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Importance of Physico-chemical Cycling of Nutrients and Carbon in Marine Transitional Zones (Nuts & Bolts)

Authors: Peter Croot, Rachel Cave, Sheena Fennell, Maija Heller, Tiernan Henry,Nadeeka Rathnayake, Fatimatuj Zohara Sonny and Dagmar Stengel

Lead organisation: University of Galway

Rialtas na hÉireann
Government of Ireland

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Environmental Protection Agency

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2. Office of Environmental Enforcement
3. Office of Evidence and Assessment
4. Office of Radiation Protection and Environmental Monitoring
5. Office of Communications and Corporate Services

The EPA is assisted by advisory committees who meet regularly to discuss issues of concern and provide advice to the Board.

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What did this research aim to address?

The Nuts & Bolts project examined the physical and chemical controls on the biogeochemistry and bio-optics at four sites representing different exemplars of Irish marine transitional zones (MTZs): (i) the Shannon estuary; (ii) Kinvara Bay – a site influenced by submarine groundwater discharge; (iii) Lough Furnace – a meromictic lagoon; and (iv) the outflow plume of the River Corrib in Galway Bay.

For the purpose of determining good environmental status (GES) in the context of the Marine Strategy and Water Framework Directives, Nuts & Bolts was designed to address knowledge gaps regarding the action of multiple environmental stressors on Irish MTZs. The approach included (i) application of flow cytometry to study pico- and nanoplankton distribution, abundance, growth and mortality rates; (ii) inclusion of coloured dissolved organic matter and fluorescent dissolved organic matter as tracers of carbon cycling; (iii) measurement of in situ bio-optical properties along salinity gradients to assess light quality and impact on primary production; and (iv) application of membrane inlet mass spectrometry to determine climate-relevant dissolved gases (O2, CO2, Ar and dimethyl sulfide). Data from Nuts & Bolts provide essential baseline information for evidence-based decision-making on key environmental aspects of Irish MTZs related to management and governance.

What did this research find?

Nuts & Bolts generated new datasets on the abundance and distribution of bacteria and pico- and nanoplankton, along with information on their growth rates, grazing rates and how they may respond to changes in light, nutrients and temperature. This information fills a critical gap from a management and governance perspective in Ireland, and allows for picoplankton in MTZ ecosystems to be given more attention in planning for GES and the Marine Strategy Framework Directive, including their impact on the descriptors for biodiversity, non-indigenous species and food webs and on eutrophication and harmful algal blooms.

New biogeochemical data on the environmental stressors impacting Irish MTZs, including nutrient and metal fluxes, in situ light field and potential greenhouse gas fluxes, were also obtained. Viewing the MTZ as a whole ecosystem, as is done now in river management by examining the whole catchment, allows for a more complete picture of the bio-optical and biogeochemical processes occurring in MTZs. This work thus helps to inform management decisions in each of the MTZs and creates a framework for future studies in other important Irish MTZs.

How can the research findings be used?

The new data allow us to improve the ability to predict the impact of climate change and other anthropogenic stressors (e.g. eutrophication, coastal darkening) on GES in MTZs by including biogeochemistry and bio-optics in the modelling of Irish MTZs. This should be done as part of the National Marine Monitoring Programme, co-ordinated by the EPA. An approach to this would be to start with the LOICZ box model methodology for each of the Irish MTZs. For MTZs with existing hydrodynamic models (e.g. Galway Bay, Shannon estuary), implementation of the PISCES biogeochemical model would greatly improve the ability to forecast future ecosystem changes. Critical to this is improving assessment of the nitrogen balance in MTZs by examining all relevant nitrogen species and the fluxes between them, including benthic source and sinks. It is also strongly recommended that existing harmful algal bloom surveys include a flow cytometric analysis of bacteria and pico- and nanoplankton abundances. The findings of Nuts & Bolts should be followed up and broadened to include other MTZs, most notably those in the south and east of Ireland, which are more heavily impacted by agricultural run-off.

EPA RESEARCH PROGRAMME 2021–2030

Importance of Physico-chemical Cycling of Nutrients and Carbon in Marine Transitional Zones (Nuts & Bolts)

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EPA Research Report

Prepared for the Environmental Protection Agency

by

University of Galway

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This report is based on research carried out from 1 February 2020 until 29 February 2024. More recent data may have become available since the research was completed.

The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Executive Summary

Marine transitional zones (MTZs) act as important material filters in the transfer of elements, and chemical species, from the land to the sea. Through physico-chemical and biological processes, nutrients and trace metals are removed (e.g. primary productivity or coagulation) or chemically altered (e.g. photochemistry, denitrification) in MTZs, resulting in a reduced flux of materials to the coastal zone. MTZs are also regions that contain high levels of biodiversity and provide key ecosystem services. Recent, and predicted, changes in freshwater inputs and nutrient loads due to anthropogenic activities (e.g. hydrological cycle, eutrophication), along with changes in other environmental stressors (e.g. temperature, light, oxygen), can impact MTZs in complex ways because of non-linear changes in the responses of the ecosystem to the combination of the individual environmental stressors encountered.

The goal of this project was to improve our understanding of the impact of multiple environmental stressors in Irish MTZs by carrying out biogeochemical and bio-optical investigations at four sites, each providing a different MTZ exemplar: (i) the Shannon estuary, (ii) Kinvara Bay – a site influenced by submarine groundwater discharge, (iii) Lough Furnace – a meromictic lagoon and (iv) the outflow plume of the River Corrib in Galway Bay.

The approach taken in the Nuts & Bolts project was designed to address specific gaps in our knowledge of biogeochemical cycling in Irish MTZs, which included several novel aspects for studies of Irish MTZs: (i) application of flow cytometry to study pico- and nanoplankton distribution, abundance and growth and mortality rates; (ii) inclusion of coloured dissolved organic matter and fluorescent dissolved organic matter as tracers of carbon cycling in the MTZs; (iii) measurement of *in situ* bio-optical properties along salinity gradients in the MTZs to assess light quality and impact on primary production; and (iv) application of membrane inlet mass spectrometry to determine dissolved gases (O_2, CO_2, Ar and dimethyl sulfide) in the MTZs that are climate relevant. The information collected in this study provides essential information for the environmental aspects of decision-making related to management and governance of Irish MTZs.

1 Introduction

1.1 Importance of Marine Transitional Zones

Marine transitional zones (MTZs) are both defined and characterised as the interface between land and sea. Historically, this was viewed as a unidirectional system, with material flowing from the land via rivers to the sea, whereupon the vast size of the ocean rendered it invisible. We now know that this is only part of a great cycle in the ocean and that pollutants from the land can accumulate in the ocean, altering ecosystems and climate. MTZs are also critically important zones for nutrient and elemental cycling, primary productivity, fisheries recruitment and human well-being. However, MTZs are also now increasingly vulnerable to multiple stressors (Gruber, 2011) that can act in different ways on ecosystems, requiring careful assessment of the action of each stressor on the key biogeochemical processes occurring in MTZs.

MTZs trap significant quantities of materials and act as filters between the land and the oceans (Lisitsyn, 1995; Schubel and Kennedy, 1984). In this way, estuaries, via physical and biological processes, can substantially reduce the export of nutrients (Asmala *et al.*, 2017), trace metals (Sholkovitz, 1976) and carbon (Najjar *et al.*, 2018) to coastal waters. Estuaries in particular are effective traps for riverine sediment due to physical and chemical processes occurring in the mixing zone between freshwater and seawater, resulting in turbidity maxima (Burchard *et al.*, 2018) in the upper reaches of MTZs.

To assess the impact of future changes in climate on the cycling of nutrients and carbon in MTZs, it is necessary to understand how the current ecosystem functions. It is also important to recognise that catchment areas in Europe, and globally, are likely to have been substantially altered from a pre-industrial baseline due to land use changes and run-off from agricultural and urban environments, and so the definition of good environmental status (GES) requires an assessment of what is a sustainable system. Evaluation of GES for MTZs then requires information on not only abundances but fluxes as well, so that sources and sinks can be determined. Recently, MTZs have been more widely recognised as providing critical ecosystem services and contributions to human well-being and as such are considered a key aspect of the United Nations Sustainable Development Goals (Singh *et al.*, 2018).

1.2 Multiple Environmental Stressors in Marine Transitional Zones

Human activities on land are resulting in changes to the type and severity of environmental stressors on MTZ ecosystems (Glibert *et al.*, 2022). The impacts of multiple stressors on MTZ environments cannot be assessed singularly as they act non-linearly due to complex interactions, and a new approach to assessing the key drivers is required. In this project we utilised the Multiple Environmental Driver Design Lab for Experiments (MEDDLE; Boyd *et al.*, 2019) as a toolbox for assessing experimental design to examine multiple environmental stressors in Irish MTZs.

1.2.1 Temperature – seasonal cycles and global warming

Global warming is rapidly becoming a major stressor on MTZ ecosystems as land and seas warm, resulting in many phytoplankton and zooplankton species moving poleward, altering the basis of food chains (Stocker *et al.*, 2013). Warmer waters are thought to favour smaller cells, as small cells may more efficiently harvest light and nutrients and maintain their position in the euphotic zone (Finkel *et al.*, 2010). Globally, ocean temperatures are increasing, as is their variability, leading to an increase in the frequency and duration of marine heat waves (Hobday *et al.*, 2016), defined as a discrete, prolonged (over 5 days in duration) warm water (>90th percentile) event. The recent marine heat wave in North-east Atlantic waters, from July to September 2023, is suggested to have resulted in a 50–60% decrease in phytoplankton concentrations compared with the climatological mean (1998–2018) (von Schuckmann *et al.*, 2023). In western Irish waters, sea surface temperatures were up to 4°C above the average (McCarthy *et al.*, 2023); however, this was confined to a shallow layer near the sea surface, most likely due to the very settled conditions experienced at the time. Overall temperatures in Irish waters have been cooling (Nolan *et al*., 2023), possibly due to changes in the Atlantic Meridional Overturning Circulation (Caesar *et al*., 2018). A recent cooling trend (2005–2021) has also been observed in the ocean heat content of the Iberian–Biscay–Ireland regional seas, after an apparent warming over the depth ranges 0–700 m and 0–2000 m during the period 1993–2005 (de Pascual-Collar *et al*., 2023).

Higher surface temperatures result in increased water column stability and in turn a shallower mixed layer depth, altering species distributions (Reynolds, 2006). Increased stratification can favour flagellates that are able to swim to obtain nutrients from deeper waters or are able to obtain their nutrients through the ingestion of particulates (e.g. mixotrophs (Stoecker *et al*., 2017)). With enhanced stratification, there is a reduced flux of nutrients to the surface, thus potentially reducing further phytoplankton growth. This reduced flux, combined with warmer temperatures, should favour the success of phytoplankton that are more dependent on regenerated sources of N and less dependent on NO_3, including cyanobacteria and dinoflagellates (Glibert *et al*., 2016); thus, new production should decline in the absence of external nitrate sources (eutrophication).

1.2.2 Sea level rise – salinity and hydrological changes

Sea level rise is also predicted to have significant impacts on shallow MTZs via coastal erosion processes, increasing tidal current amplitudes, energy dissipation and salinity intrusions, with subsequent changes in vertical mixing (Stocker *et al*., 2013). Salinity intrusions will have implications for freshwater resources in coastal zones and where **submarine groundwater discharge** (SGD) is occurring (Glibert *et al*., 2022). Other recent global studies indicate that the extent of SGD is currently underestimated in terms of water supply (Gleeson *et al*., 2016) and impact on coastal biogeochemistry (Moore, 2006). Increases in both the frequency and volume of flooding due to increased rainfall can lead to increased run-off from agricultural and urban soils, resulting in increased fluxes of dissolved and particulate loads of nutrients and other elements to MTZs.

1.2.3 Light – photochemistry and coastal darkening

Changes in coloured dissolved organic matter (CDOM) levels and sediment loadings alter the light field in the water column, shifting the hyperspectral light field at depth and possibly altering phytoplankton pigment composition or community as the organisms adapt, impacting the whole ecosystem (Garnier *et al*., 2023). A recent trend observed in many northern European rivers and coastal seas is brownification or **coastal darkening**, as CDOM and iron levels have increased in riverine run-off with time, most likely related to climate change and increased precipitation (de Wit *et al*., 2016). Coastal darkening has been shown to impact both pelagic and benthic communities along the Norwegian Skagerrak coast (Frigstad *et al*., 2023). Alterations to the hyperspectral light field can lead to changes in the phytoplankton community, as different species may be advantaged or disadvantaged according to their pigment composition (Stomp *et al*., 2004, 2007). A critical knowledge gap related to Irish MTZs is that there have been no major studies on the bio-optics of MTZs in Ireland and how sediment load and type impacts the underwater hyperspectral light field, primary productivity and phytoplankton community speciation currently, how it impacted them in the past, and what might happen in the future. Thus, information on the spectral light quality and the light that the phytoplankton community is absorbing is critical to developing productivity-based ecosystem models in MTZs.

1.2.4 Carbon system – pH and ocean acidification

Increasing concentrations of atmospheric CO_2 lead to higher pCO_2 and lower pH in MTZs (Glibert *et al*., 2022), which may impact primary productivity and marine ecosystem services by favouring some phytoplankton species over others, resulting in trophic shifts as the ecosystem adapts (Flynn *et al*., 2015). Calcite- and aragonite-forming species in MTZs with low riverine alkalinity will be impacted more quickly due to the lower buffering capacity of these waters, with implications for the siting of aquaculture facilities. For Irish MTZs, alkalinity data for Irish rivers (McGrath *et al*., 2016) provides a starting point for assessing the impact of this stressor.

1.2.5 Nutrients – eutrophication and land use change

Over the last three decades, eutrophication in MTZs and the coastal zone due to increases in the fluxes of nitrogen and, to a lesser extent, phosphorus from rivers draining agricultural regions utilising fertilisers has become a major issue. A key problem with eutrophication of the coastal zone was the occurrence of large surface blooms of algae, which would ultimately sink and be respired by bacterial processes in bottom waters and sediments, resulting in oxygen-deficient bottom waters and causing fish mortalities (Diaz and Rosenberg, 2008). To address this problem, major efforts have been made in limiting nitrogen fluxes to MTZs, most notably in Europe via the Nitrates Directive (1991) and the Water Framework Directive (2000). However, over the last decade there has been recognition that controlling only N fluxes is not enough (Conley *et al*., 2009), that both N and P fluxes need to be addressed, and that consideration of both the river and ocean fluxes is required (Paerl, 2009). Analysis of the limiting nutrient in marine and freshwater systems has revealed similarities regarding the overall N:P ratio of the system, but has also highlighted the need to have information on the concentration and bioavailability of dissolved organic nitrogen and dissolved organic phosphorus (Ptacnik *et al*., 2010).

Putting this directly into an Irish context, a recent review of the last 20 years of monitoring, as part of EPA work and the OSPAR Commission's Comprehensive Study on Riverine Inputs and Direct Discharges (O'Boyle *et al*., 2016), noted that considerable reductions in the riverine loading of N and P to the coast had occurred during this time in response to the policy directives, but that the overall N:P loading had almost doubled in some locations. As Irish coastal waters are typically N-limited in the summer, with some phosphate still present, the influx of high N:P river water supplies the missing N and allows algal growth to continue. It has been suggested that changes in nutrient supply from Irish rivers have caused changes in macroalgal abundance in some locations (Ní Longphuirt *et al*., 2015), partly in response to changing land practices (Ní Longphuirt *et al*., 2016). In regions where high N:P is maintained, this may favour the growth of mixotrophic species that may attain their phosphate by grazing on picoplankton or bacteria.

This highlights that most studies assessing ecological status in MTZs typically neglect the lower trophic levels (Beiras, 2016), most notably pico- and nanoplankton, which play an important role as primary producers.

The linkages between mixotrophs and picoplankton in Irish MTZs are currently poorly understood, and there are almost no published data on these species, with the exception of mixotrophic harmful algal bloom (HAB)-forming species (e.g. *Dinophysis*).

1.2.6 Oxygen – deoxygenation

Deoxygenation is increasing in the coastal and open ocean, primarily due to human-induced global warming and nutrient run-off from land, and modelling projections indicate that the ocean will continue losing oxygen as global warming continues. The impacts of deoxygenation in the ocean and in MTZs include biodiversity loss and changes in the distribution and abundance of species, including in economically important fisheries. Oxygen is a controlling factor in many biogeochemically important redox processes, resulting in changes to the biogeochemical cycling of key elements including carbon, nitrogen, sulfur and iron, with impacts on greenhouse gas fluxes and water quality. Ocean deoxygenation directly affects marine ecosystems, but also indirectly impacts ecosystem services supporting local communities, regional economies and tourism (Grégoire *et al*., 2023).

While deoxygenation is not currently seen as a major problem in Irish MTZs (O'Boyle and Nolan, 2010), there are a few notable locations, such as Lough Furnace and Lough Hyne, where, due to constraints on mixing, suboxic waters can build in the bottom waters over summer. This has in the past led to anoxic events in the surface waters, resulting in fish mortality when bottom waters turned over (Kelly *et al*., 2018).

1.3 Study Sites

As each Irish MTZ has its own unique bathymetry and catchment, with distinct properties related to geology, land use and rainfall, it is likely that each MTZ will respond differently to any combination of external stressors (e.g. warming, ocean acidification). In this project we preselected study sites on the basis of existing information on the catchment with regard to their vulnerability key stressors. Four MTZs were studied according to their overall importance or as a case study of key processes (Figure 1.1).

The selected sites were as follows:

1. Shannon estuary – Ireland's largest river, most likely supplying the biggest flux of nutrients to the coastal shelf.
2. Lough Furnace – fed by Lough Feeagh and the Burrishoole catchment. Low oxygen concentrations in the bottom waters are a feature of the perennially stratified basin.
3. Galway Bay – with a focus on two sites: (i) the outflow plume of the Corrib river, Ireland's second largest river by flow rate, and (ii) Kinvara Bay, County Clare, as an example of a well-described SGD source.

1.3.1 Impact of the Covid-19 pandemic on field sampling and laboratory work

The global Covid-19 pandemic impacted many aspects of this study (2019–2023), as the government-ordered lockdowns, which began in late March 2020 and continued through to the final easing of all restrictions in February 2022, prevented access to field sites and laboratory facilities. Field sampling was limited in 2020 and the first part of 2021 in particular.

1.3.2 Shannon estuary

The Shannon estuary (Figure 1.2) is the largest in Ireland at 500 km^2 (Nash *et al*., 2014) and extends some 100 km from Limerick, where the Shannon river enters the estuary, to the open sea. The Shannon

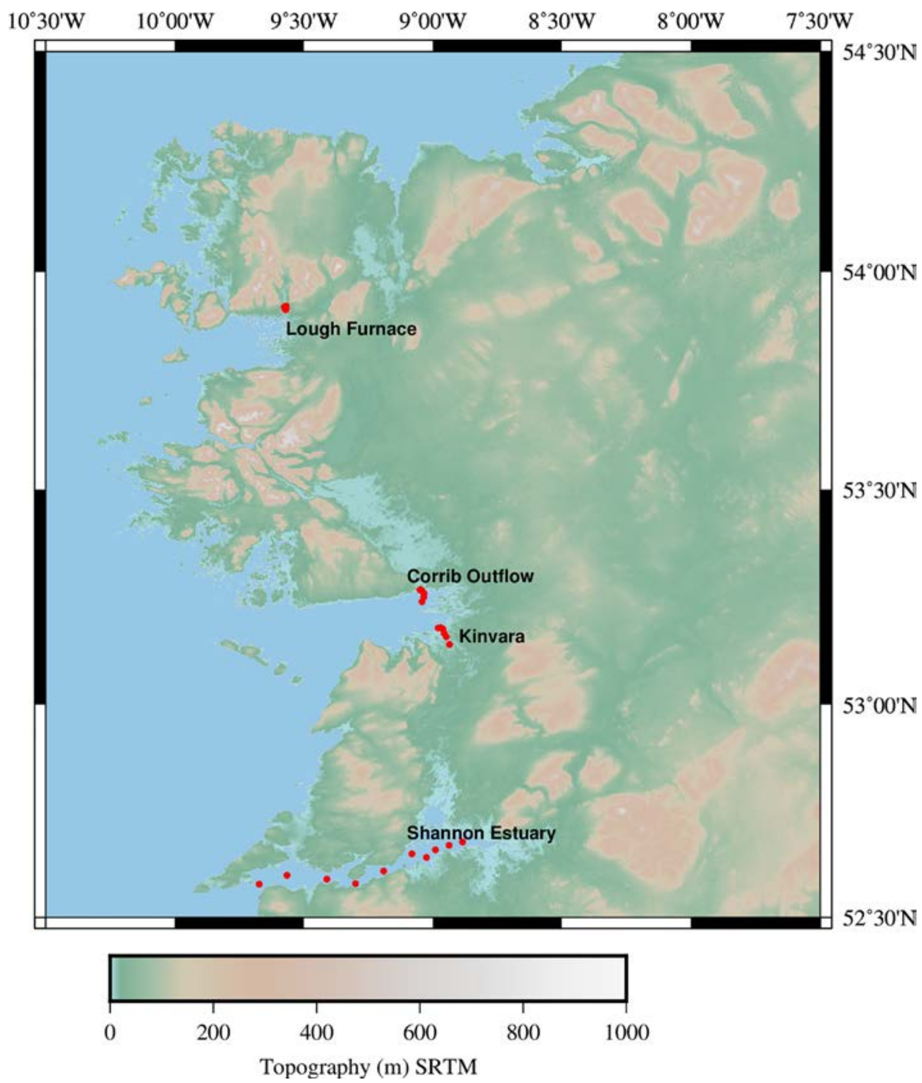

Figure 1.1. Location of sampling stations (red dots) in Irish MTZs. Topography data are from the Shuttle Radar Topography Mission.

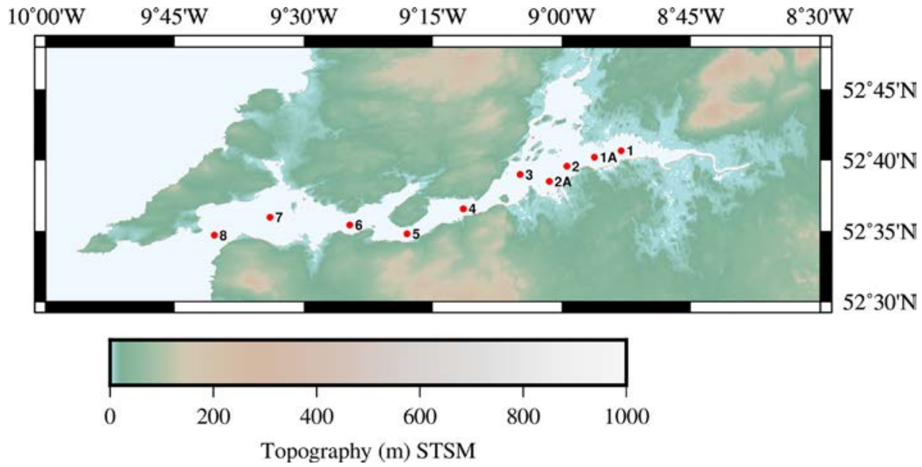

Figure 1.2. Sampling stations occupied in the Shannon estuary during this work. Topography data are from the Shuttle Radar Topography Mission.

river is the longest river in Ireland and has the greatest catchment (11,639 km^2), draining the greater part of the Irish midlands (McMahon *et al*., 1992). The overall water quality status in the estuary is considered high and its nutrient levels are much lower than those of other major European estuaries (O'Boyle *et al*., 2015). Residence time estimates for the upper and lower Shannon estuary are estimated at 16.6 and 53.9 days, respectively (O'Boyle *et al*., 2015). A strong halocline is found throughout the estuary and a turbidity maximum is often observed where salinity is 3–5 (McMahon *et al*., 1992).

1.3.3 Galway Bay – Kinvara Bay and Corrib river plume

Galway Bay receives diffuse and point freshwater discharge around its perimeter, most notably from the Corrib river in the north-eastern corner of the bay, which discharges water from Lough Corrib, through Galway city, at an annual average of 104 $m^3 s^{-1}$ (Mockler *et al*., 2017), making it the second largest river in Ireland by flow rate despite its short length (6 km). The outflow plume of the Corrib is typically found as a thin (1 m) surface lens of low-salinity water along the north shore of the bay west of the Corrib river outfall (Figure 1.3). Circulation in the bay is net inward along its southern part; net outflow is westward along the north shore and the residual circulation is anticlockwise within the bay (Booth, 1975).

Kinvara Bay is a semi-enclosed bay in the south-eastern corner of Galway Bay. It is approximately 4.5 km long with an average depth of 4.2 m and the daily tidal range is between 2 m (neap tide) and 4.5 m (spring tide). Freshwater inputs to the bay are dominated by SGD (Rocha *et al*., 2015; Schubert *et al*., 2015), with two primary inputs, both located in the inner bay near Dunguaire Castle; smaller, more diffusive inputs are likely to be found throughout the bay.

Groundwater contamination with nitrate is frequently reported in the adjacent karst system (McCormack *et al*., 2016) and this frequently results in discharges of elevated total oxidised nitrogen (i.e. nitrite plus nitrate) to the bay via SGD (Cave and Henry, 2011; McCormack *et al*., 2014; Rocha *et al*., 2015; Smith and Cave, 2012). These elevated nutrient levels are likely to be responsible for the extensive phytoplankton blooms that have been recorded previously in Kinvara Bay, most notably that of *Prorocentrum micans* (Gregory *et al*., 2024; Pybus, 1990). Major blooms of other dinoflagellate species have also been reported (Pybus *et al*., 1986). Kinvara has frequently failed in the past to meet the ecological quality status for biological oxygen demand (BOD) for surface waters, as it was often below the threshold of 4 mg/L O_2 (125 µM).

1.3.4 Lough Furnace

Lough Furnace is a lagoonal estuary with a surface area of 1.5 km^2, a maximum depth of 21 m and mean depth of 7.9 m. Lough Furnace receives freshwater input from upstream Lough Feeagh

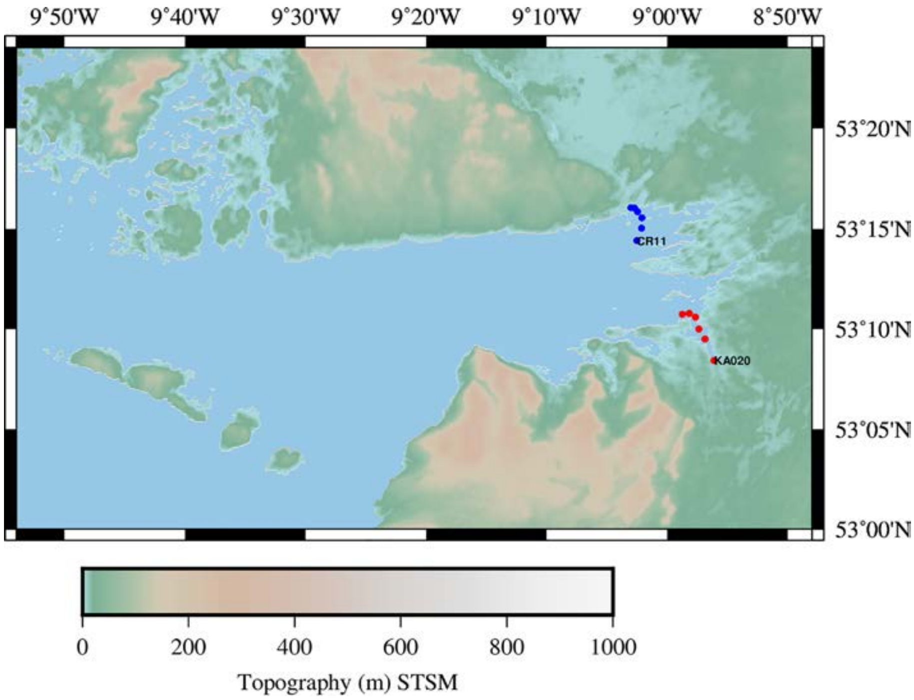

Figure 1.3. Sampling stations in Galway Bay: Corrib outflow (blue dots) and Kinvara Bay (red dots). Topography data are from the Shuttle Radar Topography Mission.

through two connecting channels: the natural Salmon Leap river and the man-made Mill Race river. Lough Feeagh drains the Burrishoole catchment, which is a small upland peat oligotrophic catchment measuring 100 km^2. Tidal input of coastal seawater comes via the Burrishoole estuary, a 1-km-long shallow, constricted channel connecting the southern part of Lough Furnace to Clew Bay.

The inner Furnace basin is notable for its strong saline stratification and deep anoxia, resembling a meromictic saline lake, although ventilation of the bottom water by dense tidal inflows occurs irregularly (Kelly *et al*., 2020).

Lough Furnace is protected as a coastal lagoon habitat under both the EU Habitats Directive (Council Directive 1992/43/EEC on the conservation of natural habitats and of wild fauna and flora) and Water Framework Directive (Council Directive 2000/60/EEC on establishing a framework for community action in the field of water policy), and forms part of the Clew Bay Special Area of Conservation (SAC Site Code 001482; National Parks and Wildlife Service 2011).

1.4 Aims and Objectives of this Study

The Nuts & Bolts project was designed around four work packages that addressed the critical knowledge gaps in Irish MTZs in the context of multiple environmental stressors identified prior to the start of the project. The overall aim of the project was to gather new data on the MTZ ecosystems to identify the key environmental stressors and drivers, now, and in the future.

The four key objectives of the study were to:

1. estimate the fluxes of bio-relevant elements from Irish surface waters and groundwaters to MTZs and the continental shelf;
2. quantify the impact on picoplankton growth rate under different combinations of environmental stressors;
3. determine the bio-optical properties in the mixing zone in Irish MTZs;
4. quantify the sources and sinks of climate-relevant gases in Irish MTZs.

2 Elemental Concentrations and Fluxes in Irish Marine Transitional Zones

2.1 Hydrography

2.1.1 Shannon estuary

Sampling along the length of the Shannon estuary was conducted on four occasions during the period October 2019 to September 2021. Salinities were lowest at the riverine end and were related to the river flow rate (as recorded at Banagher (Office of Public Works) and at Ardnacrusha (ESB)). The lowest flow rates (80 $m^3 s^{-1}$), in September 2021, corresponded to high salinity throughout the estuary (19.4–33.7), while the highest flow rate, in February 2021, saw a shift to low salinities (6.7–26) along the estuary. Temperatures ranged from 15.9°C (seawater end) and 19.0°C (riverine end) in September 2021 to 7.5°C (riverine end) and 7.9°C (seawater end) in February 2021. During all sampling transects there was a distinct halocline present, as has been observed earlier (McMahon *et al*., 1992).

2.1.2 Galway Bay – Corrib plume and Kinvara Bay

Transects across the Corrib plume were carried out on three occasions between March 2021 and March 2022. Strong horizontal and vertical salinity gradients were always present, with salinities at the seaward end approaching that of Galway Bay. Temperatures were always warmest at the riverine end (11.3–16.9°C). Full transects along the length of Kinvara Bay were carried out 12 times between May 2019 and July 2022. Throughout the bay there was always a strong halocline present in the vertical gradient and a persistent horizontal gradient in salinity from the SGD source points to Galway Bay. The lowest temperatures were recorded in March 2021 (7.8°C) and the highest in July 2022 (19.3°C).

2.1.3 Lough Furnace

Lough Furnace was sampled on seven occasions between July 2019 and March 2022. The hydrography in Lough Furnace is quite different to that of the other MTZs investigated in this project, as there is almost no horizontal salinity gradient in the surface waters. This is because there is little to no mixing between river water and seawater in the surface waters. The strong halocline found in the deeper part of the lagoon shows that the seawater confirmed earlier findings that there is little exchange across the pycnocline (Kelly *et al*., 2018). In the deep water, salinity (21.5–21.7) and temperature (12.4–13.3°C) were relatively constant throughout the year.

Surface temperatures on the lough ranged from 6.1°C (February 2022) to 18.1°C (July 2019).

2.2 Nutrient Concentrations and Fluxes

Calculating nutrient fluxes through individual MTZs is complicated by the spatial and temporal variability in sources and sinks that may exist throughout the MTZ. Sources may be represented by natural weathering processes in the catchments soils, along with additional inputs from anthropogenic activities (Nedwell *et al*., 1999). Sink terms in MTZs can include coagulation, precipitation, uptake by phytoplankton and biogeochemical changes (e.g. denitrification). Recent work (Lyons *et al*., 2021) examining total nitrogen (TN = nitrate + nitrite) concentrations in Irish rivers found that streams draining the Connemara bogs, the Caher river, the Corrib tributaries and Shannon river have similar concentrations (26–45 μM), while concentrations in other major Irish rivers is approximately double, at 93 μM. It was noted, however, that there were large variabilities in many of these systems, likely to be due to ongoing anthropogenic input of fixed nitrogen through pastureland agricultural activities. These authors also reported that total phosphate (TP as soluble reactive phosphorus) (< 0.1 μM) and ammonia (< 1 μM) concentrations are generally low in Irish rivers. Over the last three decades there have been significant reductions in the excess nutrient load being transported by Irish rivers to the coast (O'Boyle *et al*., 2016). In Irish MTZs, Mockler *et al*. (2017) used a source load apportionment model to examine the sources of nitrogen and phosphorus in 16 different catchments.

Annual TN and TP loads for Irish MTZs have previously been calculated as the product of the flow-weighted annual mean concentration of TP or TN and the annual flow (O'Boyle *et al*., 2016). It is well understood, however, that the concentration and flow rate can vary over short time scales, resulting in under- or overestimation of the true flux (Nedwell *et al*., 1999). In the present work we utilised property–salinity plots (Boyle *et al*., 1974) to derive fluxes; while this approach has many limitations (García-Martín *et al*., 2021; Nedwell *et al*., 1999), it does provide a context within which to compare different MTZs.

2.2.1 Nitrate, phosphate and silicate

During the Nuts & Bolts project, TN, TP and silicate concentrations were initially measured manually using standard methods adopted for use for low-volume sampling. From October 2022, samples were measured using a SEAL AA500 AutoAnalyzer. KANSO certified reference materials and National Institute of Standards and Technology traceable standards were used for calibration purposes throughout. Along the Shannon estuary (Figure 2.1), TN was found to behave quasi-conservatively, with concentrations being lowest at the seawater end (4–5 μM) and highest at the riverine end (~60 μM), consistent with recent measurements of TN in the Shannon river (Lyons *et al*., 2021).

Silicate concentrations were conservative along the Shannon estuary and were highly correlated to TN concentrations. Phosphate concentrations (data not shown) were somewhat conservative in the lower Shannon estuary but not in the upper Shannon estuary.

TN and Si concentrations were found to be quasi-conservative in the Corrib plume with a riverine source. Phosphate concentrations were also conservative in the Corrib plume, but in this case the seawater endmember was the main source. The conservative behaviour of the major nutrients in the Corrib plume is likely to be due to the very short temporal and spatial scales involved, where mixing dominates over biological drawdown.

In contrast, in Kinvara Bay, TN concentrations were frequently non-conservative (Figure 2.2), suggesting loss of nitrate either by phytoplankton uptake or via benthic dissimilatory nitrate reduction to ammonia or denitrification (Rocha *et al*., 2015). Cave and Henry (2011) previously suggested that the TN concentration flux from SGD in Kinvara could be similar in magnitude to that from the Corrib, although, as noted by Rocha *et al*. (2015), the longer residence time of the SGD inputs in Kinvara Bay compared with that of the Corrib, and substantial benthic-pelagic coupling in Kinvara, contributes to a high retention of TN in Kinvara Bay and only a small amount of TN from SGD being exported to Galway Bay. Silicate was found to behave more conservatively than TN in Kinvara Bay. Phosphate concentrations were generally low (< 0.2 μM) throughout Kinvara Bay and, similarly to the Corrib plume, the seaward endmember was often the highest concentration. The high N:P conditions in

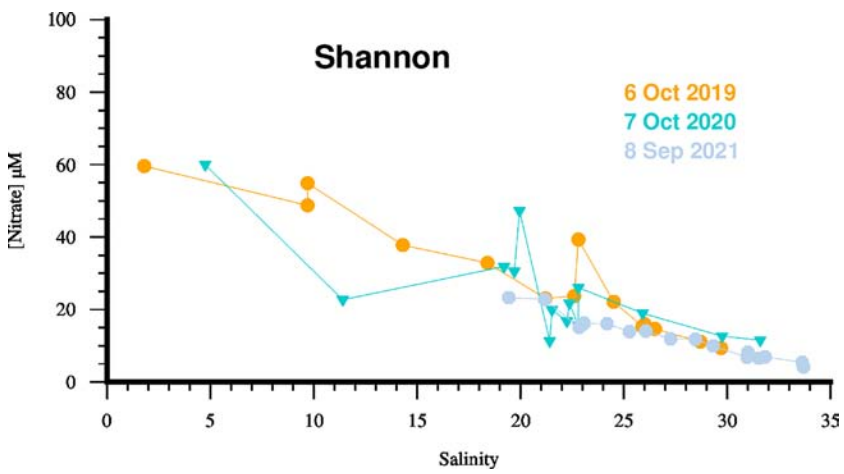

Figure 2.1. Salinity vs nitrate plot for transects along the Shannon estuary.

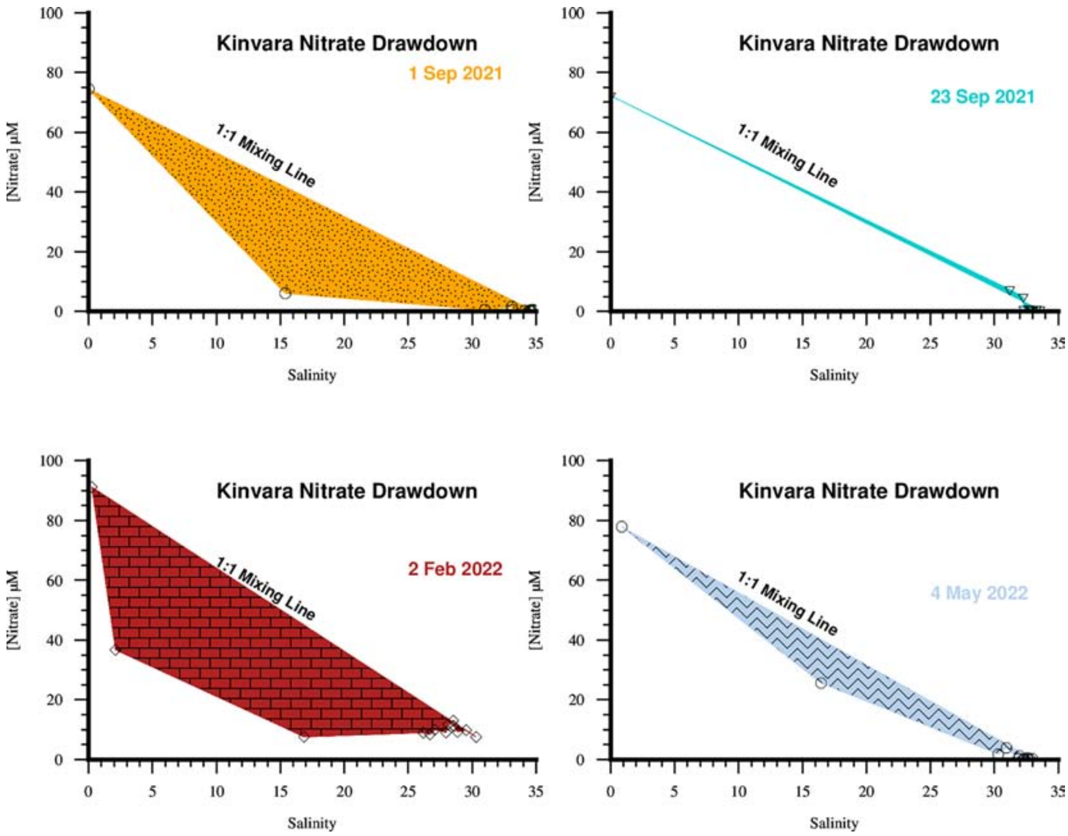

Figure 2.2. Non-conservative behaviour of nitrate along transects in Kinvara Bay on 1 September 2021 (top left), 23 September 2021 (top right), 2 February 2022 (bottom left) and 4 May 2022 (bottom right). The theoretical 1:1 mixing line is also shown and the apparent missing nitrate is indicated in the filled polygon beneath.

Kinvara Bay suggest that primary productivity at this site was phosphate limited.

Earlier measurements of TN and TP concentrations from unfiltered water samples in Lough Furnace (de Eyto *et al*., 2019) found average values of 16.4 µmol L^{-1} and 0.25 µmol L^{-1}, respectively. No vertical profiles were presented. Source waters into Lough Furnace, from the freshwater Lough Feeagh (average TP concentration 0.24 µmol L^{-1}) and from marine waters from Clew Bay (average TP concentration 0.52 µmol L^{-1}), were also found to be low in TP. In the present study we observed low concentrations of TN, phosphate and silicate in the surface waters. Below the surface waters in the deep part of the lagoon there were strong increasing gradients of phosphate and silicate present. Phosphate concentrations were strong in the deep waters at over 14 µM, while TN concentrations were highest in surface waters and decreased with depth, as has been observed previously in other meromictic lakes (Fuchs *et al*., 2022), suggesting a strongly reducing benthic environment with release of phosphate from the dissolution of sedimentary iron oxides.

2.2.2 *Ammonia and nitrite*

To provide more information on aspects of the nitrogen cycle in each of the MTZs, a limited set of measurements was taken for ammonia and nitrite concentrations using manual analytical methods adapted for low-volume sampling.

Within the Shannon estuary, ammonia (0.06–1.6 µM) and nitrite (0.1–0.67 µM) often behaved quasi-conservatively in surface waters with a riverine source term, but concentrations of ammonia were often elevated in the bottom waters, suggesting a benthic source for ammonia.

Kelly (2018) previously found that there was no ammonia detectable in samples close to the SGD source in Kinvara Bay, although there was some detectable further along the bay, possibly derived from sewage sources. In the present study we found low concentrations of ammonia (0.2–1.7 μM) throughout Kinvara Bay, with no apparent relationship to salinity.

Ammonia concentrations were typically low (0.2–0.4 μM) in the surface waters of Lough Furnace, with a strong increase to 1–2 μM in the suboxic/anoxic deep waters.

2.3 Metal Concentrations and Fluxes

Nuts & Bolts represents the first attempt to examine metal distributions along a transect in an Irish MTZ. Previously, data on metal concentrations in EPA and Marine Institute databases consisted of single point measurements from one site in the MTZ. The data were also often reported as the lowest detectable value (quantification limit) with little accompanying ancillary data (e.g. salinity) or metadata on the methods used. This prevents any application of property–property plots as an aid to determining processes impacting elements with similar biogeochemical behaviour. In the Nuts & Bolts project, data were collected from each of the studied MTZs for determination by inductively coupled plasma mass spectrometry after pre-concentration of the metals and removal of salts prior to analysis using a seaFAST pico S2 system. Unfortunately, because of logistical problems associated with the wider impacts of the Covid-19 pandemic, only a limited set of metals was examined at this time.

2.3.1 Aluminium and iron

Dissolved (0.2 μm) metals were analysed for aluminium (Al) by fluorescence with lumogallion and for iron (Fe) by spectrophotometry with ferrozine using standard methods.

Al (Figure 2.3) and Fe (Figure 2.4) concentrations were both relatively conservative throughout the Shannon estuary. This was somewhat surprising, as riverine iron is typically removed upon mixing with seawater (Sholkovitz *et al*., 1978), although the high organic content of the Shannon may stabilise Fe and Al concentrations to coagulation and precipitation.

The highest Al and Fe concentrations in the estuary were related to high river flow rates and near conservative mixing throughout the estuary. During periods of low flow rate, as observed on 8 September 2021 (Figures 2.3 and 2.4), Al and Fe concentrations were significantly lower and more likely to be the result of resuspension of suspended sediments.

Data from the Corrib were similar to data from the Shannon, while in Kinvara Bay there was considerable loss of Al and Fe at low salinity.

In Lough Furnace, very high concentrations of Fe were present in the surface waters (2.0–3.5 μM), with

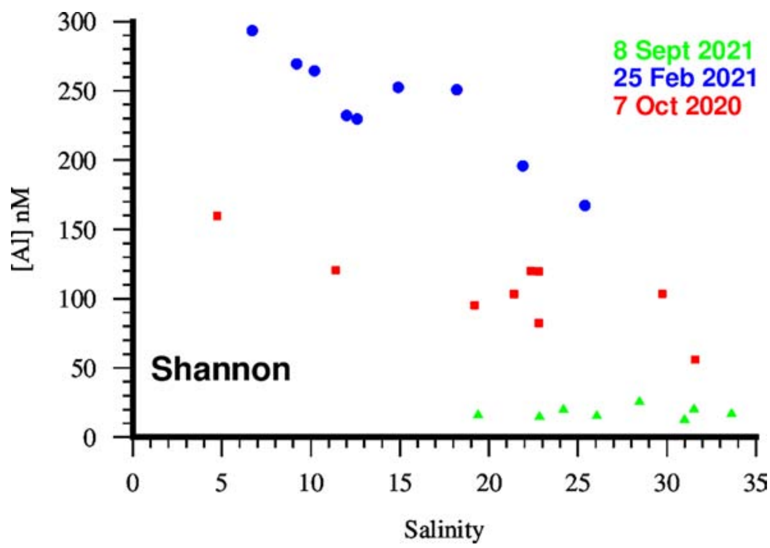

Figure 2.3. Concentration of dissolved Al along the salinity gradient in the Shannon estuary on three different sampling days.

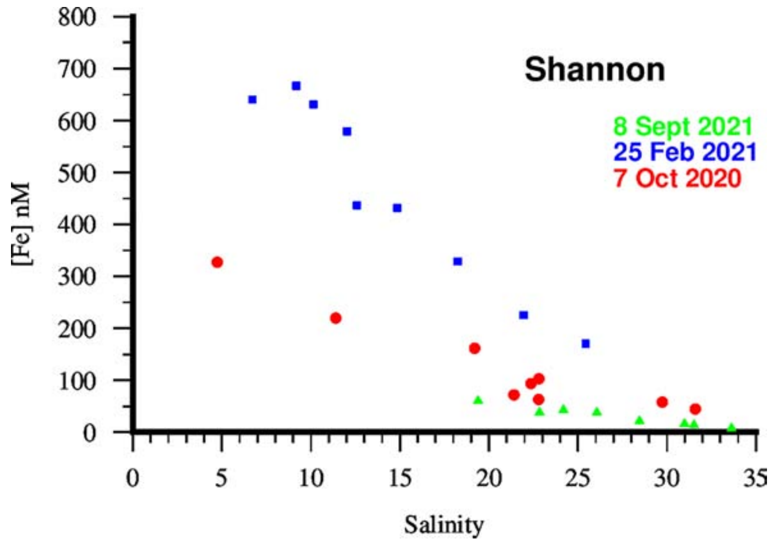

Figure 2.4. Concentration of dissolved Fe along the salinity gradient in the Shannon estuary on three different sampling days.

a midwater minimum before increasing again near the bottom (2.0–8.6 μM).

2.3.2 Manganese

Mn concentrations (measured by inductively coupled plasma mass spectrometry) in Lough Furnace were lowest in the surface waters (200–400 nM) and then rapidly increased with depth and proximity to the sediment level (25–30 μM, approximately 1 m above the sediment). This is consistent with previous work in meromictic systems (Landing *et al*., 1991), where particulate Mn was reduced to soluble Mn^{2+} in a low O_2 environment.

2.3.3 Cadmium, cobalt, copper and nickel

Voltametric analysis of seawater from Kinvara indicated concentrations of Cu (1–3 nM), Co (100–700 pM), Ni (2–6 nM) and Cd (1–10 pM) in seawater there. Cd concentrations were previously found to be slightly elevated in the bottom waters (18–41 pM) compared with surface waters (7.5–21 pM) in Lough Furnace.

3 Picoplankton in Irish Marine Transitional Zones

One of the major knowledge gaps identified at the start of this project was the lack of data on picoplankton (0.2–2.0 µm in diameter) and nanoplankton (2–20 µm in diameter) abundance and distribution in Irish MTZs. While there is now a significant time series for microplankton (< 20 µm in diameter) found in Irish coastal waters due to the long-term HAB survey conducted by the Marine Institute (Nolan *et al*., 2023), these include only a few nanoplankton species and no picoplankton species. Previous reviews of phytoplankton in Irish MTZs and coastal waters (O'Boyle and Silke, 2010; Oliver, 2005) did not consider picoplankton.

3.1 Observations of Picoplankton and Bacterial Abundance in Irish Marine Transitional Zones

Flow cytometry (FCM) is a powerful technique used to obtain information on the abundance and fluorescence characteristics of individual cells as they pass through a focused excitation light where the scattered light (forward and side scatter) and cell fluorescence are measured simultaneously (Sosik *et al*., 2010). In marine and freshwater studies, FCM has been applied widely to the study of pico- and nanoplankton abundance since the mid-1980s (Marie *et al*., 2014; Sosik *et al*., 2010). In combination with specific fluorescent stains, such as for DNA, analysis has also been extended to bacteria and protozoans (Marie *et al*., 2014; Rose *et al*., 2004).

In the present study, FCM was performed on an Accuri C6 with the following standard set-up:

- FSC-H forward scatter – blue laser (488 nm) – proxy for cell size;
- SSC-H side scatter – blue laser (488 nm) – proxy for cell size and shape;
- FL1-H green fluorescence – blue laser (488 nm) – filter 533/30 – used with LysoTracker Green and SYBR Green stains;
- FL2-H orange fluorescence – blue laser (488 nm) – filter 584/40 – phycoerythrin (PE);
- FL3-H red fluorescence – blue laser (488 nm) – filter 670 LP – chlorophyll *a*;
- FL4-H red fluorescence – red laser (640 nm) – filter 675/25 – phycocyanin (PC).

Every day sets of six and eight peak rainbow beads (Spherotech) were run to check the overall performance of the system. Counting beads (Spherotech) were also used to calibrate the fluidics and assess the overall counting efficiency. Analysis of the flow cytometer data was undertaken using FlowJo software. Gating the different phytoplankton populations distinguishable in the samples based on the six measurable parameters was done based on previous experience and aided by the recent work of Thyssen *et al*. (2022) (denoted below as TH22). Figure 3.1 shows the different populations identified by the flow cytometer during this study.

Below is a short description of the different populations identified by FCM in this study.

3.1.1 Picoplankton

Syn-PE. These are PE-containing *Synechococcus*, identifiable by their orange fluorescence (FL2-H) (TH22: OraPicoProk).

Syn-PC. These are PC-containing *Synechococcus*, identifiable by their red fluorescence (FL4-H) (TH22: RedRedPico).

PE1Pop1. This population is possibly related to *Ostreococcus*, based on its extremely small size (Marie *et al*., 2010). *Ostreococcus* sp. have been identified in the Celtic Sea and English Channel (Tragin and Vaulot, 2019).

PE1Pop2. This population is similar to that of the small picoeukaryote *Micromonas.* We frequently see this population in Galway Bay and along the west coast. We isolated a strain of *Micromonas* in 2015 (identified by 18S at the Roscoff Culture Collection in France) and have been growing it continuously since then in the laboratory in Galway.

PE2Pop1. It is not immediately clear what species may be represented by this population, as it has high

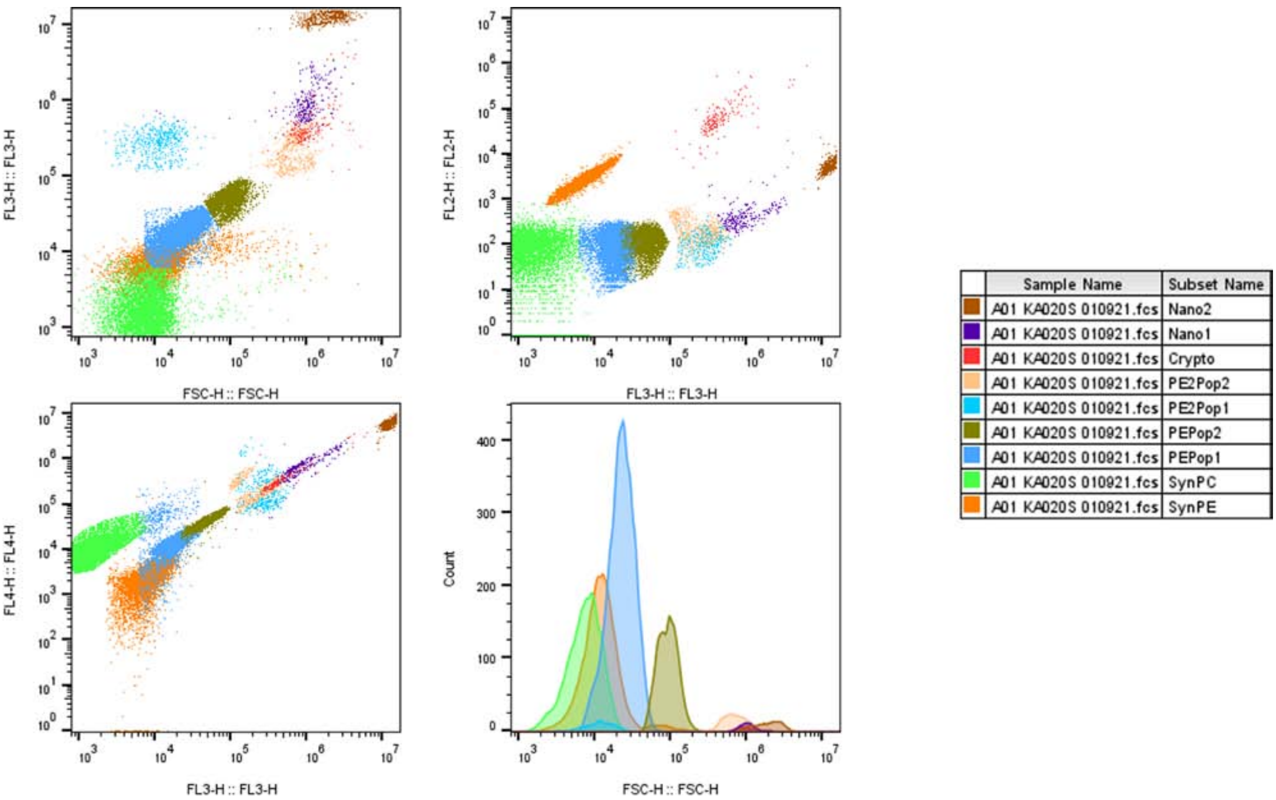

Figure 3.1. (Top left) FSC-H (forward scatter) vs FL3-H (red fluorescence); (top right) FL3-H (red fluorescence) vs FL2-H (orange fluorescence); (bottom left) FL3-H (red fluorescence) vs FL4-H (red fluorescence) and (bottom right) histogram of FSC-H (forward scatter).

FL3-H and FL4-H concentrations but is a similar size to *Synechococcus.*

PE2Pop2. This population is on the border between pico- and nanosize classes. The composition of this group is currently unknown.

3.1.2 Nanoplankton

Crypto. These are most likely cryptophytes, identifiable by their high level of orange fluorescence. They could also be mixotrophic nanoplankton that have ingested Syn-PE cells (TH22: OraNano).

Nano1. A population of small nanoplankton, likely to include haptophytes (e.g. coccolithophorids) based on comparison with cell cultures of phytoplankton isolated from Galway Bay (TH22: RedNano).

Nano2. This population represents larger nanoplankton (or small microplankton) and would include dinoflagellate (e.g. *Prorocentrum micans*) and diatom species. Roughly equivalent to TH22: RedMicro.

3.1.3 DNA stains and flow cytometry – bacterial abundance and cell cycle

Most marine bacteria lack pigmentation that would cause them to fluoresce when passing through a flow cytometer. In order to be able to enumerate them, a nucleic acid stain, such as SYBR Green, is commonly employed (Veldhuis *et al*., 1997). In the present work we used the nucleic acid stain SYBR Green and counted the cells using standard FCM protocols (Marie *et al*., 1997). Using this approach, the apparent DNA content of both prokaryote and eukaryote cells can be determined. This method can also distinguish between different stages of the cell cycle (G_1, S and G_2), which is often tightly regulated to a diel cycle in marine picoplankton such as *Prochlorococcus* and *Synechococcus* (Jochem and Meyerdierks, 1999; Liu *et al*., 1999).

Initial studies employing nucleic acid stains and FCM to determine bacterial abundance in natural waters found that marine bacteria could be divided into low nucleic acid content (LNA) bacteria and high nucleic acid content (HNA) bacteria (Gasol *et al.*, 1999; Lebaron *et al.*, 2001; Li, 1995). LNA bacteria were initially considered to be dead or inactive (Gasol *et al.*, 1999) but subsequent work indicates that they are physiologically active, although with lower cell-specific metabolic activity than HNA bacteria (Schattenhofer *et al.*, 2011). They have smaller cell size and genomes and may be better adapted to oligotrophic conditions (Hu *et al.*, 2022). LNA bacteria have been found to contribute between 20% and 90% of the total bacterial abundance across a wide range of marine and freshwater environments (Andrade *et al.*, 2004; Hu *et al.*, 2022; Schattenhofer *et al.*, 2011). Filtration through 0.45 µm membrane filters has been used to separate and isolate LNA bacteria for culturing (Wang *et al.*, 2009).

3.1.4 Staining with LysoTracker Green – abundance of heterotrophic nanoflagellates

Heterotrophic nanoflagellates (HNFs) are important grazers of bacteria and picoplankton in the marine environment, despite their small size (2–5 µm) and modest abundance of 1×10^5 to 1×10^6 cells L^{-1} compared with the more abundant bacteria (Christaki *et al.*, 2011). HNF grazing is a key process in the heterotrophic bacterial C transfer towards higher trophic levels (Sherr and Sherr, 1994) and is responsible for significant nutrient remineralization (Goldman *et al.*, 1985; Sherr and Sherr, 2002). The enumeration of HNF is complicated, as research has shown that many species live only a few hours in freshly collected samples (Gifford and Caron, 2000). LysoTracker Green is a pH-specific fluorescent stain that has been applied to the flow cytometric enumeration of HNF, as it has affinity for the acidic organelles of eukaryotes, including intracellular food vacuoles, lysosomes and chloroplasts (Rose *et al.*, 2004). An alternative approach to determining HNF on fixed samples is to stain the HNF with SYBR Green and run the flow cytometer at high flow rates to collect sufficient counts and then separate the autotrophs from heterotrophs based on green (LysoTracker) and red (chlorophyll) fluorescence (Christaki *et al.*, 2011). In the present work we used the LysoTracker Green method of Rose *et al.* (2004) to identify HNF in Irish MTZs.

3.1.5 Shannon estuary

Surprisingly, there has been very little previous work published on phytoplankton in the Shannon estuary. Jenkinson (1990) reported data on the biomass and diversity of microplankton (10–200 µm) in the Shannon, Deel and Robertstown estuaries from May 1979 to May 1980. In the Deel estuary, the largest biomasses were found in July when the community was dominated by the dinoflagellate *Glenodinium foliaceum*. The only other study of note is that of McMahon *et al.* (1992), who made measurements of chlorophyll *a*, suspended matter and light attenuation in the Shannon estuary from 1988 to 1990. They found very high chlorophyll *a* levels (up to 70 µg L^{-1}) at the freshwater end of the estuary but provided no information on the species responsible.

Using FCM we found that the freshwater end of the Shannon estuary was dominated numerically by PC-containing *Synechococcus*, while the PE-containing *Synechococcus* dominated the marine waters. This pattern has also been observed in the estuarine and coastal waters of Hong Kong (Liu *et al.*, 2014), where it indicated that the PC-rich *Synechococcus* is able to dominate in turbid waters, most noticeably during the wet season, as PC absorbs in the red, which is more prevalent in turbid waters (Stomp *et al.*, 2004, 2007; see also section 4.4).

Small picoeukaryotes were also present throughout the transects along the Shannon estuary, and, while nanoplankton were less abundant numerically, they contributed significantly to the chlorophyll fluorescence signal observed using FCM (Figure 3.2).

Bacterial abundance in the Shannon estuary generally decreased moving downstream towards the Atlantic for all of the sampling transects performed in this study. The highest bacterial abundances were found in February 2021, with highest values of 4.5×10^9 cells L^{-1} being found at station 2, decreasing to 2.0×10^9 cells L^{-1} at station 7. In October 2019, bacterial abundances ranged from 1.7×10^9 cells L^{-1} at station 1 to 0.5×10^9 cells L^{-1} at station 7. For the transect sampled in October 2020, bacterial abundances were more uniform, ranging from 0.5 to 1.0×10^9 cells L^{-1}. In September 2021, bacterial abundances varied from 2.1×10^9 cells L^{-1} at station 1 to 0.9×10^9 cells L^{-1} at station 7. Across all of the surveys, LNA cells made up 45–62% of the total bacterial abundance.

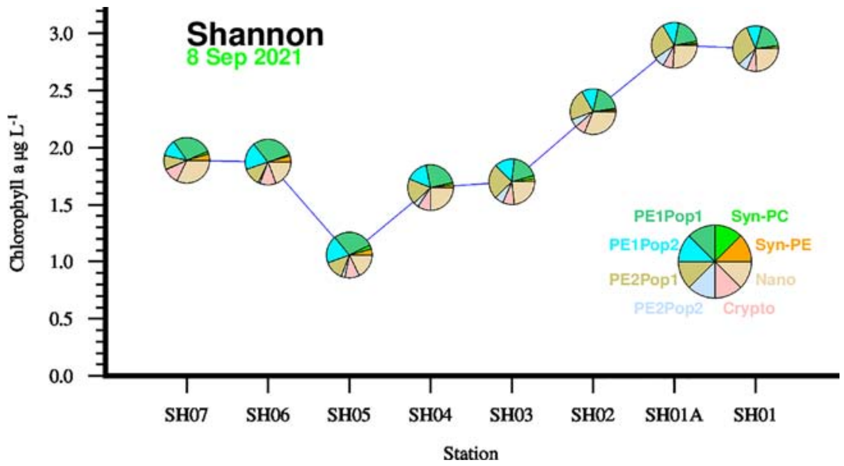

Figure 3.2. Distribution of chlorophyll *a* along a transect in the Shannon estuary in September 2021. Superimposed onto the chlorophyll distribution is a pie chart of the apparent contribution of pico- and nanoplankton species to the chlorophyll signal identified by FCM.

3.1.6 Galway Bay – Kinvara and Corrib

There have been a number of studies on the microphytoplankton and zooplankton found in the Galway Bay region, although most reports are related to incidences of non-toxic red tides. Pybus (1996) examined the distribution and abundance of diatoms throughout Galway Bay during 1974 and 1975 and observed that the spring bloom (February/March) was dominated by *Skeletonema* sp. in 1974 and *Thalassiosira* sp. in 1975. Later, Roden (1984) conducted a seasonal survey in Connemara during 1980/1981 and observed that the spring bloom arrived in late April and was dominated by diatoms. Large blooms of the dinoflagellate *Ceratium tripos* were found in autumn and it was suggested that these were related to freshwater run-off. This species of dinoflagellate was also found in unusually high concentrations throughout Galway Bay in October 1980 (Pybus, 1984). Also in 1980 and again in 1981, dense populations of the dinoflagellate *Prorocentrum micans* were found throughout Galway Bay (Pybus, 1990), with cell concentrations reaching up to 11×10^6 cells L^{-1}, staining the water a bright red colour. Red water was also observed in Lough Atalia, near the outflow of the Corrib, in April 1982 due to high concentrations of the dinoflagellate *Glenodinium foliaceum* (Pybus *et al*., 1984). The seasonal cycle of microphytoplankton and zooplankton in the Dunkellin estuary, adjacent to Kinvara Bay, was observed from December 1984 to July 1986 (Byrne and O'Mahony, 1993; O'Mahony, 1992). The highest concentrations of phytoplankton were observed from May to August. Chlorophyll concentrations were lowest in winter (0.1 µg L^{-1}), with the highest concentration in April 1985 (14.5 µg L^{-1}). Further blooms of *Prorocentrum micans* have been reported in Kinvara in recent years (Gregory *et al*., 2024) but not in the nearly adjacent Aughinish Bay, which receives no SGD.

During the Nuts & Bolts project, an extremely dense bloom of *Prorocentrum micans* (as identified by microscopy) was observed in Kinvara at station KA020 on 1 September 2021, with chlorophyll concentrations reaching 90 µg L^{-1} (Figure 3.3). This bloom was likely to have been initiated due to a combination of recent rainfall after a dry spell, driving SGD, and a neap tide, which limits the movement of water in the inner bay and creates a retention area suitable for intense dinoflagellate blooms (Gregory *et al*., 2020). FCM data indicated that, numerically, the bloom was dominated by picoplankton, most noticeably PE1Pop1 (33.4 cells µL^{-1}), Syn-PE (21.0 cells µL^{-1}) and Syn-PC (19.3 cells µL^{-1}), with concentrations in the Nano2 grouping, which contained *Prorocentrum micans*, being significantly lower at 1.47 cells µL^{-1}. In terms of chlorophyll, however, the Nano2 grouping was by far the greatest component present.

Cells from the phytoplankton bloom at station KA020 in September 2021 were maintained in our laboratory using standard culturing conditions (15°C, 100 µEin m^{-2} s^{-2} in Salinity 35 Red Sea Media), and,

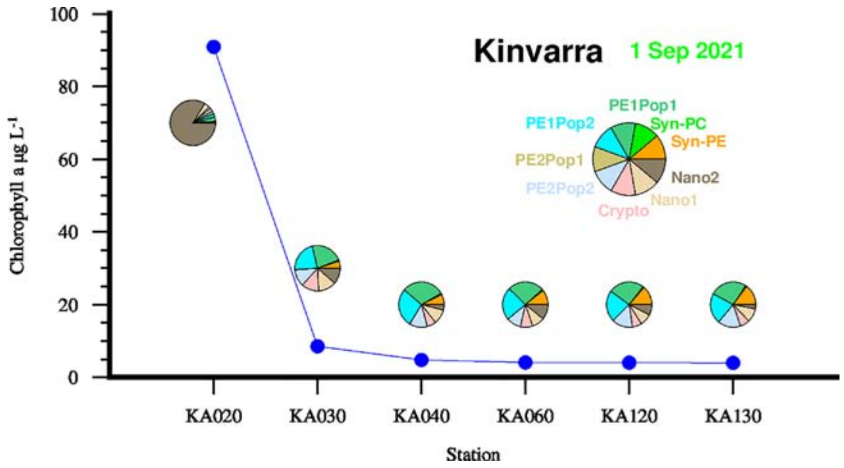

Figure 3.3. Distribution of chlorophyll *a* along a transect in Kinvara Bay in September 2021. Superimposed onto the chlorophyll distribution is a pie chart of the apparent contribution of pico- and nanoplankton species to the chlorophyll signal identified by FCM.

from these, we were able to isolate cells from samples taken from KA020S (surface) and KA020B (deep). The cultures were not initially monoalgal, and efforts to isolate pure cultures are ongoing. It was anticipated that the KA020S sample would lead to an isolation culture dominated by *Prorocentrum micans* (Nano2); however, this was not the case, as a smaller cell from the Nano1 population grew quicker and came to dominate the culture.

Further classification of the isolated algae was undertaken at the Roscoff Culture Collection. KA020S was revealed by Basic Local Alignment Search Tool (BLAST) analysis of the 18S rRNA gene to be most likely a *Pavlova* or *Rebecca* species. Further 18S rRNA gene analysis using Geneious suggests that KA020S is a *Rebecca* sp. nov. Ovoid, spherical and pear-shaped cells were observed. *Rebecca* is a genus of photosynthetic, flagellated marine haptophytes in the family *Pavlovaceae* that prefer saltwater estuaries with muddy or turbulent waters (Bendif *et al*., 2011; Edvardsen *et al*., 2000; Véron *et al*., 2023).

KA020B was indicated to be a likely PC-containing *Synechococcus* sp. (identified as Syn-PC using FCM). The *Synechococcus* sp. was around 1 µm in diameter; however, the sample also contained a small heterokont (Stramenopile) of around 3 µm, which complicated further identification. We had previously isolated PE-containing *Synechococcus* (identical to Syn-PE using FCM) from Galway Bay and the west coast of Ireland, and continue to maintain these isolates in culture.

Similarly to the Shannon estuary, the PC-containing *Synechococcus* sp. (Syn-PC) was mostly found in the turbid, low-salinity waters, while the PE-containing species were associated more with the clearer coastal waters. At station KA030 (Figure 3.4), PE1Pop1 was the most abundant picoplankton during the summer of 2021, but in the following summer Syn-PE was more abundant. Both species showed maxima in the summer, with lower concentrations in the winter. Syn-PC and PE1Pop2 also appear to have summer maxima in their abundance.

Bacterial abundance at KA030 (Figure 3.5) was dominated by HNA for most of the year, with total bacterial numbers peaking at different times each year, suggesting bacterial abundance may be more related to SGD events than seasonal cycles.

3.1.7 Lough Furnace

Earlier work in Lough Furnace (de Eyto *et al*., 2019) had examined the deep chlorophyll maximum that exists typically 3–5 m below the surface and often below the 1% light level (photosynthetically active radiation). The authors observed that freshwater diatoms were frequent in the freshwater layer throughout the year and formed the bulk of the spring bloom. Some marine diatom species, *Thalassiosira* sp. and *Skeletonema* sp., were also present. A cryptophyte nanoflagellate, *Chromonas acuta*, was present year-round, while the dinoflagellate *Hetercapsa triguetra* and the cryptophyte *Teleaulax*

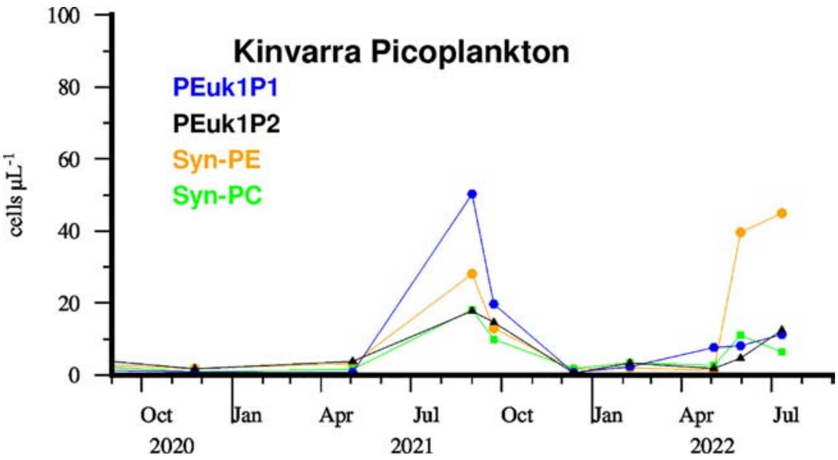

Figure 3.4. Time course of Syn-PE, Syn-PC, PEuk1P1 and PEuk1P2 picoplankton in Kinvara Bay at station KA030.

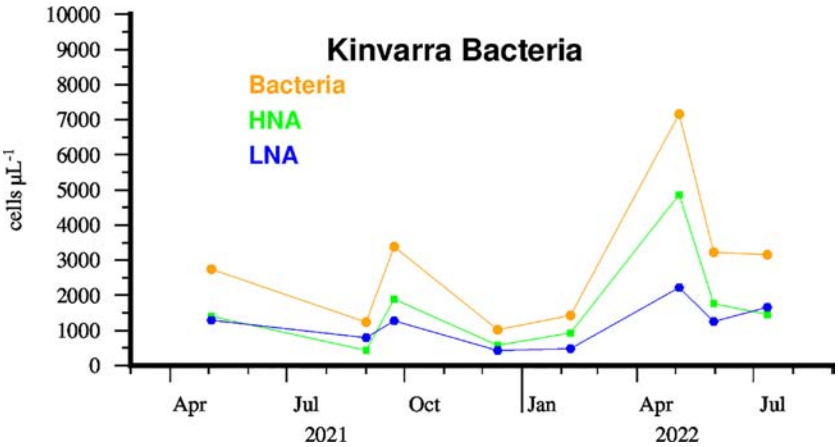

Figure 3.5. Time course of total, HNA and LNA bacterial abundance in Kinvara Bay at station KA030.

acuta were also abundant at times. Haptophytes *Pavlova* sp. and *Pseudopedinella* sp. were also present on occasion. A bloom of *Prorocentrum minimum* was observed in August–September 2013. Other dinoflagellates identified in Lough Furnace included the dinoflagellates *Prorocentrum micans* and *Dinophysis* sp. The authors suggested that phytoplankton blooms observed in Lough Furnace appeared to be related to inflow from marine waters under high tidal inputs.

In the present study, FCM data from Lough Furnace indicated that there were relatively few chlorophyll-containing cells in the freshwater layer (< 1 cell µL^{-1}), and what was there was dominated by a few nanophytoplankton and PC-containing *Synechococcus*, although PE-containing *Synechococcus* were also present. In the deeper saline waters, FCM revealed the presence of a large population of small cells (~1 µm) that had a high level of red fluorescence but were not consistent with *Synechococcus* sp. (Figure 3.6), as they had no significant PC or PE fluorescence.

Based on the environmental conditions, and the FCM signals, the cells are identified here as **green sulfur bacteria** (GSB), most likely the brown-coloured *Chlorobium* species (Casamayor *et al*., 2007); for more details on this identification, see section 4.3. *Chlorobium* are rod-shaped cells (0.6–0.8 µm wide, 1.3–2.7 µm long) that contain the pigments bacteriochlorophyll *e* and isorenieratene as their main antenna pigments. GSB perform **anoxygenic photosynthesis**, in which H_2S is used instead of H_2O and elemental sulfur is formed instead of oxygen. *Chlorobium* cells are **anaerobes**, thus not tolerant of oxygen, and are frequently found with sulfur globules outside the cells.

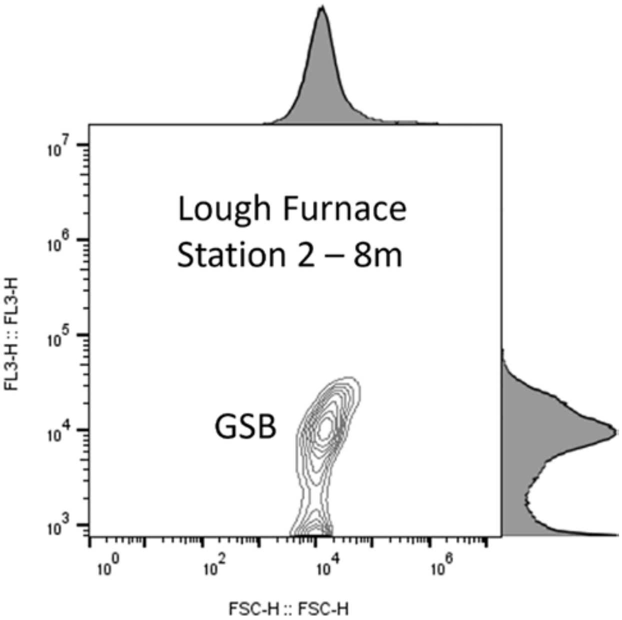

Figure 3.6. Flow cytogram of a water sample from Lough Furnace, station 2 at 8 m, from 16 March 2022.

Samples from the deep saline waters in Lough Furnace taken in July 2020 showed no indications of a substantial population of GSB. However, when sampling from November 2020 through to March 2022, GSB were found to be the most numerically abundant phototrophic organisms in Lough Furnace, with maximum concentrations at around 10 m depth (Figure 3.7). It does appear that GSB abundance was also increasing during this time, as GSB numbers were four to five times lower in the deep waters in November 2020 than they were in March 2022, but the dataset is too small to really draw any major conclusions from this.

It is interesting to note that the earlier work of de Eyto *et al*. (2019) reports a time series of vertical profiles of chlorophyll in Lough Furnace using a standard chlorophyll fluorescence sensor, which was optimised for chlorophyll *a* fluorescence and may not have detected the bacteriochlorophyll *e* fluorescence signal that is found in the infrared. As GSB are able to exist only in anoxic or extremely low-oxygen environments, it is likely that they are removed during ventilation events (Kelly *et al*., 2020) and, once the oxygen is removed, they can grow back to significant numbers. We had previously sampled in Lough Furnace in September 2013, and at this time no significant population of GSB was evident, consistent with a reoxygenation event that had occurred early in 2013

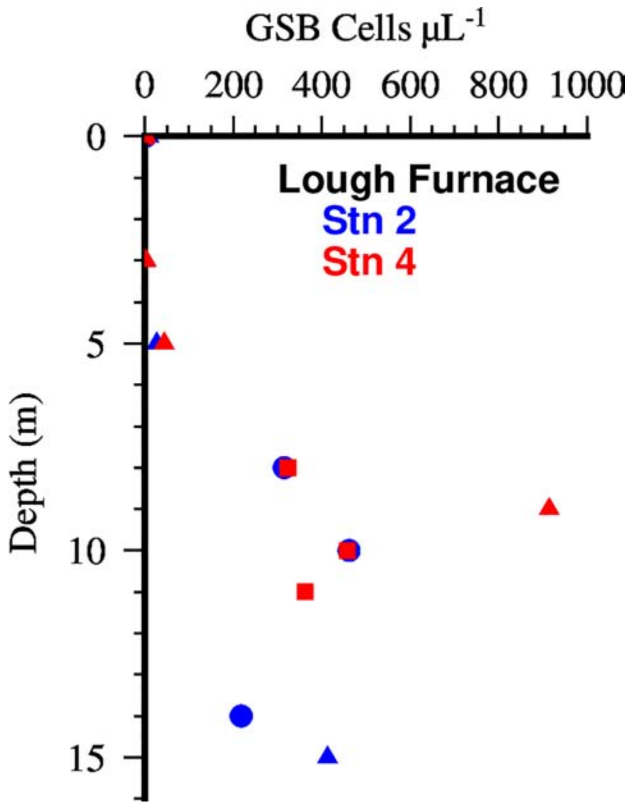

Figure 3.7. Lough Furnace vertical distribution of GSB. Blue symbols, station 2; red symbols, station 4; circle, 16 March 2022; triangle, 22 November 2021.

(de Eyto *et al*., 2019; Kelly *et al*., 2018). While oxygen levels were decreasing by the time we sampled, they were not low enough for GSB to exist.

Bacterial numbers in Lough Furnace, not including GSB, increased with depth throughout the basin, with the highest numbers in the anoxic waters. In some deep locations, GSB were more abundant than non-photosynthetic bacteria. Surface waters in the lough were dominated by LNA bacteria, with as low as 10% HNA, while deeper waters were around 40% HNA bacteria. This is consistent with other recent studies of freshwater systems (Proctor *et al*., 2018).

3.2 Dilution Grazing Experiments – Obtaining Picoplankton Growth and Grazing Rates in Irish Marine Transitional Zones

In dilution grazing experiments, filtered (0.2 µm) seawater is used to create a gradient pressure on phytoplankton, allowing the estimation of both the growth rate and the grazing rate of bacteria or phytoplankton (Landry *et al*., 1995). It is noted, however, that a dilution grazing experiment is not a natural state of affairs for the plankton involved, as the experiments can be unrealistic (Agis *et al*., 2007) because of cells being stressed due to bottle effects (Berg *et al*., 1999), trophic cascades (Calbet and Saiz, 2013) and mixoplankton grazers being present (Duarte Ferreira *et al*., 2021). During the Nuts & Bolts project we performed 10 such 24-hour dilution grazing experiments (Kinvara Bay, $n = 8$; Lough Furnace, $n = 1$; and Shannon estuary, $n = 1$), without additional nutrients, to gain insight into the growth and grazing rates of pico- and nanoplankton in the selected Irish MTZs.

Unfortunately, the Lough Furnace dilution grazing experiment using surface water had too few chlorophyll-containing cells for statistical analysis. In the experiment using surface water from the most seaward Shannon estuary station (SH07), the cyanobacteria and picoeukaryotes did not grow but significant bacterial growth and grazing occurred (Table 3.1).

Bacterial growth rates in Kinvara Bay ranged from 1.01 to 2.33 d^{-1}, with the highest growth rates found in an experiment with virus-free seawater (10 kDa ultrafiltration), indicating that viral lysis was also a significant mortality pathway for bacteria at this time. Bacterial grazing rates were often higher or similar to growth rates. HNA bacteria grew quicker than LNA bacteria (except in virus-free seawater) but were also more likely to be grazed quicker.

Syn-PE often did not grow in the grazing experiments, and when it did it appeared to be limited to a growth rate of one division per day (0.69 d^{-1}), most likely as its growth is driven by a strong diel cycle, with cell division occurring at night, as is the case with many marine picoplankton, although some are capable of ultradian growth (> 0.69 d^{-1}) under certain conditions (Jacquet *et al*., 2001). Syn-PC appears to be more robust than Syn-PE when it comes to laboratory experiments, although it too was similarly limited in growth rate, but did manage to grow at 1.42 d^{-1} in August 2020. Grazing rates on Syn-PE were often higher than the growth rates, while Syn-PC was often not grazed significantly at all (grazing < 30% of growth).

The picoeukaryote species PE1Pop1 (Table 3.2) was able to grow (0.3–1.1 d^{-1}) significantly faster than Syn-PE, with which it competes for numerical abundance in Kinvara Bay. In most experiments the fraction of PE1Pop1 that was being grazed was less than 30% of the growth rate. PE1Pop2 had the fastest growth rates (0.37–2.30 d^{-1}) of all of the observed FCM populations. In all experiments this species was grazed only slightly during the course of the experiments. PE2Pop1 also grew at significant rates (0.71–1.26 d^{-1}) and was often minimally grazed.

The dilution grazing experiments conducted during the Nuts & Bolts project (Tables 3.1 and 3.2) provide the first insights into the growth and grazing of key bacteria and picoplankton species in Irish MTZs. These data will help inform future modelling efforts.

3.3 Heterotrophic Nanoflagellates

Using FCM and the stain LysoTracker Green (Rose *et al*., 2004), samples were measured at various times during the course of this project for the presence of HNF. It is also noted that FCM data collected with staining using SYBR Green (see section 3.2) can provide an alternative method to estimate HNF (Christaki *et al*., 2011).

In Kinvara, the highest concentrations of HNF were found at station KA020 during the large phytoplankton bloom that was observed there on 1 September 2021, with values of 35×10^5 cells L^{-1} and two distinct populations (based on size) being distinguishable. Values were more uniform throughout the rest of the transect at around $4–6 \times 10^5$ cells L^{-1}. At all other times cell counts were too low to identify distinct groups. There was no apparent relationship between HNF and bacterial numbers (data not shown).

In general, HNF abundance was low, at $\sim 2–4 \times 10^5$ cells L^{-1}, and uniform in the surface waters of the October 2019 transect in the Shannon estuary. Deeper samples collected at stations SH01 (30×10^5 cells L^{-1}) and SH02 (16×10^5 cells L^{-1}) were significantly elevated in HNF, though bacterial numbers were not. Along the Corrib transect taken in August 2021, values were also relatively uniform at $1–6 \times 10^5$ cells L^{-1}. No reliable counts of HNF in Lough Furnace were obtained due to a combination of the low numbers of phytoplankton found there and the high number of natural particles, which gave a high background when the LysoTracker stain was added.

Table 3.1. Summary of dilution grazing experiments: prokaryotes – bacteria and *Synechococcus* (rates are d^{-1})

Location/nutrient	Bacterial growth	Bacterial grazing	LNA growth	LNA grazing	HNA growth	HNA grazing	Syn-PE growth	Syn-PE grazing	Syn-PC growth	Syn-PC grazing
Kinvara										
9 July 2020	1.79 ± 0.06	1.00 ± 0.09	1.40 ± 0.06	0.61 ± 0.09	2.15 ± 0.05	1.35 ± 0.08	0.58 ± 0.05	0.14 ± 0.07	0.36 ± 0.05	0.01 ± 0.33
6 August 2020	1.18 ± 0.09	1.52 ± 0.13	0.88 ± 0.07	1.10 ± 0.11	1.48 ± 0.13	2.06 ± 0.19	0.65 ± 0.09	0.41 ± 0.13	1.42 ± 0.04	0.43 ± 0.06
23 September 2020	1.10 ± 0.12	1.00 ± 0.18	0.69 ± 0.05	0.77 ± 0.07	1.56 ± 0.19	1.29 ± 0.27	–0.05 ± 0.06	0.28 ± 0.09	0.10 ± 0.06	0.04 ± 0.08
24 November 2020	1.91 ± 0.19	1.16 ± 0.28	2.66 ± 0.17	1.00 ± 0.24	2.43 ± 0.06	1.78 ± 0.09	0.32 ± 0.08	0.36 ± 0.12	n.d.	n.d.
(Virus free[a])	2.33 ± 0.11	1.28 ± 0.16	3.26 ± 0.15	1.40 ± 0.22	2.71 ± 0.10	1.65 ± 0.15	0.14 ± 0.02	0.53 ± 0.03	n.d.	n.d.
1 September 2021 (KA020)	1.80 ± 0.14	2.15 ± 0.21	1.28 ± 0.14	1.66 ± 0.21	2.12 ± 0.15	2.44 ± 0.23	0.05 ± 0.08	0.48 ± 0.12	0.35 ± 0.12	0.10 ± 0.17
1 September 2021 (KA060)	0.73 ± 0.20	0.78 ± 0.29	0.34 ± 0.20	0.44 ± 0.29	1.18 ± 0.20	1.10 ± 0.29	0.14 ± 0.08	0.35 ± 0.11	0.61 ± 0.10	0.29 ± 0.15
7 September 2021	1.01 ± 0.04	0.40 ± 0.06	0.55 ± 0.02	0.23 ± 0.03	1.25 ± 0.06	0.49 ± 0.08	0.14 ± 0.04	0.08 ± 0.06	0.58 ± 0.08	0.43 ± 0.11
Shannon										
8 September 2021	0.96 ± 0.14	0.51 ± 0.20	0.46 ± 0.12	–0.17 ± 0.17	1.27 ± 0.19	0.69 ± 0.27	n.d.	n.d.	n.d.	n.d.

[a]Data indicate values for virus-free seawater.

n.d., not determinable.

Table 3.2. Summary of dilution grazing experiments – pico- and nanoeukaryotes (rates are d^{-1})

Location/nutrient	PE1Pop1 growth	PE1Pop1 grazing	PE1Pop2 growth	PE1Pop2 grazing	PE2Pop2 growth	PE2Pop2 grazing
Kinvara						
9 July 2020	0.53 ± 0.02	0.19 ± 0.02	2.26 ± 0.09	0.95 ± 0.13	1.26 ± 0.09	0.68 ± 0.14
6 August 2020	1.10 ± 0.10	0.70 ± 0.15	1.89 ± 0.06	0.62 ± 0.09	0.96 ± 0.01	0.07 ± 0.02
23 September 2020	0.30 ± 0.02	0.43 ± 0.02	1.71 ± 0.04	0.33 ± 0.06	0.71 ± 0.05	0.11 ± 0.08
24 November 2020	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
(Virus free[a])	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1 September 2021 (KA020)	0.76 ± 0.11	0.26 ± 0.16	0.48 ± 0.17	0.02 ± 0.25	0.85 ± 0.05	0.05 ± 0.07
1 September 2021 (KA060)	0.68 ± 0.06	0.13 ± 0.08	0.37 ± 0.09	0.03 ± 0.14	0.54 ± 0.11	0.39 ± 0.16
7 September 2021	0.63 ± 0.02	0.19 ± 0.02	2.30 ± 0.05	0.48 ± 0.07	1.23 ± 0.07	0.63 ± 0.11
Shannon						
8 September 2021	n.d.	n.d.	0.15 ± 0.10	0.36 ± 0.15	n.d.	n.d.

[a]Data indicate values for virus-free seawater.

n.d., not determinable.

3.4 Mixotrophy

As noted earlier, dinoflagellates are a common feature in the late autumn in Irish waters. In particular, a common bloom-forming species in Galway Bay is the dinoflagellate *Prorocentrum micans* (Pybus, 1990); this species has been shown in laboratory studies to be able to act as a mixotroph, with the result that the overall carbon uptake of the dinoflagellate was reduced compared with autotrophic growth (Jeong *et al.*, 2016).

Laboratory studies during this work revealed that the *Rebecca* sp. isolated from Kinvara stains with LysoTracker Green, indicating the presence of an acidic organelle, possibly used for phagotrophic uptake. Experiments are ongoing currently to determine if *Rebecca* sp. do actively graze bacteria or the PC-containing *Synechococcus* that is also found in the freshwater endmembers of estuaries.

4 Bio-optical Properties of Irish Marine Transitional Zones

The attenuation of light in the water column plays a significant role in the primary productivity and zonation of ecosystems in MTZs. Light is essential for macroalgal and phytoplankton photosynthesis, and the amount and spectral distribution of light that penetrates into the water column is impacted by the absorption and scattering of light by dissolved organic matter (DOM) and particles within the water column. CDOM, also known as *gelbstoff*, is the fraction of DOM that absorbs the ultraviolet (UV) and visible wavelengths of light and is a major control on the optical properties of most natural waters. CDOM is also an important component in many biogeochemical and photochemical processes, impacting the cycling of carbon, nitrogen, sulfur and oxygen in the marine environment. Light is also absorbed and scattered by suspended particles, most notably phytoplankton and their pigments.

Light is an important control on primary productivity in MTZs, and its attenuation in the water column and reflectance can provide significant bio-optical and biogeochemical information on processes occurring there. Upon entering the water light (photons) can either be *absorbed*, where energy is converted into another form such as heat or breaking a chemical bond, or *scattered*, where it changes direction or wavelength. The absorption and scattering properties of natural waters are described by its inherent optical properties (IOPs) (Preisendorfer, 1976). IOPs are properties of the media and do not depend on the ambient light field. Apparent optical properties depend on both the properties of the water (IOPs) and the geometric radiance distribution (sun location, cloud cover, waves, etc.). A major objective of the current project was to investigate the factors controlling light in the water column of Irish MTZs and obtain baseline data for IOPs.

The downwelling diffuse attenuation coefficient (K_d units m^{-1}) can be calculated for each wavelength at each sampling site from the irradiance (E_d) observations collected by least squares regression to fit the following equation:

$$\ln E_{d,z} = -K_d z + \ln E_{d,0}$$

where $E_{d,z}$ is the irradiance at depth z (m).

The contribution of absorption and scattering to the observed attenuation coefficient is typically split into separate components:

$$K_{d,\lambda} = a_\lambda + b_\lambda$$

The absorption at each wavelength can be further separated into components related to absorption due to pure water, CDOM, phytoplankton and non-algal particles, as discussed above.

$$a_\lambda = a_{CDOM,\lambda} + a_{ph,\lambda} + a_{pa,\lambda} + a_{pw,\lambda}$$

Backscatter (b_λ) was not measured directly in this work, but the overall contribution of backscatter can be resolved by calculating the difference between K_d and a_λ. Different components (e.g. ions, colloids, bubbles, particles) in seawater can contribute to the overall backscatter observed (Stramski *et al.*, 2004).

CDOM is the main factor controlling the attenuation of UV light in natural waters (Kirk, 1994). CDOM is photoreactive and can be efficiently degraded to non-chromophoric DOM and CO_2 on exposure to solar radiation. Absorption spectrums for CDOM show the highest absorbance in UV wavelengths and decrease approximately exponentially with increasing wavelength (Twardowski *et al.*, 2004). Further analysis of the observed CDOM absorption spectra can be made by fitting absorption data to the equation:

$$a_\lambda = a_{\lambda ref} e^{-S(\lambda - \lambda ref)}$$

where a = absorption coefficient (m^{-1}), λ = wavelength (nm), λ_{ref} = reference wavelength (nm) and S = spectral slope (nm^{-1}) (Twardowski *et al.*, 2004).

CDOM absorbance in the UV is often highly correlated to the concentration of dissolved organic carbon (DOC) (Fichot and Benner, 2011; Peacock *et al.*, 2014). Studies have shown that the slope ($S_{275-295}$) of the CDOM absorption spectrum in the UV (275–295 nm) is a good proxy for the apparent molecular weight (MW) of the DOM and can give information about the source (terrestrial or marine, autochthonous or allochthonous, natural or anthropogenic) of the CDOM and its exposure to potential degradation via solar radiation (photobleaching) (Fichot and Benner, 2012; Helms *et al*., 2008). Studies frequently also utilise the spectral slope ($S_{350–400}$) to calculate the slope ratio or S_R (Helms *et al*., 2008), $S_R = S_{275–295}/S_{350–400}$, as this index provides further information on changes in DOM MW and composition.

Many chemical species within CDOM also have fluorescent properties, and this subset of CDOM is referred to as fluorescent dissolved organic matter (FDOM). Work on characterising FDOM in natural waters has grown rapidly since the 1990s with the development of excitation emission matrix (EEM) spectroscopy (Coble, 1996; Coble *et al*., 1990). Typical EEMs of FDOM from natural waters include fluorophores with characteristics of proteins and humic/fulvic acids. Quantification and identification of fluorophore species has been greatly facilitated by the application of the parallel factor (PARAFAC) procedure (Bro, 1997; Murphy *et al*., 2013; Stedmon and Bro, 2008), allowing researchers to examine the sources, sinks and distribution of individual fluorophores in natural waters and waste waters (Ishii and Boyer, 2012).

In the present work we undertook a suite of bio-optical measurements to compare the major controls on light attenuation in our study locations. Measurements of CDOM absorbance and FDOM fluorescence were performed using a HORIBA Aqualog following standard protocols for sample collection and filtering (0.2 µm filter). Raw absorbance data collected on the Aqualog were converted to absorption coefficients as follows:

$$a_\lambda = 2.303 * \frac{A}{l}$$

where *A* is the raw absorbance and *l* the pathlength (1 cm).

Fluorescence data processing for 3D EEMs was performed using established PARAFAC routines (Murphy *et al*., 2013; Stedmon and Bro, 2008). Particular absorption measurements were made using the filter pad technique (Roesler *et al*., 2018) using a QFT-1 filter holder (World Precision Instruments) connected to an Ocean Optics spectrophotometer. Hyperspectral measurements of the underwater light field were made *in situ* with a RAMSES hyperspectral irradiance sensor (TriOS GmbH) equipped with a pressure and inclination sensor.

4.1 Coloured Dissolved Organic Matter as a Control on Light Attenuation in Irish Marine Transition Zones

There has been very little work in Ireland investigating CDOM absorbance in Irish coastal waters or rivers. Some data do exist for UV absorbance in Galway Bay (Monahan and Pybus, 1978), along with a series of transects for measurements of yellow substance (440 nm) made across the Corrib plume using the red–green–blue output from a digital camera (Goddijn and White, 2006). More recently, Kelly (2018) investigated CDOM absorbance and FDOM fluorescence in Kinvara Bay as part of a study into carbon cycling there. Globally, however, there have been a large number of studies examining the distribution and absorbance characteristics of CDOM in MTZs (Helms *et al*., 2008; Osburn *et al*., 2016). While in many MTZs CDOM behaves conservatively, in some turbid UK estuaries CDOM has been seen to behave non-conservatively, with substantial removal in the upper estuary (Spencer *et al*., 2007; Uher *et al*., 2001), presumably due to flocculation of humic and suspended materials during mixing with seawater (Sholkovitz *et al*., 1978).

During our investigations in the Shannon estuary (Figure 4.1 and Table 4.1) we found that at high flow rates (> 300 $m\,s^{-1}$, October 2019 and February 2021) CDOM behaved apparently conservatively, but was apparently non-conservative at low salinities (October 2020) when flow rates were lower (160 $m\,s^{-1}$). The non-conservative behaviour is likely to reflect a range of processes occurring at this time, such as CDOM removal at the low salinity end, with possible CDOM inputs from the sediments and/or other tributaries to the estuary at intermediate salinities. In the case of the lowest river flow rate encountered (80 $m\,s^{-1}$) in September 2021, CDOM again appeared to behave conservatively throughout the estuary when salinities were elevated throughout.

Observed values of the spectral slope $S_{275–295}$ for the Shannon estuary (Figure 4.2) indicate that there is a gradual progression from lower values at the freshwater end to higher values in marine waters.

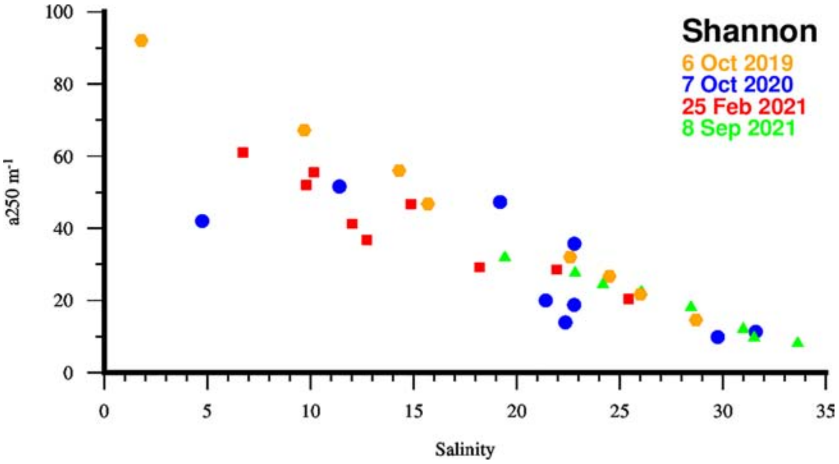

Figure 4.1. CDOM absorbance ($a_{CDOM,250}$ m^{-1}) as a function of salinity for transects in the Shannon estuary.

Table 4.1. Observed values of $a_{CDOM,\lambda}$ (m^{-1}) and S_{275} (nm^{-1}) for the MTZs sampled

Location/nutrient	$a_{CDOM,250}$ (m^{-1})	$a_{CDOM,350}$ (m^{-1})	$S_{275–295}$ (nm^{-1})	$S_{350–400}$ (nm^{-1})
Kinvara				
SGD	49.7 ± 19.4	12.7 ± 4.7	0.0142 ± 0.0017	0.0165 ± 0.0070
Seawater	9.4 ± 3.9	1.4 ± 1.1	0.0262 ± 0.0104	0.0279 ± 0.0213
Corrib				
River	31.7 ± 2.9 (2)	6.4 ± 1.6	0.0185 ± 0.0026	0.0170 ± 0.0024
Seawater	12.1 ± 5.3	1.8 ± 0.9	0.0230 ± 0.0017	0.0285 ± 0.0021
Lough Furnace				
Surface	72.2 ± 20.3 (64)	20.4 ± 6.3	0.0133 ± 0.0008	0.0164 ± 0.0012
Deep	41.8 ± 11.1 (43)	10.8 ± 3.2	0.0148 ± 0.0011	0.0178 ± 0.0024
Shannon				
River	56.8 ± 26.5 (4)	12.7 ± 6.4	0.0158 ± 0.0011	0.0182 ± 0.0004
Seawater	17.2 ± 6.2 (4)	3.4 ± 1.4	0.0191 ± 0.0040	0.0210 ± 0.0071

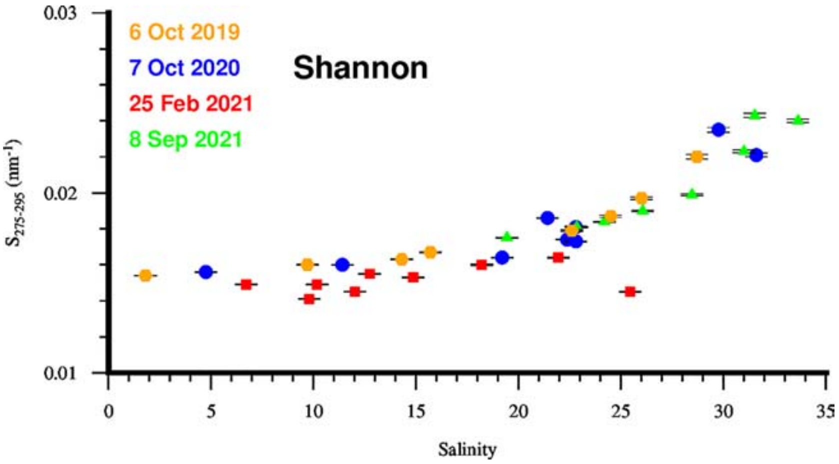

Figure 4.2. Spectral slope $S_{275–295}$ as a function of salinity for transects in the Shannon estuary. The error estimates for the spectral slope are included as vertical bars.

This is consistent with global observations where the increase in $S_{275–295}$ is interpreted as being due to photobleaching of the organic material and a shift in a portion of the FDOM composition from high MW to low MW.

Data from Kinvara (Figure 4.3) are complicated to assess, as the full range of salinity was not always observed. Overall, though, it does seem that there is significant removal of CDOM supplied from SGD into the bay. Non-conservative behaviour of CDOM in Kinvara Bay was also a finding in the earlier work of Kelly (2018), although comparison with that study is complicated, as no transects along the bay were made, because data were collected over tidal cycles at fixed sites and the author chose to use CDOM absorbance at 360 nm as their proxy for DOC.

Spectral slope $S_{275–295}$ values in Kinvara Bay (Figure 4.4) were similar to those found in an earlier study (Kelly, 2018).

Variations in $S_{275–295}$ at the seawater end of Kinvara Bay are likely to represent mixing of surface waters from Galway Bay that have had longer exposure times to sunlight, becoming progressively more photobleached with time, as the highest values of $S_{275–295}$ were usually found during late summer.

CDOM data from the Corrib plume (not shown here) were similar to those from the Shannon estuary and Kinvara Bay, and showed near-conservative behaviour along the plume and an increase in $S_{275–295}$ seaward.

CDOM data in Lough Furnace (not shown) were remarkably similar throughout the lough, mostly due

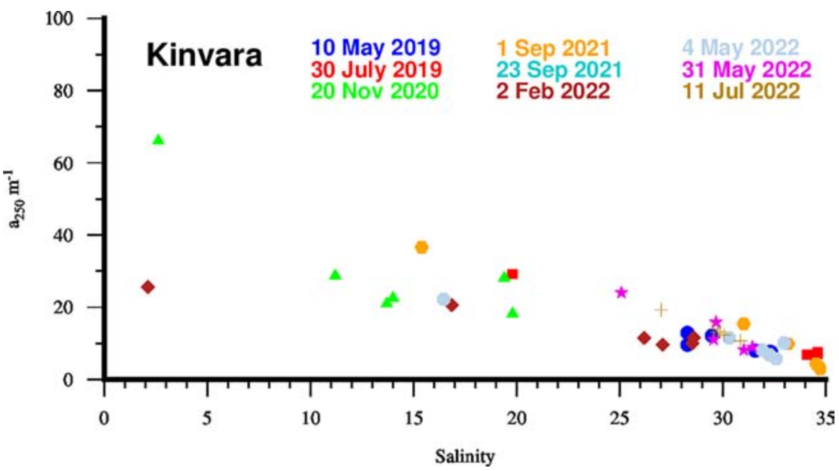

Figure 4.3. CDOM absorbance ($a_{CDOM,250}$ m^{-1}) as a function of salinity for transects in Kinvara Bay.

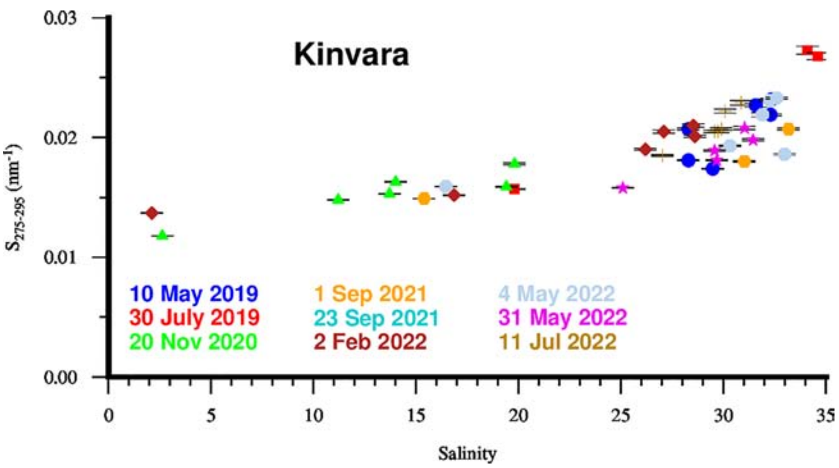

Figure 4.4. Spectral slope $S_{275–295}$ as a function of salinity for transects in Kinvara Bay. The error estimates for the spectral slope are included as vertical bars.

to the lack of any horizontal salinity gradient, as the incoming freshwater from Lough Fee does not mix with seawater in the surface waters of Lough Furnace. Values of $a_{CDOM,250}$ were high throughout the surface waters, as expected for a humic-rich environment. While there was a distinct vertical gradient in $a_{CDOM,250}$, with highest values in the surface waters, $S_{275–295}$ varied very little (0.014 ± 0.001), indicating that riverine humic material dominated above and below the halocline and that there was little photobleaching occurring, likely to be consistent with the deep waters of the halocline receiving no UV or near-UV light.

4.2 Fluorescent Dissolved Organic Matter as a Tracer of Organic Matter in Irish Marine Transition Zones

Sample fluorescence was normalised to daily measurements of the Raman-induced fluorescence of water (excitation 350 nm) (Stedmon *et al*., 2003). The normalised EEMs were analysed using the PARAFAC procedure in MATLAB using the DOMFluor toolbox (Stedmon and Bro, 2008). No samples were removed from the dataset. Data from this project are presented in Raman units using the Raman fluorescence of pure water (Heller *et al*., 2013; Lawaetz and Stedmon, 2009).

Using split half analysis, three components were validated (Figure 4.5).

Component 1 (ex 320, em 420) was similar to peak M identified in earlier works (Coble, 1996; Stedmon *et al*., 2003) as a humic-like component. Component 2 (ex 375, em 475) was similar to peak C. The third component was similar to protein-like fluorescence from tryptophan but could also include some contributions from tyrosine-like fluorescence that was not adequately resolved.

Table 4.2 shows the humic flouresence components identified from the Nuts & Bolts dataset.

Table 4.3 shows the typical values for the components identified by PARAFAC analysis.

The distribution of the three FDOM components identified by PARAFAC analysis are shown in Figures 4.6 (Shannon estuary) and 4.7 (Kinvara Bay). The two humic species, C1 and C2, both exhibited quasi-conservative behaviour, with higher levels of

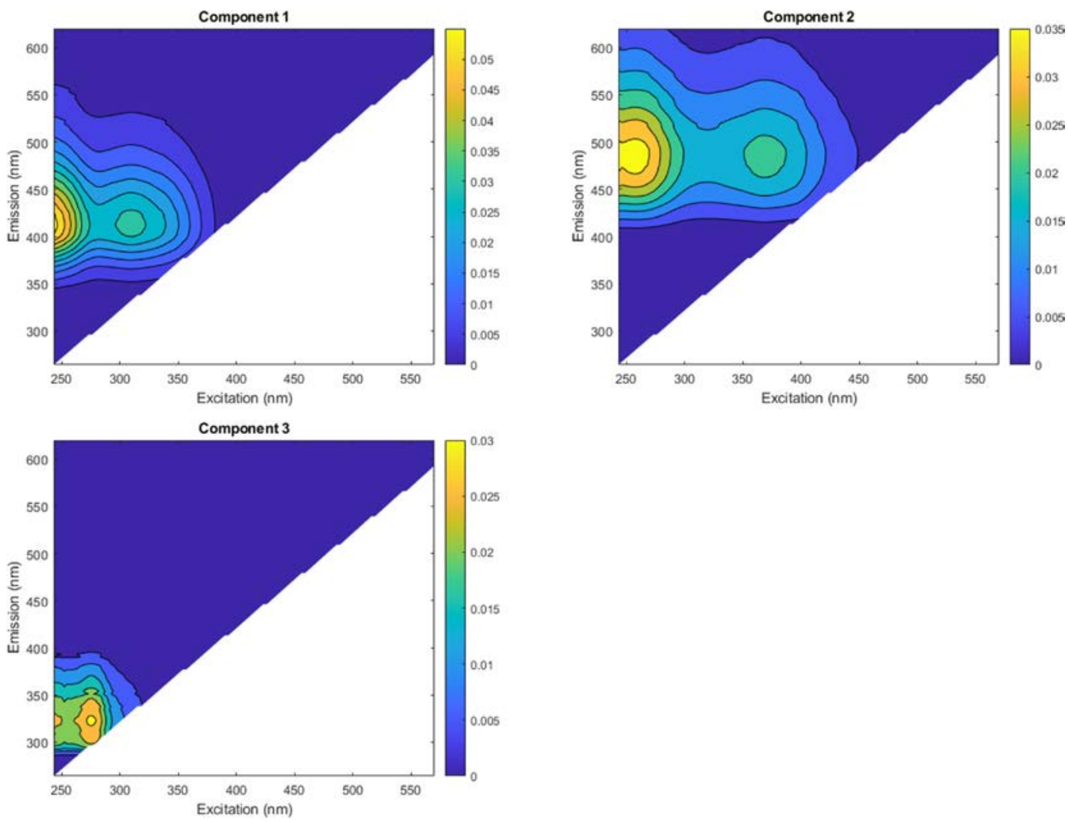

Figure 4.5. Optimised FDOM components from PARAFAC analysis of the Nuts & Bolts dataset (n = 324 – one sample omitted).

Table 4.2. Humic fluorescence components identified from the Nuts & Bolts dataset ($n=325$)

Component (ex/em)	Fluorescence characteristics (ex/em)	Description and probable source
C1 <240/410	290–310/370–410	Marine fulvic “M” peak (Coble, 1996)[a]
310/410	340/420	Marine humic material “C2” (Murphy *et al*., 2010)[b]
	315/418	
C2 250/490	<260/400–460	Terrestrial humic substance “C” peak (Coble, 1996)[a]
370/490		
C3 276/320	275/340	Tryptophan-like peak “T” (Coble, 1996)[a]
	280/328	
	280/330	

[a]Manual EEM interpretation.
[b]PARAFAC analysis.

Table 4.3. Typical values (Raman units) for the components identified by PARAFAC analysis in the different MTZ study regions

Location/nutrient	Component 1	Component 2	Component 3
Kinvara			
($n=106$)	0.264±0.261	0.147±0.170	0.110±0.066
Corrib			
($n=28$)	0.270±0.315	0.112±0.132	0.109±0.081
Lough Furnace			
($n=129$)	0.958±0.299	0.665±0.229	0.199±0.129
Shannon			
($n=62$)	0.734±0.529	0.391±0.299	0.184±0.132

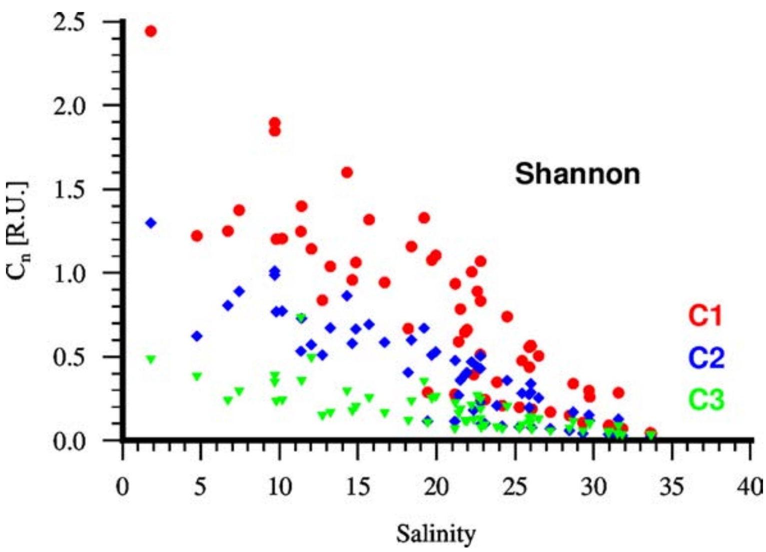

Figure 4.6. Distribution of the three FDOM components identified by PARAFAC analysis for the Shannon estuary.

FDOM in the Shannon estuary than in Kinvara Bay. Component C3 also appears to be quasi-conservative in the Shannon estuary with a riverine source, but not so in Kinvara Bay, where the sea endmember is often highest. Previous work in Kinvara (Kelly, 2018) also applied the PARAFAC procedure to FDOM

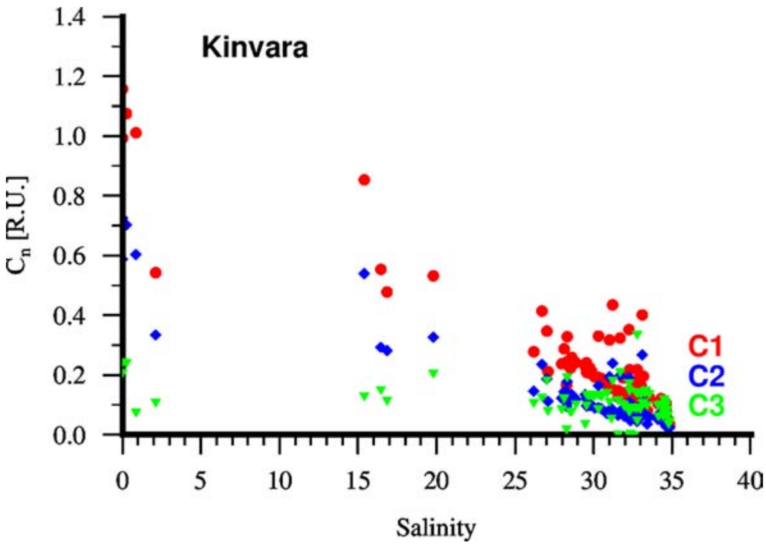

Figure 4.7. Distribution of the three FDOM components identified by PARAFAC analysis for Kinvara Bay.

measurements made there and identified three humic/fulvic components. The authors also found some protein-like fluorescence included in the 3D EEM for their component 3. That study also found that, while the overall DOC signal appeared to be dominated by humics, the maximum protein-like signal was found at the same time as maximum DOC concentrations (March and May), probably corresponding to times when fertilisation and agricultural wastes may have percolated into the groundwaters. Previous work has shown that the tyrptophan-like component can also be derived from zooplankton grazing.

4.3 Light Absorption by Particulate Material in Irish Marine Transitional Zones

Particulates in MTZs often dominate the variability in IOPs and apparent optical properties in these environments. Determination of the particulate absorption (a_p) via the quantitative filter technique (QFT) (Mitchell, 1990) provides an approach to gathering hyperspectral information on the absorption characteristics of particles. To calculate a_p the absorbance spectrum of the particles collected on filters is corrected for the pathlength amplification effect (β) (Mitchell, 1990; Roesler *et al*., 2018; Stramski *et al*., 2015). In the present work we used a 25 mm-diameter GF/F filter to collect the particles on after passing a known volume of sample. The absorbance of the filter was measured using a QFT holder (World Precision Instruments) in accordance with standard protocols. Our measurements of a_p include contributions from pigments and non-pigmented particles.

$$a_p = a_{ph,\lambda} + a_{pa,\lambda}$$

We are aware of only one previous study on particulate absorption in Irish MTZs (Darecki *et al*., 2003), where some samples from Galway Bay were reported. In that study the absorption was dominated by phytoplankton pigments, while in the Nuts & Bolts project most spectra had small contributions from phytoplankton pigments, as evidenced by small peaks at 670 nm due to chlorophyll *a*.

The utility of using the QFT approach became apparent when examining samples from the deep waters in Lough Furnace, where the usual chlorophyll peak at 670 nm was not found (Figure 4.8), and instead a strong absorption was seen at 720 nm. The peak at 720 nm is from the bacteriochlorophylls contained in the GSB found at this location (see section 3.1.3).

After measurement, all particulate filters were extracted in 90% acetone at –20°C overnight, and the resulting solution was measured using spectrophotometry. The chlorophyll concentration in the extract was calculated using the equations of Jeffrey and Humphrey (1975). For the samples from Lough Furnace where GSB were present, the chlorophyll *a* peak (663 nm) was no longer present and instead a new peak at 653 nm occurred (Figure 4.9). This peak is consistent with that of

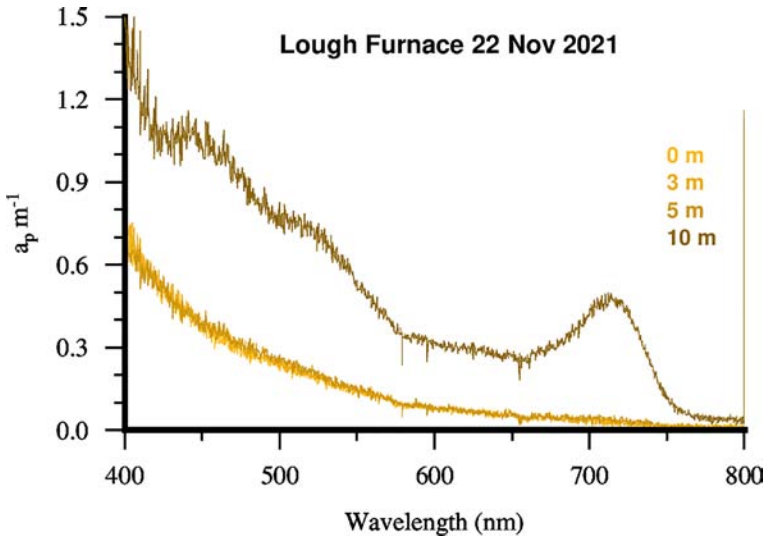

Figure 4.8. Particulate filter absorption at depth for station 2 in Lough Furnace.

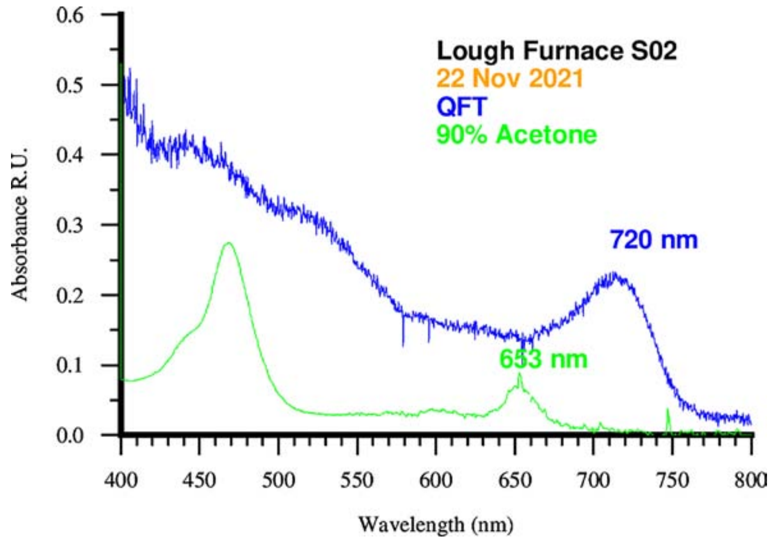

Figure 4.9. Lough Furnace – S02 sample from 10 m. Particulate absorption spectrum (blue) and spectrum for pigments extracted in 90% acetone (green).

bacteriochlorophyll *e* (Borrego *et al*., 1999) in GSB (brown), which have previously been identified in Lake Cadagno (Posth *et al*., 2017) and in the Black Sea (Manske *et al*., 2005). Estimates of the bacteriochlorophyll *e* concentrations were made using the dichromatic equations of Overmann and Tilzer (1989) for chlorophyll *a* and bacteriochlorophylls *d* and *e*, although it is noted that this probably overestimates the true concentrations (Henderson, 2015).

There are some indications based on the FCM counts for GSB, a_p measurements and extracted pigments that during the course of sampling the number of GSB was increasing, presumably as there were no reoxygenation events during this time (Kelly *et al*., 2020).

4.4 Hyperspectral Measurements of Light Attenuation in Irish Marine Transitional Zones

In this work the light attenuation in the water column was measured at each of the study sites using a TriOS RAMSES hyperspectral radiometer (ACC-2 VIS: 320–950 nm) equipped with pressure and inclination sensors. The sensor was lowered by hand through the water column while connected to a protective frame via cables, and the irradiance at each wavelength ($W\,m^{-2}\,nm^{-1}$) was recorded via a real-time interface on a deck unit. Data were collected from all sites but some difficulties were encountered at highly turbid sites due to the combination of rapid attenuation of light in the water column and/or wave action, which resulted in insufficient resolution of data in the vertical being obtained. In particular, in low salinity waters where the turbidity maximum was located, K_d values were often greater than $5\,m^{-1}$ (first optical depth 20 cm), which represents an upper level of detection for the system we employed during the Nuts & Bolts project. Figure 4.10 shows an example of the hyperspectral determination of K_d from the seaward end of the Shannon estuary. By combining the measurements of a_{CDOM}, a_p and a_{pw}, it is possible to estimate the contribution of each IOP to the value of K_d.

We are not aware of any previous hyperspectral studies in Irish MTZs; a study was carried out previously in the Shannon estuary (McMahon *et al*., 1992) and found that the depth attenuation coefficient for photosynthetically available radiation (400–700 nm) was highly correlated to the suspended matter concentration. In the present work (Figure 4.11) we found that K_d increased as salinity decreased and that the major contributor was backscatter b_λ in the photosynthetically available radiation range and a_{CDOM} in the UV.

FCM data in the Shannon revealed that at low salinity Syn-PC dominated, while at high salinity Syn-PE were dominant. Previous work (Stomp *et al*., 2004, 2007) had suggested that this distribution was in part due to changes in the light field. Figure 4.12 shows that this is also likely to be the case in the Shannon estuary, as the wavelength of maximum light penetration is red shifted moving landward and that there is a shift from favouring PE to PC and allophycocyanin.

For the major phytoplankton bloom detected at KA020 in Kinvara Bay in September 2021 (Figure 4.13), a_p and a_{CDOM} dominated over backscattering at this time. In general, backscattering made a much smaller contribution to K_d in Kinvara Bay (Figure 4.14).

The average Secchi depth in Lough Furnace was previously found to be 1.75 m by de Eyto *et al*. (2019). The chlorophyll maximum was found below the Secchi depth at 4 m, and in that study it was dominated by the dinoflagellates *Heterocapsa triquetra* and *Oxyrrhis marina*. During the Nuts & Bolts project the high concentrations of CDOM (humics) and suspended particles made hyperspectral measurements difficult due to the high values of K_d. Figure 4.15 shows results from the surface waters at station LF02 in November 2021. In contrast to most other marine environments, in Lough Furnace, red light was able to penetrate the deepest in the water column.

This in part explains the presence of GSB in Lough Furnace, as they were observed to have

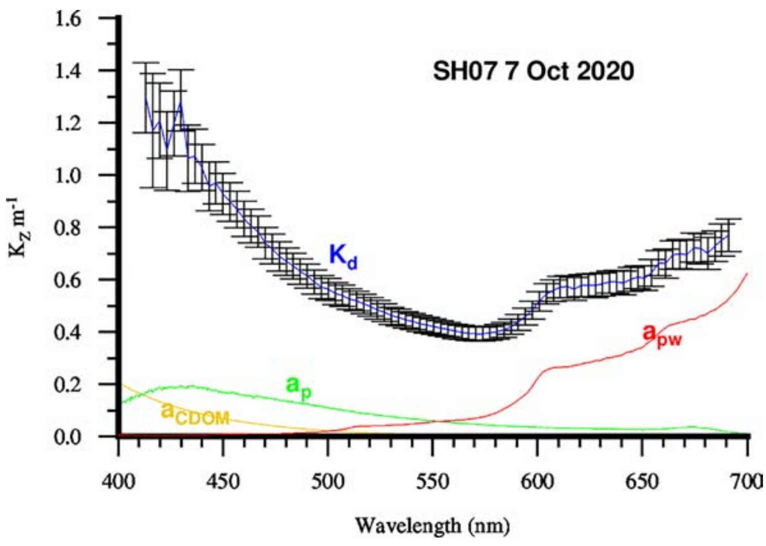

Figure 4.10. Diffuse attenuation coefficient (K_d) as a function of wavelength for the downward irradiance at SH07 in the Shannon estuary (7 October 2020). The contributions to K_d from a_{CDOM} (yellow), a_{pw} (red) and a_p (green) are also shown.

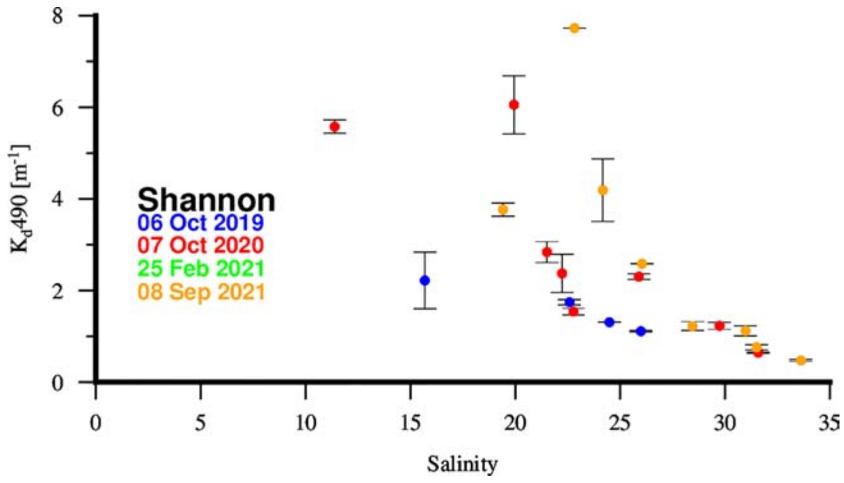

Figure 4.11. Diffuse attenuation coefficient (K_d) as a function of wavelength for the downward irradiance at SH07 in the Shannon estuary (7 October 2020). The contributions to K_d from a_{CDOM} (yellow), a_{pw} (red) and a_p (green) are also shown.

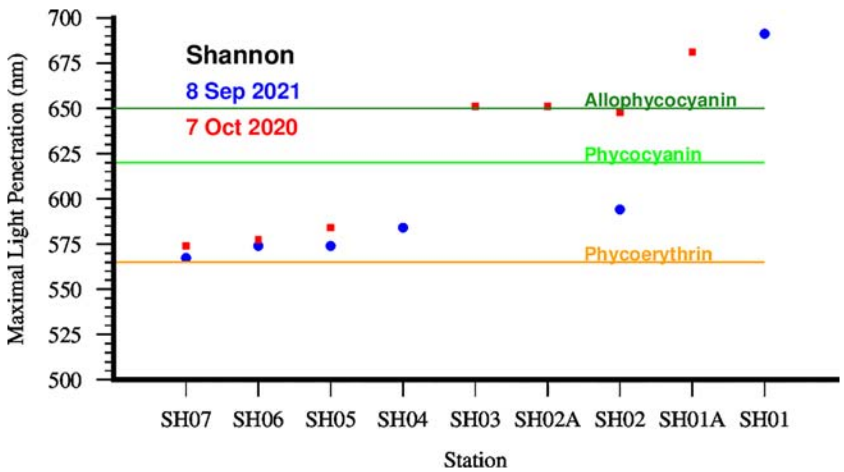

Figure 4.12. Wavelength of the maximum light penetration along the Shannon estuary. The horizontal lines represent the wavelength of maximum absorbance for the three accessory pigments found in *Synechococcus* sp.

maximum absorption at 720 nm via the pigment bacteriochlorophyll *e*, and so they are able to occupy a specialised niche in this meromictic lagoon. Using the data collected here, we can use the observed value of K_{720} 1.55 ± 0.21 m^{-1}, which would imply a light level of 1.9 × 10^{-7} of that at the surface at 10 m depth, considerably below the usual definition of a 1% light level for the euphotic depth. However, this is likely to be an underestimate, as the saline layer has less suspended material and CDOM content.

Under precisely controlled laboratory conditions, an enriched green sulfur bacterium culture proved to be capable of exploiting light intensities as low as 0.015 µmol quanta m^{-2} s^{-1} for photosynthetic $^{14}CO_2$ fixation (Manske *et al*., 2005). Calculated *in situ,* doubling times of the green sulfur bacterium range between 3.1 and 26 years depending on the season, and anoxygenic photosynthesis contributes only 0.002% to 0.01% to total sulfide oxidation in the chemocline. These authors noted that the population of GSB in the Black Sea chemocline represented the most extremely low-light-adapted and slowest-growing type of phototroph known to date (Manske *et al*., 2005). It is likely, then, that the GSB found in Lough Furnace are also slow-growing, and further work is needed to ascertain how they are impacted during reoxygenation events and how quickly they can build back up to a stable population.

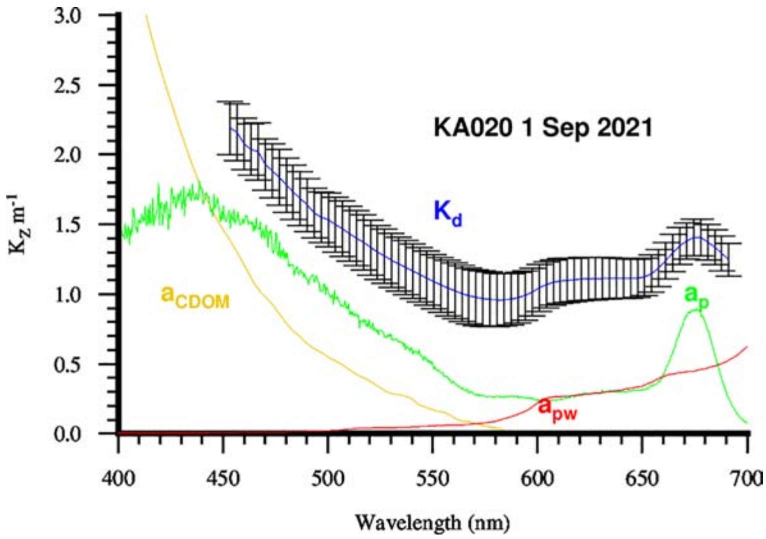

Figure 4.13. Diffuse attenuation coefficient (K_d) as a function of wavelength for the downward irradiance at KA020 in Kinvara Bay (1 September 2021). The contributions to K_d from a_{CDOM} (yellow), a_{pw} (red) and a_p (green) are also shown.

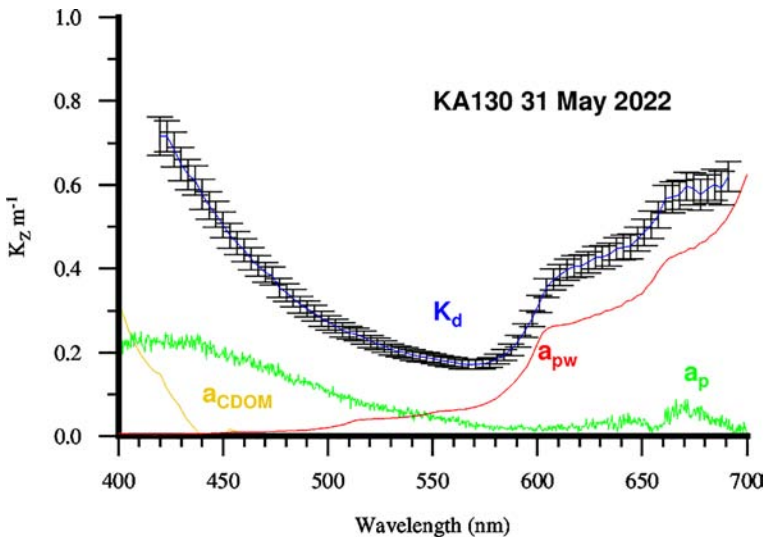

Figure 4.14. Diffuse attenuation coefficient (K_d) as a function of wavelength for the downward irradiance at KA130 in Kinvara Bay (31 May 2022). The contributions to K_d from a_{CDOM} (yellow), a_{pw} (red) and a_p (green) are also shown.

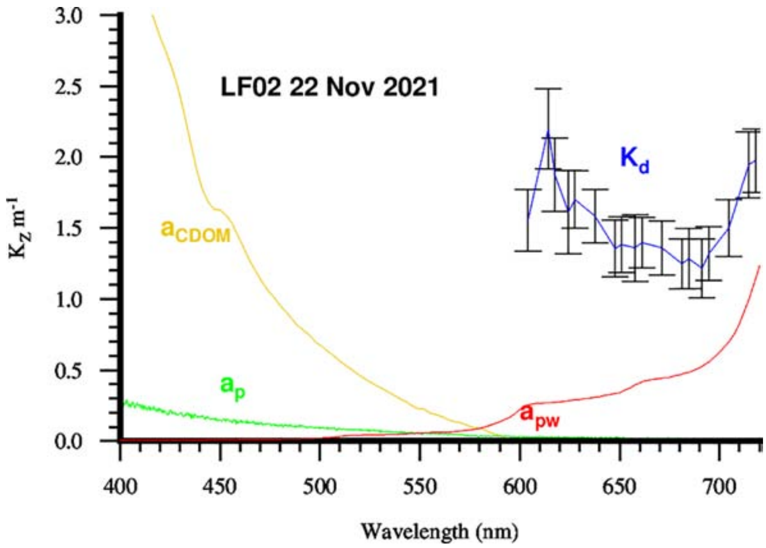

Figure 4.15. Diffuse attenuation coefficient (K_d) as a function of wavelength for the downward irradiance at LF02 in Lough Furnace (22 November 2021). The contributions to K_d from a_{CDOM} (yellow), a_{pw} (red) and a_p (green) are also shown.

5 Climate-relevant Gases in Irish Marine Transitional Zones

MTZs are very important zones for the biogeochemical cycling, and air–sea exchanges, of climate-relevant gases (Upstill-Goddard, 2011). In the present study we undertook a preliminary investigation of the abundance and distribution of some climate-relevant gases found in our study regions using a Hiden HPR-30 DSA membrane inlet mass spectrometer (MIMS). MIMS is a well-established technique that can directly analyse volatile compounds in aqueous solutions. It employs a thin polymer membrane that separates the liquid sample and the vacuum of a mass spectrometer. The volatile compounds permeate through the membrane and evaporate into the vacuum (vapour phase), where they are detected by a quadrupole mass spectrometer (Burlacot *et al*., 2020; Kana *et al*., 1994).

Sampling for dissolved gases was performed as per GO-SHIP protocols for oxygen sampling at sea. In brief, samples were drawn first from the Niskin bottles as soon as they were recovered, before taking any other samples. A flexible plastic drawing tube was connected to the nipple of the petcock on the water sampler and then pushed in to start the flow of water. Any air bubbles within the tubing were dislodged by squeezing or tapping the plastic tube. A 12-mL Labco Exetainer (www.labco.co.uk) was then filled by inserting the end of the drawing tube into the bottom of the Exetainer. The sample was rinsed twice and then allowed to overflow to the top. The cap was then screwed on to the top of the Exetainer and the sample was checked for any bubbles present. Samples were then returned to the laboratory as soon as possible. Exetainers used in this way have been found to be acceptable for storing most climate-relevant gases for short time periods (Faust and Liebig, 2018; Sturm *et al*., 2015). Once back in the laboratory, samples were kept in a refrigerator until just prior to analysis, when they were placed in a 25°C water bath. The top of the vial of the Exetainer was opened immediately prior to insertion of the MIMS probe into the sample.

In the course of the present work we investigated a number of methods for preserving the gas composition of the samples collected in the Exetainers. Due to the transit times from some of the sampling sites to the laboratory in Galway, we anticipated that there may be some loss of O_2 and gain of CO_2 due to respiration in the samples (i.e. from biological or chemical oxygen demand). In order to minimise risks to the sampling team we decided at this stage not to pursue the use of $HgCl_2$ to poison the samples, as is done for O_2 (Wassenaar and Koehler, 1999) and for CO_2 system samples that are to be stored (Dickson *et al*., 2007). In addition, chemical reactions resulting from the presence of reduced metal species may also impact dissolved O_2 concentrations over time and would not be altered by the use of $HgCl_2$ (Köhler *et al*., 2021). In the present work we trialled three approaches: (i) no preservation, (ii) addition of $ZnCl_2$ (Wilson *et al*., 2020) and (iii) addition of benzalkonium chloride (Gloël *et al*., 2015). We found that $ZnCl_2$ altered the pH of the samples, impacting CO_2 measurements. Benzalkonium chloride was previously found to be useful for short storage times (Gloël *et al*., 2015); this also appeared to be the case in our study using the Exetainers, but more work is needed to optimise this aspect of the sampling.

5.1 Oxygen and O_2:Ar Ratios – Net Community Production

The main application of the MIMS in this study was to investigate processes involving dissolved O_2 in MTZ waters; as O_2 is a product of photosynthesis (autotrophy) and is consumed during respiration (heterotrophy), the net balance between these processes represents whether an ecosystem is net-autotrophic (net community production (NCP) > 0) or net-heterotrophic (NCP < 0). Previously, O'Boyle *et al*. (2013) used measurements of pH and dissolved oxygen to develop a simple index for assessing the trophic status of Irish MTZs and found that their index was strongly correlated to observations of chlorophyll and BOD.

The advantage of using the MIMS here is that it is possible to measure other dissolved gases at the same time. Argon (Ar) is an inert noble gas that is not removed or produced by biology, and its concentration in natural waters is impacted only by temperature (solubility) and the physical mixing processes. By normalising O_2 concentrations to Ar, the resulting O_2:Ar ratio in water can be used for making NCP (Craig and Hayward, 1987) or respiration estimates (Barone *et al*., 2019). For example, in Kinvara Bay (Figure 5.1) in March 2021, low O_2:Ar ratios at station KA020 indicated that the waters closest to the main area of SGD were deficient in O_2 due to strong community respiration, presumably fuelled by inputs of labile DOC. Further seaward, the O_2:Ar values indicated that there was net productivity in the surface waters at the end of Kinvara Bay (KA040 and KA060), with values close to equilibrium in Galway Bay itself (KA120 and KA130).

Other recent work in Kinvara Bay (Guerra, 2022) utilised measurements of total alkalinity and dissolved inorganic carbon (DIC) as an alternative method to estimate NCP. Guerra found that there was a net export of total alkalinity and DIC to Galway Bay in autumn and winter, indicating negative NCP and a heterotrophic system, probably driven by freshwater inputs into the bay. In the spring and summer, Guerra observed a positive NCP and over an annual cycle the bay was considered to be autotrophic. Kinvara Bay has previously shown a high level of BOD and low water column oxygen concentrations during the summer (O'Boyle *et al*., 2009; Rocha *et al*., 2015).

Due to logistical constraints during the Covid-19 pandemic, no samples for analysis by the MIMS were taken from the Corrib plume. Samples from the Shannon estuary were obtained on two occasions (October 2019 and February 2021), and all showed near equilibrium values for O_2:Ar, although there was also some indication of respiration artefacts during transport in unpoisoned samples. Data from Lough Furnace indicated that the O_2:Ar ratio in surface waters was at close to equilibrium values, while samples from the deep waters had little or no O_2 present and were difficult to quantify at these low levels due to the uptake of O_2 from the air into the sample over the course of the analysis. Further work is under way in the laboratory to minimise these and other sampling and analysis issues indicated here.

A small pilot study was also undertaken into the applicability of measuring the isotopic composition of dissolved oxygen (i.e. m/z 32 $^{16}O^{16}O$, m/z 33 $^{16}O^{17}O$, m/z 34 $^{16}O^{18}O$ and m/z 36 $^{18}O^{18}O$) as a means to assess different processes impacting O_2 cycling in MTZs and coastal waters (Mader *et al*., 2017). Isotope enrichment experiments using $H_2{}^{18}O$ were also performed to assess gross primary productivity via the formation of $^{16}O^{18}O$. Future work will focus on applying these techniques more regularly to work in Irish MTZs.

5.2 Dissolved CO_2 and CH_4 – Greenhouse Gases

The MIMS also allows the measurements of two important greenhouse gases, CO_2 and CH_4. Globally, MTZs are thought to be net sources of CO_2 (Cai, 2011) and CH_4 (Middelburg *et al*., 2002) to the atmosphere.

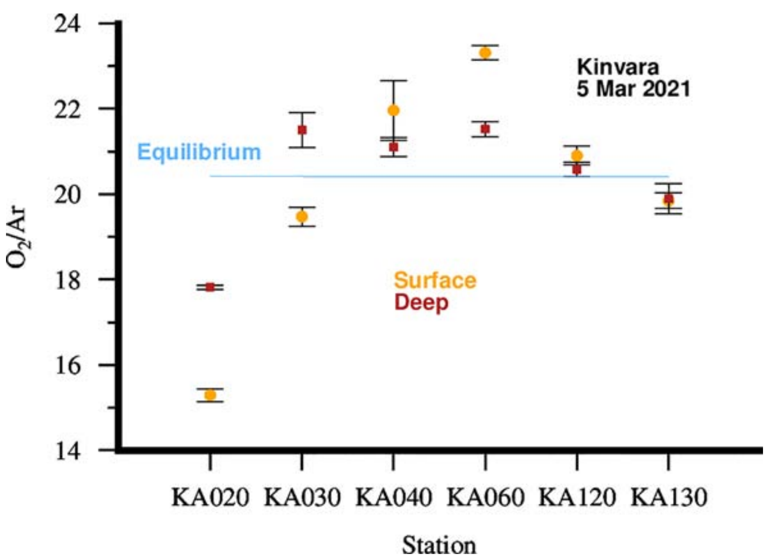

Figure 5.1. O_2:Ar values in Kinvara (5 March 2021).

The MTZs examined here all appeared to be sources of CO_2 to the atmosphere, as CO_2 concentrations measured in the water all exceeded the estimated concentration for equilibrium with the atmosphere. Figures 5.2 and 5.3 show the results of transects in the Shannon estuary for dissolved CO_2 concentrations and indicate that throughout the length of the estuary the water is oversaturated with CO_2 relative to the atmosphere (equilibrium concentrations range from 14.8 to 16 μmol L^{-1}), and the degree of oversaturation decreases moving seaward.

The Shannon estuary has previously been shown to be a CO_2 source to the atmosphere in winter based on measurements of alkalinity and DIC (TCO_2) (McGrath *et al.*, 2016, 2019). Further work is ongoing to provide estimates of the CO_2 flux to the atmosphere during each transect.

Lough Furnace (Figure 5.4) surface waters were also found to be a source of CO_2 to the atmosphere. CO_2 concentrations were elevated in the deep, high-salinity waters, as expected due to low-oxygen conditions. Samples of CO_2 from below the halocline were also observed to have more negative $\delta^{13}C$ values based on lower ratios for m/z 45 to m/z 44. A shift to more negative $\delta^{13}C$ has been observed in other marine meromictic systems such as Jellyfish Lake in Palau (Lyons *et al.*, 1996). Future work will look to better quantify this effect using appropriate reference materials.

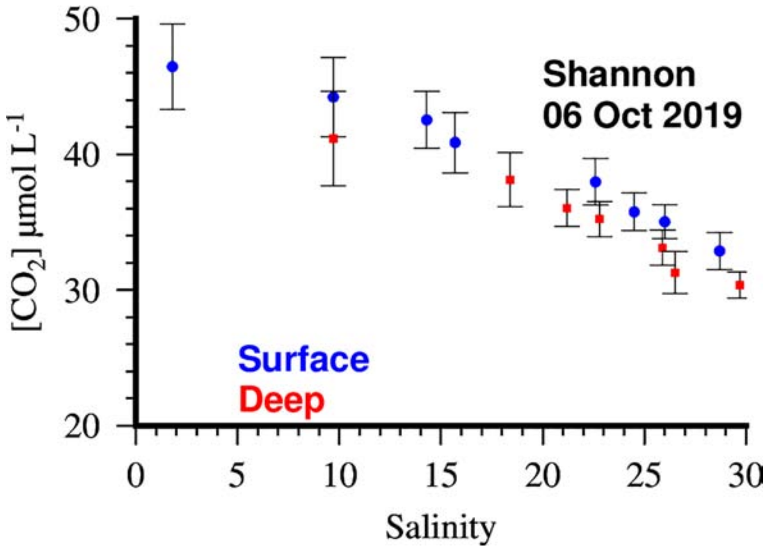

Figure 5.2. Dissolved CO_2 in the Shannon estuary (October 2019).

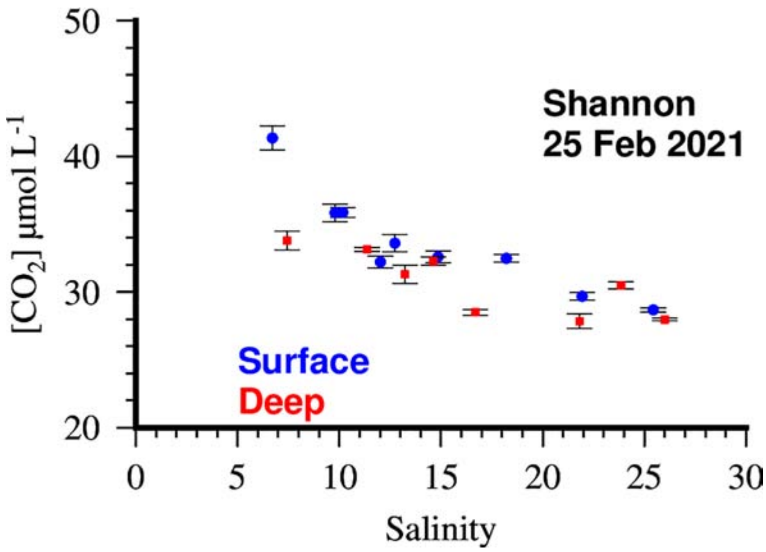

Figure 5.3. Dissolved CO_2 in the Shannon estuary (February 2021).

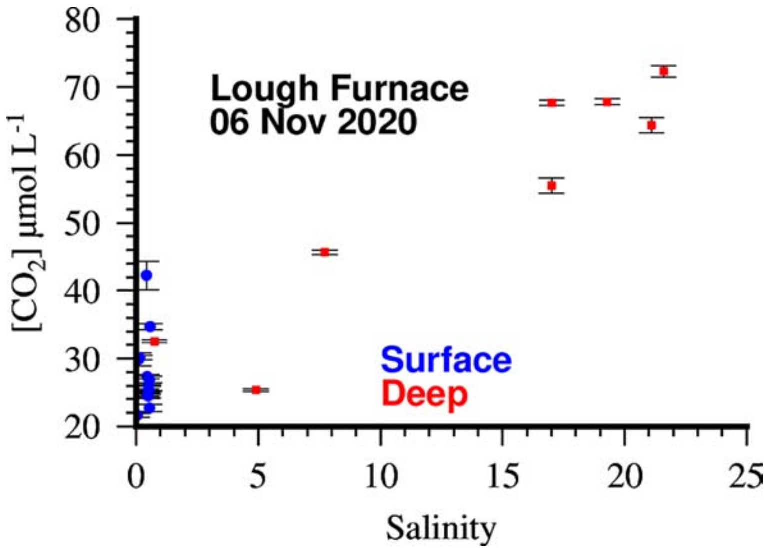

Figure 5.4. Dissolved CO_2 in Lough Furnace (November 2020).

Previous studies of pCO_2 in surface waters of Lough Feeagh, which feeds into Lough Furnace, have shown a seasonal late summer peak related to the export of DOC from the surrounding catchment (Doyle *et al*., 2021).

CH_4 was not detected in any samples during the course of the Nuts & Bolts project. The detection limit using the MIMS set-up employed during Nuts & Bolts was approximately 100 nM. CH_4 and CO_2 are typically found at significant concentrations in meromictic lakes (Fuchs *et al*., 2022), and CH_4 bursts are common when such lakes turnover during "blue tide" events (Sasaki *et al*., 2022). Measurements using the same MIMS system on peatbog waters have been able to detect CH_4 at µM levels. Recent improvements to the sampling system have lowered the detection limit for CH_4 to nM levels. It is highly likely that there is significant production of CH_4 in Lough Furnace, but this will require a more dedicated study in the future, as concentrations probably go to near-zero after reoxygenation events and then build up again over time as oxygen levels decline again.

5.3 Dimethyl Sulfide and Methanethiol

Dimethyl sulfide (DMS; CH_3SCH_3) and methanethiol (CH_3SH) are volatile sulfur compounds produced by many marine organisms and are key components of the marine biogenic sulfur cycle (Jackson and Gabric, 2022). DMS accounts for up to 80% of global biogenic sulfur emission, and its influence on cloud microphysics led to the "CLAW" hypothesis of Charlson *et al*. (1987), in which it was hypothesised that a temperature-driven change in marine phytoplankton growth would increase DMS emissions to the atmosphere. The DMS would be oxidised to form sulfate aerosol, which could potentially form more cloud condensation nuclei and brighter clouds. This increase in clouds would then act to cool the Earth's surface and act as a climate feedback mechanism against perturbations due to greenhouse gas warming. However, there is still no consensus over the global validity of this hypothesis (Ayers and Cainey, 2007; Jackson and Gabric, 2022).

Samples collected from the Shannon estuary showed no detectable DMS (m/z 62) or methanethiol (m/z 47). In Kinvara there were detectable DMS concentrations in July 2019 (Figure 5.5), with the highest concentrations (~100 nM) seen at the freshwater end of the bay (station KA020), coincident with a high concentration of nano-/microplankton, as evidenced by FCM (Nano2 abundance 1.5×10^6 cells L^{-1}). The *Rebecca* species that was isolated from this location in 2021 does release DMS in culture and so it is likely that the source of this DMS was the algae in the water column, although other sources such as macroalgae are also possible (Van Alstyne and Puglisi, 2007). There was no detectable DMS (< 5 nM) in Kinvara in March 2021.

A strong m/z 47 (methanethiol) signal was observed at KA020S on 13 September 2019, coincident

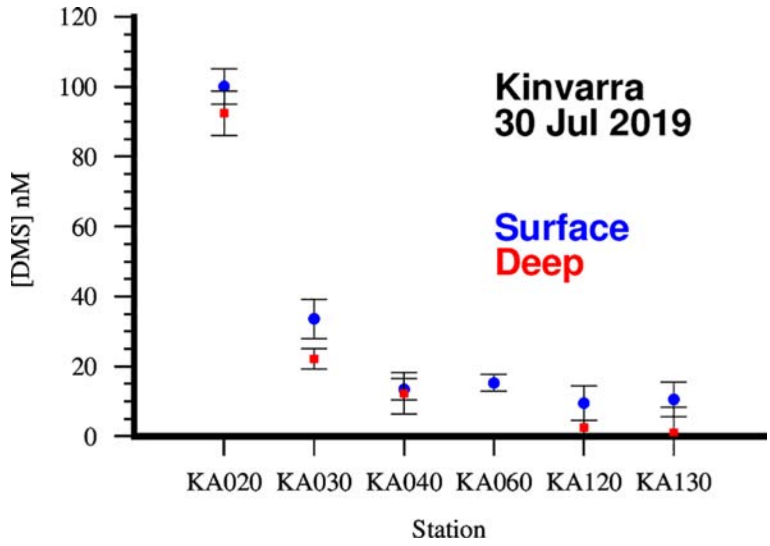

Figure 5.5. DMS in Kinvara, 30 July 2019.

with brown-coloured water; however, there was no significant large nano-/microplankton population or an accompanying DMS signal. Salinities at this time were quite low (8.9 at KA020 in the surface waters), indicating strong input of SGD and a likely lowering of the water pH. The likely increase in pCO_2 would have resulted in a greater contribution for m/z 47 from $^{13}C^{16}O^{18}O$. This is also what we suspect was the reason for elevated m/z 47 in Lough Furnace (Figure 5.6). We are currently looking into using the related isotopes m/z 44 ($^{12}C^{16}O^{16}O$) and m/z 45 ($^{13}C^{16}O^{16}O$) to separate this effect out.

We had initially expected to detect reduced thiols effluxing from the sediments in the low-oxygen waters of Lough Furnace, but no significant concentrations of DMS were observed. We did, however, detect some trace amounts of H_2S (m/z 34) upon acidification of the samples, which may complicate detailed studies of O_2 isotopes (m/z 34 $^{16}O^{18}O$). It is likely that this H_2S is a key sulfur source for the GSB found in these low-oxygen waters. Recent upgrades to our MIMS system has enabled lower detection limits for all species, and in the future we may be able to detect other trace sulfur gases, including COS and CS_2.

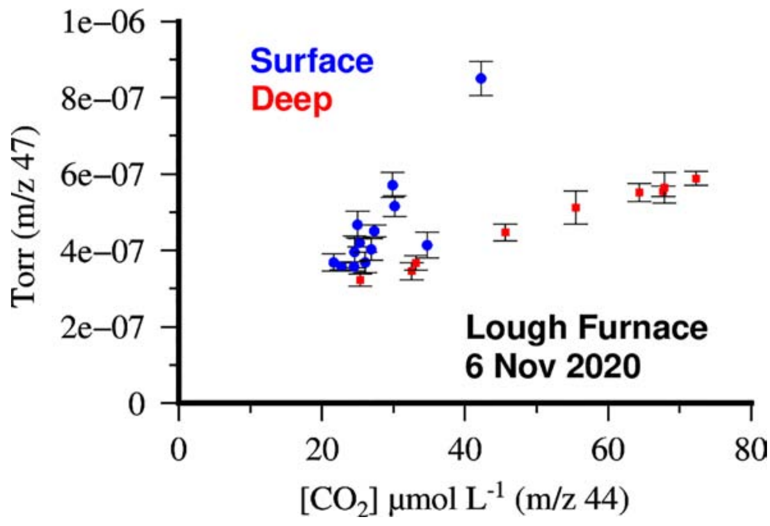

Figure 5.6. Variation of CO_2 (m/z 44) with m/z 47 in Kinvara in November 2020.

6 Knowledge Transfer Activities in Nuts & Bolts

During the Nuts & Bolts project a range of knowledge transfer activities took place as part of the project's outreach programme.

6.1 Schools

In 2022 and 2023, outreach to several schools in the west of Ireland based on aspects of the Nuts & Bolts project was facilitated by Benny Joyce. Benny introduced the students to the role of light in water and showed them how to construct and use a Secchi disk.

Examples of these outreach activities can be found online at https://www.nickerns.com/2023/01/30/marine-science-with-benny-joyce/.

The Nuts & Bolts project team is extremely grateful to Benny for his help and support with these activities.

6.2 Webpage and Social Media

The Nuts & Bolts webpage (http://nutsandboltsproject.ie/) and Twitter/X feed (@Riverflux2sea) provided a public-facing portal to what was going on in the project, particularly during field sampling campaigns. Both the webpage and X feed will continue to report on Nuts & Bolts findings and related activities after the project has formally finished.

6.3 Sustainability Lecture

In October 2022, the Nuts & Bolts project team hosted a public seminar at the University of Galway, led by Professor Karen Wiltshire, vice-director of the Alfred Wegener Institute in Germany, entitled "Coastal seas in the fast lane of climate change: resilience and adaptation for a sustainable future". This lecture was the third in the Royal Irish Academy Sustainability Series held in 2022. The lecture was held in collaboration with Integrated Marine Biosphere Research (IMBeR) and was a contribution to the United Nations Decade of Ocean Science for Sustainable Development (2021–2030).

6.4 Workshop on Sustainability of Marine Transitional Zones in Ireland

The Nuts & Bolts project team hosted an in-person workshop, the "Sustainability of marine transitional zones in Ireland", at the Aula Maxima at the University of Galway on 19 January 2024. The workshop was well attended, with 35 participants from all over Ireland, and included 11 presenters, each examining different aspects of pressures or activities in Irish MTZs. The workshop was an opportunity to present new results from the Nuts & Bolts project and include other researchers involved in MTZ work. Panel sessions on the "Sustainability of observations in the coastal environments in Ireland" (panellists: Brian Ward (University of Galway), Sean Cullen (Department of the Environment, Climate and Communications) and Deirdre Brophy (Atlantic Technological University)) and "Current and future governance of coastal zones – societal impacts" (Liam Carr (University of Galway), Ruairí Ó Conchúir (Local Authority Waters Programme) and Colm O'Dowd (CuanBeo)) were very lively and informative, with strong engagement from the other participants present.

The first panel discussion focused on the sustainability of observations in the coastal environment in Ireland. The general consensus was that we need better coordination of efforts in the coastal environment. Changing baselines require more detailed assessment of past conditions; this could be achieved by utilising natural archives that exist in sediment cores, otoliths, statoliths, shells, etc., and linking them to biogeochemical models. The application of artificial intelligence to issues related to MTZs has enormous potential, but this requires good data management, with an urgent need to improve and integrate datasets nationally and globally. Panellists also identified that more effort is needed to improve infrastructure and capacity-building nationally and to better utilise existing resources. Securing long-term funding and coordination support was a key challenge identified in supporting coastal observations. This includes making data open access and ensuring there are enough highly trained and qualified people to support the development of operational oceanography tools and services that we need in the future.

The second panel discussion examined the governance of coastal zones.

A common thread that emerged here was the lack of a government minister for the marine, and that within government there are multiple departments that have oversight of marine activities, with the result that there is a greater burden of bureaucracy, red tape and paperwork. This also often results in slow response times on the marine issues connected to climate change, despite the effects of climate change happening now and becoming more visible every year. Ireland needs to do more for coastal communities, as they are disempowered, and there is a clear need to provide better mechanisms to support local volunteers. Actions supporting resilience in MTZs should be "guided by science, driven by communities". A further theme that emerged was related to governing MTZs and looking to practices that were occurring on the land, as some of the bigger drivers of change in the MTZs occurred on land rather than taking place in the MTZs themselves.

A full report on the workshop was sent to IMBeR and the EPA. The participants at the workshop agreed to look to make this an annual event going forward, and this will be a legacy of the Nuts & Bolts project.

7 Conclusions and Recommendations

7.1 Summary and Conclusions

The Nuts & Bolts project has provided new data on picoplankton in Irish MTZs, which form an important part of the ecosystem that has been overlooked until now. Further to this, new information on the environmental stressors impacting Irish MTZs has been gathered, most notably on nutrient and metal fluxes, light field and potential greenhouse gas fluxes.

Examining the MTZ as a whole ecosystem, in an analogous fashion to the recent shift in river management to examining the whole catchment, provides a more complete picture of the bio-optical and biogeochemical processes occurring in MTZs. This work will therefore help to inform management decisions in each of the MTZs and allow a framework for future studies in other important Irish MTZs.

One specific example from the Nuts & Bolts project, with respect to improving management and governance of MTZs, comes from the finding that light plays a strong role in shaping phytoplankton and bacterial communities in Irish MTZs. While CDOM was a major contributor to the light attenuation in the MTZs we investigated, suspended sediment was also important. Continuous monitoring of CDOM absorbance and suspended sediment (i.e. via turbidity) levels in MTZs, using low-cost sensors, could provide real-time data for predicting hyperspectral light levels and phytoplankton abundance throughout the year. This would allow for more accurate assessment of the impacts of flooding events and storm surges on the coastal zone. It would also provide valuable data on the transport and deposition of sediment within Irish MTZs and enable the calculation of mass balances to assess the impact of climate change, most notably sea level rise, on the likely changes in geomorphology within MTZs in the future.

Changes to biogeochemical and bio-optical baselines are occurring now as a result of climate change, but these changes also have to be viewed within the prism of what has occurred previously due to human activity during the industrial age. Globally, it has been observed that the damming of rivers leads to a retention of silicate in the newly formed lake, which, by itself or in combination with eutrophication (due to run-off of nitrogen and phosphorus from the land), typically leads to a reduction in the flux of silica to MTZs and the coastal environment (Humborg *et al*., 2000). This loss of silica may tip the phytoplankton community towards a reduction in diatom abundance, allowing other species to move in. For the Shannon river, which has had a dam in place since 1929, the impact of this on the biogeochemical fluxes to the MTZ is unknown at present, and what the baseline was before that is even less clear. Information on pre-industrial age baselines for MTZs are important for understanding what we aspire to for GES, but they must be viewed in the context of the environmental stressors that were reduced or absent then.

The Nuts & Bolts project has provided, for the first time in Ireland, a view into the abundance and distribution of bacteria and pico- and nanoplankton and provided information on their growth rates, grazing rates and how they respond to changes in light, nutrients and temperature. From a management and governance perspective in Ireland, this information fills in a critical gap and allows for the inclusion of this important facet of MTZ ecosystems in planning for GES.

7.2 Recommendations for Future Monitoring and Research Gaps

The results of this study have highlighted the importance of a number of environmental stressors and processes that are not yet well elucidated or quantified for Irish MTZs. The following recommendations are made to address the critical gaps identified in the research:

- Include flow cytometric analysis of bacteria and pico- and nanoplankton abundances in HAB surveys, as also suggested recently for the UK (McQuatters-Gollop *et al*., 2024).
- Undertake eDNA or genomic assessment of the picoplankton and bacteria found in Irish MTZs. Use this approach to better identify the different FCM populations found in this study.

- Include biogeochemistry in ongoing modelling efforts for MTZs. A good approach would be to start with the LOICZ box model approach (Gordon *et al*., 1996; Swaney *et al*., 2011) for each of the Irish MTZs. For MTZs with existing hydrodynamic models (e.g. Galway Bay, Shannon estuary), look to implement the PISCES biogeochemical model or similar.
- Improve assessment of the nitrogen balance in MTZs by including all relevant nitrogen species and the fluxes between them, including benthic sources and sinks.
- Undertake quantitative studies of the transport of sediment, and its contribution to backscattering, in Irish MTZs in the context of sea level rise and coastal darkening.
- Utilise riverine and lake sediments to provide a recent history of changes to riverine fluxes of dissolved nutrients and suspended sediment to MTZs.
- Establish sentinel sites within Irish MTZs and establish time series and repeat transects for monitoring changes.

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Abbreviations

BOD	Biological oxygen demand
CDOM	Coloured dissolved organic matter
DIC	Dissolved inorganic carbon
DMS	Dimethyl sulfide
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EEM	Excitation emission matrix
FCM	Flow cytometry
FDOM	Fluorescent dissolved organic matter
GES	Good environmental status
GSB	Green sulfur bacteria
HAB	Harmful algal bloom
HNA	High nucleic acid content
HNF	Heterotrophic nanoflagellates
IOP	Inherent optical property
LNA	Low nucleic acid content
MIMS	Membrane inlet mass spectrometer
MTZ	Marine transitional zone
MW	Molecular weight
NCP	Net community production
PARAFAC	Parallel factor
PC	Phycocyanin
PE	Phycoerythrin
QFT	Quantitative filter technique
SGD	Submarine groundwater discharge
TN	Total nitrogen
TP	Total phosphate
UV	Ultraviolet

An Ghníomhaireacht Um Chaomhnú Comhshaoil

Tá an GCC freagrach as an gcomhshaol a chosaint agus a fheabhsú, mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaol a chosaint ar thionchar díobhálach na radaíochta agus an truaillithe.

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

Rialáil: Rialáil agus córais chomhlíonta comhshaoil éifeachtacha a chur i bhfeidhm, chun dea-thorthaí comhshaoil a bhaint amach agus díriú orthu siúd nach mbíonn ag cloí leo.

Eolas: Sonraí, eolas agus measúnú ardchaighdeáin, spriocdhírithe agus tráthúil a chur ar fáil i leith an chomhshaoil chun bonn eolais a chur faoin gcinnteoireacht.

Abhcóideacht: Ag obair le daoine eile ar son timpeallachta glaine, táirgiúla agus dea-chosanta agus ar son cleachtas inbhuanaithe i dtaobh an chomhshaoil.

I measc ár gcuid freagrachtaí tá:

Ceadúnú

- Gníomhaíochtaí tionscail, dramhaíola agus stórála peitril ar scála mór;
- Sceitheadh fuíolluisce uirbigh;
- Úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe;
- Foinsí radaíochta ianúcháin;
- Astaíochtaí gás ceaptha teasa ó thionscal agus ón eitlíocht trí Scéim an AE um Thrádáil Astaíochtaí.

Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil

- Iniúchadh agus cigireacht ar shaoráidí a bhfuil ceadúnas acu ón GCC;
- Cur i bhfeidhm an dea-chleachtais a stiúradh i ngníomhaíochtaí agus i saoráidí rialáilte;
- Maoirseacht a dhéanamh ar fhreagrachtaí an údaráis áitiúil as cosaint an chomhshaoil;
- Caighdeán an uisce óil phoiblí a rialáil agus údaruithe um sceitheadh fuíolluisce uirbigh a fhorfheidhmiú
- Caighdeán an uisce óil phoiblí agus phríobháidigh a mheasúnú agus tuairisciú air;
- Comhordú a dhéanamh ar líonra d'eagraíochtaí seirbhíse poiblí chun tacú le gníomhú i gcoinne coireachta comhshaoil;
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaol.

Bainistíocht Dramhaíola agus Ceimiceáin sa Chomhshaol

- Rialacháin dramhaíola a chur i bhfeidhm agus a fhorfheidhmiú lena n-áirítear saincheisteanna forfheidhmithe náisiúnta;
- Staitisticí dramhaíola náisiúnta a ullmhú agus a fhoilsiú chomh maith leis an bPlean Náisiúnta um Bainistíocht Dramhaíola Guaisí;
- An Clár Náisiúnta um Chosc Dramhaíola a fhorbairt agus a chur i bhfeidhm;
- Reachtaíocht ar rialú ceimiceán sa timpeallacht a chur i bhfeidhm agus tuairisciú ar an reachtaíocht sin.

Bainistíocht Uisce

- Plé le struchtúir náisiúnta agus réigiúnacha rialachais agus oibriúcháin chun an Chreat-treoir Uisce a chur i bhfeidhm;
- Monatóireacht, measúnú agus tuairisciú a dhéanamh ar chaighdeán aibhneacha, lochanna, uiscí idirchreasa agus cósta, uiscí snámha agus screamhuisce chomh maith le tomhas ar leibhéil uisce agus sreabhadh abhann.

Eolaíocht Aeráide & Athrú Aeráide

- Fardail agus réamh-mheastacháin a fhoilsiú um astaíochtaí gás ceaptha teasa na hÉireann;
- Rúnaíocht a chur ar fáil don Chomhairle Chomhairleach ar Athrú Aeráide agus tacaíocht a thabhairt don Idirphlé Náisiúnta ar Ghníomhú ar son na hAeráide;
- Tacú le gníomhaíochtaí forbartha Náisiúnta, AE agus NA um Eolaíocht agus Beartas Aeráide.

Monatóireacht & Measúnú ar an gComhshaol

- Córais náisiúnta um monatóireacht an chomhshaoil a cheapadh agus a chur i bhfeidhm: teicneolaíocht, bainistíocht sonraí, anailís agus réamhaisnéisiú;
- Tuairiscí ar Staid Thimpeallacht na hÉireann agus ar Tháscairí a chur ar fáil;
- Monatóireacht a dhéanamh ar chaighdeán an aeir agus Treoir an AE i leith Aeir Ghlain don Eoraip a chur i bhfeidhm chomh maith leis an gCoinbhinsiún ar Aerthruailliú Fadraoin Trasteorann, agus an Treoir i leith na Teorann Náisiúnta Astaíochtaí;
- Maoirseacht a dhéanamh ar chur i bhfeidhm na Treorach i leith Torainn Timpeallachta;
- Measúnú a dhéanamh ar thionchar pleananna agus clár beartaithe ar chomhshaol na hÉireann.

Taighde agus Forbairt Comhshaoil

- Comhordú a dhéanamh ar ghníomhaíochtaí taighde comhshaoil agus iad a mhaoiniú chun brú a aithint, bonn eolais a chur faoin mbeartas agus réitigh a chur ar fáil;
- Comhoibriú le gníomhaíocht náisiúnta agus AE um thaighde comhshaoil.

Cosaint Raideolaíoch

- Monatóireacht a dhéanamh ar leibhéil radaíochta agus nochtadh an phobail do radaíocht ianúcháin agus do réimsí leictreamaighnéadacha a mheas;
- Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as taismí núicléacha;
- Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta;
- Sainseirbhísí um chosaint ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

Treoir, Ardú Feasachta agus Faisnéis Inrochtana

- Tuairisciú, comhairle agus treoir neamhspleách, fianaise-bhunaithe a chur ar fáil don Rialtas, don tionscal agus don phobal ar ábhair maidir le cosaint comhshaoil agus raideolaíoch;
- An nasc idir sláinte agus folláine, an geilleagar agus timpeallacht ghlan a chur chun cinn;
- Feasacht comhshaoil a chur chun cinn lena n-áirítear tacú le hiompraíocht um éifeachtúlacht acmhainní agus aistriú aeráide;
- Tástáil radóin a chur chun cinn i dtithe agus in ionaid oibre agus feabhsúchán a mholadh áit is gá.

Comhpháirtíocht agus Líonrú

- Oibriú le gníomhaireachtaí idirnáisiúnta agus náisiúnta, údaráis réigiúnacha agus áitiúla, eagraíochtaí neamhrialtais, comhlachtaí ionadaíocha agus ranna rialtais chun cosaint chomhshaoil agus raideolaíoch a chur ar fáil, chomh maith le taighde, comhordú agus cinnteoireacht bunaithe ar an eolaíocht.

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil

Tá an GCC á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóir. Déantar an obair ar fud cúig cinn d'Oifigí:

1. An Oifig um Inbhunaitheacht i leith Cúrsaí Comhshaoil
2. An Oifig Forfheidhmithe i leith Cúrsaí Comhshaoil
3. An Oifig um Fhianaise agus Measúnú
4. An Oifig um Chosaint ar Radaíocht agus Monatóireacht Comhshaoil
5. An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tugann coistí comhairleacha cabhair don Ghníomhaireacht agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

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