.7	U-1-1	16.5
01-2023 F 5-2	11.1	01-2023 U 1-2	13.6	01 -2023 C 13-1	16.1	F 2-7	13.5	U-1-2	2.9	C-13-2	11.8	F-5-11	18.7	U-1-2	18.9
01-2023 F 5-3	12.1	01-2023 U 1-3	2.5	01-2023 C 13-5	16.0	F 2-8	13.5	U-1-3	7.9	C-13-3	12.1	F-5-12	18.1	U-1-3	18.3
01-2023 F 5-4	11.5	01-2023 U 2-1	12.7	01-2023 C 13-6	16.4	F 2-9	14.7	U-1-4	15.7	C-13-5	11.9	F-5-13	18.2	U-1-4	20.0
01-2023 F 5-5	11.0	01-2023 U 2-2	12.7	01-2023 C 13-7	17.9	F 2-10	11.3	U 1-5	15.8	C-13-6	12.3	F-5-14	16.5	U-1-5	17.8
01-2023 F 5-6	11.2	01-2023 U 2-3	11.6	01-2023 C 13-8	14.3	F 2-11	13.2	U 1-6	-2.7	C-13-7	12.5	F-5-8	19.1	U-1-6	21.1
01-2023 F 5-9	11.6	01-2023 U 3-1	1.3	01-2023 CC 13-3	16.3	F 2-12	13.8	U 2-1	16.9	C-13-8	12.4	F-5-9	18.5	U-2A-1	18.6
01-2023 F 6-10	13.7	01-2023 U 3-2	-0.6	01-2023 CC 13-4	16.9	F 2-13	14.7	U 2-4	18.0	C-16-1	10.5	F-6-13	18.7	U-2A-2	20.2
01-2023 F 6-11	11.6	01-2023 U 3-3	1.3	01-2023 C 14-1	18.6	F 6-1	13.5	U 2-5	17.4	CC-16-3	8.5	F-6-14	17.4	U-2A-3	17.8
01-2023 F 6-12	12.3	01-2023 U 4-1	10.5	01-2023 C 14-2	12.9	F 6-3	12.2	U 2-6	16.0	CC-16-4	9.4	F-6-15	19.5	U-2A-4	19.5
01-2023 F 6-4	13.6	01-2023 U 4-2	8.4	01-2023 C 14-3	16.1	F 6-5	11.8	U 3-1	14.2	C-18-4	14.3	F-6-16	17.8	U-2B-1	3.0
01-2023 F 6-5	10.7	01-2023 U 4-3	9.8	01-2023 C 14-4	14.7	F 6-7	13.2	U 3-2	11.4	CC 19-9	9.0	F-6-17	22.6	U-2B-2	2.8
01-2023 F 6-6	14.1	01-2023 U 5-10	12.8	01-2023 CC 14-5	16.4	F 6-9	12.2	U 3-3	11.5	C 19-12	8.8	F-6-2	22.0	U-2B-3	2.3
01-2023 F 6-7	11.6	01-2023 U 5-7	13.0	01-2023 C 15-2	15.3	F 6-10	14.0	U 3-4	13.4	C 20-3	8.2	F-6-3	16.8	U-3-1	19.6
01-2023 F 6-8	15.3	01-2023 U 5-8	12.3	01-2023 C 15-3	18.7	F 6-11	13.2	U 4-1	13.9	C-24-10	11.7	F-6-4	19.0	U-3-2	19.2
01-2023 F 7-1	10.0	01-2023 U 6-1	12.7	01-2023 CC 15-1	18.9	F 6-12	13.5	U 4-2	12.5	CC-24-11	16.4	F-6-5	20.0	U-3-3	19.0
01-2023 F 7-11	10.7	01-2023 U 6-13	12.7	01-2023 CC 15-4	15.9	F 6-13	12.4	U 4-3	10.4	C-29-18	11.9	F-7-10	18.5	U-4-1	6.0

01-2023 F 7-12	12.0	01-2023 U 6-2	12.5	01-2023 C 16-1	14.7	F 7-1	14.7	U 4-4	14.6		F-7-11	14.1	U-4-2	3.4
01-2023 F 7-13	12.5	01-2023 U 6-3	8.7	01-2023 C 16-2	15.5	F 7-2	14.1	U 4-5	12.6		F-7-12	18.4	U-4-3	7.4
01-2023 F 7-2	12.3	01-2023 U 6-9	12.1	01-2023 C 16-4	14.5	F 7-3	14.5	U 5-1	9.1		F-7-13	16.7	U-4-4	8.4
01-2023 F 7-3	11.4	01-2023 U 7-10	7.2	01-2023 CC 16-3	22.2	F 7-4	12.8	U 5-2	9.1		F-7-14	16.3	U-4-5	0.0
01-2023 F 7-4	10.1	01-2023 U 7-14	11.6	01-2023 CC 16-5	23.3	F 7-5	11.7	U 5-3	9.1		F-7-7	19.3	U-4-6	2.4
01-2023 F 7-5	12.1	01-2023 U 8A-1	7.2	01-2023 CC 16-6	22.8	F 7-6	13.5	U 5-4	7.9		F-7-8	18.4	U-4-7	1.9
01-2023 F 7-6	11.0	01-2023 U 8A-2	10.1	01-2023 C 17-1	12.0	F 7-7	12.6	U 5-5	9.5		F-7-9	18.1	U-5-1	18.4
01-2023 F 7-7	11.4	01-2023 U 8A-3	9.3	01-2023 C 17-2	13.9	F 7-8	12.5	U 6-2	14.2		F 8B-12	13.1	U-5-15	17.8
01-2023 F 7-8	10.6	01-2023 U 8A-4	6.9	01-2023 C 17-3	15.1	F 7-9	11.6	U 6-4	17.2		F-8B-11	14.1	U-5-16	18.7
01-2023 F 7-9	10.0	01-2023 U 8B-7	8.5	01-2023 C 18-1	15.1	F 7-10	12.6	U 6-6	17.4		F-8B-5	14.1	U-5-2	18.7
01-2023 F 8B-1	13.7	01-2023 U 8B-9	9.9	01-2023 C 18-3	13.2	F 8B-1	11.5	U 6-8	16.8		F-8B-6	13.1	U-5-3	18.0
01-2023 F 8B-2	11.6	01-2023 U 9-4	16.8	01-2023 C 18-4	10.3	F 8B-2	13.7	U 6-14	17.0		F-8B-7	12.6	U-5-4	20.1
01-2023 F 8B-3	11.6	01-2023 U 19-2	8.1	01-2023 CC 18-2	12.6	F 8B-3	10.8	U 8A-1	13.8		F-8B-8	13.6	U-5-5	19.8
01-2023 F 8B-4	12.7	01-2023 U 19-3	7.7	01-2023 CC 18-5	15.5	F 8B-4	10.9	U 8A-2	10.3		F 9-1	10.1	U-5-6	21.1
01-2023 F 8B-5	13.1	01-2023 U 19-4	8.9	01-2023 C 19-1	6.8	F 8B-9	11.9	U 8A-3	8.2		F 9-2	9.1	U-5-7	18.3
01-2023 F 8B-6	11.3	01-2023 U 20-1	5.3			F 9-1	10.7	U 8A-4	8.6		F 9-3	8.0	U-6-1	19.0
01-2023 F 8B-8	11.9	01-2023 U 20-2	4.6			F 9-2	12.2	U-8B-5	9.6		F 9-4	12.4	U-6-10	19.2
01-2023 F 9-1	13.9	01-2023 U 20-3	5.3			F 9-3	11.3	U 8B-6	15.3		F 9-5	16.3	U-6-11	20.3

01-2023 F 9-2	11.8	01-2023 U 23-1	12.4
01-2023 F 9-3	11.8	01-2023 U 23-2	13.2
01-2023 F 10-1	9.5	01-2023 U 24-15	13.9
01-2023 F 10-2	10.8	01-2023 U 24-6	12.7
01-2023 F 10-3	8.6	01-2023 U 24-7	14.8
01-2023 F 10-4	6.4	01-2023 U 24-8	12.7
01-2023 F 10-5	7.6	01-2023 U 24-9	13.1
01-2023 F 10-6	9.8	01-2023 U 25-1	12.7
01-2023 F 10-7	10.1	01-2023 U 25-2	12.5
01-2023 F 10-8	5.2	01-2023 U 26-2	10.3
01-2023 F 10-9	9.1	01-2023 U 26-3	11.4
01-2023 F 10-10	9.6	01-2023 U 26-7	11.7
01-2023 F 11-1	10.2	01-2023 U 27-11	11.5
01-2023 F 11-3	10.0	01-2023 U 27-12	12.4
01-2023 F 11-4	11.9	01-2023 U 27-13	13.0
01-2023 F 11-5	9.5	01-2023 U 27-14	13.7
01-2023 F 11-6	8.7	01-2023 U 29-3	-2.2
01-2023 F 11-7	9.1	01-2023 U 29-4	-0.4

F 9-4	11.9	U 8B-7	14.8
F 9-5	9.3	U 8B-8	13.4
F 9-6	11.9	U 9-10	16.0
F 10-1	10.1	U 9-11	18.7
F 10-2	12.1	U 9-12	18.8
F 10-3	11.9	U 10-11	18.3
F 10-4	12.5	U 10-12	18.3
F 10-5	10.9	U-10-13	12.9
F 10-6	13.1	U-10-14	19.7
F 10-7	11.9	U-10-15	14.7
F 10-8	12.5	U-10-16	15.6
F 10-9	11.3	U-12-1	13.0
F 10-10	11.4	U-12-8	15.9
F-11-1	12.1	X-12-17	13.7
F-11-2	11.4	U-12-18	12.4
F-11-3	12.3	X-12-19	13.5
F-11-4	12.5	U-12-20	9.7
F-11-5	12.4	U-12-21	10.8

F 9-6	12.4	U-6-12	19.7
F 9-7	13.8	U-6-6	20.0
F 9-8	14.6	U-6-7	19.8
F 10-1	13.2	U-6-8	19.9
F 10-19	16.9	U-6-9	19.3
F 10-2	13.3	SL-7-4	18.1
F 10-3	12.7	SL-7-5	17.3
F 10-4	7.6	SL-7-6	17.5
F 10-5	11.4	U-7-1	17.3
F 10-6	11.5	U-7-2	16.2
F 10-7	10.3	U-7-3	17.3
U 10-11	18.9	U-8A-1	6.3
U 10-12	14.3	U-8A-2	7.3
U 10-14	19.1	U-8A-3	9.3
U 10-17	19.5	U-8A-4	6.1
F 11-4	6.2	U-8A-5	5.9
F 11-5	6.2	U-8B-1	17.4
F 11-6	8.5	U-8B-10	18.2

01-2023 F 11-8	8.5	01-2023 U 29-5	3.6
01-2023 F 12-1	8.5	01-2023 U 29-6	4.4
01-2023 F 12-2	4.2	01-2023 U 30-10	10.0
01-2023 F 12-3	7.1	01-2023 U 30-14	13.4
01-2023 F 12-4	4.8	01-2023 U 31-11	9.0
01-2023 F 12-5	7.9	01-2023 U 31-12	12.3
01-2023 F 12-6	5.4	01-2023 U 31-13	13.4
01-2023 F 12-7	3.4	01-2023 U 32-13	9.2
01-2023 F 12-8	6.9	01-2023 U 32-14	12.1
01-2023 F 22-1	13.1	01-2023 U 32-15	11.3
01-2023 F 22-2	13.0	01-2023 U 33-17	13.5
01-2023 F 22-3	13.6	01-2023 U 33-18	11.3
01-2023 F 22-4	12.7	01-2023 U 33-2	11.9
01-2023 F 22-5	12.9	01-2023 U 33-3	9.0
01-2023 F 22-6	13.2	01-2023 U 33-6	11.2
01-2023 F 22-7	12.7	01-2023 U 33-7	12.7
01-2023 F 22-8	14.5	01-2023 U 34-11	13.6
01-2023 F 22-9	12.9	01-2023 U 34-4	12.1

F-11-6	12.3	U-12-22	16.1
F-11-7	12.0	U-13-1	12.4
F-11-8	12.5	U-14-1	13.5
F-11-9	11.0	U-14-2	9.6
F-11-10	10.6	X-14-3	13.3
F-12-3	11.9	U-14-4	14.3
F-12-4	13.5	U-14-5	14.4
F-12-5	13.6	U-14-6	13.6
F-12-6	13.0	X-14-7	13.0
F-12-7	13.0	X-14-8	12.7
F-12-9	12.4	U-15-1	13.1
F-12-11	12.4	U-15-2	15.2
F-12-12	13.8	U-15-3	15.3
F-12-14	14.2	U-16-2	14.8
F 19-3	12.5	U-16-5	15.6
F 19-4	14.3	U-17-1	11.3
X 19-5	15.1	U-17-2	11.0
F 19-6	13.6	U-17-3	13.7

FS 11-1	2.8	U-8B-2	18.4
FS 11-2	4.3	U-8B-3	15.1
FS 11-3	5.6	U-8B-4	17.9
FS 11-7	6.7	U-8B-9	17.0
F-12-10	13.4	U 10-11	18.9
F-12-12	10.2	U 10-12	14.3
F-12-18	10.7	U 10-14	19.1
F-12-21	7.4	U 10-17	19.5
F-12-9	13.9	U-12-14	8.6
FS-12-13	8.9	U-12-15	5.2
FS-12-16	8.2	U-12-20	4.0
FS-12-19	10.8	U-12-3	8.3
F 20-1	13.4	U-12-4	12.2
F 20-10	12.3	U-12-5	13.9
F 20-11	12.9	U-12-6	15.1
F 20-12	10.6	U-12-7	14.9
F 20-13	11.1	U-12-8	15.4
F 20-15	13.9	C-13-1	13.0

01-2023 F 23-10	14.1	01-2023 U 35-3	11.6
01-2023 F 23-11	13.5	01-2023 U 35-8	11.9
01-2023 F 23-12	12.5	01-2023 U 35-9	12.3
01-2023 F 23-13	13.1		
01-2023 F 23-14	11.9		
01-2023 F 23-3	12.7		
01-2023 F 23-4	11.9		
01-2023 F 23-5	14.0		
01-2023 F 23-6	12.8		
01-2023 F 23-7	13.0		
01-2023 F 23-8	12.1		
01-2023 F 23-9	12.5		
01-2023 F 24-1	13.8		
01-2023 F 24-10	12.8		
01-2023 F 24-11	13.8		
01-2023 F 24-12	10.5		
01-2023 F 24-13	13.4		
01-2023 F 24-14	13.0		

X 19-7	13.3	U-17-4	12.8
F 19-8	11.0	U-18-1	14.4
FS 19-10	12.6	U-18-2	13.7
F 19-11	13.3	U-18-3	12.9
F 19-13	13.0	U-18-5	14.5
F-19-14	13.0	U-19-1	15.8
F 21-6	11.4	U-19-2	11.8
F 21-7	11.7	U 19-18	14.8
F 21-8	11.3	U 20-1	9.1
F 21-9	12.1	U 20-2	7.5
F 21-10	13.3	U 21-1	15.0
F 21-11	12.3	U 21-2	15.5
F 21-12	12.9	U 21-3	16.7
F 21-13	12.0	U 21-4	17.6
F 21-14	12.2	U 21-5	18.0
F 22-1	13.8	U 21-15	14.0
F 22-3	12.9	U 22-2	13.9
F 22-4	11.7	U 22-8	15.9

F 20-16	10.2	C-13-2	13.0
F 20-3	12.7	C-13-5	12.2
FS 20-2	6.3	U-13-3	13.9
F 22-12	15.3	U-13-4	14.0
F 22-13	18.1	U-14-1	13.8
F 22-14	16.1	U-14-15	12.2
F 22-15	17.7	U-14-17	11.3
F 22-17	15.2	U-14-2	12.9
F 22-8	15.1	U-14-3	12.3
F 22-9	17.8	U-14-4	12.0
F 23-1	11.3	U 16-1	15.8
F 23-2	12.2	U 16-2	13.5
F 23-3	12.5	U 16-3	14.2
F 23-4	12.0	U 16-4	17.7
F 23-5	12.1	U 16-5	12.8
F 24-11	13.3	U 17-1	13.2
F 24-12	14.5	U 17-2	12.3
F 24-13	13.9	U 17-3	14.0

01-2023 F 24-2	13.7			F 22-5	12.1	U 22-9	19.1		F 24-14	13.2	U 17-4	12.7
01-2023 F 24-3	12.4			F 22-6	12.5	U 22-13	13.6		F 24-15	13.7	U 18-1	9.9
01-2023 F 24-4	12.4			F 22-7	11.5	U 23-5	16.5		F 24-4	14.6	U 18-2	8.8
01-2023 F 24-5	13.8			F 22-10	11.6	U 23-6	15.9		F 24-5	13.2	U 18-3	10.3
01-2023 F 25-10	13.8			F 22-11	10.8	U 23-9	14.2		F 24-6	14.0	U 18-4	9.5
01-2023 F 25-3	11.4			F 22-12	12.7	U-23-14	15.7		F 25-1	12.6	U 18-5	11.4
01-2023 F 25-4	11.6			F 22-14	11.0	U-24-1	12.6		F 25-10	14.6	U 19-1	11.6
01-2023 F 25-5	13.6			F 23-1	11.6	U-24-12	14.1		F 25-11	14.4	U 19-2	10.5
01-2023 F 25-6	13.9			F 23-2	11.0	U-24-13	13.5		F 25-2	13.5	U 19-3	10.0
01-2023 F 25-7	12.6			F 23-3	11.4	U-24-15	14.0		F 25-3	13.7	U 20-14	12.4
01-2023 F 25-8	12.8			F 23-4	12.0	U-25-10	10.6		F 25-4	15.0	U 20-7	15.5
01-2023 F 25-9	12.7			F 23-7	11.6	U-25-11	11.2		F 25-5	13.8	U 20-8	15.6
01-2023 F 26-1	13.6			F 23-8	11.4	U-25-12	11.0		F 25-6	12.4	U 20-9	14.5
01-2023 F 26-10	12.4			F 23-10	12.0	U-26-13	15.1		F 25-7	13.4	U 21-1	0.5
01-2023 F 26-11	11.6			F 23-11	12.0	U-26-14	15.7		F 25-8	14.8	U 21-2	0.2
01-2023 F 26-12	13.5			F-23-12	11.0	U-26-17	19.0		F-27-1	14.7	U 21-3	5.9
01-2023 F 26-13	14.1			F-23-13	12.0	U-27-10	14.0		F-27-16	14.6	U 22-1	16.5
01-2023 F 26-4	12.4			F-23-15	11.2	U-27-11	11.4		F-27-17	13.6	U 22-11	16.1

01-2023 F 26-5	13.0	F-24-2	13.3	U-27-12	12.3	F-27-18	10.2	U 22-2	15.8
01-2023 F 26-6	12.8	F-24-3	13.5	U-27-16	10.3	F-27-2	12.9	U 22-3	17.7
01-2023 F 26-8	14.0	F-24-4	11.7	U-28-10	13.5	F-27-3	14.7	U 22-4	17.9
01-2023 F 26-9	14.0	F-24-5	11.1	U-28-12	14.2	F-27-4	14.4	U 22-5	15.3
01-2023 F 27-1	13.3	F-24-6	11.9	U-28-15	14.2	F-27-5	14.6	U 22-6	17.0
01-2023 F 27-10	12.8	F-24-7	12.2	U-29-11	14.0	F-27-6	14.4	U 22-7	16.7
01-2023 F 27-2	13.1	F-24-8	13.7	U-29-14	14.3	F-27-7	14.8	U 23-10	13.6
01-2023 F 27-4	13.3	F-24-9	12.9	U-29-15	12.4	F-28-1	13.6	U 23-11	16.4
01-2023 F 27-5	12.5	F-24-14	13.9	U-29-16	15.5	F-28-2	13.3	U 23-12	16.0
01-2023 F 27-6	14.1	F-24-16	13.2	U-29-17	15.3	F-28-3	12.2	U 23-13	16.6
01-2023 F 27-7	13.7	F-24-17	12.4	U-29-19	13.4	F-28-4	13.2	U 23-6	14.4
01-2023 F 27-8	11.9	F-25-1	10.6	U-29-20	13.9	F-28-5	13.3	U 23-7	17.0
01-2023 F 27-9	12.7	F-25-2	10.6	U-30-15	12.6	F-28-6	13.9	U 23-8	17.1
01-2023 F 29-1	3.2	F-25-3	11.1	U-30-16	13.9	F-28-8	12.3	U 23-9	15.1
01-2023 F 29-2	-1.8	F-25-4	8.3	U-30-18	13.1	F-28-9	13.7	U 24-1	14.7
01-2023 F 30-1	13.2	F-25-5	10.1	U-30-21	13.3	F 29-11	14.6	U 24-10	15.6
01-2023 F 30-12	9.9	F-25-6	10.2	U-31-7	12.6	F 29-2	14.0	U 24-2	16.3
01-2023 F 30-2	13.5	F-25-7	10.4	U-31-14	11.8	F 29-3	13.9	U 24-3	15.4

01-2023 F 30-3	15.2			F-25-8	10.3	U-31-15	11.0		F 29-4	15.4	U 24-7	15.1
01-2023 F 30-4	12.7			F-25-9	9.9	U-31-17	11.6		F 29-5	13.5	U 24-8	14.8
01-2023 F 30-5	12.7			F-26-1	12.5	U-31-19	11.3		F 29-6	15.4	U 24-9	13.8
01-2023 F 30-6	14.3			F-26-2	10.1	U-31-21	11.6		F 29-7	15.3	U 25-9	17.1
01-2023 F 30-7	13.8			F-26-3	11.5				F 29-8	15.0	U 25-10	17.5
01-2023 F 30-8	13.2			F-26-4	11.9				F 30-1	14.3	U 25-11	16.7
01-2023 F 30-9	14.0			F-26-5	11.4				F 30-2	15.1	U 25-12	16.2
01-2023 F 31-1	14.4			F-26-6	12.1				F 30-3	13.0	U 25-13	17.5
01-2023 F 31-10	12.5			F-26-7	10.7				F 30-4	13.5	U 25-14	16.2
01-2023 F 31-2	12.6			F-26-9	11.3				F 30-5	13.4	U 26-1	14.3
01-2023 F 31-3	13.6			F-26-10	11.6				F 30-6	13.8	U 26-2	8.5
01-2023 F 31-4	11.9			F-26-12	12.9				F 30-7	13.7	U 26-3	4.2
01-2023 F 31-5	13.5			F-27-2	11.8				F 30-8	13.5	U 26-4	11.8
01-2023 F 31-6	13.9			F-27-3	13.5				F 31-1	11.3	U-26-5	8.6
01-2023 F 31-7	13.5			F-27-4	10.7				F 31-11	10.8	U-26-6	8.9
01-2023 F 31-8	14.0			F-27-5	11.7				F 31-2	12.2	U-26-7	11.0
01-2023 F 31-9	12.4			F-27-6	12.5				F 31-3	11.4	U-27-10	15.2
01-2023 F 32-1	14.1			F-27-7	10.9				F 31-4	10.8	U-27-11	13.3

01-2023 F 32-11	14.1			F-27-8	11.8			F 31-5	12.1	U-27-12	12.7
01-2023 F 32-12	13.5			F-27-13	10.5			F 31-6	11.8	U-27-13	13.7
01-2023 F 32-2	12.4			F-27-15	10.4			F 31-8	11.6	U-27-14	13.1
01-2023 F 32-3	13.4			F-28-1	12.8			F 31-9	12.1	U-27-15	12.9
01-2023 F 32-4	13.5			F-28-2	13.2			F 32-1	10.8	U-28-10	14.5
01-2023 F 32-5	12.4			F-28-3	11.8			F 32-10	11.7	U-28-11	11.5
01-2023 F 32-6	13.8			F-28-4	12.8			F 32-3	10.5	U-28-12	13.2
01-2023 F 32-7	12.5			F-28-5	12.3			F 32-4	10.8	U-28-13	13.1
01-2023 F 32-8	14.1			F-28-6	11.9			F 32-5	12.0	U-28-14	13.5
01-2023 F 32-9	13.2			F-28-8	12.4			F 32-6	10.5	U-28-15	13.9
01-2023 F 33-1	14.8			F-28-11	11.0			F 32-7	10.8	U-28-7	12.8
01-2023 F 33-10	15.0			F-28-14	12.5			F 32-8	11.1	U 29-12	15.6
01-2023 F 33-11	15.0			F-28-16	12.9					U 29-13	13.5
01-2023 F 33-12	14.2			F-28-17	13.3					U 29-14	15.4
01-2023 F 33-13	14.3			F-29-1	10.4					U 29-15	15.8
01-2023 F 33-16	13.6			F-29-2	10.8					U 29-16	15.5
01-2023 F 33-4	14.2			F-29-3	11.2					U 29-17	15.5
01-2023 F 33-5	14.3			F-29-4	10.8					U 30-10	13.6

01-2023 F 33-8	14.1			F-29-5	10.2			U 30-11	14.6
01-2023 F 33-9	14.4			F-29-6	11.1			U 30-12	13.7
01-2023 F 34-1	13.1			F-29-7	11.6			U 30-13	13.8
01-2023 F 34-13	12.1			F-29-8	11.1			U 30-14	13.4
01-2023 F 34-14	12.4			F-29-9	12.7			U 30-15	13.7
01-2023 F 34-15	13.8			F-29-12	10.3			U 30-16	14.2
01-2023 F 34-16	12.6			F-30-1	11.6			U 30-9	13.9
01-2023 F 34-17	11.8			F-30-2	11.9			U 31-10	11.1
01-2023 F 34-18	12.5			F-30-3	12.9			U 31-13	12.0
01-2023 F 34-19	12.4			F-30-4	8.3			U 31-15	12.9
01-2023 F 34-20	13.7			F-30-5	9.8			U 31-16	11.3
01-2023 F 34-7	12.8			F-30-6	10.8			U 31-17	9.8
01-2023 F 34-8	11.6			F-30-7	11.1			U 31-18	12.7
01-2023 F 34-9	12.3			F-30-8	10.8			U 32-12	8.3
01-2023 F 35-1	10.6			F-30-9	10.8			U 32-13	10.0
01-2023 F 35-10	9.2			F-30-10	11.6			U 32-14	10.8
01-2023 F 35-11	10.3			F-31-1	8.4			U 32-15	10.9
01-2023 F 35-5	10.0			F-31-2	8.6			U 32-16	10.0

01-2023 F 35-6 10.3 01-2023 F 35-7 9.4			F-31-3 9.8 F-31-4 9.5 F-31-5 9.9 F-31-6 10.6 F-31-9 9.3 F-31-10 9.1				U 32-17 9.6 U 32-2 10.6 U 32-9 10.2

Appendix 4: Chapter 4 Data Set

Dock Information:

Dock	Dock Areas (m^2)	Depths (m)	Volume (m^3)
Albert	28510	6	171060
Canning	15833	4.5	71248.5
Canning Half Tide	9722	6	58332
Salthouse	24450	4.5	110025
Dukes	10914	4.5	49113
Wapping	22348	4.5	100566
Queens	70196	4.5	315882
Coburg	27132	4.5	122094
Brunswick	62414	4.5	280863
South Docks Total	271519		1279183.5

Herbaria Macroalgae (River Mersey):

Year	Species	Location	$\delta^{15}N_{macroalgae}$ (AIR) ‰
1821	*Enteromorpha compressa*	Liverpool	13.4
1821	*Enteromorpha compressa*	Liverpool	5.1
1822	*Polysiphonia elongata*	Liverpool	6.4
1823	*Polysiphonia elongata*	Liverpool	8.8
1823	*Ceramium rubrum*	Liverpool	7.4
1824	*Ceramium rubrum*	Liverpool	12.7
1830	*Dictyota dichotoma*	Liverpool	7.8
1831	*Ectocarpus amphibius*	Liverpool	5.2
1834	*Pylaiella littoralis*	Liverpool	6.2
1842	*Cladophora sericea*	Liverpool	8.7
1844	*Ulva lactuca*	Liverpool	11.1
1845	*Halidrys siliquosa*	Liverpool	7.5
1845	*Pelvetia canaliculata*	Liverpool	4.9
1849	*Enteromorpha intestinalis*	Bootle	21.5
1850	*Ulva linza*	Bootle	7.1
1851	*Polysiphonia elongata*	Liverpool	6.4
1853	*Enteromorpha compressa*	Liverpool	4.7
1863	*Ectocarpus granulosus = Hincksia*	New Brighton	4.7
1936	*Halidrys siliquosa & small red*	Birkdale	9.1
1936	*Halidrys siliquosa & small red*	Birkdale	6.2
1937	*Fucus ceranoides*	Birkdale	2.9
1938	*Fucus serratus*	Birkdale	4.7
1949	*Fucus spiralis*	Mersey	4.2
1953	*Fucus serratus*	New Brighton	11.3
1953	*Fucus serratus*	New Brighton	14.6
1967	*Pylaiella litoralis*	Seacombe	10.3

1968	*Fucus vesiculosus*	Otterspool	-4.0
1978	*Fucus vesiculosus*	Eastham	31.5
1978	*Ceramium rubrum*	New Brighton	19.8
1978	*Ulva compressa*	New Brighton	16.7
1979	*Fucus vesiculosus*	Speke	2.9
1982	*Fucus vesiculosus*	Eastham	23.0
1983	*Enteromorpha intestinalis*	Leasow & Meols	12.6
1990	*Fucus vesiculosus*	Grassendale	12.7
1993	*Ceramium rubrum*	New Brighton	14.3
1997	*Fucus Vesiculosus*	Grassendale	4.7
1998	*Chondrus crispus*	New Brighton	8.5
2001	*Ulva linza*	New Brighton	13.1
2002	*Cladophora vagabunda*	New Brighton	14.2
2005	*Fucus Vesiculosus*	Speke	16.5
2008	*Pelvetia canaliculata*	Ainsdale	8.6
2008	*Polysiponia fucoidiies*	New Brighton	11.7
2010	*Pelvetia canaliculta*	Hall Road	13.0
2012	*Pylaiella littoralis*	New Brighton	10.9
2013	*Cladophora sericea*	Hall Road	14.6
2013	*Pylaiella littoralis*	New Brighton	9.1

Herbaria Macroalgae (Liverpool Docks):

Year	Species	Location	$\delta^{15}N_{macroalgae}$ (AIR) ‰
1846	*Fucus vesiculosus*	Princes Pier	8.2
1981	*Prasiola calophylla*	Sandon Dock	13.2
1988	*Cladophora vagabunda*	Albert Dock	15.6
1989	*Cladophora vagabunda*	Queens Dock	9.2
1991	*Polysiphonia urceolata*	South Docks (Albert)	8.8
1993	*Ceramium rubrum*	Princes Dock	20.4
1999	*Cladophora vagabunda*	Queens Dock	8.6
2000	*Polysiphonia stricta*	Albert Dock	15.4
2003	*Bryopsis hypnoides*	Queens Dock	14.7
2004	*Bryopsis hypnoides*	Queens Dock	15.2
2004	*Polysiphonia stricta*	Queens Dock	15.2
2012	*Polysiphonia stricta*	Queens Dock	18.3
2012	*Ulva intestinalis*	Salthouse Dock	13.8
2012	*Polysiphonia stricta*	Wapping Dock	17.4
2014	*Ulva rigida*	Albert Dock	12.8
2014	*Ulva intestinalis*	Albert Dock	9.0
2014	*Ceramium diaphanum*	Albert Dock	17.5
2014	*Cladophora vagabunda*	Collingwood Dock	10.4
2015	*Cladophora vagabunda*	Albert Dock	3.9
2015	*Ceramium diaphanum*	Albert Dock	14.2

2015	*Polysiphonia stricta*	Queens Dock	16.9
2015	*Cladophora vagabunda*	Salthouse Dock	15.8
2015	*Polysiphonia stricta*	Salthouse Dock	17.6
2018	*Polysiphonia stricta*	Queens Dock	13.4

2022 $\delta^{15}N$ data:

Year	Species	Location	$\delta^{15}N_{macroalgae}$ (AIR) ‰
2022	*Fucus Vesiculosus*	River Mersey	14.5
2022	*Fucus Vesiculosus*	River Mersey	14.8
2022	*Fucus Vesiculosus*	River Mersey	15.8
2022	*Fucus Vesiculosus*	River Mersey	15.3
2022	*Fucus Vesiculosus*	River Mersey	15.8
2022	*Fucus Vesiculosus*	River Mersey	13.9
2022	*Fucus Vesiculosus*	River Mersey	15.9
2022	*Fucus Vesiculosus*	River Mersey	15.0
2022	*Fucus Vesiculosus*	River Mersey	15.7
2022	*Fucus Vesiculosus*	River Mersey	13.6
2022	*Fucus Vesiculosus*	River Mersey	15.8
2022	*Fucus Vesiculosus*	River Mersey	16.7
2022	*Fucus Vesiculosus*	River Mersey	18.1
2022	*Fucus Vesiculosus*	River Mersey	19.7
2022	*Fucus Vesiculosus*	River Mersey	17.5
2022	*Fucus Vesiculosus*	River Mersey	17.7
2022	*Fucus Vesiculosus*	River Mersey	18.3
2022	*Fucus Vesiculosus*	River Mersey	17.4
2022	*Fucus Vesiculosus*	River Mersey	19.7
2022	*Fucus Vesiculosus*	River Mersey	17.2
2022	*Fucus Vesiculosus*	River Mersey	18.7
2022	*Fucus Vesiculosus*	River Mersey	22.8
2022	*Fucus Vesiculosus*	River Mersey	17.9
2022	*Fucus Vesiculosus*	River Mersey	19.2
2022	*Fucus Vesiculosus*	River Mersey	21.9
2022	*Fucus Vesiculosus*	River Mersey	17.0
2022	*Fucus Vesiculosus*	River Mersey	17.1
2022	*Fucus Vesiculosus*	River Mersey	16.4
2022	*Fucus Vesiculosus*	River Mersey	15.8
2022	*Fucus Vesiculosus*	River Mersey	12.8
2022	*Fucus Vesiculosus*	River Mersey	13.8
2022	*Fucus Vesiculosus*	River Mersey	13.6
2022	*Fucus Vesiculosus*	River Mersey	17.5
2022	*Fucus Vesiculosus*	River Mersey	15.7
2022	*Fucus Vesiculosus*	River Mersey	17.9

2022	*Fucus Vesiculosus*	River Mersey	17.5
2022	*Fucus Vesiculosus*	River Mersey	15.3
2022	*Fucus Vesiculosus*	River Mersey	17.9
2022	*Fucus Vesiculosus*	River Mersey	15.8
2022	*Fucus Vesiculosus*	River Mersey	16.2
2022	*Fucus Vesiculosus*	River Mersey	17.2
2022	*Fucus Vesiculosus*	River Mersey	16.0
2022	*Fucus Vesiculosus*	River Mersey	15.1
2022	*Fucus Vesiculosus*	River Mersey	15.6
2022	*Fucus Vesiculosus*	River Mersey	15.5
2022	*Fucus Vesiculosus*	River Mersey	16.5
2022	*Fucus Vesiculosus*	River Mersey	17.1
2022	*Fucus Vesiculosus*	River Mersey	14.8
2022	*Fucus Vesiculosus*	River Mersey	14.6
2022	*Fucus Vesiculosus*	River Mersey	15.2
2022	*Ulva* sp.	River Mersey	16.7
2022	*Ulva* sp.	River Mersey	18.3
2022	*Ulva* sp.	River Mersey	19.6
2022	*Ulva* sp.	River Mersey	14.5
2022	*Ulva* sp.	River Mersey	13.8
2022	*Ulva* sp.	River Mersey	13.7
2022	*Ulva* sp.	River Mersey	15.0
2022	*Ulva* sp.	River Mersey	14.8
2022	*Ulva* sp.	River Mersey	14.4
2022	*Ulva* sp.	River Mersey	16.7
2022	*Ulva* sp.	River Mersey	15.1
2022	*Ulva* sp.	River Mersey	16.7
2022	*Ulva* sp.	River Mersey	15.3
2022	*Ulva* sp.	River Mersey	16.1
2022	*Ulva* sp.	River Mersey	17.9
2022	*Ulva* sp.	River Mersey	17.7
2022	*Ulva* sp.	River Mersey	17.5
2022	*Ulva* sp.	River Mersey	7.4
2022	*Ulva* sp.	River Mersey	8.0
2022	*Ulva* sp.	River Mersey	7.8
2022	*Ulva* sp.	River Mersey	18.5
2022	*Ulva* sp.	River Mersey	18.3

Year	**Species**	**Location**	**$\delta^{15}N_{macroalgae}$ (AIR) ‰**
2022	*Callithamnium corymbosum*	South Docks	10.0
2022	*Callithamnium corymbosum*	South Docks	8.6

2022	*Cladophora*	South Docks	6.3
2022	*Cladophora*	South Docks	5.7
2022	*Cladophora*	South Docks	7.2
2022	*Cladophora*	South Docks	7.3
2022	*Cladophora*	South Docks	3.6
2022	*Cladophora*	South Docks	14.1
2022	*Cladophora*	South Docks	8.3
2022	*Cladophora*	South Docks	15.1
2022	*Cladophora*	South Docks	13.3
2022	*Ulva* sp.	South Docks	15.2
2022	*Ulva* sp.	South Docks	11.8
2022	*Ulva* sp.	South Docks	12.0
2022	*Ulva* sp.	South Docks	9.8
2022	*Ulva* sp.	South Docks	10.8
2022	*Ulva* sp.	South Docks	8.5
2022	*Ulva* sp.	South Docks	13.4
2022	*Ulva* sp.	South Docks	8.8
2022	*Ulva* sp.	South Docks	10.2
2022	*Ulva* sp.	South Docks	12.1
2022	*Ulva* sp.	South Docks	8.4
2022	*Ulva* sp.	South Docks	15.9
2022	*Ulva* sp.	South Docks	15.4
2022	*Ulva* sp.	South Docks	12.2
2022	*Ulva* sp.	South Docks	10.3
2022	*Ulva* sp.	South Docks	11.1

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Diffuse and concentrated nitrogen sewage pollution in island environments with differing treatment systems

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Macroalgae is an under-utilised tool as a bioindicator of anthropogenic nitrogen loading to the coastal environment in the UK. This study compared two island systems—Jersey (Channel Islands) and St Mary's (Isles of Scilly) to assess how differing sewerage infrastructure affects nitrogen loading. A total of 831 macroalgae samples of *Fucus vesiculosus* and *Ulva* sp. were analysed for nitrogen isotopes ($\delta^{15}N$). Elevated $\delta^{15}N$ values were recorded for Jersey (> 9‰) in St Aubin's Bay—caused by the outflow of the Bellozanne Sewerage Treatment Works (STW). $\delta^{15}N$ isoplots maps indicate low diffusion of nitrogen out of St Aubin's Bay. St Mary's produced a varied $\delta^{15}N$ isoplot map in comparison. $\delta^{15}N$ was typically lower and is attributed to a smaller population and inefficient STW. Outflow of sewage/effluent at Morning Point, Hugh Town and Old Town produced elevated $\delta^{15}N$ values in comparison to the island average. St Mary's inefficient sewerage treatment and reliance on septic tanks/soakaways complicates $\delta^{15}N$ interpretation although it still indicates that nitrogen pollution is an island-wide issue. Future sewerage development and upgrades on islands are required to prevent similar effluent environmental issues as recorded in St Aubin's Bay. This study advocates the use of macroalgae as a bioindicator of nitrogen effluent in the marine environment.

Anthropogenic activity has altered the modern nitrogen cycle primarily through the increased influx of sewage to marine environments[1]. It has been estimated that biologically available nitrogen to the ocean has doubled in rate between 1960 and 1990 and hence, is no longer a limiting factor on marine ecosystem productivity[2–4]. Sewage outfalls have been linked to multiple instances of eutrophication, whereby enhanced nutrient uptake by algae results in blooms and hypoxia in the marine environment[5]. Monitoring sewage pollution extent, severity and point source is critical to minimise the risk to public and environmental health[1,5].

Traditional nitrogen isotope monitoring techniques rely on analysing the dissolved inorganic nitrogen content of seawater, which is time-consuming and expensive in comparison to nitrogen isotope analysis of macroalgae[6]. Nitrogen isotope ratios (expressed as $\delta^{15}N$) from macroalgae have been shown to accurately record the nitrogen isotope composition of seawater[1,7]. In fact, nitrogen uptake in macroalgae has successfully traced the influence of sewage/effluent up to 24 km from a point source[3]. The ability to distinguish nitrogen pollution sources come from the premise that sewage/effluent $\delta^{15}N$ exhibits more elevated values compared to chemical industry sources[7,8]. This is primarily due to physical, chemical, and biological processes (denitrification[9]) that occur during the treatment of sewage at wastewater treatment plants. The process of denitrification preferentially removes ^{14}N from the effluent, hence producing a discharge with more positive $\delta^{15}N$ values > + 8‰[3,7,10]. When sewage/effluent is released without any denitrification processes the $\delta^{15}N$ will reflect the source (e.g., artificial fertiliser, animal/human sewage; see below for discussion)[8]. Typically, coastal environments that are affected by sewage range between + 4‰ to + 19‰[8,11].

Savage and Elmgren[12] recommend that values below ~ + 4 ‰ reflect artificially produced nitrate that uses atmospheric nitrogen (~ 0‰) as its nitrogen source. Most agricultural fertilisers use artificially produced products and thus, typically exhibit low or negative $\delta^{15}N$ values[7]. Therefore, macroalgae in a coastal environment dominantly affected through fertilizer runoff will exhibit near zero or negative $\delta^{15}N$ values. When animal and/or human sewage/effluent reaches the coastal environment unprocessed (i.e., raw) or processed (denitrification),

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the $\delta^{15}N$ value of coastal macroalgae will be elevated (since herbivore/human $\delta^{15}N$ values for the British Isles range between + 4‰ to + 8‰[13]) in comparison to artificial fertilizers and/or background marine signatures (e.g., artificial fertilizers < + 4‰, unpolluted/background + 4‰ to + 6‰, sewage/effluent/manure polluted = > + 6‰). However, coastal and/or estuarine environments influenced by wastewater treatment plants will exhibit much higher $\delta^{15}N$ values (e.g., > + 10‰[3]) in comparison to untreated sewage (e.g., > + 6‰). Large-scale, coastal macroalgae $\delta^{15}N$ isoplot maps will help differentiate and determine point sources of pollution, which can then influence societal practices and policies in that region[14].

The macroalgae, *Fucus vesiculosus* (bladder wrack) and *Ulva* sp. are commonly used in nitrogen isotope studies of the coastal environment[15,16]. *Ulva* sp. are more opportunistic and will bloom when concentrations of nitrogen in the water column become elevated[17]. Both macroalgae are found around the coastlines of Europe, with *F. vesiculosus* commonly found in the intertidal zone of rocky shores, while *Ulva* sp. prefers sheltered habitats[18,19]. Dissolved inorganic nitrogen is incorporated within 13–19 days in *F. vesiculosus*, whereas in *Ulva* sp. it has been reported to be within as little as 48 h[6,11,16,20]. Although the use of nitrogen isotopes in macroalgae is cheaper and quicker, it is not widely used[15] and there are only a handful of studies on UK coastlines[6,11,15]. This is surprising considering the exponential increase of unregulated release of sewage/effluent into UK rivers since leaving the European Union (i.e., Brexit)[21].

In this study, we performed nitrogen isotope analysis of *F. vesiculosus* and *Ulva* sp. (simply referred to as *Fucus* and *Ulva* hereafter) from two contrasting island environments in terms of wastewater treatment processes: Jersey has a centralised wastewater treatment facility that is currently being upgraded, and St Mary's (Isles of Scilly) has no major wastewater treatment facility but relies on continuous outflow, septic tanks and soakaway systems.

Study sites

Jersey is the largest of the Channel Islands located 14 miles off the coast of Normandy, France. As of 2019 the population was 106,800 increasing by ~ 1200 persons per year, and the majority of the population resides in St Helier and St Aubin's Bay (Fig. 1A)[22]. Jersey relies solely on the Bellozanne Sewerage Treatment Work (STW)

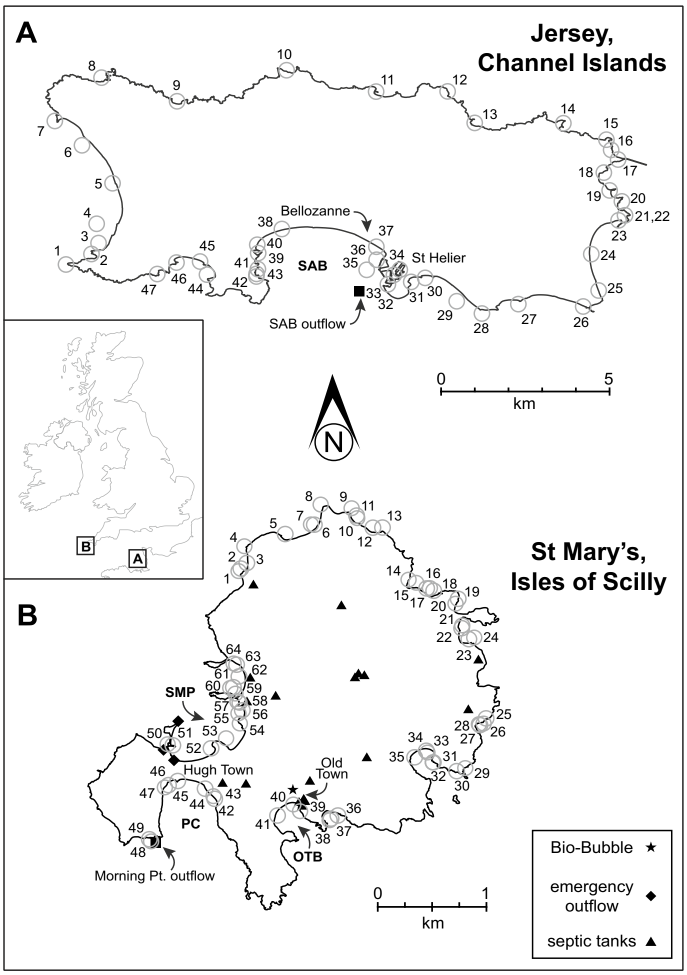

Figure 1. Map of the islands investigated in this study: (**A**) Jersey, Channel Islands; (**B**) St Mary's, Isles of Scilly. Sample locations are indicated by numbered grey circles. Key locations discussed in text are also indicated. *SAB* St Aubin's Bay, *SMP* St Mary's Pool, *PC* Porth Cressa, *OTB* Old Town Bay. Note different scales for each island.

(Fig. 1A) for all sewage treatment on the island. Prior to its commission in 1959, untreated sewage was discharged directly into the sea. The STW was built for a population of 57,000 and so continuous improvements have been required as the population has grown and environmental standards have changed[23]. Despite continued improvements, surveys have shown that St Aubin's Bay continues to exhibit high levels of nutrient loading from effluent[24,25]. Trophic status reports carried out by the Centre for Research into Environmental Health (CREH) in 1997 suggest winter hyper-nutrification in St Aubin's Bay; despite the predicted 2% to 21% reduction in chlorophyll concentration from nutrient removal by the Bellozanne STW[25]. A 2010 reassessment shows considerable reduction in nitrogen load compared to 1997 values, and modelling indicates no potential eutrophication in the bay[26]. However, the STW has continued to fail the Total Nitrogen limit set at 10 mg/l, instead recording between 11 and 63 mg/l with an average of 31.3 mg/l between 2009 and 2015[27,28]. No further upgrades are possible for the Bellozanne STW and it cannot support the Jersey population as it currently stands. Sewage effluent entering the bay is causing increased nitrogen loading that is resulting in substantial *Ulva* growth and eutrophication in St Aubin's Bay[27,29]; the Jersey Government report that *Ulva* growth in St Aubin's Bay will always persist[29]. The construction of a new STW at Bellozanne began in 2018 and will be operational in 2023 and aims to reduce discharge of partially treated sewage by 97%[30]. The new STW will support a population size of 118,000 and aims to also reduce nitrogen loading to St Aubin's Bay by an additional 10–15% compared to the current STW outflow.

The Isles of Scilly (IOS) are a group of islands situated 27 miles southwest off the coast of the UK (Fig. 1B). The islands sit at the end of the North Atlantic Current and the Gulf Stream producing a milder climate compared to mainland UK, with winter surface temperatures of ~ 10 °C[31]. Two tidal jets transport water around the isles, the primary operates in a clockwise direction transporting water south, the secondary current transports water northwards[32]. St Mary's has an area of 6 square miles and is the largest island in the IOS. The permanent population is ~ 1800, although this increases to > 6000 in summer months[33]. Most of the island's population lives in the southwest in Hugh Town (Fig. 1B). The northwest supports small-scale agriculture (6–7 hectares) with no intensive agriculture, and as such, the coastal waters are generally classified as pristine[34]. There is no wastewater treatment facility on St Mary's—instead the island relies on several smaller wastewater infrastructures. There is a 3 km sewerage network in Hugh Town supporting ~ 800 properties and connects to the Morning Point outflow (Fig. 1B). Morning Point is the only permitted outflow on the IOS where untreated raw sewage is discharged into the Atlantic[35]. Two emergency outflows intermittently discharge sewage into the Hugh Town harbour in the case of flood events (Fig. 1B)[35]. Old Town is serviced by a combination of a Bio-Bubble facility (i.e., an advanced aeration system, https://www.bio-bubble.com) connected a soak-away draining to Old Town Bay, the Morning Point network, and three septic tanks[33,35]. Septic tanks serve as the main sewage treatment process for properties outside of Hugh Town and Old Town. The Environment Agency does not provide a full list of all septic tanks on the island; thus, it must be noted that more septic tanks than those presented in Fig. 1B are likely to be in operation[36].

The private company South West Water (SWW) has taken over responsibility for sewage treatment on the IOS from the local council. SWW aim to reduce sewage pollution by 2030 and and report that St Mary's sewerage network is in need of major repair[35,37]. Poor record keeping by the IOS council has resulted in a lack of information regarding sewerage leaks and septic tank conditions across the island[35]. By 2030, SWW aims to build a resilient treatment facility and upgrade the current Bio-Bubble[37]. Initial plans by SWW[35] considered closing the Morning Point outflow, with discharge redirected to the emergency outflow into St Mary's Pool (Fig. 1B). The emergency outflow pipeline would be upgraded, and households encouraged to connect to the sewerage network and thus, reducing the reliance on septic tanks[37].

Materials and methods

Macroalgae from Jersey was collected during December 2020 and January 2021 by one of us (LPW) during the Covid pandemic. 372 samples were collected from 47 sites around Jersey (Fig. 1A) consisting of 336 *Fucus* and 36 *Ulva* samples. Macroalgae from the IOS was collected by one of us (NB) during the same time period. 459 macroalgae samples were collected from 62 sites (Fig. 1B) consisting of 429 *Fucus* and 30 *Ulva* samples. Sample sites from both locations were selected primarily on the presence of macroalgae and ease of accessibility for collecting. The outermost non-fertile tip of *Fucus* was sampled from several different specimens, whereas a 4 cm-square area of *Ulva* was sampled and compressed to remove all seawater. Each sample was placed in a small brown envelope, labelled and subsequently dried in their envelopes soon after collection in a domestic oven set between 45 and 60 °C. Sub-samples of compressed *Ulva* and the margin of the non-fertile tip of *Fucus* were weighed into tin capsules for subsequent stable isotope analysis following the protocols outlined in Gröcke et al.[6] and Bailes and Gröcke[11]. QGIS software was used to produce the isoplot maps of δ^{15}N around Jersey and St Mary's.

Results

The average δ^{15}N value from Jersey for *Fucus* is + 7.0‰ ± 1.9‰ (n = 336) and + 7.1‰ ± 1.3‰ (n = 36) for *Ulva*, which are above natural background levels reported between + 4‰ and + 6‰[8,12]. δ^{15}N values of *Fucus* range between + 2.9‰ (Sites 1 and 13) and + 12.1‰ (Site 36) showing elevated δ^{15}N in St Aubin's Bay for both *Fucus* and *Ulva* (Fig. 2A). Site 40 recorded the greatest elevation in δ^{15}N for *Fucus* (+ 11.0‰ ± 0.6‰, n = 7) and the five most elevated δ^{15}N (> + 9.0‰) sites (33, 34, 36, 38 and 40) are found in St Aubin's Bay. *Ulva* recorded a δ^{15}N range between + 4.3‰ and + 9.8‰ (Fig. 2A). Lower average δ^{15}N values were recorded in *Fucus* for the north coastline (+ 5.1‰ ± 1.3‰, n = 57), the west coastline (+ 5.7‰ ± 1.4‰, n = 78) and for the east coastline (+ 6.5‰ ± 1.2‰, n = 69) in comparison to the southern coastline (+ 8.3‰ ± 1.8‰, n = 160). Although there are less data for *Ulva* it recorded higher δ^{15}N average values for each coastline compared to *Fucus* for the north, east and west coastlines (+ 5.6‰ ± 1.1‰, n = 7; + 6.7‰ ± 0.5‰, n = 10; and + 7.1‰ ± 0.6‰, n = 6, respectively). However, the south coast showed a depleted δ^{15}N average (+ 7.8‰ ± 1.4‰, n = 14) compared to *Fucus*. Macroalgae in St Aubin's

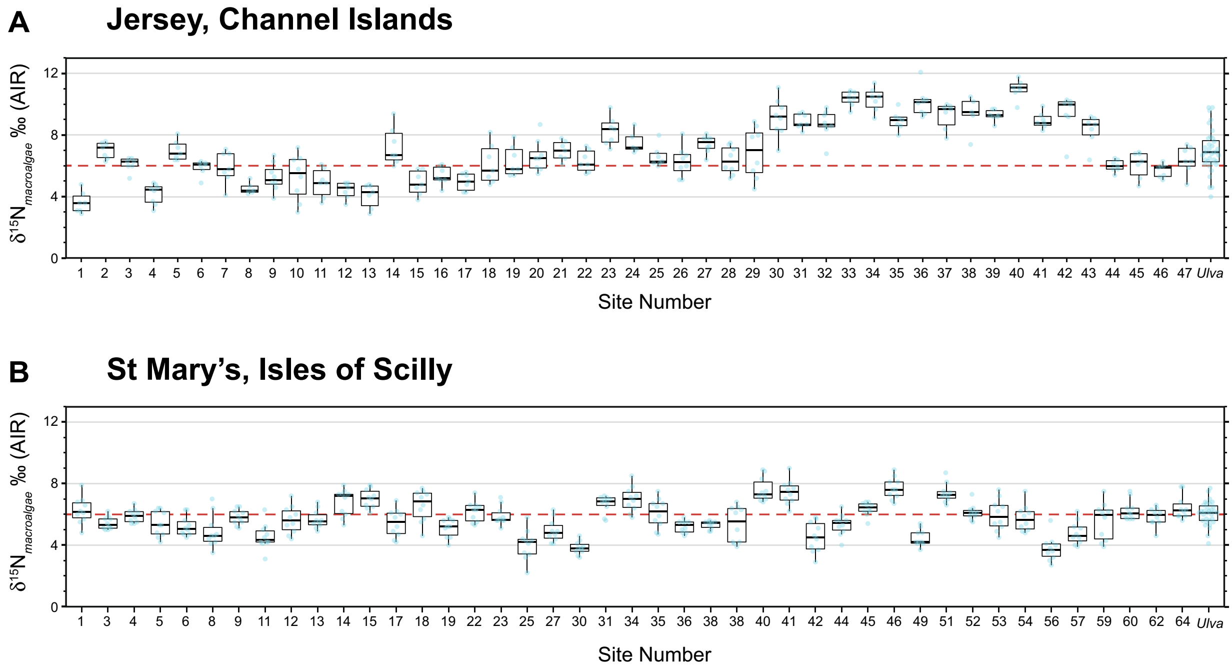

Figure 2. Box and whiskers plots of macroalgae δ^{15}N from Jersey (**A**) and St Mary's (**B**). All numbered sites represent *Fucus* results. All *Ulva* results for the islands have been grouped as one dataset and are presented on the farthest right of each graph. δ^{15}N values above the dashed red line represent coastal environments that are affected by sewage/effluent. Macroalgae δ^{15}N elevation is recorded for St Aubin's Bay (Sites 30–43) linked to the Bellozanne STW outflow.

Bay produced a significantly more elevated δ^{15}N signature in comparison to the whole island average; this is observed in both *Fucus* (+ 9.5‰ ± 1.1‰, *n* = 85) and *Ulva* sp. (+ 8.3‰ ± 1.2‰, *n* = 8) (with a *p* value < 0.05; see Supplementary Fig. 1).

From the IOS, *Fucus* recorded an average δ^{15}N value of + 5.8‰ ± 1.2‰ (*n* = 429), and *Ulva* recorded an average δ^{15}N value of + 6.2‰ ± 0.7‰ (*n* = 30). IOS seagrass has an average δ^{15}N value of ~ + 5‰ which is on the low side of our dataset[38]. *Fucus* ranged between + 2.2‰ (Site 25) and + 9.0‰ (Site 41), whereas *Ulva* varied between + 4.1‰ and + 7.7‰ (Fig. 2B). δ^{15}N values are slightly skewed towards more elevated δ^{15}N values along the southern (+ 5.9‰ ± 1.4‰, *n* = 99), western (+ 5.8‰ ± 1.2‰, *n* = 99) and northern coastlines (+ 5.8‰ ± 1.0‰, *n* = 140) compared to the eastern coastline (+ 5.5‰ ± 1.3‰, *n* = 90). Site 46 recorded the most elevated average δ^{15}N value from the IOS (+ 7.7‰ ± 0.7‰, *n* = 10), which is located at Porth Cressa. The three other most elevated δ^{15}N (> 7.0‰) sites are also in the southwest of the island (Sites 40, 41, 51) (Fig. 2B). Average δ^{15}N values decrease in distance away from Morning Point (see Supplementary Fig. 2). No correlation exists between site average δ^{15}N values and proximity to septic tanks (see Supplementary Figs. 3 and 4). Sites 30 and 56 recorded the lowest δ^{15}N average value for *Fucus* (+ 3.8‰ ± 0.4‰, *n* = 10 and + 3.8‰ ± 0.8‰, *n* = 10, respectively). *Ulva* was found to have the lowest δ^{15}N values along the east coastline (+ 5.6‰ ± 0.4‰), which is in line with results from *Fucus*.

Discussion

Jersey: focused point-source effluent pollution. The δ^{15}N values from macroalgae around the Jersey coast demonstrate a clear geospatial pattern with more elevated δ^{15}N values in St Aubin's Bay compared to the remainder of the island (Fig. 3A). Multiple elevated δ^{15}N values > + 6.0‰ indicate nitrogen loading from an anthropogenic effluent source[1,12,39]. The position of the Bellozanne STW corresponds with these elevated δ^{15}N sites (Fig. 3A for *Fucus* and Fig. 3B for *Ulva*). Such elevated δ^{15}N values are in line with previous studies of macroalgae near STW[1,39]. δ^{15}N of groundwater sampled from Jersey in 1995 also show elevated values (+ 11.8‰ to + 18.4‰ in the St Aubin's Bay coastal margin[40]. These elevated δ^{15}N values correspond to the lowest nitrate concentrations in the groundwater and are interpreted as a result of denitrification processes in the deep groundwater system. Quantitative information on the mixing zone between groundwater and seawater is not available for St Aubin's Bay and thus, we are uncertain of the influence of deep groundwater δ^{15}N on macroalgae δ^{15}N. Although data on Total Dissolved Solids for Jersey indicate that saline intrusion occurs around St Aubin's Bay and other low-lying regions, and thus would imply little influence from deep groundwater[41].

The lack of elevated δ^{15}N values elsewhere around Jersey strongly suggests that the release of sewage effluent nitrogen in St Aubin's Bay is not transported around the island through oceanic currents. St Aubin's Bay has a large tidal range (on the order of 10 m) and is sheltered by the westerlies and Atlantic swell, thus causing reduced mixing with the open ocean. Limited water exchange with the open ocean reduces the capacity for effluent diffusion as well as reducing δ^{15}N via ammonia volatisation[3,42,43]. Macroalgae δ^{15}N data from Jersey shows that the geographic position of STWs and the placement of outflow pipes require a thorough environmental assessment, considering—at least—prevailing wind and oceanic and tidal currents prior to any planning and development.

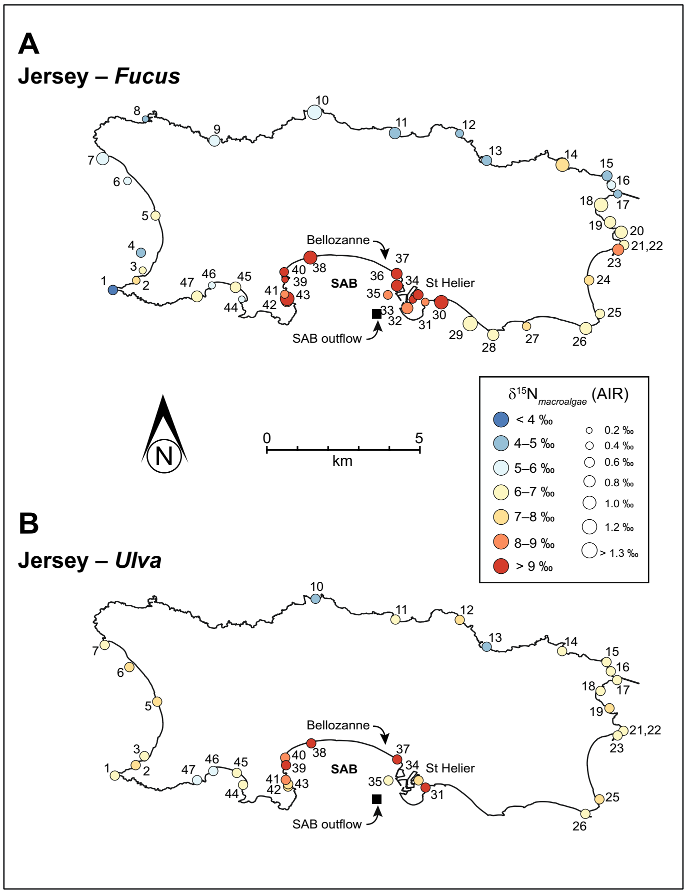

Figure 3. $\delta^{15}N$ isoplot map for the macroalgae, *Fucus* (**A**) and *Ulva* (**B**) around Jersey, Channel Islands. Colour range represent distinct $\delta^{15}N$ value ranges, while the size of the circle represents the standard deviation of $\delta^{15}N$ from each site. Elevated macroalgae $\delta^{15}N$ values are recorded for St Aubin's Bay (Sites 30–43) and is associated to the Bellozanne STW outflow. Normal background macroalgae $\delta^{15}N$ values are located on the west and north coastlines (blue circles). For abbreviations refer to Fig. 1.

If such investigations were conducted in Jersey, St Aubin's Bay may not be the environmental disaster it has become[44,45]. The north, east and west coastlines of Jersey reflect $\delta^{15}N$ values typical of natural background levels, suggesting minimal anthropogenic nitrogen sources (effluent, fertilizer, industrial sources) (Fig. 3A) and/or that oceanic currents, tidal ranges and bay openness are dissipating any nitrogen loading into the open ocean.

St Mary's: dispersed point-source effluent pollution. In comparison to Jersey, St Mary's in the IOS exhibits more complex nitrogen loading and geospatial variation in $\delta^{15}N$ around the coastline. The main difference causing this complexity is the difference in sewerage treatment approaches between the islands: a single, principal sewage treatment plant *versus* a spatial array of septic tanks and two isolated sewerage systems in disrepair around the island. Several sites around St Mary's coastline record elevated $\delta^{15}N$ values in comparison to normal, healthy marine values: Sites 41, 46 and 51 are located in three separate bays (Fig. 4A). Nitrogen pollution point sources on St Mary's can be separated into three main infrastructures: Morning Point discharge, septic tanks/soakaways and the Hugh Town emergency outflow. Figure 4A reveals that the two most elevated $\delta^{15}N$ sites correspond to Morning Point discharge and the Hugh Town emergency outflow. Site 46 has the most elevated $\delta^{15}N$ values on the island and is the closest sampled point to the sewage outflow at Morning Point, discharging untreated and semi-treated sewage into Porth Cressa (Fig. 4A,B)[33]. A steady decline in $\delta^{15}N$ values is observed across a 160 m profile at Porth Cressa, indicating diffusion of nitrogen loading from the Morning Point source into the bay: even with limited data this pattern is also reflected in *Ulva* (see Supplementary Fig. 2). A lowering of macroalgae $\delta^{15}N$ away from a source outflow would also suggest that Porth Cressa is not well-mixed, allowing the opportunity for the effluent to be absorbed by the local environment (e.g., macroalgae, microalgae, microflora, sediment). The relationship between decreasing $\delta^{15}N$ and distance from a nitrogen source has been recorded in other macroalgae investigations[1,12,20].

A significant proportion of the population (residential and tourist) on St Mary's rely on septic tanks and soakaways as a method for sewage processing, storage and release. As a result of sewage storage in septic tanks, the septic tank environment would start with nitrification processes (e.g., ammonia oxidisation to nitrate) in an oxygenated empty tank and then shift to anaerobic denitrification once oxygen becomes depleted; at that point

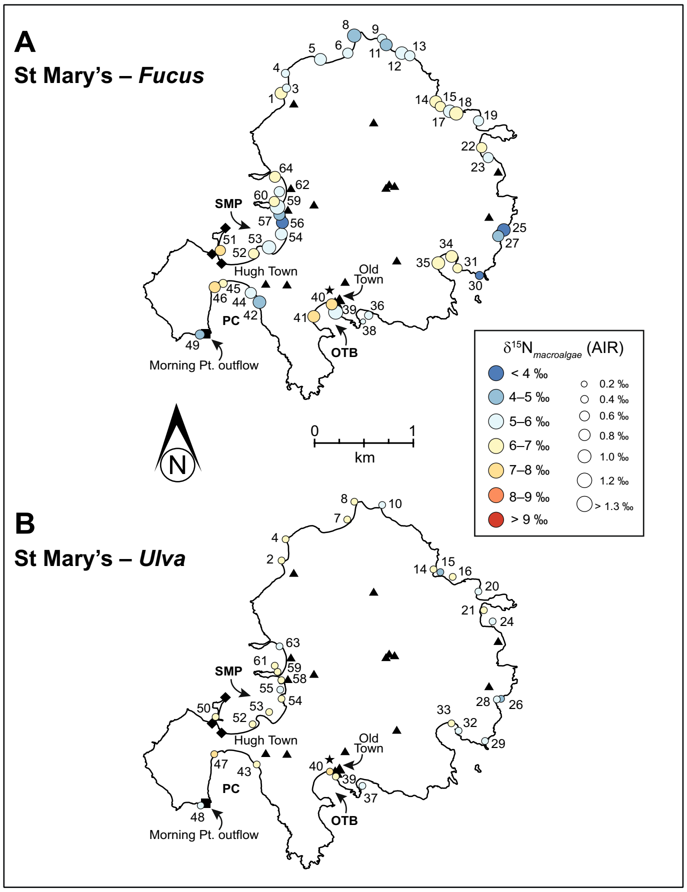

Figure 4. $\delta^{15}N$ isoplot map for the macroalgae, *Fucus* (**A**) and *Ulva* (**B**) around St Mary's, Isles of Scilly. Colour range represent distinct $\delta^{15}N$ value ranges, while the size of the circle represents the standard deviation of $\delta^{15}N$ from each site. In comparison to Jersey, macroalgae $\delta^{15}N$ values are not as elevated (e.g., with dark orange or red circles) on St Mary's: most likely related to the lower population size, release of untreated sewage/effluent and/or the islands reliance on a septic tank/soakaway system. Elevated macroalgae $\delta^{15}N$ values are recorded for Morning Point, Hugh Town and Old Town regions. For abbreviations refer to Fig. 1.

denitrification would further elevate the $\delta^{15}N$ signature of the sludge and effluent[46,47]. Reduced regulation and monitoring of septic tanks will lead to their disrepair, allowing them to leak nutrients (nitrogen and phosphorus) into the groundwater system and eventually to the coastal environment.

Old Town has a soakaway delivering effluent from the Bio-Bubble to Old Town Bay. Although this effluent may have slightly elevated $\delta^{15}N$ (e.g., animal/human signature[13]), the aeration process (i.e., nitrification processes) would not elevate it further. Site 41 at Old Town Bay has the highest individual $\delta^{15}N$ value (+9.0‰) for the entire island. Therefore, elevated $\delta^{15}N$ at Old Town Bay may be attributed instead to the three septic tanks located nearby (see Fig. 4). Sites around St Mary's that are located near septic tanks exhibit large standard deviations (>0.7‰), but there is no trend with distance from the source (Sites 39–41, 56–64) (see Supplementary Figs. 3 and 4). Although legislation on St Mary's does not allow for the discharge of effluent from septic tanks the maintenance of these tanks is not regulated: septic tanks are meant to be emptied and the contents transported to the mainland of England for processing[33,48]—although it is known by one of us (NB) that emptied tanks are not being transported back to the mainland. Farmers may be managing their own septic tank systems, such as those on Bryher, IOS[21]. In addition, many septic tanks on the island have soak-aways that may have some degree of contact with oxygen causing nitrification processes to occur (e.g., lowering $\delta^{15}N$ values)[12]. Elevated $\delta^{15}N$ values recorded at the above-named sites may be caused from poorly maintained septic tank systems or from groundwater denitrification processes at depth producing less distinct source point pollution areas with elevated $\delta^{15}N$ signatures[40,47,48].

Another issue that complicates the interpretation of macroalgae $\delta^{15}N$ around St Mary's is the geographic position of the island and surrounding islands, the structure of the coastline and ocean circulation/currents. Viana and Bode[43] showed that open coastal waters along the coast of Spain recorded lower $\delta^{15}N$ values in macroalgae compared to enclosed bays. For example, elevated $\delta^{15}N$ values at Sites 31–35 for *Fucus* and *Ulva* are potentially caused by the shape of the bay restricting water movement, causing ^{15}N enrichment. There are no obvious sewage/effluent sources to the eastern coastline. A direct comparison between our macroalgae $\delta^{15}N$ data with IOS seagrass $\delta^{15}N$ data[38] is not possible since latitudinal information was not provided for the seagrass data. However, the seagrass $\delta^{15}N$ data is typically < + 5‰, suggesting that the nitrogen isotope signature of effluent is not reaching the seagrass community further out from the coastline. Sewage nitrogen trapped in bays has a longer time to become isotopically fractionated, and therefore could lead to an elevated $\delta^{15}N$ signature that is subsequently incorporated into macroalgae[3]. The enclosed nature of Porth Cressa, may also be preserving and/or enhancing the elevated $\delta^{15}N$ values in macroalgae at this site. Another complicating factor is the clockwise movement of oceanic water around the IOS, which has the potential for transporting effluent-contaminated waters away from the north-west coastline producing less elevated macroalgae $\delta^{15}N$ values on the isoplot map[32].

To treat or not to treat: effluent pollution in island environments. As shown in this study, a single, operational wastewater treatment plant for an entire island has the potential to concentrate effluent release to a single point/area, resulting in elevated coastal macroalgae $\delta^{15}N$ values. The principal issue in Jersey is that the outflow of the Bellazonne STW is directed solely into St Aubin's Bay. This causes elevated nitrogen loading in the bay which has a direct impact on the health of the ecosystem. The nature of the oceanic conditions in the region and the fact that coastal bays are natural traps means that nitrogen loading is intensified, causing significant environmental issues—for example, green tides, high levels of nitrogenous compounds and obvious water discolouration[44,45,49,50]. In direct contrast, St Mary's has no centralised wastewater treatment plant and therefore effluent is either/or; (1) directly released into the ocean with no and/or limited treatment, and unregulated emptying of septic tanks; and/or (2) an antiquated, sewerage system in need of significant investment to upgrade it. The lack of adequate wastewater treatment on St Mary's may be the cause behind lower macroalgae $\delta^{15}N$ values compared to Jersey. Although the $\delta^{15}N$ values may be lower, indicating less denitrification, this does not equate to less nitrogen loading in the coastal environment around St Mary's. In fact, groundwater nitrate concentrations from 1995 on St Mary's averaged 58 mg/l[51], which exceeds the World Health Organisation guideline value: no current nitrate or Total Nitrogen is publicly available for St Mary's. Irrespectively, nitrogen chemical analyses (i.e., nitrogen pollution) would need to be monitored around the entire island of St Mary's, compared to Jersey which could focus on the one region (i.e., St Aubin's Bay), to monitor effluent contamination. Therefore, despite the significantly smaller population size on St Mary's nitrogen loading may equally be having an impact on the coastal environment and ecosystems (e.g., on eelgrass, *Zostera marina*, populations and genetic diversity around the IOS[52]).

In the future, wastewater treatment plants require more thorough development and planning on islands, specifically in terms of a more comprehensive environmental assessment that considers oceanic circulation, modelling the effect of swell, geography and bathymetry of the coastline, tidal ranges, and any other ocean–atmosphere environmental conditions; this must all occur prior to placement of an outflow pipe. St Aubin's Bay clearly demonstrates a lack of foresight when positioning the outflow, since it is now causing environmental issues in that bay all-year round[27–29,44,45]. The Morning Point outflow on St Mary's is also positioned incorrectly, as evidenced by the elevated $\delta^{15}N$ values at Porth Cressa: this beach also encounters problems associated with abundant macroalgae and *Ulva* sp. reflecting nitrogen-rich waters. A more confusing geospatial pattern in macroalgae $\delta^{15}N$ values around St Mary's is thought to be caused by the island's reliance on managed and unregulated septic tanks, and an inadequate sewerage system.

Conclusions

Nitrogen isotopes of macroalgae around island coastlines can offer valuable insight into sewerage practices and effluent pollution affecting the region. This study shows that Jersey, which relies on an inadequate STW facility is unable to cope with current and projected population demands. The effluent from this STW facility drains into St Aubin's Bay which has led to nitrogen overloading and its current environmental issue: blooms of opportunistic macroalgae smothering the beaches of that bay (e.g., *Ulva* sp.). Other regions around Jersey do not show significant elevation in macroalgae $\delta^{15}N$ indicating a lack of effluent pollution and therefore, nitrogen loading. This may be the result of oceanic currents, geography and bathymetry of the coastline and/or simply a lack of effluent release to those coastline regions. On the other hand, St Mary's, IOS, records a complex pattern resulting from an array of unmonitored sewage/effluent sources from all over the island and not from a single source, into a single region (e.g., St Aubin's Bay, Jersey). Future planning and development of any wastewater treatment plant on St Mary's requires a thorough investigation of the marine system, especially oceanic currents, tidal effects, marine chemistry, and ecological assessments as standard practice. Case studies on the $\delta^{15}N$ value of macroalgae from other islands should be conducted to assess the influence and impact of effluent discharge. The use of macroalgae $\delta^{15}N$ can help identify point source effluent discharges and thus, impact policy changes to mitigate current and future environmental problems/disasters along our coastlines. This study shows that islands are not immune to the environmental issues caused by effluent pollution. This idea was aptly conveyed by John W. Gardner[53], p. 108 when he wrote, "*we cannot have islands of excellence in a sea of slovenly indifference to standards*".

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Author contributions
D.R.G. developed the concept and designed the study. L.P.W. and N.B. collected the samples and provided background information and notes on the island environments. F.A. & C.Y.L. conducted the nitrogen isotope analyses under the direction and supervision of D.R.G. The QGIS maps were generated by F.A. The manuscript was written by F.A. and D.R.G., and all authors have provided contributions to the final manuscript.

Competing interests
The authors declare no competing interests.

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