Report No.447

Macroalgal Blooms in Transitional and Coastal Waters; Management – Pressures, Policy and Solutions (MACRO-MAN)

Authors: Ricardo Bermejo, Nessa Golden, Sara Haro, Sita Karki, Michéal MacMonagail, Sara García-Poza, Teresa Navarrete-Fernández, Benedikt Brunner, Kay Knöller, Mark Healy, Owen Fenton, Per-Erik Mellander and Liam Morrison.

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Rialtas na hÉireann
Government of Ireland

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5. Office of Communications and Corporate Services

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Identifying pressures

Eutrophication of waters and consequent algal blooms place significant pressure on marine ecosystems. Reducing the nutrient load of these waters is essential for ecosystem restoration.

The aim of the MACRO-MAN project was to develop innovative methods for the assessment of the environmental quality of Irish estuaries, and to identify drivers of and management strategies for macroalgal blooms. The potential risks associated with macroalgal blooms were considered in a global change context (e.g. climate change, emerging contaminants, biological invasions) in order to investigate the impact on ecosystem functioning and services provided by Irish estuaries.

Earth observation technologies from the European Space Agency and algorithms developed during the project were used to map the spatial and temporal distribution of brown, green and red macroalgal blooms in Irish estuaries, including the reconstruction of the invasion of a red Asian seaweed (Gracilaria vermiculophylla) in the Clonakilty estuary.

Based on the nitrogen isotopic signature of the bloom-forming species, agricultural sources of nitrogen were the biggest drivers of blooms in rural locations, while effluent from wastewater treatment plants was more relevant in urban areas. Tissue nitrogen and phosphorus content indicated that anthropogenic nitrogen enrichment of aquatic environments has produced a shift from a nitrogen- to a phosphorus-limited ecosystem.

Informing policy

From a policy perspective, the use of Earth observation technologies, including the use of free satellite imagery from the EU Copernicus and Landsat programmes for the monitoring of macroalgal blooms, including the spread of invasive bloom-forming species, is an important development. These technologies fulfil Irish legal obligations and comply with the EU Water Framework Directive and the EU Marine Strategy Framework Directive in terms of monitoring and assessment of the ecological and environmental status of transitional and coastal waters.

The assessment of the nutrient status of macroalgal blooms based on the analysis of the tissue nitrogen and phosphorus content, and the nitrogen isotopic ratio, will be a direct indicator of the nutrient status of transitional and coastal waters, allowing for suitable assessment of strategies aimed at reducing and controlling eutrophication by identifying the most relevant limiting nutrient.

Developing solutions

A key recommendation from this project is that Earth observation technologies and nutrient bioassessment should be included in the monitoring of macroalgal blooms in Ireland. Furthermore, we recommended controlling the spread of the alien red seaweed Gracilaria vermiculophylla, as the presence of this species could increase the extension and persistence of macroalgal blooms. We also recommended reducing nutrient loadings from agriculture and urban sources, and enhancing nutrient removal, actively and passively. In this sense, the restoration of oyster beds and seagrass meadows, which play an important role in nutrient and carbon sequestration, might be considered a mitigation strategy.

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Prepared for the Environmental Protection Agency

by

University of Galway

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This report is based on research carried out/data from 2018 to 2022. More recent data may have become available since the research was completed.

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Executive Summary

The MACRO-MAN project (MACROalgal blooms in transitional and coastal waters; MANagement – pressures, policy and solutions) aimed to develop and test innovative methods, including Earth observation technologies and biomonitors, for the monitoring of the environmental quality of Irish estuaries and the management of macroalgal blooms, and to assess the potential risks associated with global climate change that could impact ecosystem functioning and the services provided by Irish estuaries.

Using free-access satellite imagery (EU Copernicus and Landsat programmes), the suitability of different algorithms for mapping macroalgal blooms in Irish estuaries was assessed. Overall, the results were positive, although cloud coverage limited the use of satellite imagery for environmental monitoring in Ireland. A new tool based on the Normalised Difference Vegetation Index and machine learning was developed to map macroalgal blooms and estimate biomass abundance. Furthermore, using a pixel-based maximum likelihood classifier, it was possible to reconstruct the invasion of a red Asian seaweed (*Gracilaria vermiculophylla*) in the Clonakilty estuary (Co. Cork) and to map the *Pilayella littoralis* bloom affecting Killybegs harbour (Co. Donegal).

Based on tissue nitrogen and phosphorus content, the nutrient status of different macroalgal blooms was determined on a seasonal basis. Furthermore, using the isotopic ratio between lighter and heavier nitrogen isotopes ($\delta^{15}N$), the most likely sources of nutrient enrichment were determined. In most cases, the analysis of the tissue nitrogen and phosphorus content indicated phosphorus rather than nitrogen limitation during the peak bloom season (i.e. spring–summer). The $\delta^{15}N$ during the peak bloom period indicated agricultural (Clonakilty and Argideen) or urban (Tolka) wastewaters as the most likely sources of nitrogen. Considering that primary production in cold temperate coastal and estuarine areas under limited human disturbance is limited by nitrogen rather than phosphorus during the summer, these results support the hypothesis that anthropogenic nitrogen enrichment of aquatic environments has produced a shift from a nitrogen- to a phosphorus-limited ecosystem. Lastly, based on these results, some guidelines are provided for the bioassessment of macroalgal blooms in Ireland.

Different observational and laboratory-based experiments were conducted to assess the impact of the arrival of *G. vermiculophylla* on ecosystem functioning. The fauna associated with the *G. vermiculophylla* canopy was compared with that associated with *Ulva* blooms in Clonakilty Bay, which is affected by both *Ulva* and *G. vermiculophylla* blooms. A laboratory incubation was conducted to compare the biomass decay of *G. vermiculophylla* with different native macrophytes typical of estuarine environments (i.e. *Fucus vesiculosus, Ulva* spp. and *Zostera marina* var. *angustifolia*). Furthermore, a laboratory experiment was performed to assess the biotic interaction between *G. vermiculophylla*, *F. vesiculosus* and *Ulva flexuosa*. The results showed that the biodiversity associated with *G. vermiculophylla* blooms is similar to that associated with *Ulva* blooms. This alien species had the lowest decay rates, as *G. vermiculophylla* can survive for a while after being buried. Lastly, *U. flexuosa* (a native opportunistic species) was able to outcompete *F. vesiculosus* (a native late successional species) and *G. vermiculophylla* in the context of eutrophication. These results suggested that, although the impact of *G. vermiculophylla* is not greater than that of other native opportunistic species in a eutrophication context, the arrival of this species can increase bloom size and persistence.

The short-term effects of two widely used herbicides (i.e. MCPA and glyphosate) on two native late successional macrophytes (*F. vesiculosus* and *Z. marina*), sensitive to human pressures, were assessed in the laboratory. In this case, the results indicated no negative effects for the concentrations and timescales considered. Further research is necessary to determine effect and lethal concentrations (effective concentration 50% and lethal dose 50%) in these species, and to assess the long-term effects in ecologically relevant species such as *Fucus*, *Zostera* and *Ulva* spp.

Overall, we recommend including the use of Earth observation technologies and nutrient bioassessment

in the monitoring of macroalgal blooms in Ireland; controlling the spread of *G. vermiculophylla*, as the arrival of this species can increase the size and persistence of macroalgal blooms; and reducing nutrient loadings (especially nitrogen) and enhancing nutrient removal (active – harvesting of seaweed biomass; passive – ecological restoration), as the nutrient bioassessment indicated a shift in estuaries from a nitrogen- to a phosphorus-limited ecosystem during summer.

1 Introduction

Eutrophication remains one of the most important pressures in European marine ecosystems, and the reduction of nutrient loads is a key restoration measure (Hering *et al*., 2010; Boesch, 2019). In transitional water bodies, such as coastal lagoons or estuaries, the proliferation of fast-growing opportunistic macroalgal species, mainly ulvoids (Teichberg *et al*., 2010), is indicative of eutrophication (Scanlan *et al*., 2007). These accumulations, or blooms, of seaweeds have been called seaweed tides or seaweed blooms, and their presence has negative impacts on local economies and biodiversity (Smetacek and Zingone, 2013), producing massive mortalities of organisms of commercial interest and visual and olfactory impacts (Valiela *et al*., 1997; Sfriso and Facca, 2013) because of the associated reduction in dissolved oxygen concentrations in the water when the seaweed biomass is degrading. The decomposition and remineralisation of the organic matter reduces the oxygen concentration and increases the concentration of nutrients in pore water in the sediment, acting as a nutrient reservoir fuelling subsequent blooms, perpetuating the eutrophication problem (Corzo *et al*., 2009; Garcia-Robledo *et al*., 2016).

Nutrient release from agricultural land is already a significant source of surface water pollution (EPA, 2020, 2022), and is predicted to increase with an increasing demand for food. The government’s response, envisioned in Food Wise 2025, is for agricultural intensification to satisfy domestic demand and increase exports, possibly at the expense of achieving water quality and greenhouse gas emission targets. It is likely that this issue, in combination with other aspects of global change (i.e. seawater temperature increase, biological invasions, emerging contaminants), will result in increased eutrophication and hence increased incidence and severity of extraordinary macroalgal blooms as suggested by EPA modelling approaches (Dynamic Combined Phytoplankton and Macroalgae; Ní Longphuirt *et al*., 2016b; O’Boyle *et al*., 2017) and direct observations (Ní Longphuirt *et al*., 2015; Bermejo *et al*., 2019b). An increase of between two- and threefold in the biomass density of *Ulva* during the peak bloom period was observed in the green tide affecting the Tolka estuary from 1990 to 2016 (Bermejo *et al*., 2019b), and a proliferation in the spatial extent of macroalgal blooms has also been observed in the Clonakilty estuary as a result of the arrival of an alien species (Bermejo *et al*., 2022; see Chapter 2). Seaweed tides are thus anticipated to be a much more significant problem in the future if effective management actions are not developed (Ní Longphuirt *et al*., 2015; Shore *et al*., 2016; O’Boyle *et al*., 2017).

Seagrass meadows and perennial canopy-forming seaweeds (mainly fucoids) are considered to provide a broad range of ecosystem services such as carbon and nutrient burial, mitigating nutrient enrichment and improving water quality. Community shifts from perennial canopy-forming macrophytes to opportunistic macroalgal blooms have been observed in many temperate estuaries as a consequence of eutrophication (Valiela *et al*., 1997; Viaroli *et al*., 2008), having an important impact on biogeochemical cycles (i.e. greater remineralisation and reduced nutrient and carbon burial) and other environmental conditions (e.g. increase in water turbidity, anoxic crises, habitat simplification) that could lead to non-linear and positive feedback loops, amplifying the eutrophication process and preventing seagrass recolonisation (Koch, 2001). Although there is a lot of information about the effect of nutrients and opportunistic macroalgal blooms on seagrass meadows and fucoid canopies, little is known about the impact of other emerging contaminants associated with agricultural activities (e.g. pesticides) on these habitat-forming species.

To preserve the integrity of aquatic ecosystems and the services that they provide, and prevent further degradation, the EU has developed various directives, such as the Habitats Directive (92/43/EEC), the Birds Directive (2009/147/EC), Nitrates Directive (91/676/EEC), the Water Framework Directive (WFD; 2000/60/EC) and the Marine Strategy Framework Directive (MSFD; 2008/56/EC). The large expansion in monitoring required by these directives has resulted in governments putting pressure on their regulatory agencies to reduce the costs of monitoring while maintaining coverage and effectiveness (Carvalho *et al*., 2019; Karki *et al*., 2021). Remote sensing techniques offer enormous advantages by providing cost-effective systematic observations for measurements of water quality and its ecological effects (Sent *et al*., 2021). Furthermore, the existence of historical satellite imagery (i.e. since the late 1980s) allows the setting of baselines and the temporal assessment of different environmental processes, including the development of seaweed tides or biological invasions (Bermejo *et al*., 2020b; Choi *et al*., 2020; Karki *et al*., 2021; Schreyers *et al*., 2021).

1.1 Objectives: Overall

The main objectives were to develop and test innovative methods, including Earth observation (EO) technologies and bioindicators, for the monitoring of the environmental quality of Irish estuaries and the management of seaweed tides, and assess potential risks associated with global change that could impact ecosystem functioning and the services provided by Irish estuaries. Owing to unexpected circumstances associated with the COVID-19 pandemic and other issues, the original aims and objectives were modified in consultation with the project steering committee. The objectives affected most by these circumstances were those related to seagrass restoration and the creation of a network of citizen scientists. The following objectives were modified:

- use of multispectral satellite imagery to assess bloom extension and severity, and to reconstruct the invasion of a bloom-forming red seaweed;
- determining the nutrient status of macroalgal blooms and identifying nutrient sources from tissue nutrient content and isotopic signature from bloom-forming seaweeds;
- evaluating the risks to ecosystem functioning associated with the introduction of an invasive bloom-forming red seaweed;
- assessing the impact of the pesticides most commonly used in Ireland at environmentally relevant concentrations on the biological performance of two foundational macrophytes.

2 Monitoring Macroalgal Blooms in Irish Estuaries Using Earth Observation Technologies

2.1 Background

The EU WFD (2000/60/EC) and MSFD (2008/56/EC) are two of the most ambitious initiatives to prevent further deterioration of water bodies and associated ecosystems. These directives represent a change in the scope of water management from the local to the basin scale, and are based on an ecological approach rather than traditional physicochemical assessments (Apitz *et al*., 2006). The implementation of both directives requires a large expansion in monitoring, with the use of standardised methodologies over a wide geographical range under different environmental conditions, which places increased demands on regulatory agencies to expand monitoring programmes while maintaining costs (Carvalho *et al*., 2019; Karki *et al*., 2021). The development of field surveys in coastal and estuarine environments is demanding in terms of time, labour and costs, and can frequently pose significant logistical challenges, such as coordination of field equipment, survey procedures, means of transport, field crew and safety (Scanlan *et al*., 2007; Karki *et al*., 2021), making the development of long-term monitoring at large spatial scales unaffordable.

In European transitional water bodies (i.e. estuaries), the monitoring of opportunistic macroalgal blooms is required to assess ecological status in the context of the WFD (Scanlan *et al*., 2007; Wan *et al*., 2017). EO techniques offer enormous advantages by providing affordable cost-effective systematic observations for environmental quality assessment. Seaweed tides (green, golden or red) have been assessed using satellite imagery (Bermejo *et al*., 2020b; Choi *et al*., 2020; Karki *et al*., 2021). These studies have increased considerably since the launch of the newest satellites (with higher spectral, spatial and temporal resolution). Sentinel-2A/B (12 bands, 10 m pixel resolution and 2–5 days revisit time, launched in 2015) and Landsat products (up to eight spectral bands of 30 m spatial resolution) seem optimal instruments for monitoring and investigating macroalgal blooms.

This chapter aims to develop EO tools based on free-access satellite imagery (EU Copernicus and Landsat programmes) to monitor seaweed blooms in Irish estuaries. In this case, using different classification algorithms and Landsat and Sentinel-2 imagery, green (i.e. *Ulva* spp.), red (i.e. *Gracilaria vermiculophylla*) and golden (i.e. *Pilayella littoralis*) seaweed tides affecting Irish estuaries were monitored.

2.2 Materials and Methods

2.2.1 *Mapping spatial distribution and biomass of intertidal* Ulva *blooms*

Study area

The spatial distribution and biomass of intertidal *Ulva* spp. blooms were assessed in eight estuarine areas most affected by macroalgal blooms (*Ulva* spp.) across Ireland: (i) Clonakilty, Co. Cork (346.5 ha), (ii) Courtmacsherry, Co. Cork (447.1 ha), (iii) Lower Blackwater Estuary, Co. Waterford (287.37 ha), (iv) Dungarvan, Co. Waterford (1239.9 ha), (v) Bannow Bay, Co. Wexford (984.8 ha), (vi) Tolka, Co. Dublin (113.5 ha), (vii) Malahide, Co. Dublin (407.5 ha), and (viii) Rogerstown, Co. Dublin (448.8 ha). In this study, seaweed blooms present in the estuaries and tidal flats, excluding the salt marshes, were mapped at low tides. These areas display a moderate, poor or bad ecological status, as assessed for the WFD; parameters include loss of seagrass meadows, general physicochemical properties or the development of large macroalgal blooms (EPA, 2019; https://www.catchments.ie).

Generation of an Ulva *bloom map*

The mapping of macroalgal blooms using satellite imagery from Sentinel-2, Landsat-5 and Landsat-8 comprises several steps, from data download to the generation of the map (Figure 2.1). The study used the EO data sets from the Sentinel-2 and Landsat (5 and 8) missions to acquire the temporal coverage between 2010 and 2018. To obtain better temporal

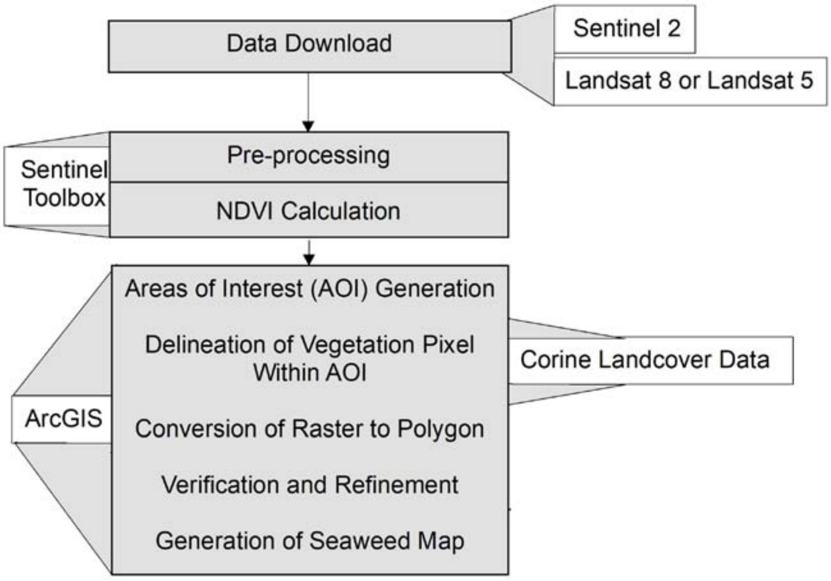

Figure 2.1. The workflow developed for mapping blooms using the NDVI delineation technique.

coverage from 2010, data sets from Landsat-5 Thematic Mapper (data availability: 1984–2012) and Landsat-8 Operational Land Imager (data availability: 2013–2018) were used.

The Corine (Coordination of Information on the Environment) land cover data set was used to define the areas of interest (i.e. intertidal mudflats) for each location. Following the Corine land cover classification,[1] the categories of interest corresponded to tidal flats (4.2.3) and estuaries (5.2.2), facilitating the removal of terrestrial vegetation and saltmarshes.

The visual inspection determined the threshold of the Normalised Difference Vegetation Index (NDVI) values that corresponded with the natural colour composite of the bloom-forming seaweed. In most cases, NDVI values greater than 0.15 and 0.20 for Sentinel-2 and Landsat images, respectively, represented *Ulva* blooms. For each location, the vegetation pixels were segregated after determining the threshold. This technique was able to segregate bloom patches bigger than a few metres.

Computation of Ulva *biomass using machine learning*

Building on the spatial coverage extracted using the NDVI delineation approach, an artificial neural network (ANN) model was developed to quantify the biomass distribution in the estuarine area. The Sentinel-1 synthetic aperture radar and Sentinel-2 multispectral instrument were used to develop the ANN model. The *in situ* biomass samples (point locations in g m^{-2}) collected as a part of the WFD monitoring programme and those obtained under the previously EPA-funded Sea-MAT Project (Bermejo *et al.*, 2019a) were compiled as a response variable for the model. The entire biomass computational process can be summarised as data acquisition (Sentinel-1) and processing (Sentinel-2), identification of determining variables, and model development and application, as shown in Figure 2.2.

The ANN model was developed using a total of 346 biomass samples, where the magnitude of biomass (g m^{-2}) was the response variable and the remaining variables (NDVI, percentage of green reflectance, percentage of red reflectance and radar backscatter) were used as determining variables. Of the total samples, 20% of the data points were used as validation data to prevent the model from overfitting due to excessive training. The fully trained ANN model was achieved with five hidden neurons when the number of epochs reached 50. The learning process was continued until the model's performance plateaued, at which point the error was minimal for both validation (20%) and training (80%) data sets. The entire process was accomplished using Google's

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[1] https://land.copernicus.eu/pan-european/corine-land-cover

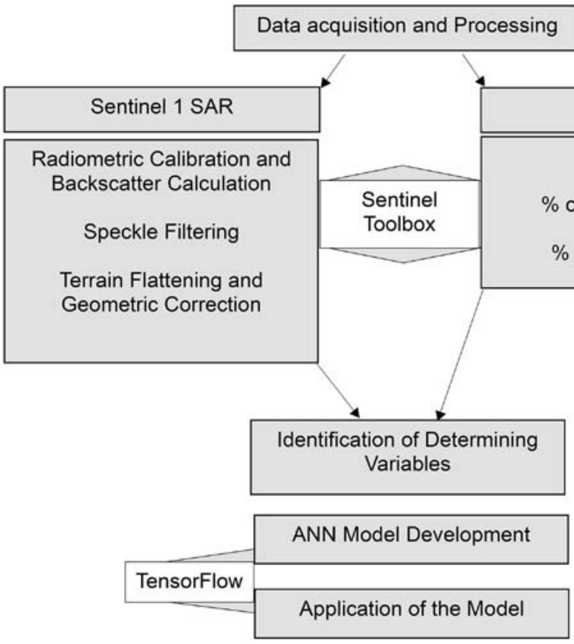

Figure 2.2. The workflow developed for computing biomass using ANN. MSI, multispectral instrument; SAR, synthetic aperture radar.

TensorFlow, a free and open-source application using Python programming language. The input data preparation was performed using ArcGIS, where a table consisting of all input variables was generated.

Application of the model

After the development of the ANN model, it was applied to generate biomass values using the input variables. To apply the model, the input data sets were compiled in table format. The TensorFlow-generated results were later converted to a geographical information system raster for mapping and further analysis. The quantity of *Ulva* spp. biomass can then be predicted for any area, if the input data sets are available for that area.

2.2.2 Reconstructing the spread of Gracilaria vermiculophylla *in Clonakilty Bay*

Study site

The Clonakilty estuary is shallow, sheltered, nutrient-enriched and located along the south-western coast of Ireland, and has been historically impacted by large intertidal macroalgal blooms of native *Ulva* spp. (see section 2.2.1, as well as Wan *et al*., 2017) and more recently by the non-native bloom-forming red seaweed *G. vermiculophylla* (Bermejo *et al*., 2020b). This alien seaweed was identified using molecular biology tools (the *rbcL* marker was amplified in six specimens of gracilarioids from the Clonakilty estuary), confirming its presence in Ireland (Krueger-Hadfield *et al*., 2017b; Bermejo *et al*., 2019a).

Maximum likelihood classifier

To reconstruct the extension of *G. vermiculophylla* and *Ulva* spp. blooms, and to assess the interaction between both bloom-forming species, satellite imagery from both Sentinel and Landsat were used. A pixel-based maximum likelihood classifier was applied to individual corrected scenes to produce both *Ulva* spp. and *G. vermiculophylla* masks. Satellite scenes from 2010 to 2018 captured during bloom proliferation, at low tide and under cloud-free conditions, were downloaded. Initial processing of satellite products was carried out using the European Space Agency Sentinel Application Platform (SNAP) toolbox (v. 6.0). The Sentinel-2 and Landsat satellite product process is presented in Figure 2.3.

The annual coverage, potential extension and overlap between native and invasive bloom-forming species were estimated using QGIS (http://qgis.osgeo.org). In this study, the potential extension of both *G. vermiculophylla* and *Ulva* spp. was defined as the

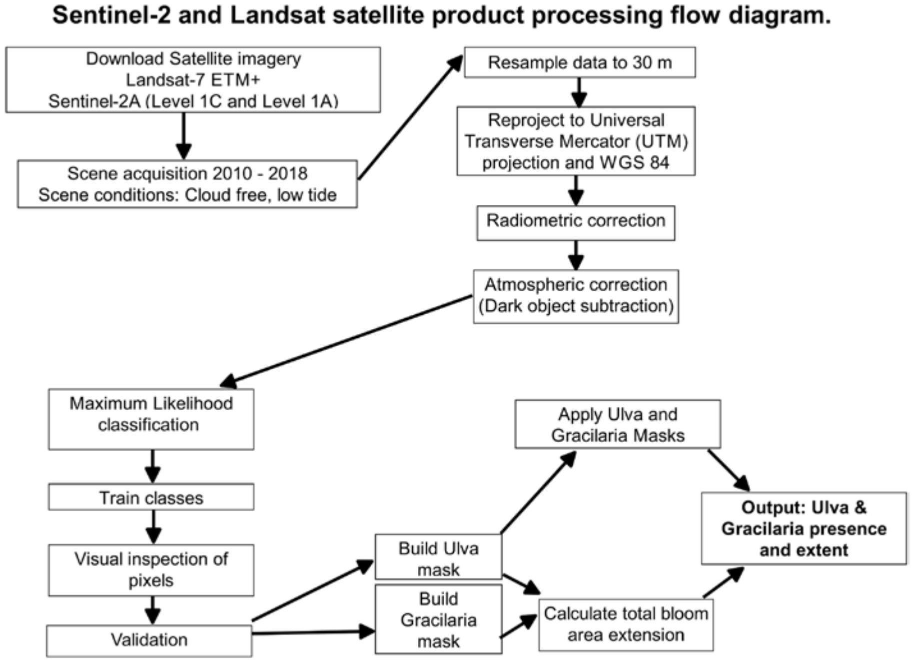

Figure 2.3. Flow diagram showing Sentinel-2 and Landsat satellite product processing.

entire area covered by these species at least once during the study period.

2.2.3 *Monitoring the* Pilayella littoralis *bloom in Killybegs Bay*

Study area

The northern area of Mc Swyne's Bay, in the surrounding area of Killybegs (Co. Donegal), is a shallow, sheltered and nutrient-enriched embayment affected by large intertidal golden tides dominated by *P. littoralis*. The taxonomical identity of this seaweed was determined using molecular biology tools (see Bermejo *et al*., 2019a, for further details).

Seaweed sampling

To validate and calibrate classification techniques, *Pilayella* samples were collected in Killybegs on 28 April 2022 during low tide while the Sentinel-2 satellite was simultaneously flying over the study area (Figure 2.4). The sampling points were georeferenced using a Garmin GPS 60 device. The biomass of *P. littoralis* was determined using 35 cm × 35 cm quadrats. Three quadrats were measured at each sampling point, and the presence or absence of *Pilayella* spp. or other seaweeds (e.g. *Ulva* spp., *Fucus* spp.), and areas of bare sediment devoid of seaweed coverage (mud or coarse sand), were recorded using the GPS device.

The Sentinel-2 images for 28 April 2022 were downloaded from the Copernicus Open Access Hub website.[2] The reflectance spectrum of *Pilayella* spp. [level 1C and level 2A (L2A)], as well as other species spectra, were extracted using the tool "pin manager" and "spectrum view" in the SNAP software. The *Pilayella* spectra were compared with the reflected light spectrum measured under controlled conditions in the laboratory using a spectroradiometer. Subsequently, the NDVI was calculated from reflectance bands (L2A), as [band 8 – band 4]/[band 8 + band 4]. The NDVI values were extracted from the sampling points where *Pilayella* spp. was present using the QGIS "Zonal statistics" plugin. Scenes were registered in the WGS 84/UTM zone 29N coordinate system (EPSG: 32629).

Random forest classifier

The L2A Sentinel-2 image for 28 April 2022 was used to train the machine learning algorithm. The image was resampled using a bilinear method to a spatial

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[2] https://scihub.copernicus.eu/dhus/#/home

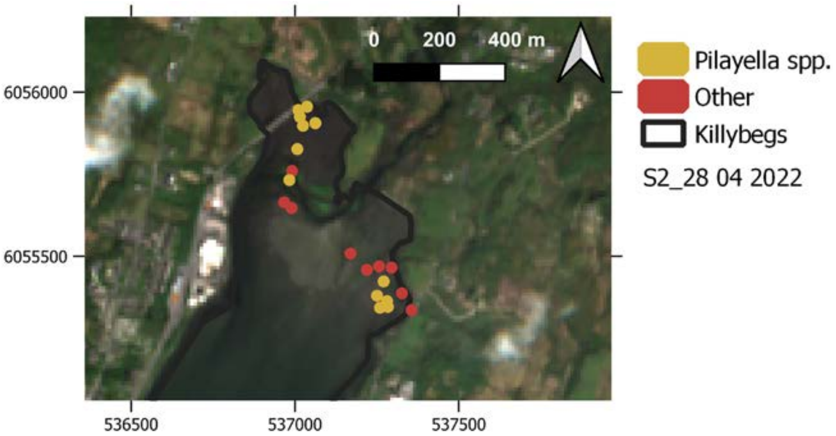

Figure 2.4. Sampling points with the presence of *P. littoralis* and other seaweeds (e.g. *Ulva* spp., *Fucus* spp.), and areas of bare sediment, 28 April 2022, in Killybegs estuary.

resolution of 10 m. A total of 87 field visualisations using RGB imagery were used to design one random forest algorithm (100 trees) to identify pixels with the presence of golden seaweed (i.e. *P. littoralis*), as well as other macroalgae (i.e. *Ulva* spp. and *Fucus* spp.), muddy or gravel sediments (without macroalgal coverage) and water (a total of six classes). The reflectance bands used (spatial resolution of 10 m) were 2, 3, 4, 5, 6, 7, 8, 8A, 11 and 12, and NDVI. Twenty-four visualisations were used to validate the random forest algorithm. The Google Earth Engine code was used to train this algorithm, the accuracy matrix was constructed, and its overall accuracy and kappa coefficient were calculated.

2.3 Results

2.3.1 *Mapping spatial distribution and biomass of intertidal* Ulva *blooms*

The extension of the macroalgal blooms from field surveys ranged between 21.3 ha (Lower Blackwater Estuary, 2017) and 543 ha (Tolka, 2017). There was a total of 22 EO estimates with corresponding field survey data, some of which are shown in Figure 2.5. A significant correlation between EO estimated coverage and survey measurements was observed, which was independent of the satellite missions used, i.e. Sentinel-2 and Landsat-8 ($p < 0.01$). All the correlation

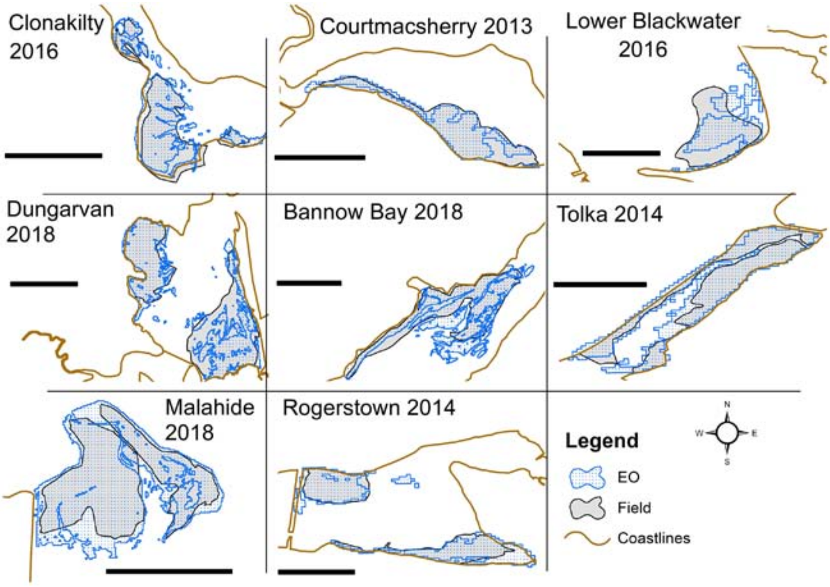

Figure 2.5. Eight locations showing selected sites with coverages from EO and the corresponding field surveys. Scale bar shows 1 km in all the locations. Reproduced from Karki *et al*. (2021); licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

analyses yielded coefficients of determination close to 1 (between 0.93 and 0.98) and a good fit between the observations. The spatial coverages from EO show more detailed delineation than those mapped on the ground, since the field campaign was more concentrated on the predominant and accessible regions of the *Ulva* blooms.

The ANN model was used to compute the biomass images for several estuarine areas. The biomass image shows the distribution of the bloom's biomass in the areas delineated by the spatial coverage mapping technique based on NDVI. Figure 2.6 displays the estimated biomass distribution for the summers of 2016 and 2018 in Clonakilty (Figure 2.6A) and Courtmacsherry (Figure 2.6B), respectively. Similarly, the computed biomasses for Malahide and Tolka for the summer of 2017 are shown in Figure 2.6C and D, respectively. The results predicted by the ANN model

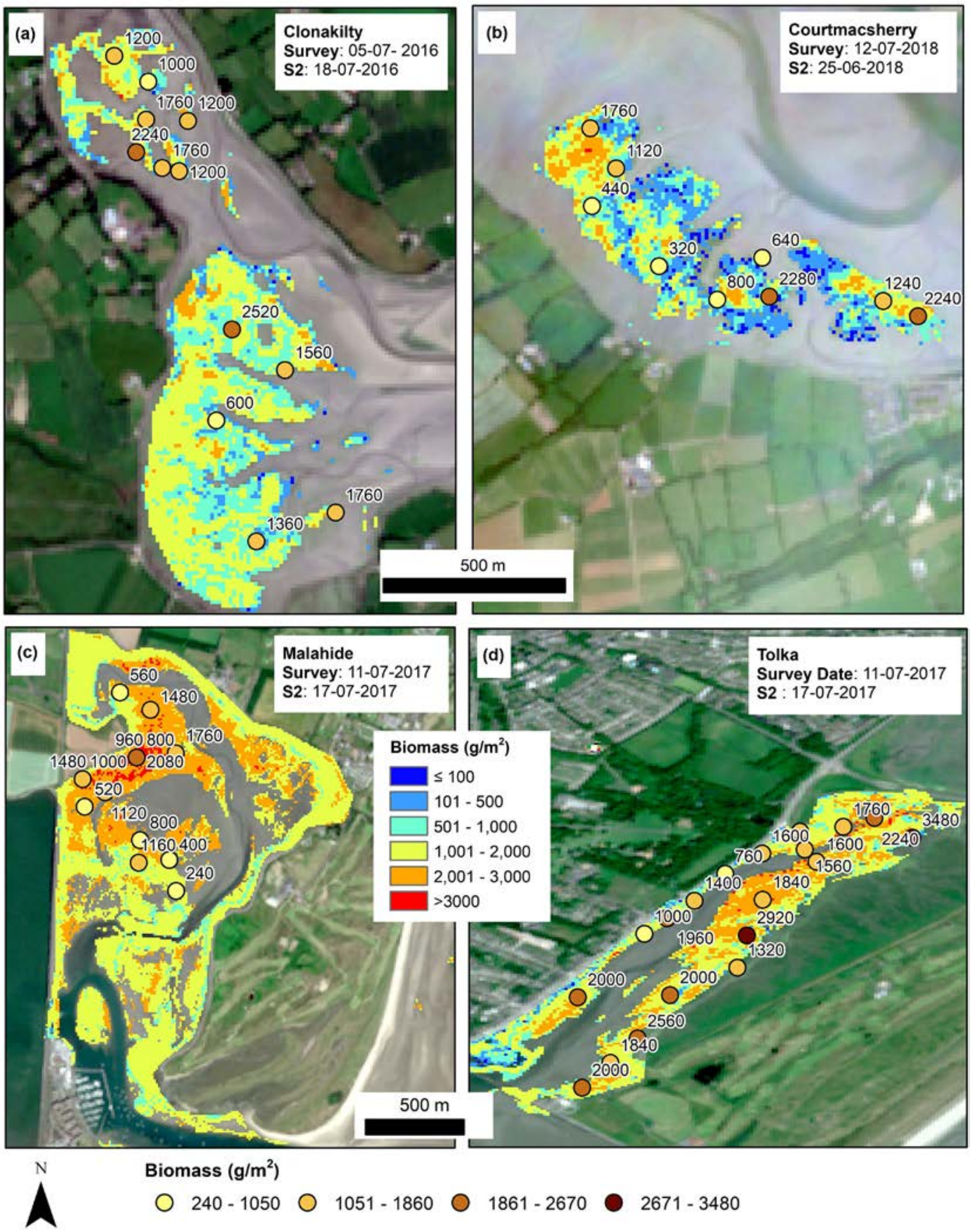

Figure 2.6. Biomass distribution for four estuaries: (a) Clonakilty, (b) Courtmacsherry, (c) Malahide and (d) Tolka. The field measured samples from the *in situ* survey are shown as points. The dates of field survey and Sentinel-2 acquisitions are also shown. Reproduced from Karki *et al.* (2021); licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

correlated well with the biomass data recorded during the field survey [root-mean-square error (RMSE) value of 471.70; adjusted $R^2 = 0.74$].

2.3.2 Reconstructing the spread of Gracilaria vermiculophylla *in Clonakilty Bay*

In the case of *Ulva* spp., the total extension of the bloom ranged from 19.6 to 50.8 ha between 2010 and 2018. The encroaching *Gracilaria* canopy is evident from 2014 to 2018, when the area colonised increased from 3.9 to 8.1 ha (Figure 2.7), with increased overlapping from 2015 to 2018 due to the colonisation of *G. vermiculophylla* in areas potentially covered by *Ulva*. *Gracilaria* colonised the northern shore, which had remained relatively bloom free prior to 2014 (Figures 2.7 and 2.8). The total extension of the estuary potentially covered by bloom-forming species increased by 6.7 ha after the arrival of *G. vermiculophylla,* with the average size of macroalgal blooms during peak bloom conditions being 1.21 times larger during the period 2014–2018 than for the period between 2010 and 2013.

2.3.3 Monitoring Pilayella littoralis *bloom in Killybegs Bay*

Biomass versus NDVI

The fresh biomass of *Pilayella* in Killybegs on 28 April 2022 varied from 0 to 3273 ± 1174 g m^{-2}. A significant linear relationship was found between the fresh biomass and the NDVI (Figure 2.9).

Random forest classifier

Figure 2.10 displays the results obtained for the random forest classifier in the Killybegs estuary, trained from the L2A Sentinel-2 image from 28 April 2022. The overall accuracy and kappa coefficient were 0.92 and 0.89, respectively, indicating an excellent match between field observations and the random forest classifier.

2.4 Discussion

The current work presents exploratory efforts to investigate the use of machine learning and artificial intelligence for seaweed tide assessment using satellite imagery. Three different machine learning algorithms (i.e. ANN model, maximum likelihood classifier and random forest classifier) were trained to map green, red and golden seaweed tides from Sentinel-2 and Landsat imagery. Both Sentinel-2 and Landsat imagery provided good coverage estimates and a good spatial match between them, despite their different spatial resolutions (10 and 30 m, respectively). The results of the green seaweed biomass computed using the ANN model showed excellent correspondence with the general distribution of the biomass survey findings (RMSE value of 471.70; adjusted $R^2 = 0.74$). In the case of the golden tide affecting Killybegs, a significant correlation was observed between the NDVI signal and the biomass measured at low tide during the field survey. The model presents significant potential for improvement with the addition of more data points. When comparing *in situ* data with trained mapping, all machine learning algorithms showed a good or excellent spatial correspondence with *in situ* observations (kappa coefficient between 0.69 and 0.96). The maximum likelihood classifier and random forest classifier distinguished red and golden seaweeds from co-occurring green tides, respectively. This distinction between green, golden and red seaweed tides was possible due to differences in the reflectance peaks for the different phyla at different wavelengths between 550 and 670 nm (Min *et al*., 2019; Xiao *et al*., 2022).

There are a multitude of vegetation indices (e.g. Enhanced Vegetation Index (EVI), Floating Algae Index (FAI), Seaweed Enhancing Index (SEI)), which are often computed to monitor seaweeds floating at the water surface in coastal environments using satellite imagery at low spatial resolution (e.g. AquaModis, Sentinel-3) (Hu, 2009; Ody *et al*., 2019; Siddiqui *et al*., 2019). In this case, as intertidal seaweed tides were monitored at low tides using higher spatial resolution images (10–30 m), the NDVI was considered for the assessment of these seaweed tides. The NDVI possesses some advantages, and it can be computed in intertidal environments at low tide, as there is little interference produced by water in the infrared region. A high level of correlation was observed between *Ulva* spp. biomass and the red reflectance because of increased reflectance in the visible and near-infrared region by green biomass (Jensen, 1986). The NDVI can also be used to estimate the golden seaweed biomass (Figure 2.9), seagrass biomass and benthic microalgal patches

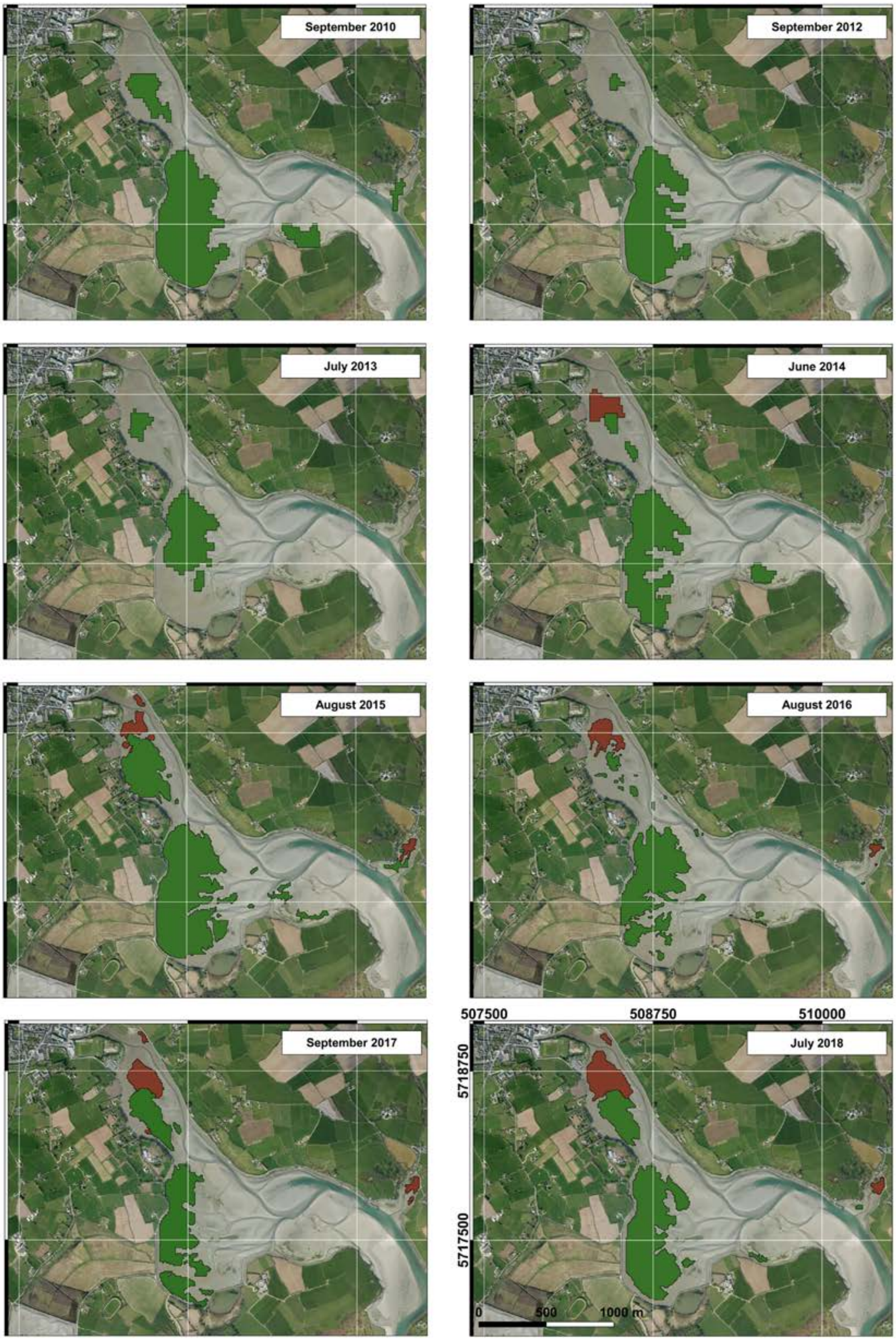

Figure 2.7. Extension of *G. vermiculophylla* (red areas) and *Ulva* spp. (green areas) in the Clonakilty estuary based on the analysis of satellite imagery obtained from 2010 to 2018. Reproduced from Bermejo *et al*. (2020b); licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

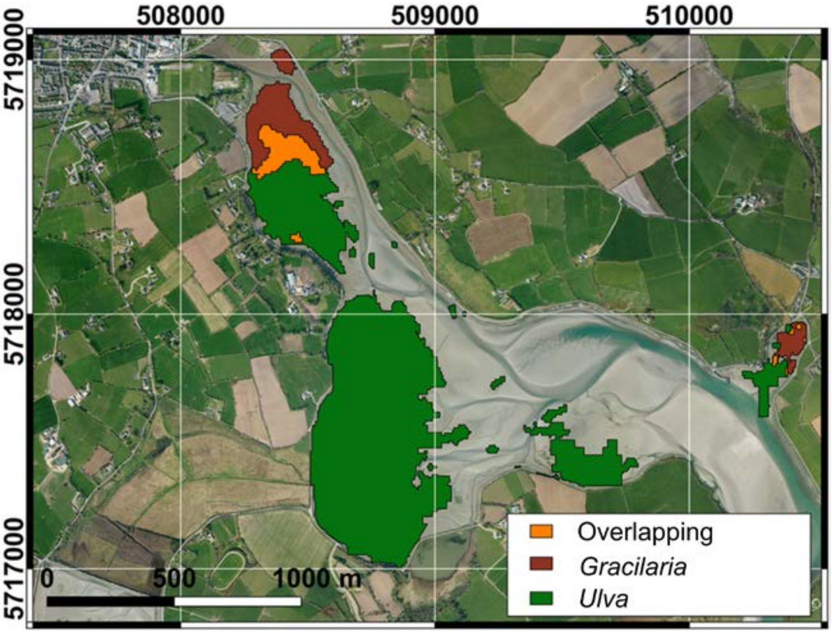

Figure 2.8. Potential extension for *G. vermiculophylla* and *Ulva*, and the intersection between both, in the Clonakilty estuary based on satellite imagery collected from 2010 to 2018. Reproduced from Bermejo *et al*. (2020b); licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

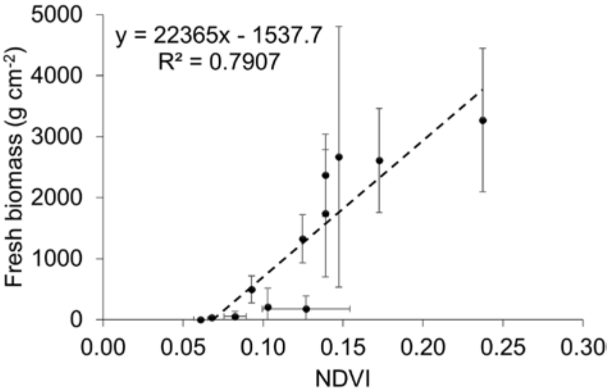

Figure 2.9. Relationship between NDVI, extracted from the L2A Sentinel-2 image for 28 April 2022 and the fresh biomass of *Pilayella* spp. measured in Killybegs. A statistically significant linear relationship between NDVI and biomass ($r = 0.89$; $n = 12$; $p < 0.05$) was found. Means ± standard deviation.

(Zoffoli *et al*., 2020; Haro *et al*., 2022). When using NDVI to assess intertidal macrophyte coverage or biomass, bottom of atmosphere reflectances were used (i.e. Sentinel-2 level 2A) to avoid underestimation.

Overall, the highest coverage and biomass of seaweed tides in Ireland were usually found in summer, independent of seaweed type and estuary. Bermejo *et al*. (2020b) and Karki *et al*. (2021) found the same spatial distribution of *Ulva* spp. in Clonakilty Bay (Figures 2.6A and 2.8). In Clonakilty, satellite data identified 2014 as the first year when *G. vermiculophylla* produced a bloom and confirmed that this species can bloom in areas of the estuary devoid of native macrophytes, i.e. *Ulva* spp., increasing the potential area of the estuary that can be affected by macroalgal blooms (*c*. 10%).

Therefore, this work validated the use of EO data processing techniques to map and assess the extension and biomass abundance of estuarine and coastal macroalgal blooms in Ireland. The main constraint for the use of EOs in Ireland was obtaining cloud-free imagery. These techniques can be easily implemented and used to support

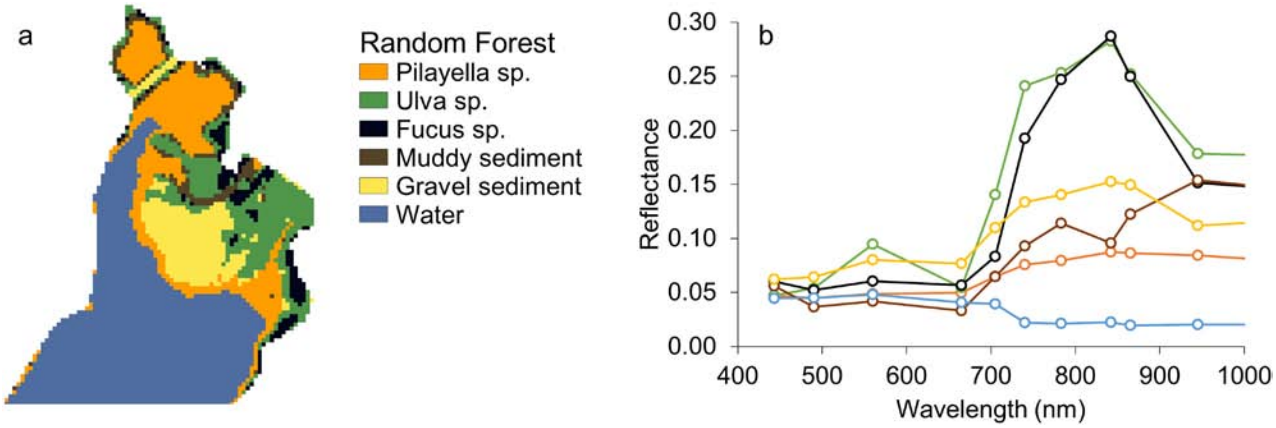

Figure 2.10. Left: Random forest classifier for Killybegs estuary trained from the L2A Sentinel-2 image for 28 April 2022. Right: Reflectance spectrum of macroalgae (*Pilayella* spp., *Ulva* spp. and *Fucus* spp.), muddy and gravel sediment (without algal coverage) and water.

environmental monitoring and assessment to understand environmental problems and inform sustainable management strategies to protect and restore our natural environment. This is especially relevant considering the current pressure put on regulatory agencies by governments to comply with the environmental monitoring requirements associated with the implementation of EU directives such as the WFD or the MSFD.

3 Biomonitoring of Nitrogen and Phosphorus Limitation in Macroalgal Tides

3.1 Background

The main environmental drivers controlling the development of primary producers, including bloom-forming seaweeds, are light, temperature and nutrients (Valiela *et al*., 1997; Lotze *et al*., 1999, 2001). In estuaries of cold temperate regions such as Ireland, primary production is limited by light and temperature during winter and by nutrients during spring and summer (Wang *et al*., 2012; Bermejo *et al*., 2019b; McGovern *et al*., 2019). Nitrogen (N) and phosphorus (P) are considered the main limiting nutrients for freshwater and coastal ecosystems (Lapointe, 1987; Howarth *et al*., 2000). Anthropogenic nutrient overenrichment of aquatic ecosystems, mainly N and P, seems to be a key factor in explaining the development of macroalgal blooms in estuarine environments (Valiela *et al*., 1997; Teichberg *et al*., 2010; Smetacek and Zingone, 2013). In order to propose effective management strategies for macroalgal bloom control, it is critical to assess the nutrient limitation status of the bloom and identify the primary sources of nutrients entering a water body (Bermejo *et al*., 2022).

The isotopic ratio between ^{14}N and the less abundant ^{15}N (i.e. $\delta^{15}N$) in marine macrophytes is often considered to assess the relative importance of nutrient sources with different isotopic signatures (e.g. Lin and Fong, 2008; Piñón-Gimate *et al*., 2017; Bermejo *et al*., 2022). These two stable isotopes can be differentially affected by physical, chemical or biological processes, leading to distinctive isotopic signatures for different N sources. Some sources of N, such as urban wastewaters, manure or terrestrial run-off, are usually more ^{15}N enriched than others, such as seawater (Costanzo *et al*., 2001; Cohen and Fong, 2006; Bateman and Kelly, 2007). Overall, the $\delta^{15}N$ value of macroalgae gives a more integrated and less transient response, reflecting the importance and variability of N sources and providing more information on nutrient enrichment than the $\delta^{15}N$ value of water samples (Viana and Bode, 2013, 2015). However, certain processes, such as the differential affinity for different forms of N (e.g. NH_4^+ vs NO_3^-) or isotopic fractionation, need to be considered when interpreting $\delta^{15}N$ values in macroalgae (Thornber *et al*., 2008; Viana *et al*., 2011; Gröcke *et al*., 2017).

The internal nutrient content in primary producers and their ratios provide more relevant information about nutrient limitation status than dissolved nutrients in water (Björnsäter and Wheeler, 1990; Fong *et al*., 1998; Lyngby *et al*., 1999). As with many biological processes, the relationship between the growth rate of primary producers and the tissue content of a limiting nutrient follows a hyperbolic curve or saturation kinetic (Hanisak, 1983) (Figure 3.1). Several ecophysiological studies (Campbell, 2001; Hernández *et al*., 2008; Pedersen and Johnsen, 2017) have determined the minimum tissue nutrient content necessary to support unrestrained growth (i.e. critical quota) and the lowest nutrient tissue content that allows growth (i.e. subsistence quota) for N and P in bloom-forming species such as *Ulva, G. vermiculophylla* or some Ectocarpales. Considering this information, it is possible to set a reference for the assessment of these nutrients as limiting factors, using tissue N and P contents and their ratio as an indicator of nutrient and relative nutrient enrichment in opportunistic

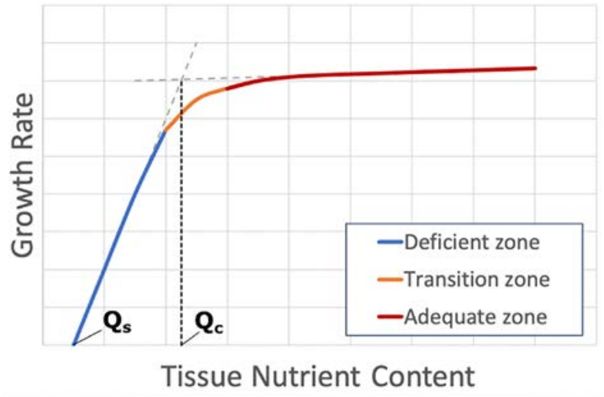

Figure 3.1. Theoretical relationship between growth and tissue nutrient content. Q_s, subsistence quota; Q_c, critical quota. Modified from Hanisak (1983). Used with permission of Elsevier, from Hanisak (1983); permission conveyed through Copyright Clearance Center, Inc.

bloom-forming seaweeds (Lyngby *et al*., 1999; Cohen and Fong, 2006; Lourenço *et al*., 2006).

This study aimed to (i) identify the most likely sources of N based on the $\delta^{15}N$ value and (ii) assess the nutrient status of macroalgal blooms based on internal nutrient content in different estuaries affected by large macroalgal blooms in Ireland.

3.2 Materials and Methods

3.2.1 *Study area*

From June 2016 to August 2017, five different seaweed blooms affecting four Irish estuaries (Figure 3.2) were monitored on six or seven sampling occasions. Three of these blooms were

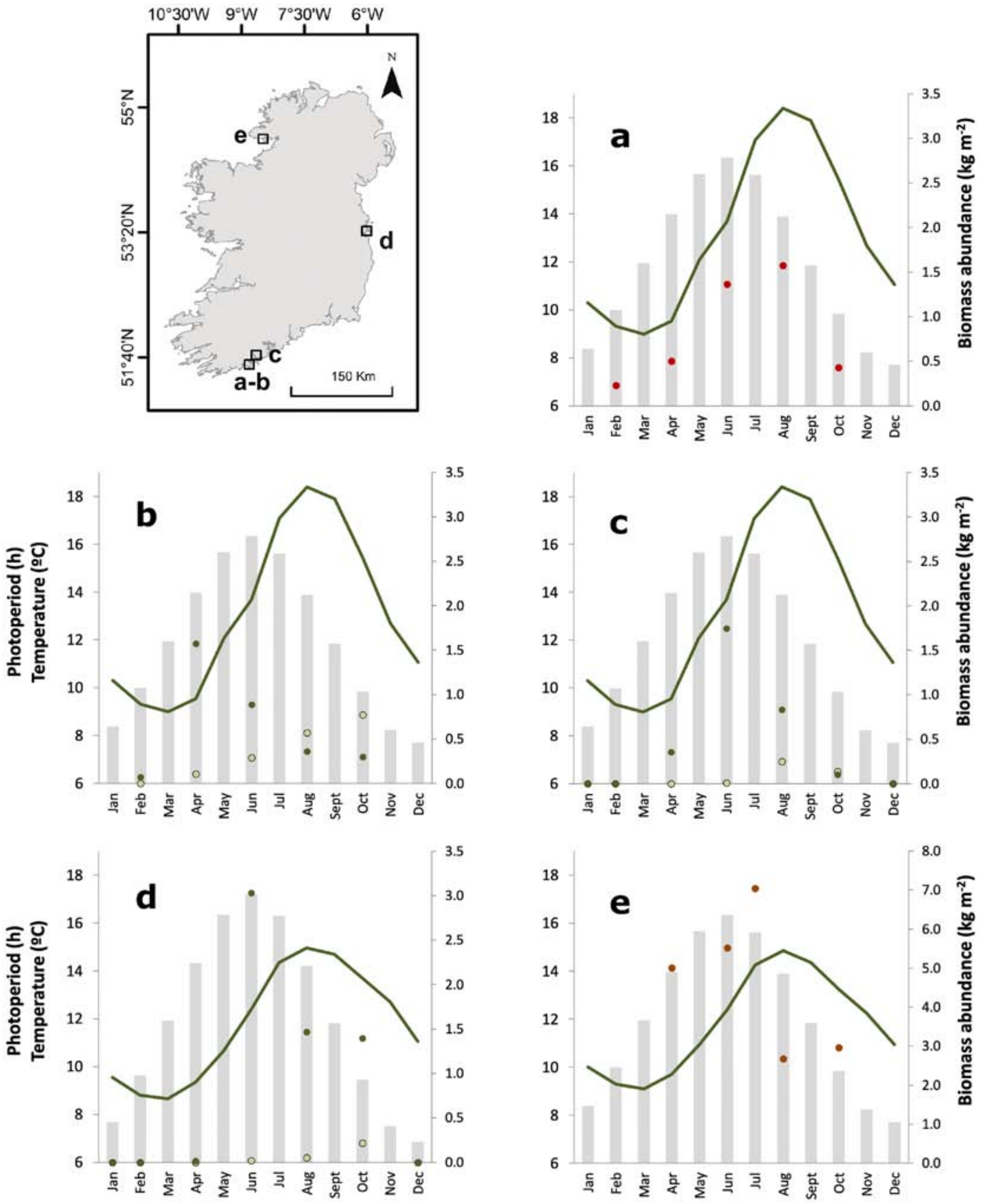

Figure 3.2. Monthly *Gracilaria* (red dots), tubular *Ulva* (dark green dots), laminar *Ulva* (pale green open circles) and *Pilayella* (brown dots) biomass abundance, photoperiod (grey bars), and sea surface water temperature (green lines) for the (a) Clonakilty, (b) Argideen, (c) Clonakilty, (d) Tolka and (e) Killybegs estuaries. Mean biomass abundances were extracted from Bermejo *et al*. (2019a), Bermejo *et al*. (2020b) and Bermejo *et al*. (2022) for the years 2016 and 2017. Photoperiod data were obtained from Met Éireann. Mean monthly sea surface temperatures for adjacent coastal waters for all estuaries were sourced from SeaTemperature.org.

dominated by *Ulva* spp. (Argideen, Clonakilty and Tolka estuaries; Bermejo *et al*., 2019a, 2022), one by *G. vermiculophylla* (Clonakilty estuary; Bermejo *et al*., 2020b) and one by *P. litoralis* (Killybegs estuary; Bermejo *et al*., 2019a). Green tides or blooms dominated by *Ulva* spp. were multispecific (up to eight species were recorded per bloom, based on molecular identification analyses), while seaweed blooms dominated by *G. vermiculophylla* (red tide) or *P. littoralis* (golden tide) were dominated by one species (monospecific). Regarding green tides, the peak of biomass in the estuaries studied was observed in early summer (April–June; Figure 3.2). In the case of *G. vermiculophylla*, the maximum biomass abundances were recorded in mid-summer (June–August; Figure 3.2a). The golden tide formed by *P. littoralis* showed the maximum biomass values during late spring and early summer (April–June; Figure 3.2) (see Bermejo *et al*., 2019a,b, 2020b, 2022, for further details).

All four estuaries were mesotidal (between 3.7 and 5 m), shallow (median depth between 3.7 and 2.1 m) and sheltered. The areas affected by macroalgal blooms were muddy and showed a relatively high percentage of organic matter (between 2.5% and 7.5%; Bermejo *et al*., 2019b). The estimated residence times were less than 1 day for Killybegs, 4 days for Argideen, 5.4 days for Tolka and 7.5 days for Clonakilty. The shape of the Argideen and Killybegs estuaries and the area affected by macroalgal blooms in the Tolka estuary (i.e. the south lagoon of North Bull Island) are elongated, being progressively wider close to the mouth. Clonakilty is like a bay or coastal lagoon, being wider in the middle and narrower at the mouth (see Figure 2.5).

N and P loadings from wastewater treatment plants (WWTPs) and freshwater inputs for each study site are presented in Table 3.1 (Irish Water, 2011, 2016, 2018; McGovern *et al*., 2020).

The catchments of the Argideen and the Clonakilty estuaries located along the south-west coast of Ireland are dominated by an agricultural landscape, and in both cases a WWTP is present. In the case of the Argideen estuary and associated rivers, nutrient loadings from the catchment are mainly from agriculture (McGovern *et al*., 2020). In Clonakilty, while there is a significant nutrient input from agriculture, nutrient loadings from the WWTPs are relatively more relevant than in the case of Argideen. The Tolka estuary is situated in Dublin Bay, a complex system comprising an open bay, estuaries and lagoons, surrounded by the metropolitan area of Dublin. Major nutrient inputs in Dublin Bay are from the Ringsend WWTP, followed by riverine inputs, with the River Liffey being the most important in terms of nutrient loadings. The primary source of N in the River Liffey is wastewater, followed by emissions from pasture (Ní Longphuirt *et al*., 2016a; McGovern *et al*., 2020). The Tolka and the Dodder rivers also contribute significantly to the total nutrient input in the bay (McGovern *et al*., 2020). The nutrient enrichment of the Tolka estuary emanates from diffuse agriculture sources (McGovern *et al*., 2020). The area studied, i.e. the south lagoon of North Bull Island, which is part of the Tolka estuary, has been historically affected by large macroalgal blooms (Jeffrey *et al*., 1995). The sheltered nature of the area and its hydrodynamic nature, with predominant currents in a clockwise direction, may favour the development of macroalgal blooms in the northern part of Dublin Bay, including the

Table 3.1. Nutrient loadings in the four estuaries studied

Estuary	Nutrient source	N loads (kg N y^{-1})	P loads (kg P y^{-1})	N:P	Reference
Argideen	Argideen and associated rivers	1,036,388	14,297	160.30	McGovern *et al*., 2020
	WWTP	1460	113	28.57	McGovern *et al*., 2020
Clonakilty	Local streams (Ashgrove and Clonakilty)	135,034	623	479.30	McGovern *et al*., 2020
	WWTP	27,112	1480	40.51	Irish Water, 2016
Killybegs	Stragar river	Not available	Not available		
	WWTP (fish processing industry and domestic)	31,107	1560	44.09	Irish Water, 2018
Tolka	Tolka river	87,994	4353	44.70	McGovern *et al*., 2020
	Dodder river	118,720	1460	179.81	McGovern *et al*., 2020
	Liffey river	825,947	10,776	169.49	McGovern *et al*., 2020
	Ringsend WWTP	2,680,900	437,256	13.56	McGovern *et al*., 2020

south lagoon of North Bull Island (Jeffrey *et al*., 1995). Finally, the area surrounding Killybegs is dominated by a mosaic of natural (mainly peat bog) and agricultural land uses. This system receives freshwater inputs from the Stragar river, and the main wastewater discharge in the area arises from the local population and the fish processing industry. There is a large seasonal factor in the fish-based discharges, which peak in late winter to early spring and fall to almost zero in summer (Irish Water, 2011).

3.2.2 Field sampling

On each sampling occasion and at each macroalgal bloom, seaweed samples were collected at two sites in the estuary, one close to the open sea (“outer”) and another approximately 1 km upstream (“inner”). Samples were collected at low tide at the inner part of the bloom during its maximum extension. Three replicates were collected per site, sampling occasion and type of seaweed. In the case of the seaweed blooms dominated by *Ulva* spp., samples of laminar and tubular morphologies were sorted and analysed independently. The biomass of each independent replicate for *G. vermiculophylla*, laminar morphologies of *Ulva* and tubular morphologies of *Ulva* and *P. littoralis* ranged from 1 to 3 g fresh weight.

3.2.3 Biomass sample processing

Once returned to the laboratory, each sample was rinsed with fresh water to remove debris, sediments and epiphytes. Samples were freeze dried and stored until further elemental analysis (i.e. tissue N and P content and δ^{15}N determination). Samples of *P. littoralis* collected in April 2017 from the inner section of Killybegs were lost during storage.

3.2.4 Tissue N and P content and δ^{15}N determination

Freeze-dried samples were ground into a fine homogeneous powder using a ball mill (TissueLyser II, QIAGEN, Hilden, Germany). The homogenised sample was divided into two subsamples: one was used for tissue P content determination and the other for tissue N content and δ^{15}N determination.

The tissue P content of samples was determined from the same dried and ground seaweed tissue after oxidation with boiling H_2SO_4 followed by spectrophotometric analysis (Murphy and Riley, 1962; Strickland and Parsons, 1968). In some replicates (six from the Clonakilty estuary and four from the Argideen estuary), it was not possible to estimate tissue P content, as there was not enough biomass after measuring tissue N and δ^{15}N. These were tubular *Ulva* from the inner section in June 2016 and from the outer section in August 2016 for the Argideen estuary; laminar *Ulva* from the outer section in August 2016 and from the inner section in June 2017; and tubular *Ulva* from the outer section in June 2016 and August 2016 for the Argideen estuary.

Regarding tissue N content and δ^{15}N determination, aliquots of the pulverised sample were weighed into tin capsules that were combusted in a Vario ISOTOPE Cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany) connected to an isotope ratio mass spectrometer (Isoprime 100, Isoprime Ltd, Cheadle Hulme, UK). The analytical precision was 0.15%. Analyses were carried out in duplicate.

3.3 Results

3.3.1 The cases of the green tides affecting the Argideen, Clonakilty and Tolka estuaries

The tissue N content of both *Ulva* morphologies was equal to or higher than the critical quota (2.45% N; one-sample *t*-test or Wilcoxon test; p-value > 0.05) on all sampling occasions and at all sites within estuaries (Figure 3.3a). The only exceptions were found in Clonakilty, where tubular *Ulva* showed a tissue N content lower than the critical quota in July 2016 and laminar *Ulva* yielded tissue N contents lower than the critical quota in July and August 2016.

Regarding phosphorus (Figure 3.3b), tissue values for this nutrient were always higher than the subsistence quota (0.05% P; one-sample *t*-test; $p > 0.05$), but lower than the critical quota on most of the sampling occasions. In the Argideen estuary, the tissue P content of *Ulva* was slightly higher than or equal to the critical quota during August 2016 in the case of tubular morphologies and during October 2016 in the case of laminar morphologies. In Clonakilty, both morphologies showed tissue P contents slightly higher than or equal

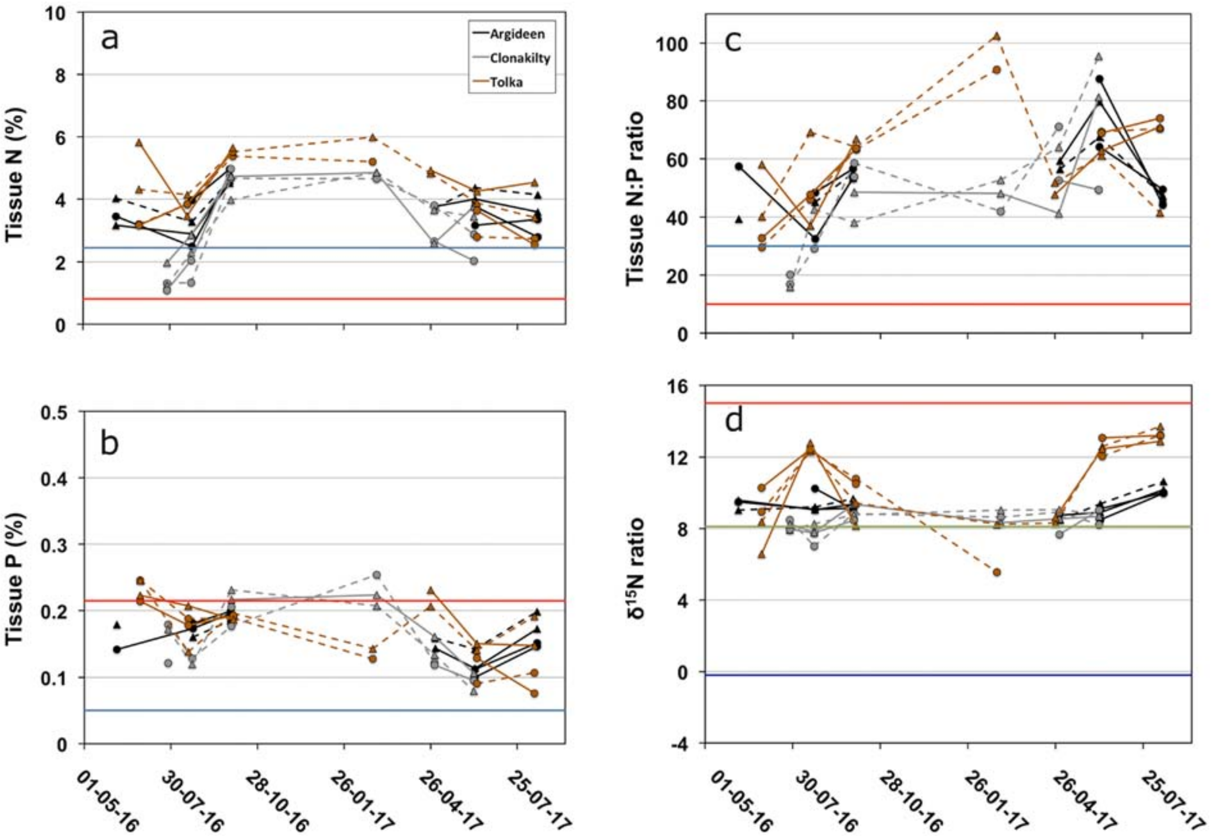

Figure 3.3. Mean values of tissue (a) N and (b) P contents, (c) δ^{15}N and (d) N:P ratios of tubular (triangles) and laminar (circles) *Ulva* for each site within each estuary and on each sampling occasion. In (a) and (b), the blue line represents the critical quota for N and P according to Villares and Carballeira (2004) and Hernández *et al*. (2008), and the red line refers to the subsistence quota for N and P according to Hernández *et al*. (2008). In (c), the red and blue lines represent the lower and higher thresholds for an equilibrated N:P ratio; values below the red line and above the blue line suggest an N or P limitation, respectively (Atkinson and Smith, 1983). In (d), the red line indicates the mean N isotope values δ^{15}N for urban wastewaters (Cohen and Fong, 2006), the green line the isotope values δ^{15}N for manure and compost (Bateman and Kelly, 2007) and the blue line the isotopic signature for synthetic fertilisers (Bateman and Kelly, 2007). Reproduced from Bermejo *et al*. (2022); licensed under CC BY-NC-ND 4.0 (https://creativecommons.org/licenses/by-nc-nd/4.0/).

to the critical quota in February 2017, as did tubular *Ulva* during October 2017 and the laminar form during August 2016. Finally, in the Tolka estuary, tissue P contents higher than or equal to the critical quota were found in June 2016 for both morphologies, as well as in October 2016 and April 2017 for tubular and laminar *Ulva*, respectively.

Overall, the atomic N:P ratio was higher than 30, suggesting nutritional disequilibrium (an N:P ratio in equilibrium is higher than 10 and lower than 30; Figure 3.3c), supporting the idea that *Ulva* had a nutritional status that tended towards P limitation rather than N limitation at all the estuaries in this study.

The δ^{15}N values for *Ulva* samples from the three estuaries investigated ranged between 4.5 and 12.5, with minimum values during winter and maximum values during summer, coinciding with the peak bloom period. The Tolka estuary yielded higher δ^{15}N values (12.50) than the Argideen (9.18) and Clonakilty (8.18) estuaries, which showed similar ratios (Figure 3.3d).

3.3.2 *The case of the red seaweed tide affecting the Clonakilty estuary*

In the case of the *G. vermiculophylla* bloom affecting the Clonakilty estuary, the tissue N content was equal to or higher than the critical quota during the entire year on both sampling sites (2.14% N; one-sample *t*-test or Wilcoxon test; $p > 0.05$; Figure 3.4a). No differences in tissue N contents were observed between sites. The highest tissue N content was recorded in February (4.68% ± 0.31%, mean ± SD; $n = 6$), coinciding with minimum standing biomass, and the lowest content was found during the summer (July 2016; 2.27% ± 0.36%; $n = 6$), coinciding with the peak bloom period (Figure 3.2).

Tissue P content was higher than or equal to the subsistence quota on all sampling occasions and sites (0.045% P; one-sample *t*-test or Wilcoxon test; $p > 0.05$; Figure 3.3b), but higher than or equal to the critical quota only on October 2016 and February 2017 (0.15% P; one-sample *t*-test or Wilcoxon test; $p > 0.05$; Figure 3.4b). The sites displayed a relatively similar seasonal trend, with maximum tissue P contents during February 2017 and minimum levels in April and June 2017. The lowest and the highest tissue P contents were observed in the outer section during April 2017 (0.082% ± 0.005%; $n = 3$) and October 2016 (0.203% ± 0.039%; $n = 3$). The atomic N:P ratio was usually between 10 and 30, suggesting nutritional equilibrium over most of the year (Figure 3.4c). In April 2017, this ratio was slightly higher than 30, supporting the idea that *G. vermiculophylla* tended towards P

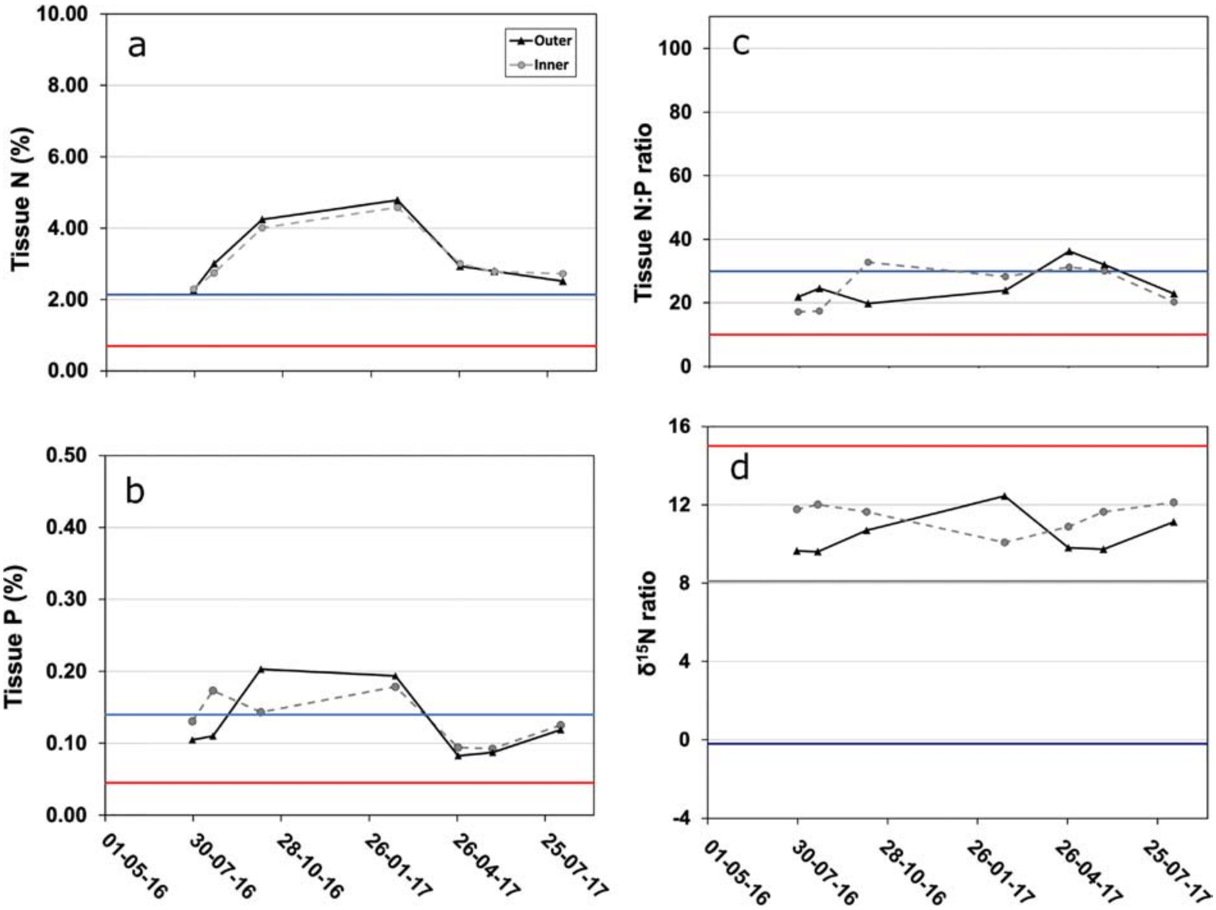

Figure 3.4. Mean values of tissue (a) N and (b) P contents, (c) N:P ratios and (d) δ^{15}N of *G. vermiculophylla* for each site and sampling occasion. In (a) and (b), the blue and red lines represent the critical and the subsistence quotas for N and P according to Pedersen and Johnsen (2017). In (c), the red and blue lines represent the lower and higher thresholds for an equilibrate N:P ratio; values below the red line and above the blue line suggest a N or P limitation, respectively (Atkinson and Smith, 1983). In (d), the red line indicates the mean N isotope values δ^{15}N for urban wastewaters (Cohen and Fong, 2006), the grey line indicates the isotope values δ^{15}N for manure and compost (Bateman and Kelly, 2007) and the blue line indicates the isotopic signature for synthetic fertilisers (Bateman and Kelly, 2007). Modified and expanded from Bermejo *et al*. (2020b); licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

limitation rather than N limitation, especially in the inner section.

The δ¹⁵N values for *G. vermiculophylla* samples from the Clonakilty estuary ranged between 9 and 12 (Figure 3.4d). In the inner section, minimum ratios were recorded during winter and maximum ratios were recorded during summer, coinciding with the peak bloom. The opposite seasonal pattern was observed for the outer section, with lower ratios in summer and higher values in winter.

3.3.3 *The case of the golden seaweed tide affecting the Killybegs estuary*

In the case of the golden seaweed tide affecting the Killybegs estuary, the tissue N content found in *P. littoralis* samples was equal to or higher than the critical quota during the entire year at both sampling sites (2.12% N; one-sample *t*-test or Wilcoxon test; $p > 0.05$; Figure 3.5a). The maximum tissue N content was recorded in April (5.99% ± 0.10%, mean ± SD; $n = 3$) and the minimum was observed during the summer (July 2016; 3.44% ± 0.81%; $n = 3$), coinciding with the peak bloom.

Regarding P (Figure 3.5b), tissue contents ranged between 0.18% and 0.26% P. The highest values were observed during early summer (April and June 2017). In this case, no reference for a critical or subsistence quota for P was found in the scientific literature for *P. littoralis* or similar species (e.g. *Ectocarpus* or *Hincksia*).

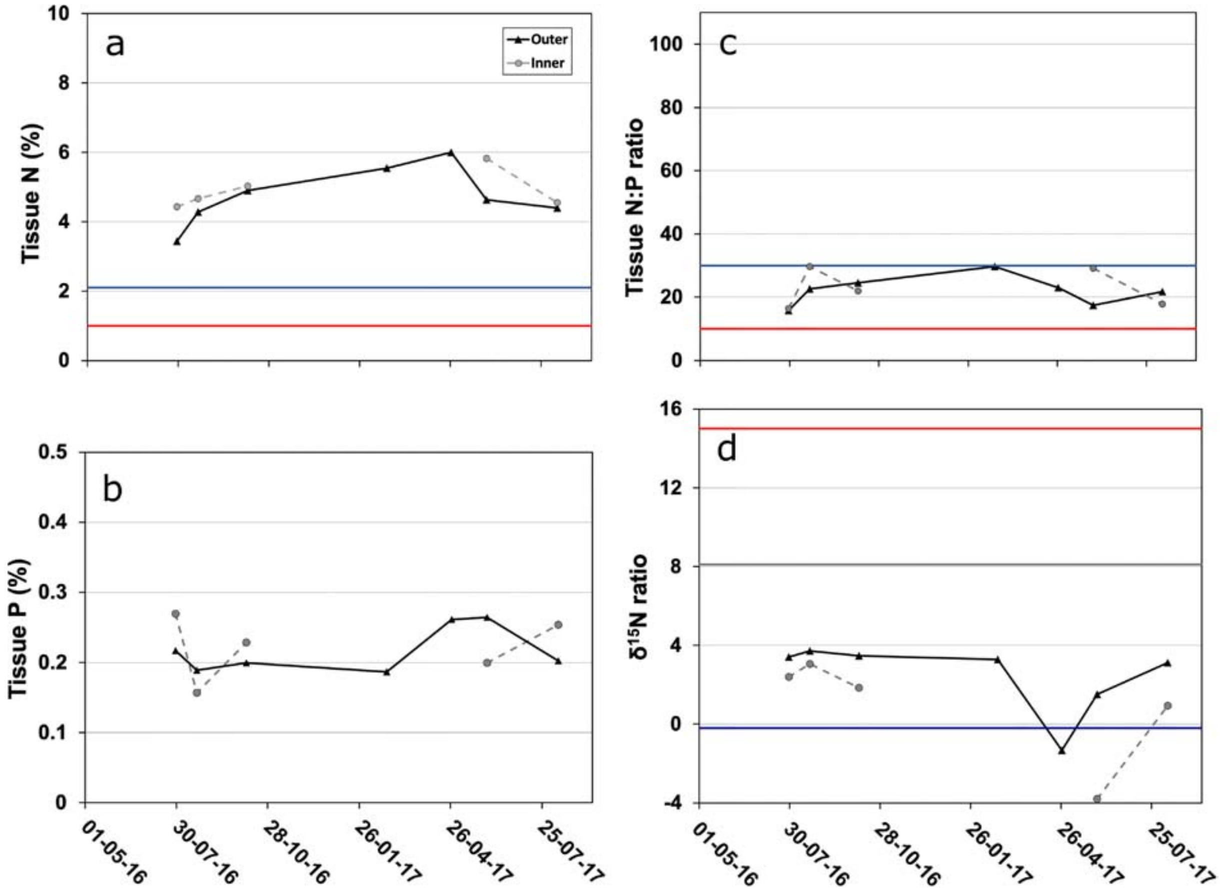

Figure 3.5. Mean values of tissue (a) N and (b) P contents, (c) N:P ratios and (d) δ¹⁵N of *P. littoralis* for each site and sampling occasion. In (a), the blue and red lines represent the critical and subsistence quotas for N, as estimated by Campbell (2001) for a similar species. In (c), the red and blue lines represent the lower and higher thresholds for an equilibrated N:P ratio; values below the red line and above the blue line suggest an N or P limitation, respectively (Atkinson and Smith, 1983). In (d), the red line indicates the mean N isotope values δ¹⁵N for urban wastewaters (Cohen and Fong, 2006), the grey line the isotope values δ¹⁵N for manure and compost (Bateman and Kelly, 2007) and the blue line the isotopic signature for synthetic fertilisers (Bateman and Kelly, 2007).

The atomic N:P ratio ranged between 15.8 and 29.5, suggesting nutritional equilibrium over the entire year (Figure 3.5c).

The $\delta^{15}N$ values for *Pilayella* samples from the Killybegs estuary ranged between –3.8 and 3.7 (Figure 3.5d), with minimum values during late spring–early summer and maximum values during summer, coinciding with the peak bloom period. In this case, the $\delta^{15}N$ was consistently lower in the inner section than in the outer one.

3.4 Discussion

The temporal and spatial assessment of tissue N content in the five seaweed blooms monitored indicated no or little N limitation across any sampling occasion, sampling site or bloom-forming species (Figures 3.3–3.5), as generally tissue N content was higher than the critical quota determined by previous studies for *Ulva* spp. (Pedersen and Borum, 1996; Villares and Carballeira, 2004; Hernández *et al*., 2008), *G. vermiculophylla* (Pedersen and Johnsen, 2017) and other Ectocarpaceae similar to *P. littoralis* (Campbell, 2001). Only once, during the summer of 2016, was the N tissue content lower than the critical quota at the green tide affecting Clonakilty, indicating N limitation (Figure 3.3a). The tissue N content showed a common seasonal pattern in all the seaweed tides. A negative correlation between total biomass abundance and tissue N content was observed for green tides ($\rho = -0.37$; $p = 0.04$) and *G. vermiculophylla* bloom ($\rho = -0.71$; $p < 0.001$), suggesting a biomass dilution effect due to the intensive growth during spring and early summer (e.g. Hernández *et al*., 2006; Pedersen and Johnsen, 2017; Bermejo *et al*., 2019c). The subsequent increase in tissue N content during the late bloom (Figure 3.2), when the abundance of biomass started to decrease, may be related to nutrient remineralisation as a consequence of biomass decay and degradation, and a slower growth of bloom-forming species after the peak bloom (e.g. Bermejo *et al*., 2020b, 2022).

However, during late spring and early summer, coinciding with the period of more active growth, tissue P content in *Ulva* and *Gracilaria* was lower than the critical quota (Hernández *et al*., 2008; Pedersen and Johnsen, 2017; Figures 3.3b and 3.4b), suggesting P limitation. Considering the elemental N:P ratio, the results indicated that the nutrient imbalance and P limitation may be more severe in *Ulva* than in *Gracilaria*. This has important relevance for management strategies as, according to the theoretical relationship between seaweed growth and tissue nutrient content proposed by Hanisak (1983), the development of *Ulva* and *Gracilaria* blooms is expected to be more responsive to a change in P availability than in N availability. These tissue P contents observed fall between the subsistence and critical quotas (i.e. deficient zone), and tissue N contents are higher than the critical quota (i.e. adequate zone; Figure 3.1). Thus, a positive linear response in *Ulva* and *G. vermiculophylla* growth rates is expected to lead to increased P availability, but a limited response is expected in the case of enhanced N availability (Hanisak, 1983; Fong *et al*., 1998). These observations partially validate the findings of McGovern *et al*. (2019), who identified P as the limiting factor constraining green tide development from April to September, and light as the factor constraining *Ulva* growth during the rest of the year, based on a modelling approach for the estuaries studied. Considering the elemental N:P ratios and tissue N contents (Figure 3.5a and c), no nutrient limitation was expected for *P. littoralis* at any time of the year, which suggests that maximum bloom size was reached. Some caution should be applied here, as no information about critical or subsistence quotas for P has been found in the scientific literature for *P. littoralis* or other Ectocarpaceae. In Killybegs estuary, further increases in dissolved nutrients in the water may lead to toxic effects in *P. littoralis* and the reduction of biomass, with unpredictable ecological consequences (e.g. shifts from macroalgal to microalgal blooms, significant increases in the amount of nutrients exported to the open sea) (Viaroli *et al*., 2008; Ní Longphuirt *et al*., 2015, 2016b).

The observed P limitation contradicted expected nutrient limitation patterns in pristine or undisturbed temperate estuaries, where N limitation rather than P limitation is expected to control primary production during spring and summer (Sfriso *et al*., 1987; Valiela *et al*., 1997; Howarth *et al*., 2000). During the last century, although P loadings have increased with the intensification of agriculture and the increase in human population, this increase was proportionally smaller than the increase in N loadings, producing a global shift in the N:P ratio of nutrient loads, affecting nutrient limitation patterns worldwide (Teichberg *et al*., 2010; Glibert, 2017; Lu and Tian, 2017). Between 1960 and 2013, the manufacture and use of N-based fertilisers expanded from 11.3 to 107.6 $\mathrm{Mt\,N\,y^{-1}}$, and the use of P-based fertilisers increased from 4.6 to 17.5 $\mathrm{Mt\,P\,y^{-1}}$ (Lu and Tian, 2017). In Ireland, for the period 2018–2020, estimated N loadings in aquatic ecosystems derived from human activities were 71,279 t, while anthropogenic P loadings were estimated at 1451 t (49.1:1 ratio; Trodd and O'Boyle, 2021). This imbalance in N and P loadings could partially explain the observed P limitation in these nutrient-enriched estuaries, as well as the increase in tissue N content and in the N:P ratio observed during the peak bloom period in the green tide in the Tolka estuary: N content increased from 2.8–3.5% in 1989–1990 (Jeffrey *et al*., 1995) to 3.8–5.1% in 2016–2017 (Bermejo *et al*., 2022), while the N:P ratio increased from 19.1–32.5 to 44.7–60.82 over the same period. In this context, control of both N and P is necessary to mitigate coastal and estuarine eutrophication (Howarth and Paerl, 2008) and reverse biogeochemical imbalances in these ecosystems.

Regarding the origin of N loadings, to assess the relevance of the most likely sources of N, the $\delta^{15}N$ value to be considered must be that observed during the peak bloom period rather than other periods (Bermejo *et al*., 2022). During peak bloom, dissolved inorganic N concentrations in water are lower (Bermejo *et al*., 2019a,b, 2020b, 2022) as a consequence of nutrient uptake by the bloom, and isotopic fractionation is expected to be less relevant (Gröcke *et al*., 2017), providing more accurate and suitable information about the N sources. Overall, the $\delta^{15}N$ values recorded followed the expected patterns, with lower $\delta^{15}N$ values observed for the Argideen and Clonakilty estuaries, and higher values for the Tolka estuary (Figures 3.3d and 3.4d). The *Ulva* samples from the Tolka and *Gracilaria* samples from the inner section of Clonakilty showed an isotopic N signature that exceeded 10‰, suggesting N enrichment from urban wastewaters (15‰) (Cohen and Fong, 2006; Piñón-Gimate *et al*., 2017). This matched expectations relatively well due to the considerable urbanisation of the Tolka catchment and the proximity of the WWTP discharge point to the *Gracilaria* bloom in Clonakilty. The low $\delta^{15}N$ values found during the peak bloom period in June 2016 in the Tolka estuary, when unusually high amounts of biomass were measured, could be related to enhanced nutrient loadings coming from sources other than urban wastewaters (Figure 3.3d). In the Argideen and the Clonakilty estuaries, where the catchment is dominated by agricultural activity, the isotopic signature of *Ulva* (8.2–9.1‰) was closer to the $\delta^{15}N$ values expected for seaweeds growing in environments enriched with N from agricultural run-off (manure and compost 8.1‰; Bateman and Kelly, 2007). At Killybegs (Figure 3.5d), the isotopic N signature observed for *Pilayella* during the peak of biomass (–3.8‰ and 3.8‰) was lower than expected. In this area, urban wastewater and fish processing waste were anticipated to be the most important sources of nutrients fuelling the development of the golden tide occurring in the surrounding area. However, the observed $\delta^{15}N$ values for *Pilayella* (–3.8‰ and 3.8‰) were far from those found in the available scientific literature for fish remains-based products (7.1‰; Bateman and Kelly, 2007) and urban wastewaters (15‰; Cohen and Fong, 2006; Piñón-Gimate *et al*., 2017). Further studies should determine the $\delta^{15}N$ of all likely sources of N in the Killybegs area, including fish-based industry, urban wastewaters and run-off from the surrounding catchment.

4 Assessing the Impact of an Invasive Red Seaweed on the Ecological Functioning of Irish Estuaries

4.1 Background

The red seaweed *G. vermiculophylla* is an invasive species native to the north-west Pacific that has proliferated in the temperate estuaries of Europe, North America and Africa (Sfriso *et al*., 2012; Krueger-Hadfield *et al*., 2017a; Bermejo *et al*., 2020b). This species can thrive in a wide range of environmental conditions (Yokoya *et al*., 1999; Nejrup and Pedersen, 2010, 2012), surviving in extremely stressful conditions (Nyberg and Wallentinus, 2009), and possesses great vegetative potential (Surget *et al*., 2017), all of which explain its rapid and successful colonisation of temperate estuaries around the world. Under conditions of nutrient overenrichment, *G. vermiculophylla* can display fast growth rates (Abreu *et al*., 2011; Pedersen and Johnsen, 2017), thriving in areas where native opportunistic species cannot (Ramus *et al*., 2017; Bermejo *et al*., 2020b). As with other seaweed blooms, the accumulation of large amounts of this red seaweed and its subsequent degradation can cause serious amenity and public health issues, as well as relevant negative impacts on ecosystem functioning and wildlife (Valiela *et al*., 1997; Sfriso *et al*., 2003; Smetacek and Zingone, 2013). However, some positive effects associated with the presence of this species have also been reported when it invades bare substrates. These positive effects were density dependent and related to the increase in environmental heterogeneity resulting from the canopy provided by this species and its metabolic activity, which can result in an enhancement of denitrification rates (Gonzalez *et al*., 2013) and a more diverse and structured associated community (Ramus *et al*., 2017).

Since 2008, when *G. vermiculophylla* was first recorded in Ireland, at Carlingford Lough (Krueger-Hadfield *et al*., 2017b), this species has spread across the entire island, with existing established populations confirmed in different estuarine environments, including Malahide (Co. Dublin), Tolka (Co. Dublin), Dungarvan (Co. Waterford), Argideen (Co. Cork), Clonakilty (Co. Cork) and Rusheen Bay (Co. Galway). To better understand the effects on ecosystem functioning of Irish estuaries, different experiments and observations were performed to (i) compare the abundance and diversity of the fauna associated with *Gracilaria* and *Ulva* blooms; (ii) assess the biotic interaction of this species with another native bloom-forming species (*Ulva flexuosa*) and a perennial foundational species (*F. vesiculosus*); and (iii) evaluate the rates of biomass decay of different marine macrophytes typical of estuarine environments.

4.2 Materials and Methods

4.2.1 Comparing the associated invertebrate community in Ulva *and* G. vermiculophylla *blooms*

Sampling area, sample collection and processing

The Clonakilty estuary (Co. Cork), located in the south-west of Ireland (51°36′45″N, 8°52′13″W), is affected by green (native *Ulva* spp.) and red seaweed blooms (non-native species *G. vermiculophylla*; Figure 2.8) (Bermejo *et al*., 2020b, 2022). By taking advantage of the co-occurrence of native and non-native bloom-forming species in this estuary, the epifaunal assemblages associated with these dominant species were compared. On four sampling occasions during 2016 and 2017 (i.e. August 2016, October 2016, February 2017 and June 2017), four locations within the Clonakilty estuary covered by large patches of *G. vermiculophylla* (site A: 51°37′08″N, 8°52′44″W; and site B: 51°36′42″N, 8°51′01″W) and *Ulva* spp. blooms (site C: 51°36′37″N, 8°52′28″W; and site D: 51°36′20″N, 8°52′24″W) were sampled at low tide (Figure 2.8). At each location, 15 to 20 25 cm × 25 cm quadrats were collected on each sampling occasion, resulting in a total of 253 samples. Invertebrates and macroalgae present in each quadrat were collected and transported in labelled plastic bags to the laboratory at the University of Galway. Once in the laboratory, seaweed biomass was rinsed with freshwater to remove adherent sedimentary and particulate material, debris and epifauna. *Ulva* biomass was separated into laminar and tubular morphologies, and the mass of each type was recorded after the removal of excess water using a manually operated low-speed centrifuge (i.e. a salad spinner). A similar procedure was applied in the case of *G. vermiculophylla*. The water used for rinsing the seaweeds was passed through a 1-mm sieve, and the retained fauna were separated from sediments, pebbles, seaweed fragments, shells and other materials. Benthic faunal biomass for each sample was individually preserved in 4% formalin until taxonomic identification. Because of the methodology employed, the most speciose groups of delicate crustacea could not be identified and these were classified as other crustacea.

Identification and abundance of benthic fauna

Before sorting and processing, samples were thoroughly rinsed with water to remove the formalin. Specimens were sorted to the lowest taxonomic level possible. Taxonomic nomenclature, identification and sorting were carried out under a dissecting microscope following Hayward and Ryland (2017) and WoRMS (2022). Once specimens were taxonomically sorted, they were weighed.

Data analysis

Prior to the statistical analyses, the algal and faunal biomasses were standardised to grams per square metre, by multiplying the weight obtained in the 25 cm × 25 cm quadrat by 16. The faunal biomass was also divided by the algal biomass, which resulted in the fauna–seaweed ratio (g_{taxon}/g_{algae}), which makes the patches of different seaweed biomass comparable (Anderson *et al*., 2005).

Statistical analyses were performed using the software R version 4.0.2 (R-Core Team, 2017). Data did not display normality or homoscedasticity even after transformation. A non-parametric test used for two-way factorial designs, the Scheirer–Ray–Hare test, was used to assess the effects of seaweed identity (two levels: *Gracilaria* and *Ulva*) and sampling occasion (four levels: August 2016, October 2016, February 2017 and June 2017) on seaweed biomass, associated faunal abundance, associated taxonomic richness and the fauna–seaweed ratio. A Venn diagram was used to depict associated fauna similarity.

4.2.2 Biotic interactions between **Ulva, G. vermiculophylla** *and* **F. vesiculosus**

Seaweed identification, collection and acclimation

Specimens of the bloom-forming species *G. vermiculophylla* and *U. flexuosa* were collected in the muddy tidal flats of the Broadmeadow estuary in Malahide (53°28′ 21.6″N, 6°8′33,11″W; Co. Dublin). Specimens of the native habitat-forming species *F. vesiculosus* were collected along the sheltered shoreline of Nimmo's Pier in Galway City (53°15′59.76″N, 9°2′50.49″W; Co. Galway). The seaweeds were transported to the laboratory wrapped in wet tissue inside a cooler box. There, thalli were rinsed with filtered artificial seawater to remove sediment, organic debris and epibiota. In the case of *U. flexuosa*, a small specimen showing a healthy visual aspect was cultivated for 2 months in the laboratory until sufficient biomass was obtained to perform the experiments. Subsequently, the biomass was maintained under low irradiance until the experiment commenced. Regarding *G. vermiculophylla* and *F. vesiculosus*, specimens were maintained in the laboratory for 3 months and 1 month, respectively, prior to the experiment [14°C; 15–30 µmol photons $m^{-2} s^{-1}$; 12 hours light–12 hours dark; salinity 32 psu (practical salinity unit); half-strength f/2 medium replaced once a month]. Previously, specimens of the three species (approx. 10 g) were cultivated separately in 5 L of aquaria for 2 weeks to acclimatise the seaweeds to the experimental conditions (14°C; 80–100 µmol photons $m^{-2} s^{-1}$; 14 hours light–10 hours dark; salinity 16 or 32 psu; half-strength f/2 medium).

Specimens of *F. vesiculosus* were identified based on morphological features. In the case of *U. flexuosa* and *G. vermiculophylla*, morphological identifications were confirmed using molecular identification tools. In both cases, the large subunit of the ribulose bisphosphate carboxylase–oxygenase gene (*rbcL*) was considered for this purpose (see Bermejo *et al*., 2019b, 2020b, for further details).

Experimental set-up

To assess the biological interaction between *U. flexuosa, G. vermiculophylla* and *F. vesiculosus* under two different salinities, a factorial design was followed. Salinity conditions were established considering field observations in areas affected by macroalgal blooms from Irish estuaries (annual mean salinity values between 16 and 31 psu; Bermejo *et al*., 2019a). In these and other cold temperate estuaries, a spatial segregation has been observed between *Ulva* and *Gracilaria* blooms (Nejrup and Pedersen, 2010; Bermejo *et al*., 2019a, 2020b), with *Gracilaria* dominating the substrate in inner areas of the estuary under hyposaline conditions, and *Ulva* blooms dominating the outer area, where the salinity is more similar to the surrounding sea. Thus, considering these observations, the two different salinities considered were 16 and 32 psu. To identify the type of biological interaction between the three seaweed species, three species combinations were considered: *F. vesiculosus* versus *G. vermiculophylla*, *F. vesiculosus* versus *U. flexuosa*, and *G. vermiculophylla* versus *U. flexuosa*. For each combination, each species was cultivated with varying biomass proportions alone (monoculture; 100%) or in co-culture with one of the other two species at two different proportions (dominant, 66%; non-dominant, 33%).

The experiments lasted for 2 weeks. Salinity treatments were performed in two different runs separated by 15 days. A treatment (*U. flexuosa* alone, 32 psu) was repeated as a control, indicating no differences between runs. The seaweeds were incubated at an initial density of 1.2 g L^{-1}. Only specimens in a healthy vegetative state (and not reproductive, full of starch grains or containing bleached areas) were used. Three replicates were run per treatment. Artificial seawater was prepared using a commercial solution for marine aquaria (Coral Pro Salt, Red Sea) and distilled water. Each replicate consisted of a 500-mL wide-mouth Erlenmeyer flask filled with 250 mL of half-strength f/2 medium without silicate at 16 or 32 psu. The medium was replaced every 2 days to avoid bacterial growth and nutrient limitation. Light was supplied from above by white cool LED light (Chihiros, A-801 LED light lamp sea reef), providing 80–100 µmol photons $m^{-2}\,s^{-1}$. The Erlenmeyer flasks were placed on an orbital shaker at 100 rpm to provide continuous agitation, facilitating nutrient uptake and limiting the accumulation of oxygen. Irradiance, salinity and water temperature were monitored daily. Irradiance was measured using a flat underwater quantum sensor connected to a data logger. A precision immersion thermometer was used to monitor temperature. Salinity was measured using a digital refractometer (ATAGO PAL-1 Digital Hand-held Pocket Refractometer). No deviations from experimental conditions were observed during the monitoring of experimental conditions. All experiments and seaweed acclimation were performed in a walk-in constant temperature room at the Ryan Institute, University of Galway.

Relative daily growth rates

The relative daily growth rate (DGR) was calculated assuming an exponential growth (equation 4.1). The fresh weights before and after the incubation period were measured using a precision weighing scale accurate to 0.0001 g. The weights were recorded to the nearest 0.001 g. Before weighing, the specimens were gently blotted with filter paper until all wet spots disappeared:

$$\mathrm{DGR}\ (\mathrm{d}^{-1}) = \ln(\mathrm{FW}_{14}/\mathrm{FW}_{0})/t \qquad (4.1)$$

where FW_0 is the initial weight (g), FW_{14} is the final weight (g) and t is the number of culture days.

Data analysis

A two-way analysis of variance (ANOVA) was performed to assess the effects of salinity (two levels) and initial co-culture proportion (three levels) in the DGR of each species and species combinations. All variables accomplished normality and homoscedasticity assumptions according to the Shapiro–Wilk and Levene tests. A post hoc Tukey's test was used to compare between levels of factors when showing a significant effect.

4.2.3 Tissue decay in common estuarine macrophytes from Irish estuaries

Marine macrophyte collection

The biomasses of five different marine macrophytes typical of estuarine environments with different ecological strategies (two competitors and three opportunistic species; Grime, 1977) were collected from the muddy tidal flats of the Broadmeadow estuary in Malahide (Co. Dublin). The opportunistic macrophytes used for the degradation experiments were *G. vermiculophylla* and tubular and laminar morphologies of *Ulva*. Regarding competitor macrophytes, the species collected were the fucoid *F. vesiculosus* and the seagrass *Zostera marina* var.

angustifolia. Between 300 and 400 g fresh weight of each macrophyte was collected. Macrophyte biomass was transported to the laboratory wrapped in wet tissue in a cooler box. There, thalli were rinsed with filtered artificial seawater to remove sediment, organic debris and epibiota. In the case of the seagrass, leaves and roots were separated. Clean biomass was kept refrigerated (5°C) wrapped in wet tissue until commencement of the experiment (less than 24 hours). Four 30-L aquaria were filled with 20 L of muddy sediment from the sampling site and 5 L of artificial seawater (32 psu). These aquaria were placed in a walk-in constant-temperature room at the Ryan Institute, University of Galway, where the temperature was set at 15°C.

Experimental set-up

To determine decay for each of the six marine macrophytes or parts of the macrophytes (i.e. *F. vesiculosus, G. vermiculophylla,* tubular *Ulva*, laminar *Ulva*, *Zostera* leaves and *Zostera* roots), biomass samples were incubated in the laboratory at different times to estimate decay by loss of biomass. Each sample or replicate consisted of approximately 2 g fresh weight of living macrophyte tissue, which was placed in a nylon mesh bag (5 cm × 7 cm; mesh size of 0.5 mm). Samples of the different macrophytes were distributed equally between the four different aquaria (eight samples per aquarium and macrophyte, except for *Zostera* leaves and roots, for which only seven and six samples were used, respectively), and these were buried in the sediment at a depth of between 4 and 5 cm. Aquaria were kept in darkness at 15°C in a walk-in culture chamber. Four samples of each macrophyte were retrieved (i.e. one per aquarium) at eight different times (0, 7, 14, 21, 28, 35, 63 and 90 days). Subsequently, samples were washed gently with filtered seawater to remove sediments, and dried to constant weight at 60°C for 5–7 days. The percentage of dry biomass remaining (DBR) was calculated following equation 4.2:

$$\mathrm{DBR}\ (\%) = 100 - 100 \times ((\mathrm{DW}_x - (\mathrm{FW}_0 \times \mathrm{FW}_m{:}\mathrm{DW}_m)/(\mathrm{FW}_0 \times \mathrm{FW}_m{:}\mathrm{DW}_m)) \qquad (4.2)$$

where FW_0 is the fresh initial weight (g), DW_x is the final dry weight (g) and $\mathrm{FW}_m{:}\mathrm{DW}_m$ is the fresh–dry weight ratio estimated for each macrophyte, considering samples at time 0.

Data analysis

A generalised linear mixed model was used to assess differences between the different macrophyte tissues at each of the eight time intervals considered. “Macrophyte” was considered a fixed factor (six levels: *F. vesiculosus*, *G. vermiculophylla*, tubular *Ulva*, laminar *Ulva*, *Zostera* leaves and *Zostera* roots) and aquarium as a random factor (four levels). A post hoc Tukey’s test was used to compare levels of the factor when the effect of the fixed factor was significant.

4.3 Results

4.3.1 Comparing associated invertebrate community in Ulva *spp. and* G. vermiculophylla *blooms*

Associated fauna

Fifteen taxa associated with *Gracilaria* and *Ulva* canopies were identified (Table 4.1). Nine (60%) of these taxa were common to both *Gracilaria* and *Ulva* canopies, four (26.7%) were exclusive to *Ulva* and two (13.3%) were present in only *G. vermiculophylla* blooms (Figure 4.1). Overall, *Ulva* showed a larger number of associated taxa than *Gracilaria* ($H_{1,245} = 45.17$; $p < 0.001$; Figure 4.2a). No effect of

Table 4.1. List of taxa associated with *Gracilaria* and *Ulva* blooms

Taxon	*Gracilaria*	*Ulva*
Abra tenuis	1	0
Cerastoderma edule	1	1
Parvicardium exiguum	1	0
Scrobicularia plana	1	1
Mytilus edulis	1	1
Hydrobia acuta	0	1
Hydrobia ulvae	1	1
Littorina littorea	1	1
Littorina saxatilis	0	1
Littorina spp.	1	1
Melarhaphe neritoides	0	1
Rissoa lilacina	0	1
Rissoa parva	1	1
Carcinus maenas	1	1
Other crustacea	1	1

1, presence; 0, absence.

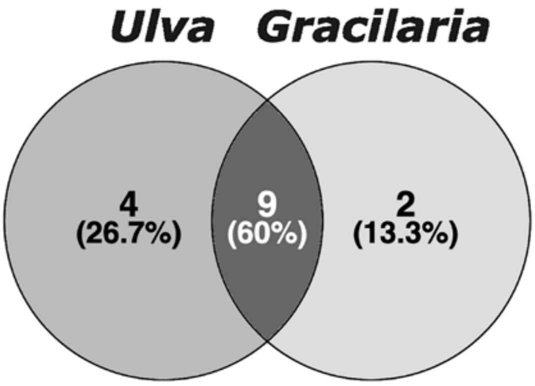

Figure 4.1. Venn diagram depicting similarities and dissimilarities in the taxonomic faunal composition associated with macroalgal blooms dominated by *Ulva* spp. and *G. vermiculophylla.*

sampling occasion was observed on associated taxonomic richness. A marginal significance was found for the interaction between seaweed identity and sampling occasion ($H_{3,245} = 7.73$; $p = 0.052$), as no differences in taxonomic richness between sampling occasions were observed for *Gracilaria*, but *Ulva* canopies depicted some seasonal variability, with higher taxonomic richness in February than in August and October.

Regarding seaweed biomass abundance, no differences were observed between *Ulva* and *Gracilaria* in the locality studied ($H_{1,245} = 0.36$; $p = 0.546$). Significant effects for sampling occasion ($H_{3,245} = 53.63$; $p < 0.001$) and for the interaction between sampling occasion and seaweed identity ($H_{3,245} = 14.46$; $p = 0.002$) on standing seaweed biomass were observed. In this case, *Gracilaria* showed a clearer seasonality biomass variability than *Ulva*, which displayed more stable biomass values throughout the year (Figure 4.2b).

Both factors, seaweed identity and sampling occasion, and their interaction showed a significant effect on faunal abundance ($p < 0.015$). Overall, *Ulva* yielded

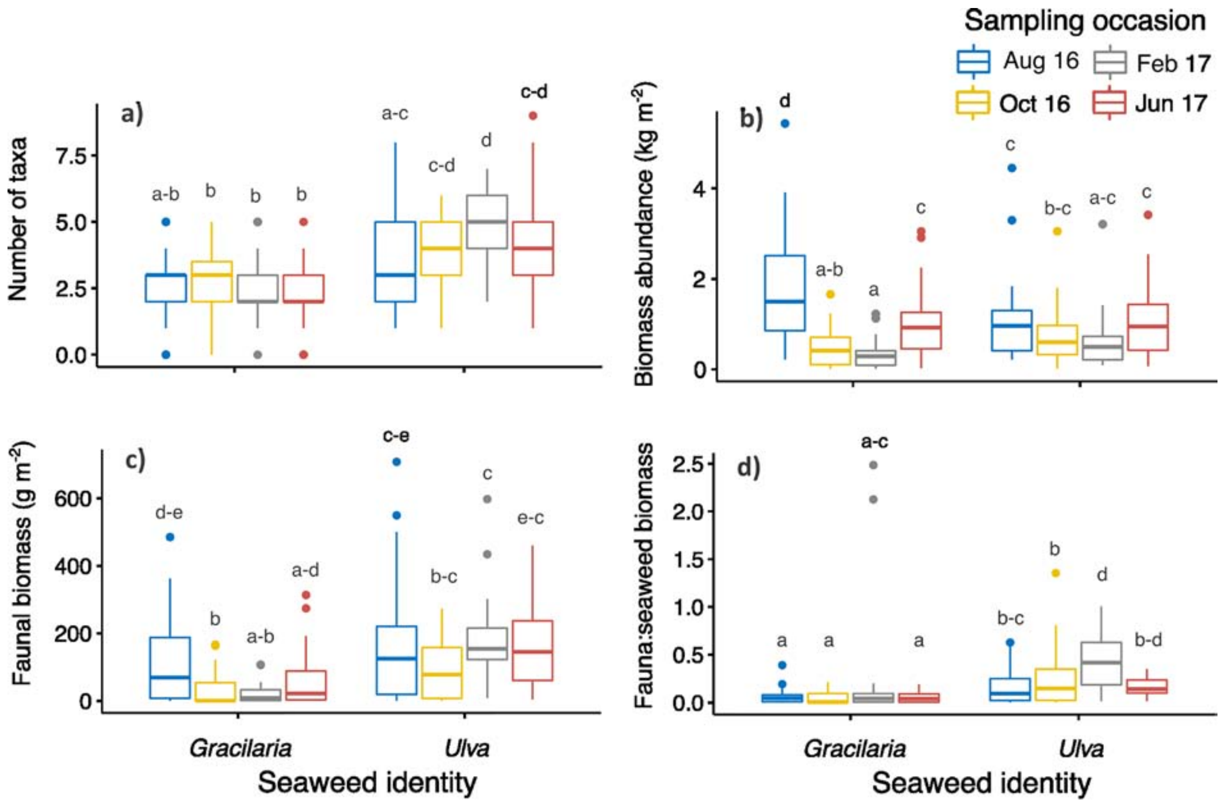

Figure 4.2. Box plot of the number of taxa associated with (a) macroalgal blooms, (b) seaweed biomass, (c) faunal biomass and (d) fauna–seaweed ratio, according to sampling occasion and seaweed identity. Box plots indicate the median (bold line near the centre), the first and third quartiles (the box), the extreme values whose distance from the box is at most 1.5 times the interquartile range (whiskers) and remaining outliers (dark dots). Letters over the box plots indicate the results of the Dunn's pairwise comparison post hoc test.

higher values of faunal biomass than *Gracilaria*. Regarding *Gracilaria*, faunal abundance was higher in August and lower in October and February. In the case of *Ulva*, no significant differences in faunal abundance were observed between the different sampling occasions (Figure 4.2c).

The fauna–seaweed ratio was significantly affected by seaweed identity ($H_{1,245} = 60.07$; $p < 0.001$) and sampling occasion ($H_{3,245} = 8.52$; $p = 0.036$), but no significant effect was found for the interaction between the two factors ($H_{3,245} = 3.97$; $p = 0.264$). No differences in faunal–seaweed ratio were observed between sampling occasions in the case of *G. vermiculophylla*. In the case of *Ulva*, this ratio showed maximum values during February and minimum values during August (Figure 4.2d).

4.3.2 Biotic interactions between Ulva, G. vermiculophylla *and* F. vesiculosus

G. vermiculophylla *versus* U. flexuosa

The results of the two-way ANOVA established that the growth of both *G. vermiculophylla* and *U. flexuosa* was significantly affected by the initial co-culture proportion and salinity (Table 4.2). This was not the case for either *G. vermiculophylla* or *U. flexuosa* (Figure 4.3).

Under seawater salinity conditions (34 psu; Figure 4.3a), the DGR of *G. vermiculophylla* ranged from 6.98% d^{-1} to 13.75% d^{-1}. For *U. flexuosa*, the DGR was greater, ranging from 12.09% d^{-1} to 20.75% d^{-1}. Under lower salinity conditions (16 psu; Figure 4.3b), the DGR of *G. vermiculophylla* was slightly higher than that of seawater, ranging from 9.78% d^{-1} to 14% d^{-1}.

Table 4.2. Results of the two-way ANOVA assessing the effects of "co-culture proportion" (proportion) and "salinity" on the DGR of *G. vermiculophylla* and *U. flexuosa*

		Gracilaria		*Ulva*	
	df	MS	*F*-value	MS	*F*-value
Salinity	1	19.735	15.806**	35.598	55.091***
Proportion	2	48.059	38.491***	126.469	195.723***
Salinity × proportion	2	3.889	3.115	0.535	0.828
Residual	12	1.249		0.646	

*******p*≤0.01; ****p*≤0.001.**

df, degrees of freedom; MS, mean square.

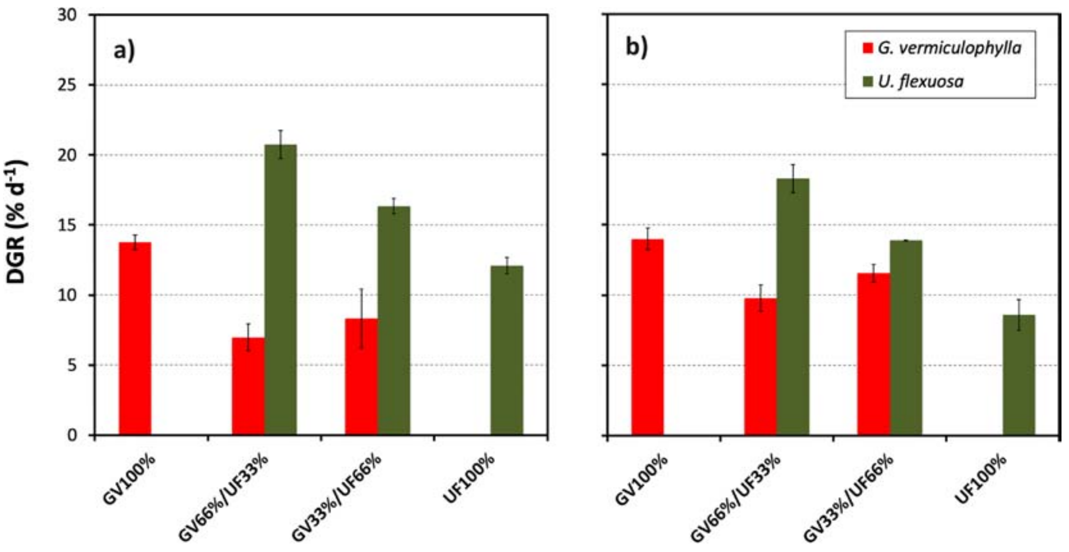

Figure 4.3. The DGR performance of *G. vermiculophylla* and *U. flexuosa* after monoculture and co-culture proportions under (a) seawater (34 psu) and (b) brackish (16 psu) salinity conditions.

For *U. flexuosa*, the DGR range was slightly lower than that under seawater salinity, ranging from 8.59% d^{-1} to 18.28% d^{-1}. In terms of overall performance (Figure 4.3), the DGR of *G. vermiculophylla* was hindered when in the presence of *U. flexuosa*, even when the species was abundant. By contrast, the DGR performance of *U. flexuosa* appeared to improve when it was co-cultivated with *G. vermiculophylla* as opposed to when cultivated alone. This was also observed even when *U. flexuosa* was not the most abundant species.

G. vermiculophylla *versus* F. vesiculosus

The two-way ANOVA results indicated a significant effect of co-culture proportion and salinity, as well as the interaction between both, on the DGR of *G. vermiculophylla* (Table 4.3 and Figure 4.4). In the case of *F. vesiculosus,* the DGR was significantly affected by co-culture proportion and salinity, but no significant interaction between factors was observed (Table 4.3).

Under seawater salinity conditions (34 psu; Figure 4.4a), the DGR of *G. vermiculophylla* was much higher than the DGR of *F. vesiculosus*. For *G. vermiculophylla*, the DGR ranged from 13.65% d^{-1} to 14.44% d^{-1}. For *F. vesiculosus*, the DGR was much lower, ranging from 3.93% d^{-1} to 6.16% d^{-1}. Under lower salinity conditions (16 psu; Figure 4.4b), the DGR of *G. vermiculophylla* was slightly higher than that observed for seawater salinity conditions (34 psu), ranging from 14% d^{-1} to 16.58% d^{-1}. For *F. vesiculosus*, the DGR range was slightly higher than that observed under seawater salinity conditions (34 psu), ranging from 4.70% d^{-1} to 6.94% d^{-1}. In terms of overall performance, the DGR of *G. vermiculophylla*

Table 4.3. Results of the two-way ANOVA assessing the effects of "co-culture proportion" (proportion) and "salinity" on the DGR of *G. vermiculophylla* and *F. vesiculosus*

		Gracilaria		***Fucus***	
	df	**MS**	***F*-value**	**MS**	***F*-value**
Salinity	1	6.130	25.642***	6.049	5.030*
Proportion	2	2.293	9.593**	7.511	6.246*
Salinity × proportion	2	3.429	14.345***	0.656	0.545
Residual	12	0.239		1.203	

$^*p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.
df, degrees of freedom; MS, mean square.

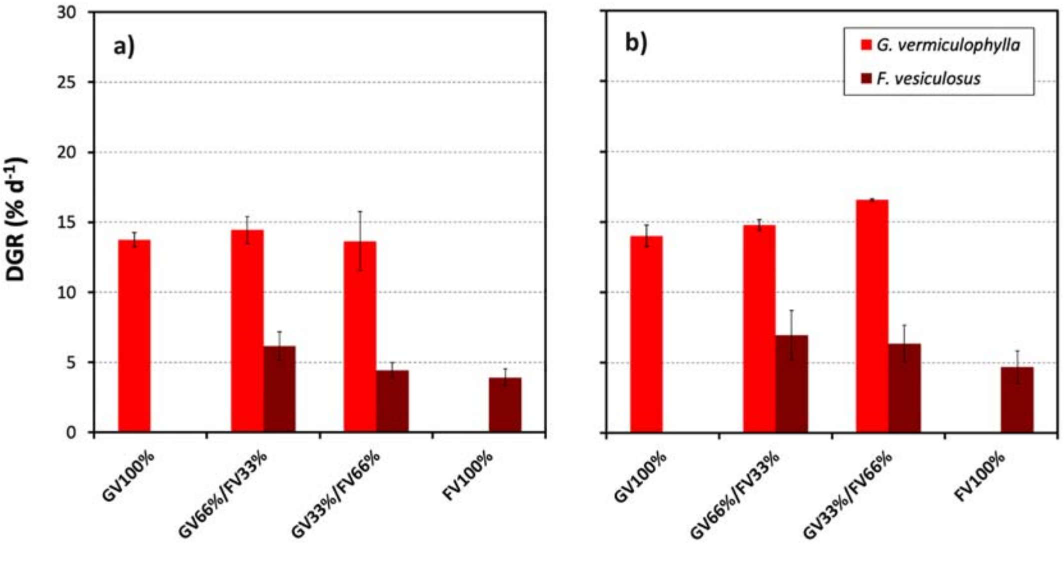

Figure 4.4. The DGR performance of *G. vermiculophylla* and *F. vesiculosus* after monoculture and co-culture proportions under (a) seawater (34 psu) and (b) brackish (16 psu) salinity conditions.

remained consistent throughout all biotic interactions under seawater conditions. Under conditions of lower salinity (16 psu), the DGR performance of *G. vermiculophylla* was enhanced slightly when in the presence of *F. vesiculosus*, even when the species was not in abundance (Figure 4.4). Such an improvement in DGR performance was also observed in the case of *F. vesiculosus*, the DGR of which increased slightly when the species was cultivated with *G. vermiculophylla*. This was the case for both salinity conditions.

U. flexuosa *versus* F. vesiculosus

The two-way ANOVA results revealed a significant effect of both co-culture proportion and salinity on the DGR performance of *U. flexuosa* (Table 4.4 and Figure 4.5). There was no significant interaction between biotic co-culture proportions and salinity conditions for *U. flexuosa*. In the case of *F. vesiculosus,* the DGR was significantly affected by salinity only. Regarding co-culture proportions, only a marginal difference was found ($p = 0.053$). No significant interaction between co-culture proportions and salinity conditions was recorded.

Under seawater salinity conditions (34 psu; Figure 4.5a), the DGR of *U. flexuosa* was significantly higher than the DGR of *F. vesiculosus*. For *U. flexuosa*, the DGR ranged from 12.09% d^{-1} to 23.99% d^{-1}. For *F. vesiculosus*, the DGR significantly varied between 2.54% d^{-1} and 3.93% d^{-1}. Under lower salinity conditions (16 psu; Figure 4.5b), the DGR of *U. flexuosa* was slightly lower than that observed for seawater salinity (34 psu), ranging from 8.59% d^{-1} to 19.08% d^{-1}. For *F. vesiculosus*, the DGR range was slightly higher at lower salinity than that observed under seawater salinity (34 psu), ranging

Table 4.4. Results of the two-way ANOVA assessing the effects of “co-culture proportion” (proportion) and “salinity” on the DGR of *U. flexuosa* and *F. vesiculosus*

		Fucus		***Ulva***	
	df	**MS**	***F*-value**	**MS**	***F*-value**
Salinity	1	8.564	18.458**	81.149	142.558***
Proportion	2	1.753	3.779	193.314	339.605***
Salinity × proportion	2	0.473	1.019	0.752	1.321
Residual	12	0.464		0.569	

$^{**}p \leq 0.01$; $^{***}p \leq 0.001$.
df, degrees of freedom; MS, mean square.

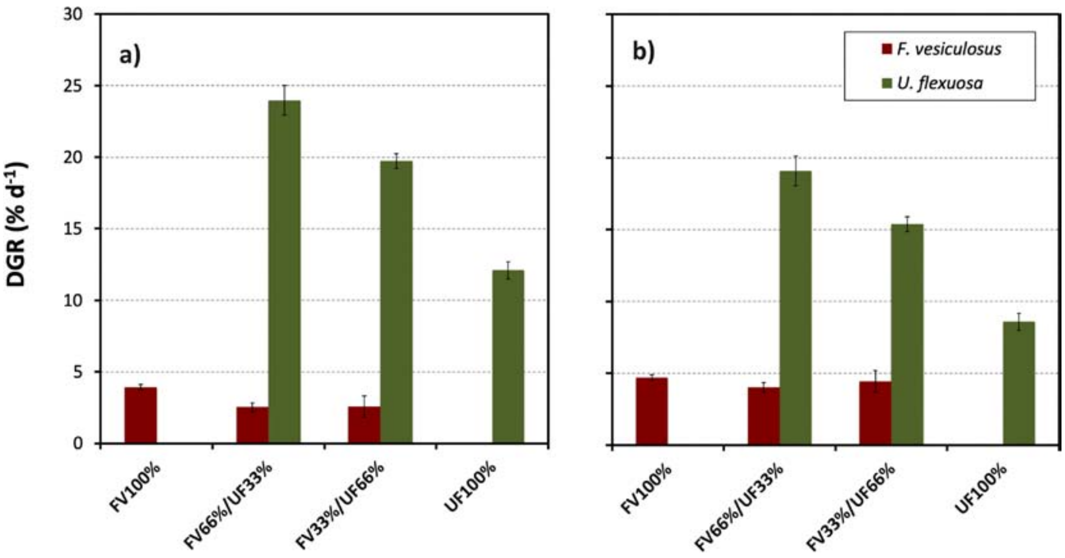

Figure 4.5. The DGR performance of *F. vesiculosus* and *U. flexuosa* after monoculture and co-culture proportions under (a) seawater (34 psu) and (b) brackish (16 psu) salinity conditions.

from 4.03% d^{-1} to 4.70% d^{-1}. In terms of overall performance, the DGR of *U. flexuosa* remained consistent throughout all biotic interactions under both seawater salinity (34 psu) conditions and lower (16 psu) salinity conditions. In contrast, for *F. vesiculosus*, the DGR performance decreased slightly when cultivated with *U. flexuosa* under seawater salinity (34 psu) conditions. Under lower salinity conditions (16 psu), the DGR performance of *F. vesiculosus* remained more or less constant throughout (Figure 4.5).

4.3.3 Decay rates in common estuarine macrophytes from Irish estuaries

All taxa showed an exponential decrease in remaining biomass throughout the experiment, with the exception of *G. vermiculophylla* (Figure 4.6), which showed an initial decrease during the first 2 weeks followed by a small increase and a subsequent decrease after 1 month. This species exhibited the slowest decay rate and the highest values of remaining biomass for all the sampling occasions considered. The thalli of *Ulva* displaying a laminar morphology yielded the lowest values for remaining biomass during the entire experiment. During the first 2 months, the rhizomes of *Z. marina* var. *angustifolia* and *F. vesiculosus* showed higher values of remaining biomass after *G. vermiculophylla*, followed by *Z. marina* leaves and tubular morphologies of *Ulva*. After 3 months, the remaining biomass for the different macrophytes ranged from the 4% of tubular *Ulva* to the 40–50% of *Z. marina* rhizomes and *G. vermiculophylla* thalli. At this time, the remaining biomass for the leaves of *Z. marina* was 27%, and ranged between 9% and 16% for tubular morphologies of *Ulva* and *F. vesiculosus*, respectively.

4.4 Discussion

The arrival of *G. vermiculophylla* in North American and European estuaries has had important impacts on the ecological functioning of mudflats (Sfriso *et al.*, 2012; Ramus *et al.*, 2017; Volaric *et al.*, 2019). When colonising bare substrates, this red seaweed adds structural complexity and environmental heterogeneity as a result of its own physical structure and its photosynthetic and metabolic activity (e.g. changes in sedimentation patterns, light gradients, increase in oxic–anoxic interfaces). The size and the type of the effects (positive or negative) on different ecosystem processes such as denitrification, sedimentation (Ramus *et al.*, 2017), habitat provision (Davoult *et al.*, 2017; Keller *et al.*, 2019) and food availability (Haram *et al.*, 2018) depend on the abundance of the seaweed biomass (Gonzalez *et al.*, 2013). Therefore, some authors have pointed out that the presence of this species enhances ecosystem multifunctionality and have stated that these effects can be considered positive for environmental management where native foundation species have been lost (Ramus *et al.*, 2017; Thomsen *et al.*, 2019). However, this approach has been considered oversimplistic by several other authors, who have highlighted that positive effects are species dependent (Haram *et al.*, 2018), mudflat

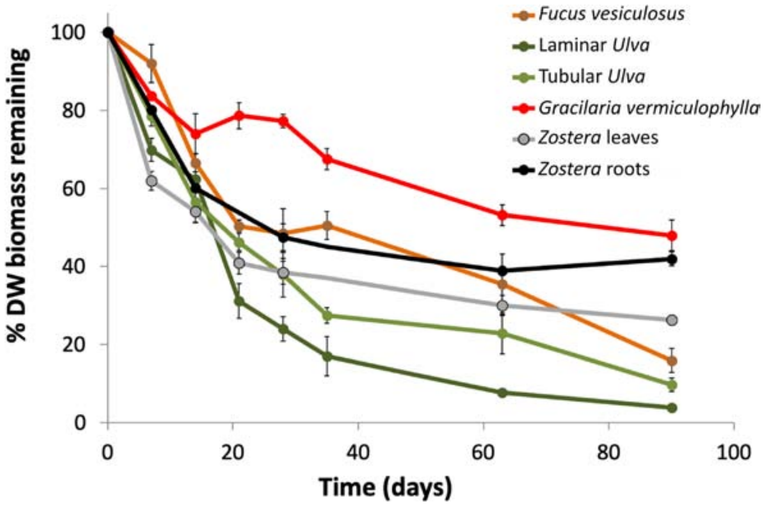

Figure 4.6. Percentage of biomass remaining during degradation experiment for different macrophytes typical of Irish estuaries. Error bars represent the standard error. DW, dry weight.

habitats harbour their own unique and diverse biota (Sotka and Byers, 2019) and *G. vermiculophylla* can lead to anoxic events and dystrophic crises after bloom occurrence (Keller *et al*., 2019; Bermejo *et al*., 2020b). The results of our study revealed that (i) the fauna associated with *G. vermiculophylla* blooms is not very different from the one associated with *Ulva* blooms in the Clonakilty estuary (Figure 4.1); (ii) other native opportunistic bloom-forming species (i.e. *U. flexuosa*) are more prone to outcompete native habitat-forming species (i.e. *F. vesiculosus*) than *G. vermiculophylla* in the context of eutrophication (Figures 4.4 and 4.5); (iii) *G. vermiculophylla* can thrive in areas previously devoid of native bloom-forming species (Figures 2.8 and 4.3); and (iv) this red seaweed can survive for several weeks buried in the sediment, which could have implications for carbon and nutrient sequestration (Figure 4.6).

This alien red seaweed acts as a habitat-forming species in areas previously devoid of vegetation, increasing habitat complexity, enhancing epibenthic diversity and ameliorating environmental conditions (Davoult *et al*., 2017; Ramus *et al*., 2017). However, other opportunistic bloom-forming species considered a nuisance and an ecological problem may play a similar role. In fact, the results revealed that *Ulva* canopies dominated by tubular morphologies in the Clonakilty estuary harbour a similar or slightly higher biomass of associated biota than *G. vermiculophylla* in relative ($g_{fauna}/g_{seaweed}$) and absolute (g_{fauna}) terms (Figure 4.2). Because of the short lifespan of these opportunistic species, following bloom occurrence a massive decline in associated fauna occurs as a consequence of anoxic events due to the decay of large amounts of biomass. These events can also affect biogeochemical processes and have long-term effects on biodiversity, which need to be assessed at the scale of decades (Gonzalez *et al*., 2013; Keller *et al*., 2019). The most pervasive mechanism producing negative effects of elevated macroalgal subsidies is the development of anoxic conditions promoting sulfide accumulation during seaweed degradation (Gonzalez *et al*., 2013; Lyons *et al*., 2014; Green and Fong, 2016).

The occurrence of anoxic events and their disruptive effects on ecosystem functioning should also be considered when assessing impacts on ecosystem functioning (Mineur *et al*., 2015; Ramus *et al*., 2017). Anoxic events due to excessive input of organic matter by decomposing *Gracilaria* and *Ulva* biomass have been previously described (e.g. Thomsen *et al*., 2006; Weinberger *et al*., 2008; Sfriso *et al*., 2012). No large anoxic events associated with fucoid assemblages or seagrass meadows have been recorded (Ramus *et al*., 2017). Therefore, the restoration of fucoids and seagrass meadows alongside nutrient abatement should be the most pertinent aim of environmental management strategies to restore ecosystem functioning of estuarine environments, and *G. vermiculophylla* should be considered another bloom-forming species.

Two relevant factors determining the occurrence of anoxic events associated with aquatic macrophyte biomass degradation are the decay rate and the standing degrading biomass. Previous studies have demonstrated that bloom-forming species show higher decay rates than late successional macrophytes such as seagrasses or fucoids (Banta *et al*., 2004; Conover *et al*., 2016; Pedersen and Johnsen, 2017). Conover *et al*. (2016) and Pedersen and Johnsen (2017) observed that *Fucus* and *Zostera* show lower decay rates than *G. vermiculophylla* and *Ulva*. These results differ from those obtained in the current study, as *G. vermiculophylla* showed the lowest decay rate (Figure 4.6) and did not follow the expected exponential biomass decrease (e.g. Pedersen and Johnsen, 2017). In this case, *Gracilaria* survived for several weeks after burial, which might explain the lower decay rates. This observation is consistent with previous experiments, which found that *G. vermiculophylla* thalli kept in darkness at 8°C for 175 days were able to survive and regrow (Nyberg and Wallentinus, 2009). Thus, these data do not permit the estimation of decay rates for *G. vermiculophylla*, and decay rates from previous studies should be considered when comparing decay rates between bloom- and non-bloom-forming species (Conover *et al*., 2016; Pedersen and Johnsen, 2017). Nevertheless, given the long-term survival of *Gracilaria* observed in this experiement, i.e. the presence of live buried thalli of *Ulva* and *Gracilaria* in the field during winter (Rinehart *et al*., 2014; Surget and Davoult, 2022; Bermejo, pers. obs., 2017), further studies should consider the implications of these findings for carbon and nutrient sequestration in estuaries affected by macroalgal blooms. Furthermore, it is likely that the survival of these thalli is also density and context dependent, as it is expected that anoxic conditions and high sulfide concentrations will limit the survival of buried thalli.

Opportunistic bloom-forming species such as *Gracilaria* and *Ulva* display short life cycles and show large seasonal variations in standing biomass as a result of alternating periods of fast growth and decomposition (Hernandez *et al*., 1997; Bermejo *et al*., 2019b, 2020b). By contrast, perennial or late successional species usually possess long life cycles and more stable standing biomasses, due to several factors such as lower productivity, higher exportation of primary production (e.g. exportation of decaying biomass because of the floatability of seagrass leaves) and more stable population dynamics without sudden and generalised die-offs, which limit the local availability of decaying biomass and the risk of anoxic events (Pedersen and Johnsen, 2017; Ramus *et al*., 2017; Bermejo *et al*., 2020b). The standing biomass of both *G. vermiculophylla* and *Ulva* blooms shows clear seasonal patterns and associated anoxic events during bloom decay when high biomass densities are reached (Valiela *et al*., 1997; Thomsen *et al*., 2006; Bermejo *et al*., 2020b). Regarding decay rates, Conover *et al*. (2016) and Pedersen and Johnsen (2017) obtained contradictory results when comparing decay rates for *Gracilaria* and *Ulva*. Considering that the freezing treatment applied by Conover *et al*. (2016) could produce a methodological artefact, Pedersen and Johnsen's (2017) findings can be considered more appropriate. In this case, the red alien seaweed *G. vermiculophylla* showed a slightly lower decay rate than alternative bloom-forming *Ulva* spp., which might slow down remineralisation cycling and act as a temporal sink for nutrients. Although the nutrient cycling may be reduced when fast-growing species such as *Ulva* spp. are replaced by *Gracilaria*, the opposite is expected when *G. vermiculophylla* replaces slow-growing species such as *Fucus* spp., *Ascophyllum nodosum* or seagrasses (Pedersen and Johnsen, 2017).

Regarding the biotic interaction of bloom-forming species with *F. vesiculosus*, the results (Figures 4.4 and 4.5) indicated that the native bloom-forming species *U. flexuosa* is more prone to outcompete and replace *F. vesiculosus* than *G. vermiculophylla*. In fact, the presence of *G. vermiculophylla* improved the growth of *F. vesiculosus* at both salinities assayed (16 and 32 psu). Hammann *et al*. (2013), in a mesocosm experiment, found a significant reduction in *F. vesiculosus* performance in the presence of dense canopies of *G. vermiculophylla* (4000 $\mathrm{g\,m^{-2}}$ fresh weight). Furthermore, these studies demonstrated that the presence of *G. vermiculophylla* provides refuge for consumers, increasing grazing pressure on *Fucus* (refuge-mediated apparent competition). The differences in biomass abundances (30–350 $\mathrm{g\,m^{-2}}$ vs 400–4000 $\mathrm{g\,m^{-2}}$) and the presence of grazers might partially explain these contrary results, and suggest that the intensity and sign of the interaction between opportunistic fast-growing and perennial slow-growing species may be density dependent. When assessing the interaction between ammonium toxicity and green tide development on the performance of *Zostera noltei,* Moreno-Marín *et al*. (2016) identified a positive effect of the presence of *Ulva* on this seagrass. The mechanism explaining this positive effect was related to the reduction in ammonium concentrations in water as result of its fast uptake by *Ulva*. A similar mechanism could explain the observed results in the current experiment, as seaweeds were cultivated under high nutrient concentrations (f/2 medium half-strength replaced every 2 days).

Thus, considering that (i) this species can bloom in areas previously devoid of native macrophytes, reaching high biomass densities that can lead to the occurrence of summer anoxic events, (ii) the future predicted temperatures for Ireland may enhance the growth of *G. vermiculophylla* in Irish estuaries and (iii) the number of estuaries affected by nutrient overenrichment as a consequence of the intensification of agriculture in Ireland is expected to increase (DAFM, 2021), the addition of this bloom-forming species to the Irish flora in a global change context could be considered a threat to biodiversity and ecosystem functioning, rather than an opportunity for the recovery of ecosystem functioning.

5 The Effects of Herbicides on Late Successional Estuarine Macrophytes

5.1 Background

Seaweeds and seagrasses provide critical ecosystem regulating services, including nutrient cycling, carbon sequestration and nursery habitats for species of commercial interest (Costanza *et al*., 1997; Nordlund *et al*., 2018; Mazarrasa *et al*., 2021). Nevertheless, these key species for ecosystem functioning are suffering significant declines in their populations globally because of human activities (Mineur *et al*., 2015; Casado-Amezúa *et al*., 2019). Intensification of agriculture remains one of the most important pressures on aquatic ecosystems worldwide because of the run-off of nutrients and associated contaminants (Hering *et al*., 2010; Glibert, 2017; Le Moal *et al*., 2019). In Ireland, agriculture is a highly valuable component of the economy, and the agri-food industry accounts for the largest exporting sector, which continues to grow on an annual basis (DAFM, 2021). There is a significant body of scientific literature on the effects of nutrient enrichment on aquatic vegetation; however, little is known about the impacts of herbicides on aquatic ecosystems (Licata *et al*., 2004; Falace *et al*., 2018), although on most occasions agricultural run-off contains both nutrients and pesticides.

It is estimated that up to 40% of the world's potential crop yield is lost every year due to pests, including weeds (FAO, 2021). Agriculture in Ireland and around the globe relies on the use of pesticides such as herbicides to guarantee and maximise yields of crops and plants. This reliance on chemical treatment resulted in over 2.6 million kg of pesticides being applied in Ireland on an annual basis from 2013 to 2020, according to the Pesticide Registration and Control Divisions of the Department of Agriculture, Food and the Marine. Adverse effects of pesticides on our aquatic systems include impairment of surface water and groundwater quality (Zhou *et al*., 2015) and non-target aquatic flora, such as seagrasses and seaweeds (Wilkinson *et al*., 2015). Once released into the environment, pesticides can move through the soil or surface water to streams and groundwater, where they can have unintended ecological effects, such as accumulation in aquatic organisms and loss of stream biodiversity (Beketov *et al*., 2013; Stehle and Schulz, 2015; Arisekar *et al*., 2019). Estuarine environments are especially sensitive to pollutants introduced into the oceans through rivers because of their hydrological and geomorphological characteristics (i.e. relatively small water bodies with low rates of water renewal), which act like a trap for nutrients and other pollutants derived from human activities (Rodrigues *et al*., 2018).

In Ireland, most pesticide use is for agricultural purposes or by local authorities to maintain common green areas. Herbicides accounted for 78% of the pesticides sold in Ireland in 2020 and the most used were glyphosate and MCPA (PCD, 2020). In 2017, an estimated 124,229 kg of glyphosate and 111,480 kg of MCPA in their commercial formulations (Roundup and Mortone, respectively) were applied to grasslands and fodder crops in Ireland, which was at least fivefold the quantities of other pesticides applied (PCD, 2020). Glyphosate is also extensively used for the control of weed growth on roadside verges and > 17,000 L has been applied across Ireland's road network since 2020 alone, at a cost of €250,000 (*The Journal*, 2021). This herbicide in its pure analytical form is depicted as "toxic to aquatic life with long lasting effects" (Merck, 2021). Among all EU Member States, Ireland exhibits the second highest detection levels of glyphosate in surface waters, at 186 $\mu g\,L^{-1}$, with concentrations $> 0.1\,\mu g\,L^{-1}$ also reported in groundwater (EFSA, 2021).

The aim of this study was to assess the effects of environmentally relevant concentrations of herbicides (glyphosate and MCPA) on the biological performance of late successional estuarine macrophytes, *F. vesiculosus* and *Z. marina*, in Ireland. Similar experiments were run with the bloom-forming species *Ulva lacinulata*; however, due to a massive sporulation event, these data were not included in this report.

5.2 Materials and Methods

Experiments were carried out to test the effects of 7 days of exposure to the herbicides glyphosate (Sigma Aldrich, PESTANAL, analytical standard) and MCPA (Sigma Aldrich, PESTANAL, analytical standard), and their commercial counterparts, Roundup (Roundup Bioactive XL) and Mortone (Hygeia, Mortone), on two late successional estuarine macrophytes, *F. vesiculosus* and *Z. marina*. One experiment tested the effects of glyphosate and Roundup, and a second tested the effects of MCPA and Mortone on *F. vesiculosus* and *Z. marina*.

5.2.1 Macrophyte collection and acclimation

Specimens of *F. vesiculosus* and *Z. marina* were collected from Galway Bay: *F. vesiculosus* was collected at South Park, Galway City, during spring 2022 and *Z. marina* was collected from an intertidal meadow in Connemara, County Galway, during the summer of 2022. The macrophytes were collected at low tide, wrapped in moist tissue paper and stored in resealable bags in a cooler box during transport to the laboratory. Both species were placed in nutrient-enriched artificial seawater (34‰; half-strength f/2 medium) in 20-L aquaria placed in a constant-temperature room at 14°C ambient temperature. Light was supplied from above by white LEDs (Chihiros A801 Professional Aquarium lights), providing 90 μmol photons $m^{-2} s^{-1}$. The photoperiod was set to 14:10 hours day–night. A pump was used to ensure water motion. Specimens were acclimatised to these conditions for 5 days prior to commencement of the experiment. Pre-experiment conditions of light, nutrient, temperature and salinity were maintained in subsequent experiments.

5.2.2 Experimental design

In the case of *F. vesiculosus*, three concentrations (22 μg L^{-1}, 220 μg L^{-1} and 2.2 mg L^{-1}) of each herbicide (glyphosate, Roundup, MCPA and Mortone) and one control (0 μg L^{-1}) were applied. This concentration range was considered appropriate and relevant to determine ecotoxicological endpoints [e.g. effect concentration 50 (EC_{50}), lethal concentration 50 (LC_{50})] and for comparative purpose with previous studies on *F. virsoides* (Falace *et al.*, 2018). Experimental treatments were replicated three times and each replicate consisted of 0.5 g fresh weight of *F. vesiculosus* with at least three apices. This biomass was placed in 500-mL Erlenmeyer flasks with a solution of herbicide and nutrient-enriched artificial seawater (250 mL, 34‰, f/2 half-strength), with the exception of control samples, which contained nutrient-enriched artificial seawater only. Culture medium was prepared and replaced every 2–3 days.

In the case of *Z. marina*, five concentrations (22 μg L^{-1}, 220 μg L^{-1}, 1.1 mg L^{-1}, 2.2 mg L^{-1} and 220 mg L^{-1}) of each herbicide (glyphosate, MCPA, Roundup and Mortone) and one control (0 μg L^{-1}) were assayed. Each replicate consisted of one apical shoot of *Z. marina* with three internodes, with maximum leaf lengths between 20 and 25 cm. Each shoot was placed in a 1.1-L volume tube filled with 900 mL of nutrient-enriched artificial seawater with one concentration of herbicide solution. Water motion was provided by bubbling with an air pump.

5.2.3 Biological performance assessment

Photosynthetic performance

The photosynthetic performance of photosystem II was assessed by chlorophyll fluorescence, using a MINI-PAM in the case of *Z. marina*. The photosynthetic yield (*Y(II)*) was used to assess photosynthetic performance. All measurements were recorded before and after experimental treatments.

Growth assessment

Relative DGRs of *Fucus* were calculated based on initial and final fresh weights, considering exponential growth (equation 5.1). The initial and final fresh weights were measured using a precision balance (Fisherbrand Precision Series Balance), accurate to 0.0001 g:

$$\text{DGR } (\%\ d^{-1}) = 100\ \ln(FW_f\ FW_0^{-1})\ t^{-1} \qquad (5.1)$$

where FW_f is the weight of each sample at the end of the experiment, FW_0 is the weight at the beginning of the experiment and t is the number of days the experiment ran for (7 days).

5.2.4 Data analyses

To assess the effects of each of the herbicides on the growth and photosynthetic performance of *F. vesiculosus* and *Z. marina*, two-way ANOVA tests were performed, considering pesticide concentration (three or five levels) and group (three levels: control group and both pesticides in each experiment) as fixed factors. Assumptions of normality and homogeneity of variance were tested using the Shapiro–Wilk test and Levene's test, respectively. A post hoc Tukey's test was used to compare levels when a significant effect was found. If a variable did not meet the assumptions of normality or homoscedasticity, a data transformation was performed. Statistical analyses were performed in SPSS (IBM SPSS Statistics version 27) software. In all statistical analyses, significance was set at $p < 0.05$.

5.3 Results

5.3.1 *Effects of glyphosate and Roundup on* F. vesiculosus

The two-way ANOVA revealed no significant effects of the pure analytical salt version of glyphosate and its commercial version, Roundup, across the different concentrations assayed on the growth (DGR) and on post-experiment measurements of photosynthetic yield *Y(II)* of *F. vesiculosus* (Table 5.1 and Figures 5.1 and 5.2).

5.3.2 *Effects of MCPA and Mortone on* F. vesiculosus

The ANOVA results for the DGR of *F. vesiculosus* revealed significant differences between the pure MCPA compound and the commercial formula, and marginal differences between the different concentrations (Table 5.2). The highest DGR ($5.53\%\ \mathrm{d}^{-1} \pm 0.09\%\ \mathrm{d}^{-1}$) was found in *F. vesiculosus* thalli cultivated at the maximum concentration ($2.2\ \mathrm{mg\ L}^{-1}$) of the commercial formulation. The

Table 5.1. Results of the two-way ANOVA assessing the effects of glyphosate and Roundup pesticides at different concentrations on the growth (DGR; $\%\ \mathrm{d}^{-1}$) and post-experiment measurements of *Y(II)* of *F. vesiculosus*

		DGR			*Y(II)*		
	df	MS	*F*-value	Probability (> *F*)	MS ($\times 10^{-5}$)	*F*-value	Probability (> *F*)
Pesticide	2	1.427	2.533	0.107	8.421	1.295	0.298
Concentration	2	0.237	0.421	0.663	7.705	1.185	0.329
Pesticide × concentration	4	0.253	0.448	0.722	6.905	1.062	0.404
Residuals	18	0.563			6.505		

df, degrees of freedom; MS, mean square.

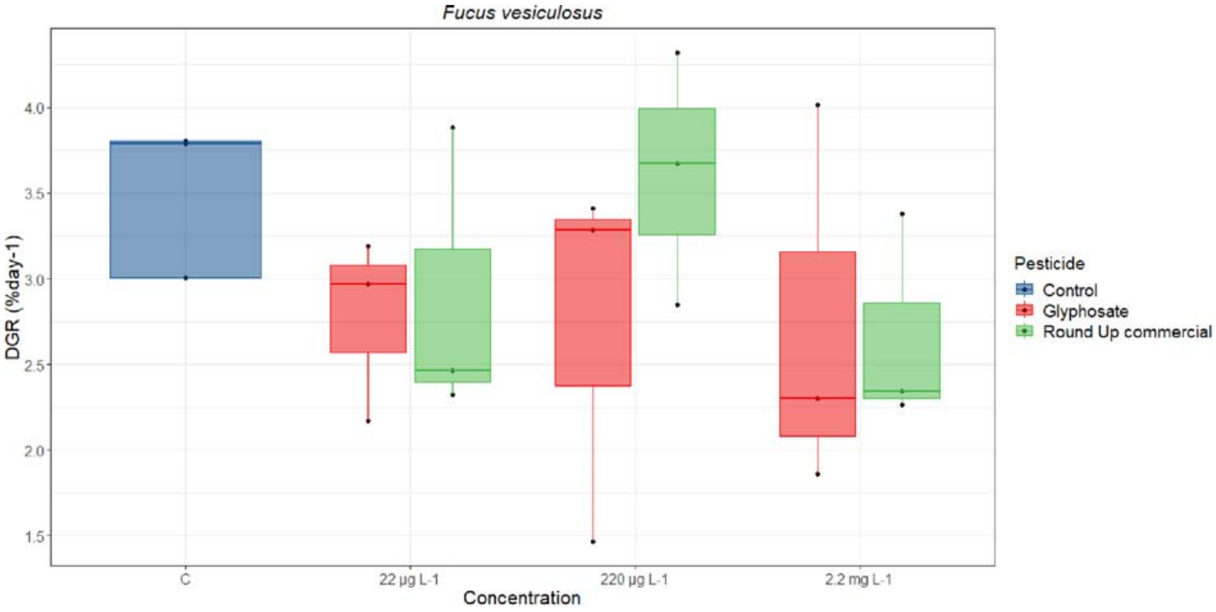

Figure 5.1. Box plot depicting the relative DGR of *F. vesiculosus* cultivated at different concentrations of the pure analytical salt version of glyphosate and its commercial version, Roundup.

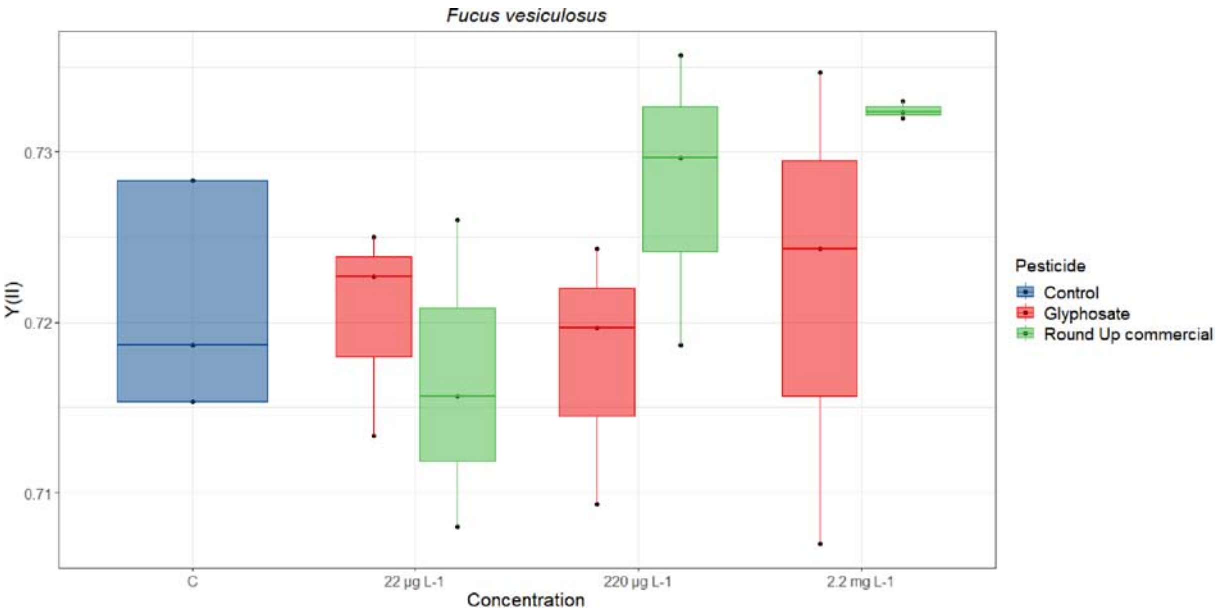

Figure 5.2. Box plot depicting post-experiment measurements of photosynthetic yield *Y(II)* of *F. vesiculosus* cultivated at different concentrations of the pure analytical salt version of glyphosate and its commercial version, Roundup.

Table 5.2. Results of the two-way ANOVA assessing the effects of MCPA and Mortone pesticides at different concentrations on the growth (DGR; % d^{-1}) and post-experiment measurements of *Y(II)* of *F. vesiculosus*

		DGR			*Y(II)*		
	df	MS	*F*-value	Probability (> *F*)	MS ($\times 10^{-3}$)	*F*-value	Probability (> *F*)
Pesticide	2	2.972	5.120	0.017	2.295	0.818	0.457
Concentration	2	1.836	3.164	0.066	10.002	3.566	0.049
Pesticide × concentration	4	0.942	1.623	0.212	8.632	3.078	0.043
Residuals	18	0.580			2.804		

df, degrees of freedom; MS, mean square.

lowest DGRs were observed for the control (3.48% d^{-1} ± 0.59% d^{-1}) and intermediate concentrations of the pure MCPA herbicide (3.18% d^{-1} ± 0.47% d^{-1}). Overall, specimens of *F. vesiculosus* cultivated in the presence of the commercial formulation of MCPA (i.e. Mortone) grew faster than specimens cultivated in absence of this pesticide or in presence of the pure MCPA compound (Figure 5.3).

With regard to the post-experiment measurements of *Y(II)* of *F. vesiculosus*, the ANOVA indicated significant differences in *Y(II)* between the different pesticide concentrations and a significant interaction between the concentration and the type of herbicide (Table 5.2). The maximum *Y(II)* values were recorded for specimens growing at the maximum Mortone concentration assayed (0.64 ± 0.01), and minimum *Y(II)* values were recorded for the intermediate Mortone concentration (0.49 ± 0.05) (Figure 5.4).

5.3.3 *Effects of glyphosate and Roundup on* Z. marina

The ANOVA results for the potential photosynthetic performance of *Y(II)* of *Z. marina* revealed significant differences between the pure glyphosate compound and the commercial formula (Table 5.3). The maximum post-experiment measurements of *Y(II)* values were recorded for specimens exposed to the intermediate assayed concentration (1.1 mg L^{-1}) of Roundup (0.70 ± 0.02), and the minimum post-experiment measurements of *Y(II)* values were recorded for

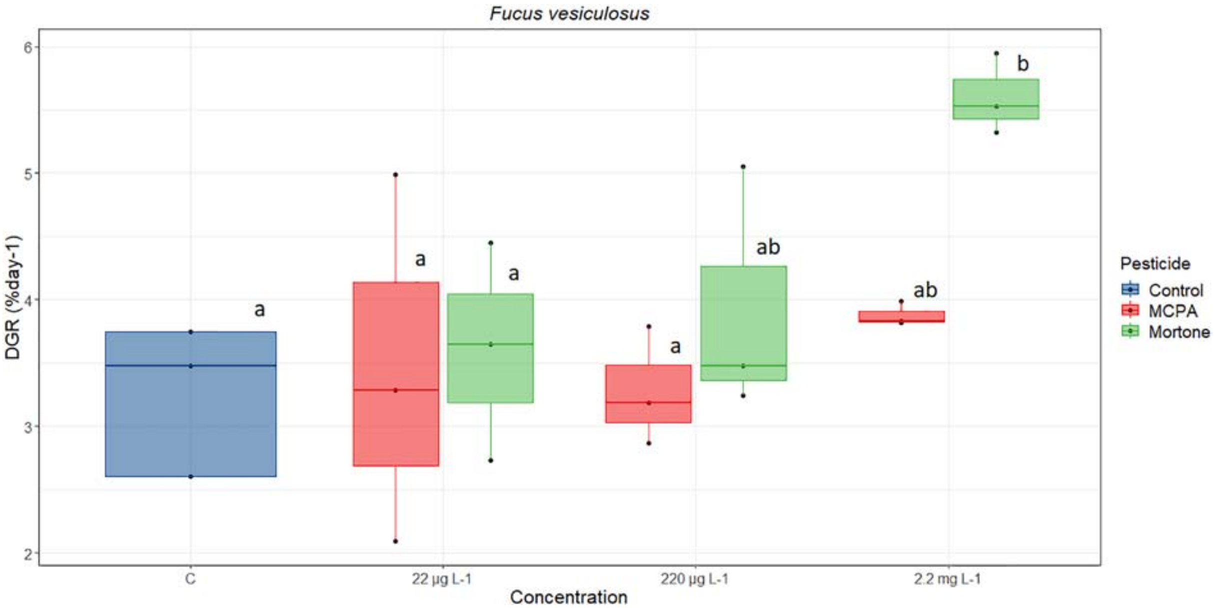

Figure 5.3. Box plot depicting the relative DGR of *F. vesiculosus* cultivated at different concentrations of pure MCPA and its commercial version, Mortone.

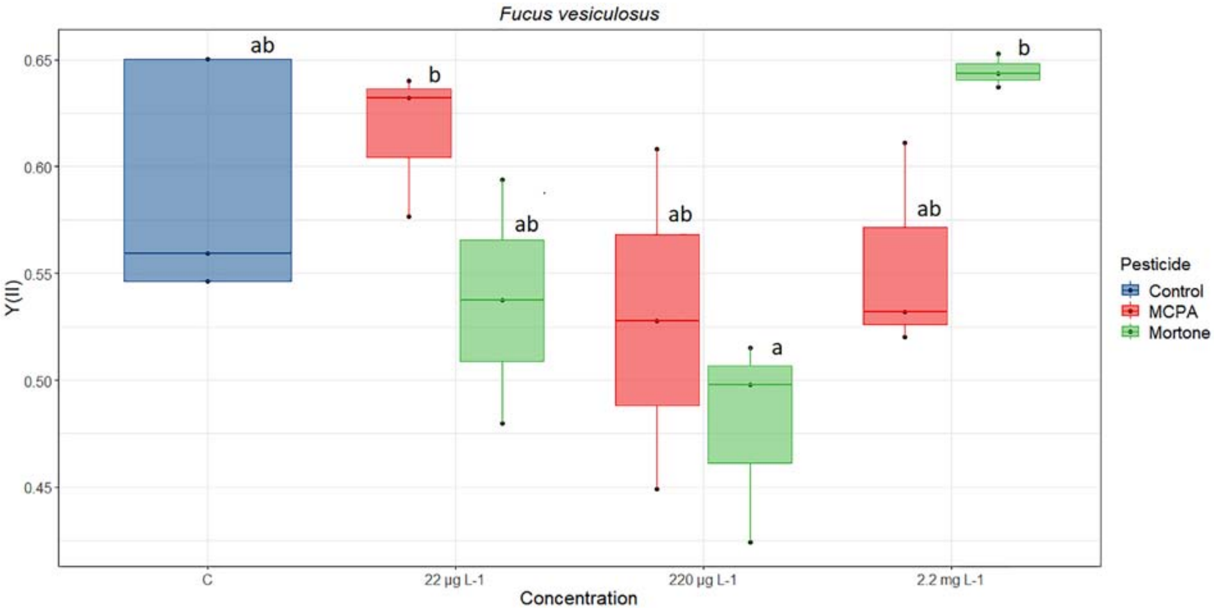

Figure 5.4. Box plot depicting the post-experiment measurements of *Y(II)* of *F. vesiculosus* cultivated at different concentrations of pure MCPA and its commercial version, Mortone.

Table 5.3. ANOVA of the effects of glyphosate and Roundup pesticides at three different concentrations in relation to the effect on post-experiment measurements of *Y(II)* of *Z. marina*

	df	MS	*F*-value	Probability (> *F*)
Pesticides	2	0.04289	10.841	0.000144
Concentration	2	0.00265	0.670	0.616
Pesticide × concentration	8	0.00431	1.089	0.388
Residuals	45	0.00396		

df, degrees of freedom; MS, mean square.

specimens exposed to the intermediate concentration (1.1 mg L^{-1}) of the pure salt of glyphosate (0.47 ± 0.12) (Figure 5.5).

5.3.4 *Effects of MCPA and Mortone on* Zostera marina

The ANOVA results for the potential photosynthetic performance of *Y(II)* of *Z. marina* revealed significant differences between the pure MCPA compound and the commercial formula (Table 5.4). The maximum post-experiment measurements of *Y(II)* values were recorded for specimens growing at the intermediate concentration (220 µg L^{-1}) of Mortone (0.71 ± 0.02), and the minimum for the intermediate concentration (1.1 mg L^{-1}) of MCPA (0.55 ± 0.09) (Figure 5.6).

5.4 Discussion

Although pesticides reduce the negative impact of pests on our food production systems, these benefits are frequently accompanied by contamination of the environment due to unintended release from the agricultural and urban sectors into non-target ecosystems (Schreiner *et al*., 2016; Chow *et al*., 2020; Mojiri *et al*., 2020). Organophosphate pesticides such as glyphosate have largely replaced organochlorines in the developed world and, despite significantly reduced toxicity compared with their predecessors, they have the potential to affect non-target organisms (Olisah *et al*., 2021).

Glyphosate and MCPA are two of the most commonly used pesticides in Ireland (Morton *et al*., 2020), and in many other regions globally (e.g. Falace

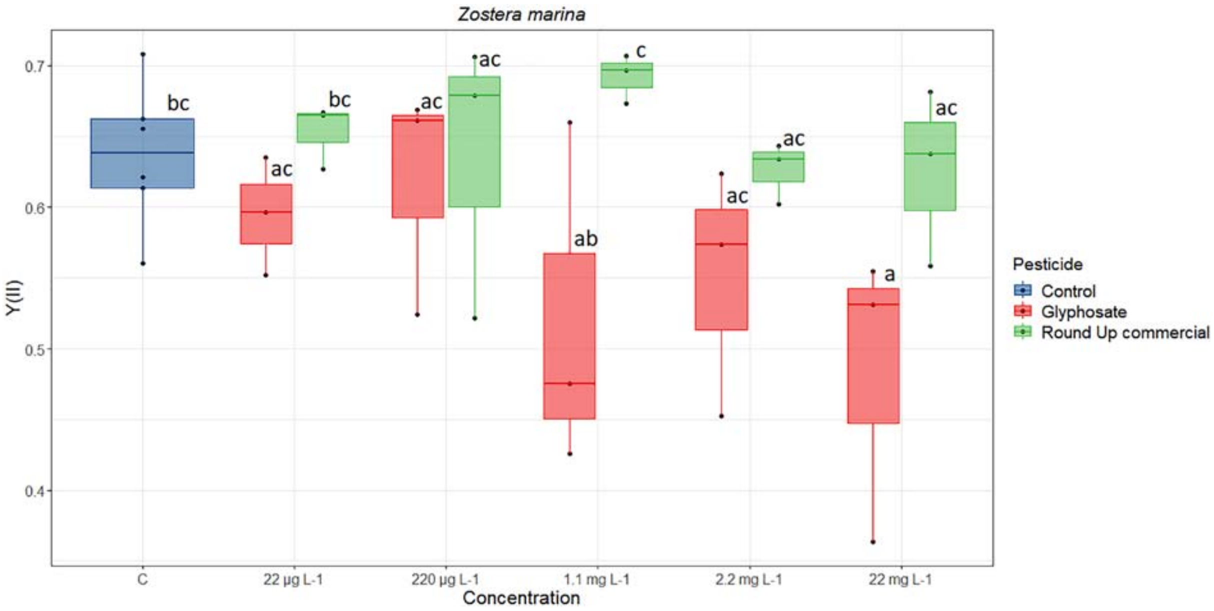

Figure 5.5. Box plot depicting the post-experiment measurements of *Y(II)* of *Z. marina* cultivated at different concentrations of the pure analytical salt version of glyphosate and its commercial version, Roundup.

Table 5.4. ANOVA of the effects of MCPA and Mortone pesticides at three different concentrations in relation to the effect on post-experiment measurements of *Y(II)* of *Z. marina*

	df	MS	*F*-value	Probability (> *F*)
Pesticides	2	0.04195	13.006	3.49e-05
Concentration	2	0.00099	0.307	0.872
Pesticide × concentration	8	0.00156	0.483	0.862
Residuals	45	0.00323		

df, degrees of freedom; MS, mean square.

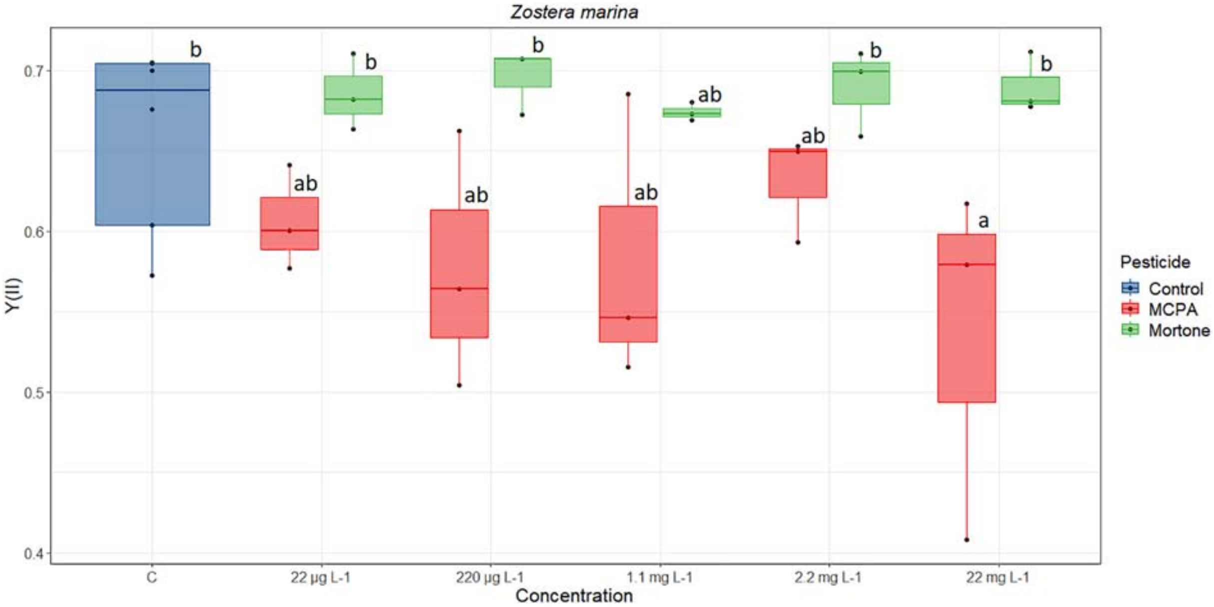

Figure 5.6. Box plot depicting the post-experiment measurements of *Y(II)* of *Z. marina* cultivated at different concentrations of the pure MCPA pesticide and its commercial version, Mortone*.*

et al., 2018; van Wyk *et al*., 2022). Glyphosate is a broad-spectrum herbicide that inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is key for the shikimate pathway (Steinrüken and Amrhein, 1980). This enzyme, found in plants, algae, fungi and some bacteria, is necessary for the synthesis of folate, aromatic amino acids and lignin; thus, its inhibition in plants and seaweeds can disrupt protein production, decrease chlorophyll content and reduce growth (Gomes *et al*., 2014). MCPA is a phenoxyalkanoic acid used to control annual and perennial weeds and is mostly used for the control of rushes (e.g. *Juncus* spp.) in Ireland (Morton *et al*., 2020). Phenoxyalkanoic acid herbicides belong to a group of chemicals related to the plant growth hormone auxin, and MCPA stimulates growth by increasing cell extension and cell differentiation (Hopkins, 1999). Although this compound can promote growth in some species, it can be toxic for others and it has been used as a selective herbicide (Morton *et al*., 2020). The widespread use of glyphosate and MCPA has impacted water quality in Ireland and has contributed to the increasing contamination of surface waters and groundwaters (Irish Water, 2021). Elevated levels of glyphosate have been detected in members of the adult Irish population with no obvious means of occupational risk or direct exposure to the substance (Connolly *et al*., 2018). In addition, it poses a serious threat to water bodies of all types, affecting non-target plants, including seagrasses and seaweeds (Wilkinson *et al*., 2015). Thus, the preliminary results obtained from these experiments will aim to bridge knowledge gaps in both seagrass and seaweed ecology from an Irish perspective, while providing valuable data on the effects of herbicides such as glyphosate and MCPA on these key habitat-forming species.

The preliminary results of the current study suggest that the two perennial macrophytes studied (i.e. *F. vesiculosus* and *Z. marina*) differ in their sensitivity to herbicides. The findings indicate that glyphosate had no short-term effects on the biological performance of *F. vesiculosus*, which contrasts with the findings of Falace *et al*. (2018) when assessing the effects of glyphosate on a closely related species (*F. virsoides*). However, MCPA appears to promote the growth of *F. vesiculosus* at elevated concentrations, which may partially be explained by this compound's role as a plant growth regulator, or because of the inorganic phosphorus content of this herbicide, which could act as a source of this limiting nutrient (Hopkins, 1999). Regarding the impact of these herbicides (MCPA and glyphosate) on the growth of *Z. marina*, Nielsen and Dahllöf (2007) conducted a short-term (3-day) experiment on this species and reported that growth (weight) was increased by almost 40% in comparison with controls for both herbicides. However, in the current study, both herbicides reduced the photosynthetic yield of *Z. marina* when the pure bioactive compound was applied. No effects were observed for the commercial formulations, which suggest a lower toxicity for *Z. marina*. Some caution should be applied when analysing these preliminary results, as these herbicides were assessed independently and over a short cultivation period (i.e. 1 week). Although our findings agree with previously published research on the impact of MCPA and glyphosate on marine macrophytes, it is worth noting that there is very little literature available on this subject. Further studies should consider a longer period of exposure, and assess the interaction with other pesticides and anthropogenic pressures, such as eutrophication (Falace *et al*., 2018) or increasing water temperatures (Gamain *et al*., 2018). Other opportunistic bloom-forming species (e.g. *Ulva* spp., *G. vermiculophylla*) should also be considered in future studies on the impacts of commonly used herbicides to assess and predict the effects of these substances on estuarine ecosystem functioning, as these preliminary results suggest species-specific effects that could lead to relevant shifts in community composition.

The concentrations assayed are in most cases higher than those reported from the aquatic environment [peak concentrations: 0.59–54 $\mu g\,L^{-1}$ for glyphosate (Mercurio *et al*., 2014) and up to 7 $\mu g\,L^{-1}$ for MCPA (Nielsen and Dahllöf, 2007)], although very few published data are available. The purpose of the experiment described here was to establish the ecotoxicological endpoints EC_{50} and LC_{50} for *F. vesiculosus* and *Z. marina.* However, these endpoints were not reached using the concentration range and exposure period used in this experiment.

6 Recommendations

Eutrophication persists as one of the most important threats to the functioning and biodiversity of aquatic ecosystems in Europe and beyond, and the reduction of nutrient loading remains the main restoration measure (Hering *et al*., 2010; Boesch, 2019). The most common limiting nutrients for primary producers in terrestrial and aquatic ecosystems are nitrogen and phosphorus (Lapointe, 1987; Howarth *et al*., 2000; Glibert, 2017), which therefore are the main components of the organic and synthetic fertilisers widely used in agriculture. These and other agriculture-related contaminants (e.g. pesticides and herbicides) are introduced into aquatic ecosystems via land run-off and urban and industrial wastewaters (Bermejo *et al*., 2022). Estuarine environments are very susceptible to pollution as a result of their hydrodynamic characteristics and size, and are the initial receiving waters for many contaminants in the land to sea pathway. In cold temperate estuaries, primary production is limited by light and temperature during the winter and by nutrients during spring and summer (Wang *et al*., 2012; Bermejo *et al*., 2019b; McGovern *et al*., 2019). In the absence of anthropogenic nutrient enrichment, nitrogen has been identified as the main limiting nutrient constraining primary production (Valiela *et al*., 1997). However, since the production of nitrogen-based synthetic fertilisers on a commercial basis since the mid-20th century, a shift from nitrogen to phosphorus limitation has been observed in many aquatic ecosystems, alongside an increased frequency of occurrence and persistence of opportunistic macroalgal blooms (Smetacek and Zingone, 2013; Glibert, 2017; Bermejo *et al*., 2022). The results obtained in this study confirmed this scenario for Irish estuaries, where phosphorus limitation, rather than the expected nitrogen limitation, was observed during conditions of peak macroalgal bloom (Bermejo *et al*., 2020a, 2022). However, the excess of nitrogen observed in Irish estuaries in combination with the arrival of a nitrophilous opportunistic bloom-forming seaweed (*G. vermiculophylla*) with different ecological requirements has led to an increase in the size of macroalgal blooms, as *G. vermiculophylla* is able to thrive in areas where native opportunistic seaweeds cannot (Bermejo *et al*., 2020b). The arrival of this species in a eutrophication context can be a threat to native perennial species that are key for nutrient cycling (Cacabelos *et al*., 2012; Hammann *et al*., 2013), as subhabitats within estuaries previously unaffected by macroalgal blooms can now be impacted. Management action to control the spread and arrival of alien species, in combination with nutrient abatement, is recommended. Although hydrological modifications (e.g. increased river flushing, reduction in water residence times, enhancing tidal currents, enhancing turbidity) or geoengineering actions (e.g. injection of aluminium in sediments) have been suggested as measures to complement the reduction in nutrient loadings to accelerate ecosystem recovery from eutrophication (Duarte and Krause-Jensen, 2018), these measures could have unpredictable ecological consequences, and therefore extreme caution should be exercised. Reducing nutrient loadings through applying best agricultural practices and improving the efficiency of wastewater treatment facilities, and restoring key species involved in nutrient regulation, should be the preferred options for eutrophication abatement in Irish estuaries. Some productive activities (e.g. shellfish and seaweed aquaculture) taking advantage of eutrophication may help to ameliorate the negative effects of eutrophication, but the final aim should be restoring the estuary to pre-impact conditions, making the ecosystem self-sustainable (Moss, 2007). Shellfish or seaweed aquaculture should be promoted to mitigate eutrophication; however, special care must be taken regarding alien species, as aquaculture has been an important vector for the introduction of these species (Zenetos *et al*., 2010; Krueger-Hadfield *et al*., 2017a). With regard to monitoring, the various tools developed based on EO technologies in this study will complement field surveys and enhance the number and quality of data collected while maintaining monitoring costs. The biomonitoring of the nutrient status of macroalgal blooms and the investigation of the effects of emerging contaminants and pressures on ecologically relevant species might be considered for the development of monitoring and management strategies. Lastly, based on our findings, we recommend the following actions:

- Reducing nutrient loading from agricultural and urban sources, maintaining N:P ratios at levels similar to those found in estuaries under little or low anthropogenic pressure. In this scenario, it is important to reduce nitrogen loadings quicker than phosphorus loadings, to avoid an imbalance in the N:P ratio, which could lead to non-linear responses favouring community shifts and harmful algal blooms (see Glibert, 2017). The development of best agriculture practices focused on reducing nitrogen loadings and improving the effectiveness of wastewater treatment facilities should be a priority.
- In terms of biomonitoring, the use of tissue nitrogen and phosphorus content in bloom-forming species is critical for the assessment of the nutrient status of opportunistic macroalgal blooms. In specific cases, this information, in combination with field incubations involving nitrogen and phosphorus enrichment and controls (no enrichment), could provide more direct information about nutrient limitation and potential pressures.
- The nitrogen isotopic composition of bloom-forming species enables the identification of the likely sources of nutrient enrichment and therefore can help to stablish more effective mitigation strategies, in the case of either agricultural or urban sources. On a regional scale, this can be a forensic tool for the identification of specific localised pressures if the isotopic signature of putative sources is also considered.
- The combination of EO technologies and traditional field monitoring techniques will complement and enhance data quality and reliability regarding the distribution and abundance of opportunistic macroalgal blooms in coastal environments. In addition, the employment of EO technologies facilitates the historical reconstruction of blooms (including alien species), which is key to establishing baselines. This might allow the assessment of the suitability of management strategies and produce information valuable for model simulations and predictions of blooms.
- The expansion of *G. vermiculophylla* can increase the areas potentially affected by macroalgal blooms, but the effects on the benthos are less important than those produced by other opportunistic bloom-forming species. In any case, there is a need to control the expansion of such blooms.
- As glyphosate and MCPA are two of the most commonly used pesticides in Ireland, these compounds have been widely detected in the aquatic environment. However, although both are considered harmful to ecosystems and organisms, their impacts and effects on marine macrophytes (bloom-forming and non-bloom-forming species) remain poorly understood. This topic requires further thorough investigation, considering the prevalence of these herbicides.

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Abbreviations

ANN	Artificial neural network
ANOVA	Analysis of variance
Corine	Coordination of Information on the Environment
DGR	Daily growth rate
EO	Earth observation
EPA	Environmental Protection Agency
EU	European Union
L2A	Level 2A
MCPA	2-Methyl-4-chlorophenoxyacetic acid
MSFD	Marine Strategy Framework Directive
NDVI	Normalised Difference Vegetation Index
PSU	Practical salinity unit
RMSE	Root-mean-square error
SNAP	Sentinel Application Platform
WFD	Water Framework Directive
WWTP	Wastewater treatment plant

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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