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Nutrient status of major Irish seaweed tides.

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Mestrado em Biologia Marinha

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Declaração de autoria de trabalho

Statement of authorship

Nutrient status of major Irish seaweed tides.

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída,

Elena Schrofner, BSc
29 September 2020, Faro, Portugal

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Abstract

Blooms of opportunistic, fast-growing macroalgae (commonly known as seaweed tides), are no novel occurrence, but evermore enhanced in a growing number of places by nutrient over-enrichment, global warming and ocean acidification. Following their first appearance several decades ago, macroalgal blooms are shifting coastal communities and hold consequences for ecosystems and shore-based activities (e.g. shifts in primary producers, habitat loss of benthic invertebrates). The invention of nitrogen-based synthetic fertilisers in the early 20th century and increasing urbanisation, including enhanced sewage release along coasts, are considered primary causes. In this thesis dissertation, the nutrient status of the main bloom-forming macroalgal species (*Ulva compressa, U. prolifera, U. rigida, Agarophyton vermiculophyllum* and *Pilayella littoralis*) in Ireland was assessed based on tissue nutrient content. Hence, biomass abundance and nitrogen status of specimens from the four estuaries affected by the largest seaweed tides in Ireland were studied between June 2016 and August 2017 over seven sampling occasions, by collecting algal biomass and determining tissue nitrogen (N) and phosphorus (P) contents. Tissue N contents were compared to in previous studies extracted cell subsistence (Qs), and critical quotas (Qc) of the studied or similar species. The obtained results show that neither of the investigated species was limited by N at any time during the study since tissue N content exceeded Qc even during the bloom pinnacle, contrasting to the predominant conception considering N the primary limiting nutrient in cold temperate estuaries. Tissue N content was highest in winter and lowest in spring and summer, coinciding with the biomass peak (i.e. June to October), and therefore negatively correlating with biomass abundance. The results further indicate that slight increases in P might enhance bloom frequency and severity, meaning P needs to be reduced in Irish estuaries to prevent future blooms and preserve pristine coastal habitats.

Keywords: Eutrophication; nutrient bioassessment; seaweed tides; nitrogen; phosphorus

Resumo

Os afloramentos de macroalgas oportunistas e de crescimento rápido (conhecidos como marés de algas) não são uma ocorrência nova. Particularmente desde a segunda parte do século XX, estas macrófitas efémeras têm devastado ecossistemas ao causar grandes quantidades de biomassa e condições anóxicas assim como enormes custos para a sua remoção. Os gastos económicos referidos ocorrem, mais especificamente, devido ao impacto que os afloramentos têm nas pescas, turismo e actividades recreativas. Desde 2007, o Mar Amarelo na China é um exemplo utilizado frequentemente no que toca a densas acumulações de biomassa de macroalgas resultantes de afloramentos, contudo, são também reportados anualmente elevados níveis de biomassa muitas vezes seguidos de perda de habitat de invertebrados bênticos e quebras na indústria pesqueira em países como a França, os EUA ou a Irlanda. As despesas que resultam da remoção da matéria algácea em decomposição são, muitas vezes, na ordem dos milhões de euros (e.g. 200 milhões de euros em Qingdao, China em 2008).

Estes eventos de afloramento costeiro são intensificados, já que os níveis de nutrientes em águas estuarinas e costeiras estão a aumentar, sendo a eutrofização um dos factores críticos que pressionam e ameaçam os ecossistemas estuarinos europeus. A invenção de fertilizantes sintéticos à base de azoto no início do século XX e o crescimento da urbanização, com o aumento das descargas de águas residuais ao longo das costas, estão progressivamente a levar à ocorrência de marés de algas mais frequentes e severas. Para além do enriquecimento excessivo das águas, o aquecimento global, a introdução de espécies não-indígenas e a acidificação dos oceanos contribuem negativamente para a situação descrita.

Com mais descargas ricas em nutrientes a chegar a estuários pristinos, os ecossistemas tornam-se desequilibrados, experienciando constantes alterações nas comunidades (e.g. produtores primários). As macroalgas presentes não são mais limitadas por nutrientes, mas sim por outros factores como a temperatura ou luz. Assim que as condições melhoram na Primavera (i.e. as temperaturas aumentam e a disponibilidade luminosa é maior), o excesso de nutrientes libertados nas águas estuarinas permite que o crescimento de macrófitas marinhas exceda em muito as acumulações de biomassa que ocorrem naturalmente. Uma vez que as macroalgas efémeras são mais susceptíveis a limitações de nutrientes em ambientes com baixa concentração dos mesmos, estas podem substituir as macrófitas perenes de crescimento lento, como ervas marinhas ou fucóides, em condições eutróficas. Por esta razão, as marés de algas são regularmente descritas como um sintoma da eutrofização.

No geral, o azoto (N) é considerado o principal factor limitante em estuários frios temperados, enquanto que o fósforo (P) limita o crescimento de macroalgas em sistemas tropicais e de água doce. Neste trabalho, foi avaliado o *status* de nutrientes da principal espécie de macroalga responsável pela formação de afloramentos na Irlanda (i.e. marés verdes: *Ulva compressa, U. prolifera, U. rigida*; marés vermelhas: *Agarophyton vermiculophyllum*; e marés douradas: *Pilayella littoralis*). O conteúdo de nutrientes nos tecidos das algas deu-se como uma ferramenta competente na determinação do *status* nutritivo de marés verdes e de outros afloramentos de macroalgas, possibilitando ainda a detecção de alterações nos níveis de nutrientes de águas estuarinas, o que os torna bioindicadores de grande utilidade. Deste modo, foram estudados o conteúdo em azoto e a abundância de biomassa de espécimes de quarto estuários afectados pelos maiores afloramentos de algas na Irlanda (i.e. os estuários de Argideen, Clonakilty, Killybegs e Tolka) em sete ocasiões de amostragem entre Junho de 2016 e Agosto de 2017 através da recolha de matéria algácea e da determinação dos conteúdos de azoto e fósforo nos tecidos após ter sido realizada uma identificação taxonómica. Mais, foi efectuada uma análise de isótopos estáveis (∂^{15}N) para identificar as fontes de nutrientes.

A alga vermelha invasiva *A. vermiculophyllum* e a alga castanha *P. littoralis* ocorrem exclusivamente nos estuários de Clonakilty e de Killybegs, respectivamente, enquanto que foram recolhidas espécies de *Ulva* nos estuários de Argideen, Clonakilty e Killybegs. Na Irlanda, os nutrientes são, normalmente, provenientes da pecuária leiteira ou de descargas urbanas, dependendo do estuário. Foram comparadas as concentrações interiores de nutrientes a valores anteriormente extraídos de outros estudos ecofisiológicos para a subsistência celular (Qs) e quota crítica (Qc) das espécies estudadas ou semelhantes. A quota crítica de determinada alga é dada pela concentração mínima de nutrientes no tecido necessária para suster taxas de crescimento máximas; por outro lado, a quota de subsistência representa a concentração mínima de um nutriente, abaixo da qual não é possível qualquer crescimento.

Os resultados obtidos demonstram que, no decorrer do estudo, nenhuma das espécies investigadas foi limitada por N, já que o teor deste nutriente nos tecidos foi superior à Qc, mesmo durante o pico de um afloramento, o que contrasta com a ideia que predomina na literatura. Na Irlanda, foi observada sazonalidade de nutrientes e biomassa em marés verdes, vermelhas e douradas. As diferenças entre estuários podem ser resultado de diferentes fontes de nutrientes ou de outras características específicas dos locais, incluindo a hidrologia, geomorfologia e factores como o tempo de residência da água. O teor de N nos tecidos foi o mais elevado no período mais frio e o mais baixo nos períodos da Primavera e Verão (i.e. Junho a Outubro), e, por isso, correlaciona-se negativamente com a abundância de biomassa. Dados acerca do teor de P só estavam disponíveis para *A. verrmiculophyllum* no estuário de Clonakilty, o que significa que não foi possível estabelecer comparações entre espécies e estuários. Ainda assim, a rodófita invasiva mostrou uma clara limitação de P no estuário durante a época de afloramentos. A análise de isótopos estáveis revelou que as fontes de nutrientes não diferiam significativamente entre diferentes morfótipos de *Ulva* spp., mas sim entre os estuários de Tolka e Clonakilty. Para além disso, não houve diferenças entre as fontes de nutrientes de *Ulva* spp. e *A. vermiculophyllum* em Clonakilty.

Os resultados indicam que tanto P como N precisam de ser reduzidos nos sistemas estuarinos irlandeses através de ações de gestão, com o objectivo de prevenir outros fenómenos de afloramento e assim preservar os habitats costeiros pristinos. Tendo em conta a limitação de P observada em *A. vermiculophyllum* no estuário de Clonakilty durante alguns meses deste estudo, pequenos aumentos em P podem levar a *blooms* mais intensos. Com esta informação relativa ao *status* de nutrientes das principais marés de algas na Irlanda, podem ser desenvolvidos e melhorados planos de gestão para fertilização agrícola e tratamento de águas residuais.

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Chapter 1: Introduction

1.1. State of the art

1.1.1. Macroalgal blooms and their drivers - an introduction

Over the past decades, the rise of macroalgal blooms, also referred to as seaweed tides, was observed in an increasing number of estuaries and coasts all around the globe (Fletcher, 1996; Smetacek and Zingone, 2013; Valiela *et al.*, 1997). Seaweed tides are commonly named after the colour of their appearance, ranging from green and red to golden tides (Bermejo *et al.*, 2019b). These mass accumulations of marine bloom-forming macroalgae are usually the consequence of enhanced nutrient loadings in coastal waters, mostly derived from anthropogenic sources, co-occurring with other significant environmental changes as the consequence of human activity (Gao *et al.*, 2017; Smetacek and Zingone, 2013). Said anthropogenic stressors include ocean acidification, rising water temperatures and extended warming periods induced by global warming, and the introduction of alien species (often summarised as global change; Bermejo *et al.*, 2019b; Gao *et al.*, 2017; Liu *et al.*, 2020). Together, they represent a significant issue concerning the health of coastal ecosystems, possibly influencing growth phases and accelerating bloom development further in the future (Gao *et al.*, 2017; Glibert, 2020).

In pristine estuarine environments, macroalgal growth is restricted by nutrient availability during spring and summer, when temperature and light conditions are optimal for growth (Clark *et al.*, 2018; Valiela *et al.*, 1997), which is omitted after an estuary reaches the eutrophic state (i.e. excess nutrients within the estuary; Cohen and Fong, 2006; Lourenço *et al.*, 2006). Then nutrient limitation no longer occurs, with the nutrient concentration exceeding the one required for maximum growth by far (Smetacek and Zingone, 2013; Valiela *et al.*, 1997). Especially in light of global change, gaining a better understanding of bloom mechanisms of different macroalgae, particularly since multi-specificity is more common in green tides than previously thought, combining effects of different species (Bermejo *et al.*, 2019b), and developing adapted management plans, is of utmost importance (Diaz and Rosenberg, 2008; Rossi, 2006; Valiela *et al.*, 1997).

1.1.2. Impact of macroalgal blooms on coastal ecosystems and shore-based activities

Worldwide, seaweed tides are shifting coastal communities through a broad variety of effects (Jones *et al*., 2020; Nelson *et al*., 2008). Despite their numerous well-studied threats (Fan *et al*., 2020; Fort *et al*., 2020; Valiela *et al*., 1997), they might as well have some advantageous effects on marine ecosystems (Lyons *et al*., 2014; Rossi, 2006). Blooming macroalgae can create habitats and food sources for benthic animals and provide shelter for juveniles (Bermejo *et al*., 2020; Lyons *et al*., 2014). A study by Lyons *et al.* (2014) suggested that blooms of ephemeral macroalgae might even mitigate eutrophication effects by removing nutrients from coastal systems through improved nutrient cycling, and additionally lead to enhanced habitat complexity. Nevertheless, macroalgal blooms often hold a series of negative consequences for coastal ecosystems and shore-based human activities, in particular after the accumulating biomass exceeds a certain tipping point, as many countries are experiencing at this very moment (see below; Smetacek and Zingone, 2013; Teichberg *et al*., 2012; Valiela *et al*., 1997; Zhang *et al*., 2019). These impacts on estuarine communities can be of a direct or indirect nature, whereby different opportunistic algal taxa implicate distinct effects, either positive or negative (Lyons *et al*., 2014).

Several factors, among them nutrient over-enrichment, are leading towards a shift in primary producers in a growing number of shallow estuaries, changing from perennial macrophytes, like seagrasses or slow-growing macroalgae, to ephemeral, fast-growing, opportunistic seaweeds (Fig. 1.1.2.; Pedersen *et al*., 2010; Pedersen and Borum, 1996). Seaweed tides are specifically made responsible for the replacement of seagrasses, such as the eelgrass *Zostera marina*, by bloom-forming macroalgae (Bittick *et al*., 2018; Campbell, 2001; Hodgkin and Hamilton, 1993; Santos *et al*., 2020). With the latter becoming dominant (e.g. through shading or light competition), interactions between biotic and abiotic components and food web structures may alter (Lyons *et al*., 2014; Smetacek and Zingone, 2013), and transitions concerning species compositions may occur in estuaries or different coastal ecosystems (Santos *et al*., 2020). Especially sessile benthic fauna was found to be somewhat unable to adapt to or to cope with these critical changes (Jones *et al*., 2020; Lyons *et al*., 2014; Paumier *et al*., 2018).

Other consequences of seaweed tides are often massive alterations in biogeochemical cycles, with increases in ammonia and sulphide concentrations, causing anoxia and unfavourable conditions for other marine macrophytes (Gonzalez *et al*., 2013; Howarth *et al*., 2011; Rossi, 2006; Santos *et al*., 2020). Eutrophication leads to enhanced organic carbon (C) inputs into estuarine bottom sediments, further changing iron cycles, and increasing sulphate reduction rates followed by higher sulphide concentrations. Less oxygen is pumped in sediments due to the changing composition of benthic animal communities to less effective sediment irrigators, as the consequence of higher anoxia or hypoxia. With these alterations, also nutrient remineralisation of nitrogen (N) and phosphorus (P) begins to shift. First, N-cycling under eutrophic conditions is marked by reduced nitrification, denitrification and anaerobic ammonium oxidation (anammox) rates. Therefore, less N is converted to N_2 gas, leading to more N staying in the estuary and thus to bloom-favouring conditions within the system. Second, less P is stored in sediments because of the said shifts in nutrient cycles (mainly iron (Fe) and sulphur (S)), possibly to decreased mixing by benthic fauna, and is instead freely available for algae in the water column, contributing to bloom development (Fig. 1.1.1.; Howarth *et al.*, 2011; McGlathery *et al.*, 2007).

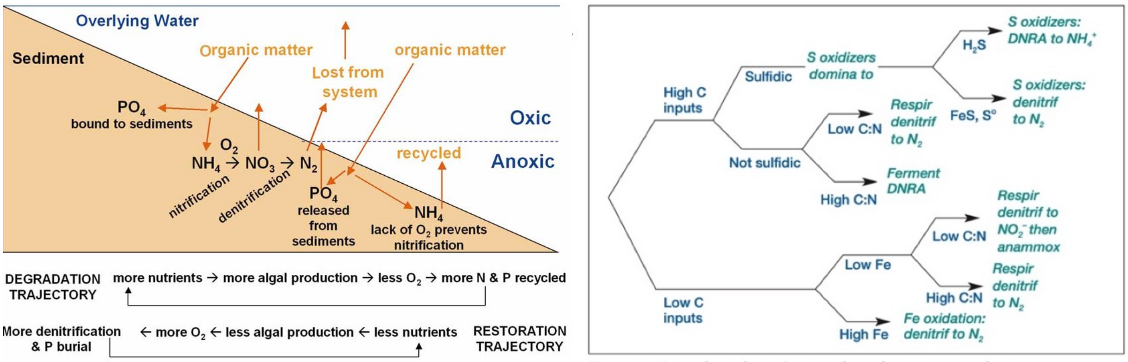

Fig. 1.1.1.: Nitrogen and phosphorus cycling under oxic and anoxic conditions in marine sediments (left; extracted from Rabalais, 2002) and other nutrient cycles in aquatic sediments (right; extracted from Howarth et al., 2011)

As soon as biomass numbers exceed a certain threshold, the coastal system might collapse, leading to colossal masses of decaying seaweed in an estuary (Teichberg *et al.*, 2012, 2009). Hypoxic conditions under the canopies are often the results (Fletcher, 1996; Smetacek and Zingone, 2013; Wan *et al.*, 2017), inducing habitat loss or populational decline of benthic fauna (Paumier *et al.*, 2018; Rossi, 2006). Several die-offs of terrestrial animals along coasts affected by macroalgal tides have been attributed to the release of hydrogen sulphide, related to the degradation of these tides (Smetacek and Zingone, 2013). Additionally, yield losses or other impacts on fisheries are reported with increasing frequency in the affected areas (Lyons *et al.*, 2014; Smetacek and Zingone, 2013). Besides, tourism is jeopardized along quite a number of coasts due to the above-mentioned decaying algal matter with its implications (Teichberg *et al.*, 2012).

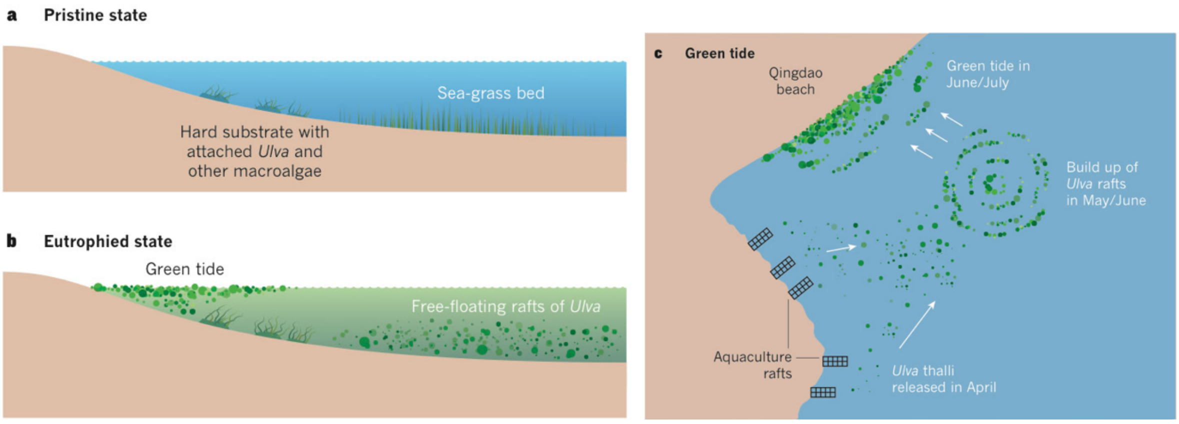

Fig. 1.1.2.: Transition from the pristine to the eutrophied state in an estuary (left) and the development of a green tide in the Yellow Sea (right, extracted from Smetacek and Zingone, 2013)

Seaweed tides vary broadly in their extent, and they are sometimes confined to constrained areas (e.g. a particular estuary or lagoon). In contrast, in other places, they take over large spaces (e.g. in the Yellow Sea; Lyons *et al.*, 2014). More and more places are being affected as the issue of global warming persists, and the problem of rising nutrient inputs into coastal waters remains unsolved (Teichberg *et al.*, 2009). Quingdao, in the Chinese province of Shandong at the Yellow Sea, represents just one example of the occurrence of massive seaweed tides. The city experienced several severe outbreaks of green tides, mainly *U. prolifera*, in the past decades, particularly from 2007 on (Fan *et al.*, 2020; Zhang *et al.*, 2019). Only between July and August 2007, for instance, around six thousand metric tons of biomass accumulated at its beaches, and not even one year later, right before the Olympic sailing competition, the largest green tide ever recorded worldwide occurred in the Yellow Sea between May and July (Fan *et al.*, 2014). There, off-shore *Pyropia* aquaculture rafts were found to be the source of the massive amounts of algal matter reaching the coast since *Ulva* spp. (often *U. prolifera*) grows attached to these rafts and is then transported to the shore by tidal currents (Fig. 1.1.2.; Han *et al.*, 2020; Jiang *et al.*, 2020; Zhang *et al.*, 2019).

Furthermore, also several other countries like France, the USA and Ireland, as the centre of this study, are facing bloom formations (e.g. by *Ulva*, *Cladophora*, *Codium*) and the resulting threats (Fig. 1.1.3.; Bermejo et al., 2019b; Green-Gavrielidis et al., 2018; Paumier et al., 2018; Teichberg et al., 2012). The high costs that arise from the removal of biomass, particularly of green tides (mostly *Ulva*; Lyons et al., 2014), represent another significant issue. Smetacek and Zingone (2013) stated that removing the beached algae amounted to approximately 30 million USD in Quingdao only in 2008.

Fig. 1.1.3.: Beached algal biomass (green tide) in Brittany, France (adapted from Smetacek and Zingone, 2013)

1.1.3. Eutrophication: causes and consequences

In literature, seaweed tides are often described as a symptom of eutrophication in estuaries (Teichberg *et al*., 2009). Eutrophication is an element of global change as the consequence of long-term human activity, especially since the industrial revolution in the 18th century. Additionally, Fritz Haber and Carl Bosch (Haber-Bosch process) were the first to invent synthetic nitrogen-based fertilisers at the beginning of the 20th century, and therefore to revolutionise agriculture (i.e. green revolution), implicating large-scale consequences for aquatic ecosystems (i.e. freshwater and marine) through agricultural run-off of nutrients (Costanzo *et al*., 2000; Glibert *et al*., 2014; Gonzalez *et al*., 2013). Apart from fertilisers, commonly named primary sources of nutrients are urban wastewaters or sewage (Fig. 1.1.4.; Costanzo *et al*., 2000; Liu *et al*., 2010; Teichberg *et al*., 2009). Transport to receiving water bodies usually takes place via streams and rivers, groundwater or direct discharge into the sea (Cohen and Fong, 2006; Zammit *et al*., 2005). Nitrogen is usually derived either from agricultural sites as run-off or from sewage-treatment facilities, insufficiently extracting nutrients (Cohen and Fong, 2006; Jeffrey *et al*., 1995; Teichberg *et al*., 2009). In the case of Phosphorus, phosphatic agricultural fertilisers are often named as the primary source for bloom-forming macroalgae (Fort *et al*., 2020; Hodgkin and Hamilton, 1993).

The so-called trophic status of an estuary, as an expression of its primary productivity (PP) and nutrient availability, can range from oligotrophic (nutrient-poor, low PP) over mesotrophic (medium nutrient availability, medium PP) to eutrophic (nutrient-rich, high PP). Water quality usually degrades from the first to the latter (Baker and Newman, 2014; Istvánovics, 2009). Despite the common conception of eutrophication being a novel human-made phenomenon, some estuaries or coastal systems can de facto become nutrient-rich due to natural processes such as close-by upwelling events, burial, river erosion or sedimentation over centuries

(Chislock *et al.*, 2013; Villares and Carballeira, 2004). Still, natural systems generally do not tend to be eutrophic to the currently observed extent (Beiras, 2018). Human-made nutrient inputs compound and accelerate this development, for example through increased nutrient release from sediments after the previous sedimentation of imported nutrients (e.g. from sewage discharge), meaning that formerly oligotrophic systems, for instance, can become eutrophic in only decades (Jeffrey *et al.*, 1995). Hence, today, scientists distinguish between natural and cultural eutrophication (also described as hyper-eutrophication; Beiras, 2018). In some areas, the natural eutrophication caused by the said factors still outweighs the nutrient over-enrichment through anthropogenic sources (Fletcher, 1996; Villares and Carballeira, 2004; Zammit *et al.*, 2005), yet, the number of culturally eutrophic estuaries is increasing worldwide.

As mentioned, in pristine coastal environments, marine macrophyte growth is usually limited by nutrient availability during the bloom season, when temperature and other environmental factors would be favourable for bloom development (Smetacek and Zingone, 2013; Valiela *et al.*, 1997). These conditions allow slow-growing seaweeds well adapted to low nutrient concentrations to thrive. As soon as nutrients are supplied from external sources, the system becomes unbalanced, and this limitation slowly disappears, giving rise to species which require higher amounts to proliferate (Fan *et al.*, 2014; Pedersen *et al.*, 2010; Pedersen and Johnsen, 2017).

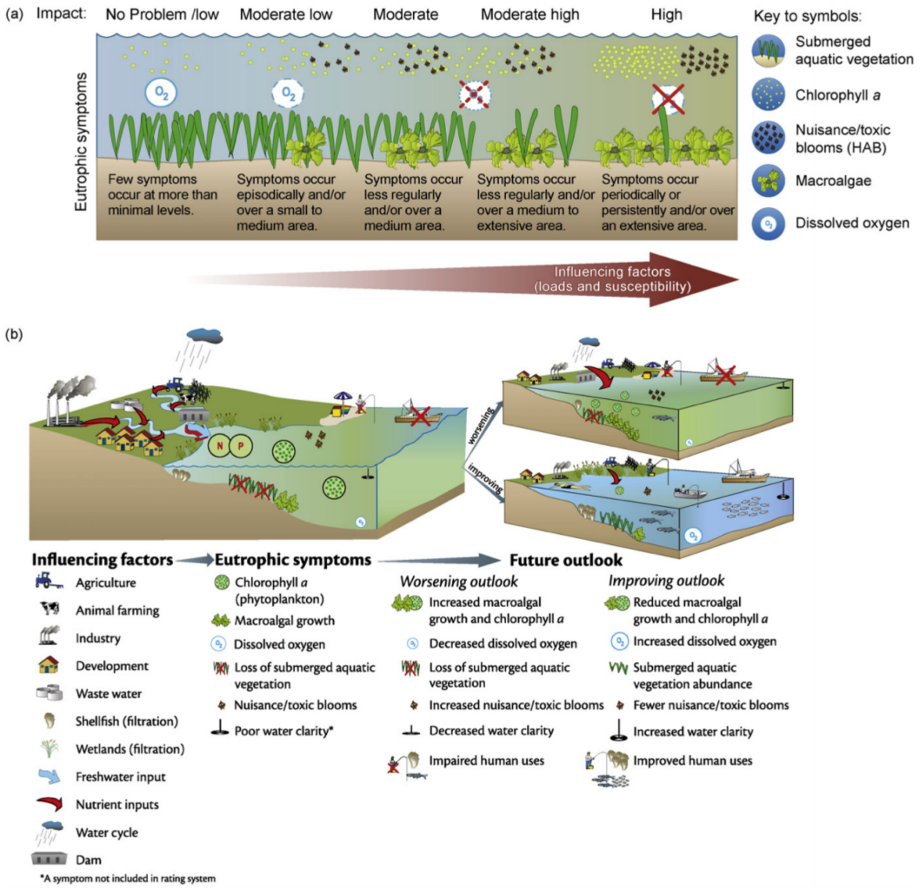

Fig. 1.1.4.: Causes and symptoms of eutrophication (extracted from Bricker et al., 2008)

1.1.4. Responsible nutrients for bloom development

Nitrogen (N) and phosphorus (P) are the two nutrients with the highest relevance for marine and terrestrial macrophytes, as they are usually found in limited amounts in aquatic environments (Fan *et al*., 2014). Supply of N and P can vary significantly within or between estuaries due to their high heterogeneity concerning mobility and spatial abundance (Fan *et al*., 2014; Valiela *et al*., 1997). Worldwide, the amounts of N accessible for marine macrophytes were found to be much larger than the ones of P (Teichberg *et al*., 2009). Both nutrients are necessary for the build-up of various cell structures. Nitrogen is mainly utilised for chlorophyll, amino acid and protein synthesis (Fan *et al*., 2014; Gao *et al*., 2017; Pedersen and Borum, 1997). Gao *et al.* (2017) stated that enhanced nitrate levels generally increase said synthesis in the case of *Ulva*. Phosphorus, on the other hand, is needed for phospholipid synthesis and energy transfer (ATP). Besides, both nutrients are crucial for DNA and RNA (Fan *et al*., 2014). The preferred form for N uptake is ammonium (NH_4^+; Fan *et al*., 2020, 2014; Fletcher, 1996), followed by nitrate (NO_3^-), whereas P is mostly taken up as phosphate (PO_4^{3-}; Teichberg *et al*., 2009). The assimilation of ammonium requires much less energy than the uptake and consequent necessary transformation of nitrate since ammonium is usually taken up via diffusion, whereas nitrate first needs to be brought into a utilisable form under energy expenditure by being reduced to nitrite (NO_2^-) and further to ammonium (Fan *et al*., 2020, 2014).

1.1.5. Nutrient limitation

As a consequence of their high importance for the algal metabolism, N and P are also the nutrients with the greatest potential to limit macroalgal growth, resulting from their often insufficient availability for algal requirements in seawater (Fan *et al*., 2014). If a nutrient is limiting for a particular seaweed can be examined based on the algae's tissue nutrient content (Fong *et al*., 1996; Lourenço *et al*., 2006; Lyngby *et al*., 1999; Villares and Carballeira, 2004). Therefore, the nutrient status is usually evaluated by conducting a tissue nutrient content analysis (Costanzo *et al*., 2000). A study by Lyngby *et al*. (1999) indicated that ambient nutrient levels positively correlate with the tissue nutrient content of seaweeds, thus providing a useful tool for detecting enhanced nutrient levels. Although physicochemical methods, including the measuring of N and P concentrations in seawater, allow an insight into the nutrient status of a water body, tissue nutrient content analysis provides more integrated information (Costanzo *et al*., 2000).

Teichberg *et al*. (2009), on the other hand, found that annual ambient DIN concentrations significantly affect macroalgal growth rates and further nutrient limitation. According to their study, low concentrations lead to control by DIN supply, whereas high concentrations induce P-controlled growth, independent of geographical or latitudinal position. They also observed that the availability of nutrients might vary spatially and temporally within an estuary and over time, leading to growth responses differing significantly between sites and seasons, particularly in temperate coastal zones. Therefore, opportunistic macroalgae are usually nutrient limited during a certain period of the year (commonly in summer during the peak of biomass in temperate estuaries; Bermejo *et al*., 2020; Lapointe, 1987; Pedersen *et al*., 2010; Teichberg *et al*., 2009).

However, as described above, growth seasonality depends not only on nutrient supply but also on abiotic factors like light intensity and temperature (Campbell, 2001; Fan *et al*., 2014; Jeffrey *et al*., 1995). Even when nutrient concentrations in the algae's environment are low, maximum growth levels can be upheld due to internal storage of nutrients (Pedersen and Borum, 1996).

Nevertheless, if the storage capacity of a species is insufficient for the supplied nutrients or external levels remain low over a longer period, a nutrient can become limiting for algal growth (Fong *et al.*, 1996; Pedersen *et al.*, 2010). There are two relevant tissue nutrient contents when talking about nutrient limitation, defined as the critical (Qc) and the subsistence quota (Qs) of a species. The critical quota is defined as the minimum interior nutrient content necessary to sustain maximum growth rates, whereas the subsistence quota marks the point below which no growth is possible at all (Pedersen and Borum, 1996; Villares and Carballeira, 2004).

In most previous studies, nitrogen was considered the primary limiting nutrient in cold temperate estuaries and coasts (Jeffrey *et al.*, 1995). Phosphorus, on the other hand, is usually made responsible for nutrient limitation in tropical coastal and freshwater ecosystems (Fan *et al.*, 2014). However, nutrient limitation depends on a series of other factors, among them said dimensions of internal nutrient pools, which can vary significantly among species (Pedersen and Borum, 1996; Teichberg *et al.*, 2009). As mentioned, requirements often differ between taxa and seasons, as well as uptake mechanisms and storage, due to differences in morphology (i.e. different surface: area ratios; Campbell, 2001; Pedersen and Borum, 1996). Small species with thin thalli, which implies a greater surface area to volume ratio, can grow and take up nutrients much faster per unit biomass and time than bigger ones with thicker thalli.

Moreover, slow-growers usually have much lower requirements, likely due to differences in tissue composition, but also higher affinities for a particular nutrient, when compared to fast-growing, opportunistic macroalgae (Pedersen *et al.*, 2010; Pedersen and Borum, 1996). According to Pedersen *et al.* (2010), fast-growing, blooming seaweeds may be more prone to nutrient limitation under nutrient-poor conditions. With an estuary reaching the nutrient-enriched state, their demands may eventually be covered, and the formation of dense canopies provoked. They further suggested that nutrient limitation might change with successful management of macroalgal blooms (i.e. temperate waters could become P-limited), which means that management plans would have to be adapted regularly. Nonetheless, a shift to P limitation could also occur if N loadings increase (Howarth *et al.*, 2011).

1.2. Seaweed tides of Ireland

1.2.1. Bloom-forming macroalgal species of Ireland

The overall number of bloom-forming macroalgal taxa is fairly small, yet, they conquered vast areas of the world (Teichberg *et al*., 2009; Valiela *et al*., 1997). Concerning Ireland, five species were found to dominate seaweed tides in the country and therefore investigated in this study, namely three species of *Ulva* spp. (*U. compressa*, *U. prolifera*, *U. rigida*), *Agarophyton vermiculophyllum* and *Pilayella littoralis* (Tables 1.1.-1.6.; Figures 1.2.1.-1.2.9.). Since morphological traits alone often do not allow taxonomic analysis, due to morphological similarity and plasticity, species determination usually takes place via the use of genetic markers (e.g. *rbc*L, see Chapter 2: Manuscript, Materials and Methods, Taxonomic identification; Bermejo *et al*., 2019b).

The frequent occurrence of the spatial and temporal succession of different species or diverse morphotypes (e.g. laminar and tubular *Ulva*) within the same genus might expand the affected area and the duration of the bloom (Bermejo *et al*., 2019a; Fong *et al*., 1996). Regarding spatial succession, this is often the case when different species are better adapted to the environmental conditions of a specific estuary than others, either through different requirements or the ability to occupy a particular ecological niche (Clark *et al*., 2018; Nelson *et al*., 2008). Especially the invasive species *A. vermiculophyllum* was found to be able to advance into areas of an estuary, where *Ulva* cannot (Bermejo *et al*., 2020; Fong *et al*., 1996).

Temporal succession, on the other hand, was discovered in laminar and tubular forms of *Ulva*: The first are more likely to be transported to the open ocean by wind and tidal currents, while the latter are anchored in the sediment, therefore having a greater local impact on the ecosystem (Bermejo *et al*., 2019a): Since tubular morphotypes attach themselves to the ground and get buried at the end of a seaweed tide, they can also help to promote favourable conditions for future blooms. By trapping organic matter in the sediment, they provide nutrients for subsequent seaweed tides, while incrementing the already existent eutrophication problem further (Bermejo *et al*., 2019a, 2019b). Moreover, several species of the genus *Ulva* were found to produce certain chemicals suppressing the growth of other organisms in their surroundings (i.e. allelochemicals) when competing with others (e.g. resources or light), for instance with phytoplankton (Harlin and Rice, 1987; Nan *et al*., 2004; Tang and Gobler, 2011). Bloom-forming species are also often less prone to grazing through chemical defence mechanisms (Bermejo *et al*., 2020; Fong *et al*., 1996).

1.2.2. *Ulva* spp.

formerly *Enteromorpha* spp.

Table 1.1.: Traits of Ulva spp. (Bermejo et al., 2019b; Bunker et al., 2017; Fan et al., 2014; Guiry and Guiry, 2020)

Type of macroalgal bloom	green tides, Chlorophyta
Description	13 *Ulva* species found in Ireland and Britain, species identification often difficult, isomorphic life cycle, rapid proliferation, broad tolerance against variable environmental conditions (e.g. salinities, temperatures)
Distribution	native to Ireland, worldwide
Morphology	tubular or laminar, light to dark green colour
Habitat	wide range of habitats worldwide, attached to substrates or free-floating

Fig. 1.2.1.: Phylogenetic tree of Ulva (extracted from Bermejo et al., 2019a)

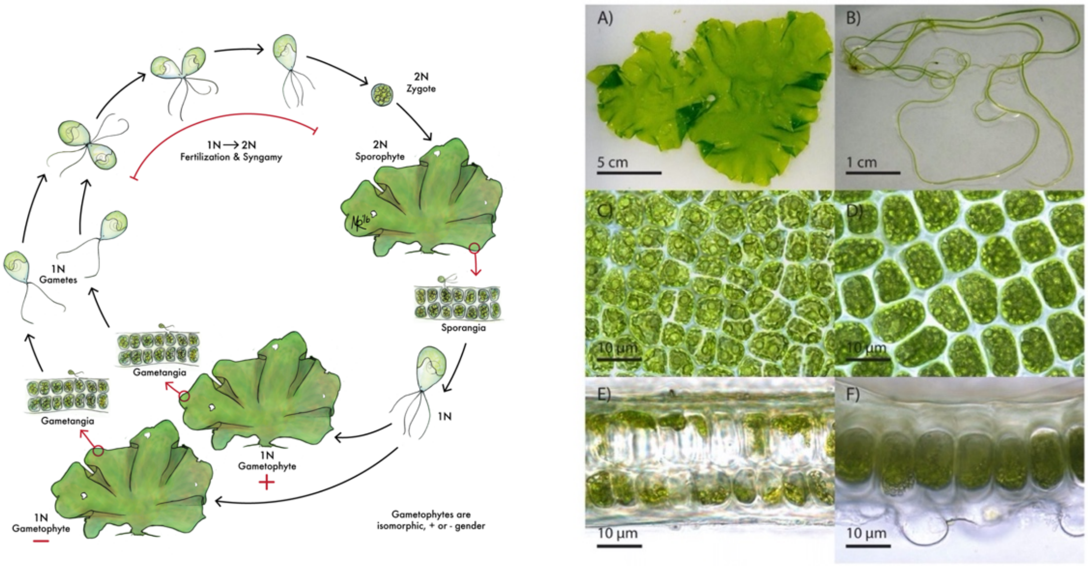

Fig. 1.2.2.: Life cycle of Ulva (left, extracted from Rocktopus, n.d.) and U. ohnoi and U. sp. light micrographs (right, extracted from Lawton et al., 2013)

Species Ulva rigida
(Rigid Sea Lettuce)

Fig. 1.2.3.: Ulva rigida (extracted from Guiry, 2020)

Table 1.2.: Traits of Ulva rigida (Bermejo et al., 2019b; Bunker et al., 2017; Guiry, 2020; Guiry and Guiry, 2020)

Description	easily confused, for instance with *U. fenestrata* and *U. gigantea*, dominant laminar *Ulva* species in Ireland
Distribution	common in the British Isles, widely distributed
Morphology	laminar, flat, sheet-like, quite stiff thallus with teeth around the margin, at times showing irregular, elliptical holes
Habitat	on rocks, stones or as an ephiphyte, also on other algae, upper shore to subtidal, sometimes free-floating

Species Ulva compressa

(Thread or Tape Weed)

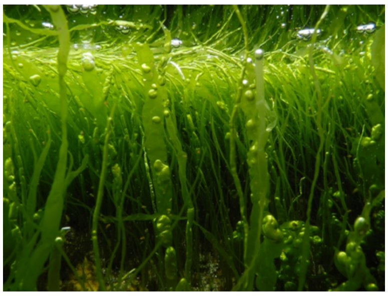

Fig. 1.2.4.: Ulva compressa (extracted from Guiry, 2020)

Table 1.3.: Traits of Ulva compressa (Bunker et al., 2017; Guiry, 2020)

Description	tolerant to a broad range of salinities and temperatures, fronds often show photosynthesis-derived oxygen bubbles, together with *U. prolifera* main species in Irish green tides
Distribution	British Isles, seemingly worldwide
Morphology	mostly tubular, but also laminar forms, compressed, flattened appearance, branched, elongated fronds (hollow)
Habitat	rock pools, sandy to rocky intertidals, on rocks, stones or as epiphyte on other algae

Species Ulva prolifera

(Proliferous Gut Weed)

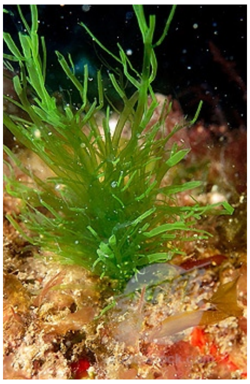

Fig. 1.2.5.: Ulva prolifera (extracted from Kipp et al., 2019)

Table 1.4.: Traits of Ulva prolifera (Bunker et al., 2017; Guiry and Guiry, 2020; Han et al., 2020; Zhang et al., 2019)

Description	together with *U. compressa* main species in Irish green tide
Distribution	British Isles, Yellow Sea, wide distribution
Morphology	tubular, branched (degree variable), soft
Habitat	marine to muddy or brackish, intertidal, genetically distinct specimes also in freshwater, epiphytic on fucoid seaweeds

1.2.3. *Agarophyton vermiculophyllum*

formerly *Gracillaria vermiculophylla*

(Worm Wart Weed or Black Wart Weed)

Fig. 1.2.6.: Agarophyton vermiculophyllum (extracted from Fofonoff et al., 2018)

Table 1.5.: Traits of Agarophyton vermiculophyllum (Bermejo et al., 2019b; Bunker et al., 2017; Fofonoff et al., 2018; Guiry and Guiry, 2020; Gurgel et al., 2018; Surget et al., 2017)

Type of macroalgal bloom	red tides, Rhodophyta
Description	invasive species, recent introduction, mainly through shipping of oysters, strongly modifies habitats with serious consequences for estuarine systems (e.g. replaces native algal species), tolerates a broad range of salinities and temperatures
Origin	Japan, Northwestern Pacific
Distribution	now worldwide, Eastern Pacific, Eastern and Northwestern Atlantic, as of recently including Ireland
Morphology	tubular, elongated, branched thallus, variable habitus, colour range from dark red to brownish or greenish
Habitat	relatively variable, ranging from mudflats, soft-bottom estuaries and seagrass beds to marshes, intertidal to shallow subtidal, grows attached to substrates or unattached

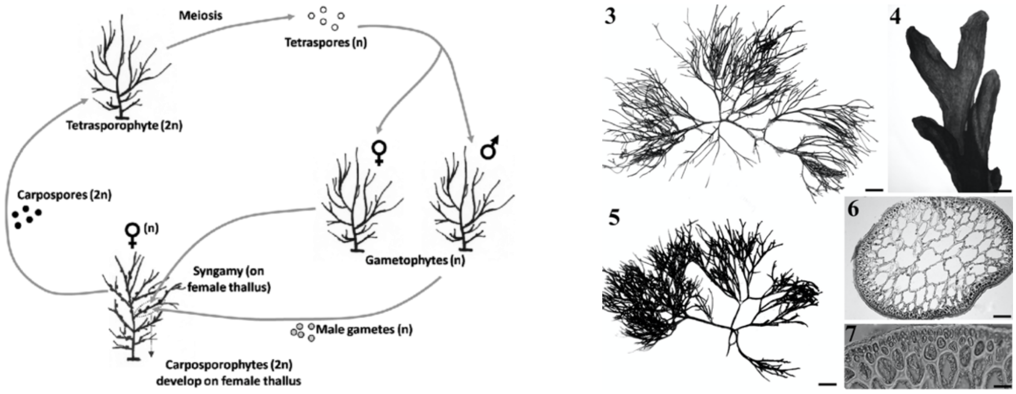

Fig. 1.2.7.: Life cycle of A. vermiculophyllum (left; extracted from Vieira et al., 2018) and microscopic analysis of Agarophyton sp. (right; extracted from Dreckmann et al., 2018)

1.2.4. *Pilayella littoralis*

(Sea Felt)

Fig. 1.2.8.: Pilayella littoralis (extracted from Fenwick, n.d.)

Table 1.6.: Traits of Pilayella littoralis (Bermejo et al., 2019b; Bunker et al., 2017; Gabrielson et al., 2012; Guiry, 2020; Guiry and Guiry, 2020; Lindeberg and Lindstrom, n.d.)

Type of macroalgal bloom	golden tides, Phaeophyceae
Description	emersion tolerant
Distribution	widely distributed from the Mediterranean Sea (introduced), over the Baltic to California and Japan
Morphology	rope-like, branched, fine, hairy, filamentous thallus, yellow to brown appearance
Habitat	marine, estuarine, intertidal to shallow subtidal, often as epiphyte (preferably on fucoids) or on rocks

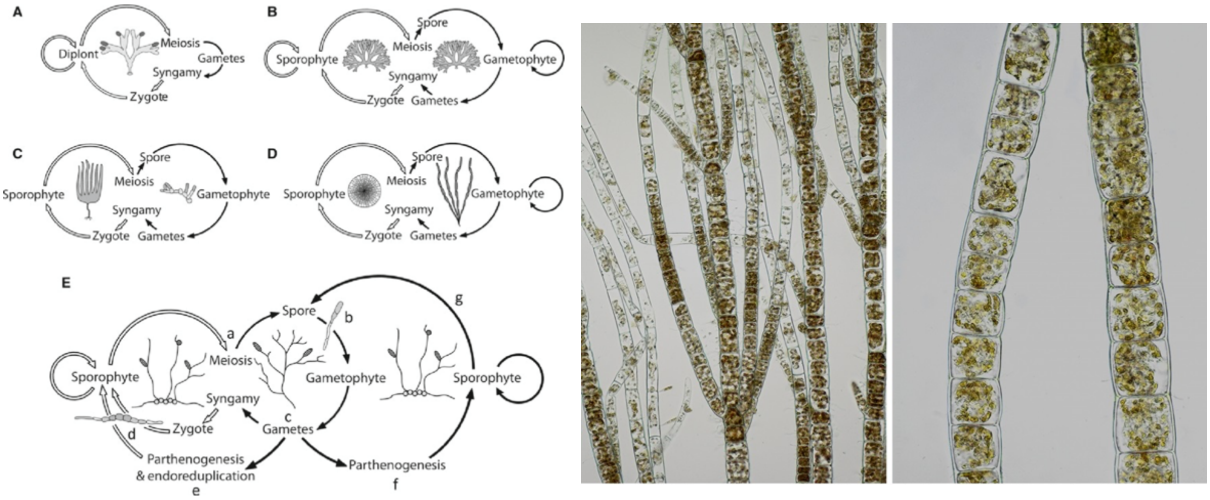

Fig. 1.2.9.: Life cycle of P. littoralis (left; extracted from Bogaert et al., 2013) and P. littoralis under the light microscope (right; extracted from Johnson and Johnson, 2017)

1.2.5. The estuary-nutrient-issue

Semi-enclosed coastal systems like bays, estuaries or lagoons are generally more constrained and thus susceptible to nutrient over-enrichment than open systems, due to slower water renewal rates and tidal influence (Sfriso *et al.*, 2019). Cohen *et al.* (2006) stated that estuaries are often marked by temporally and spatially highly variable nutrient concentrations.

Different water residence times and local hydrodynamic characteristics can lead to nutrient fluctuations in different or within estuaries, implicating effects on the nutrient availability for the algae in a specific area (Lyons *et al.*, 2014; Valiela *et al.*, 1997).

1.2.6. Ireland's most affected estuaries

High precipitation throughout the year (Fig. 1.2.10.), leading to intense freshwater influx, and the unrestrained exposure to the harsh East-Atlantic climate impact Irish estuaries to a large extent (Bermejo *et al.*, 2019b; Longphuirt *et al.*, 2016). Apart from said abiotic factors, nutrient over-enrichment affects estuarine ecosystem health in Ireland, as in many other European countries, and drives bloom development. Nutrients are either derived from agricultural run-off or urban sewage, depending on the location of a particular estuary (Bermejo *et al.*, 2019b).

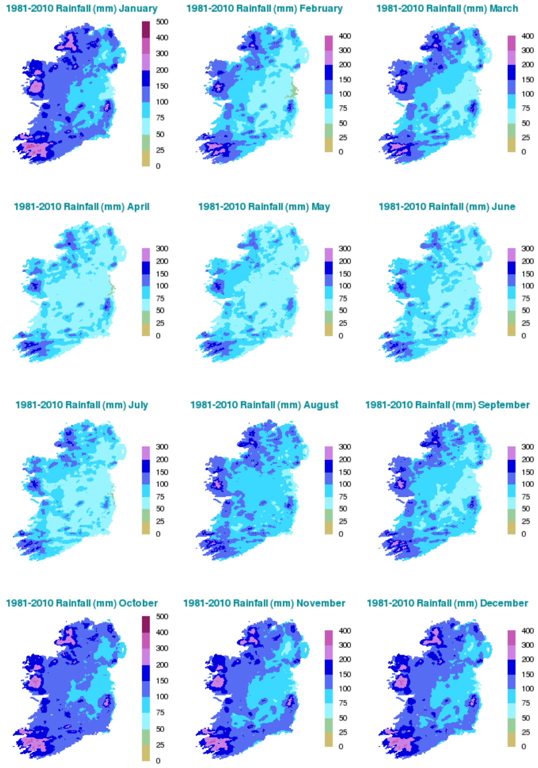

Fig. 1.2.10.: Average monthly rainfall in Ireland (1981 to 2010; extracted from Met Èireann, 2020)

Four of the most heavily by macroalgal blooms affected estuaries in Ireland were studied in the course of this work, the Argideen and the Tolka estuary (both green tides), the Clonakilty estuary (green and red tides) and the Killybegs estuary (golden tides; Bermejo *et al.*, 2019b).

1.2.7. Management and control of seaweed tides

Nutrient loadings, as well as the temperatures of coastal waters in Ireland, are expected to increase further, possibly leading to enhanced biomass of laminar morphotypes of *Ulva* spp. as a result of earlier laminar bloom development in spring (Bermejo *et al.*, 2019b). For this reason, among numerous others (e.g. decreasing ecosystem health in estuaries, economic costs), suitable management needs to be developed (Cohen and Fong, 2006; Sfriso *et al.*, 2019; Valiela *et al.*, 1997). The choice of the right management option for a particular area or estuary needs to be made carefully, and possible implications for the coastal environment and benthic communities need to be taken into consideration (Bermejo *et al.*, 2019b). Many mitigation strategies have already proven to be successful, for instance, reduced fertiliser use or improved sewage treatment (Hodgkin and Hamilton, 1993; Pedersen *et al.*, 2010).

Since lots of them focused on the reduction of P, especially in temperate zones, and mostly because N management is rather complicated and expensive (Teichberg *et al.*, 2009), P limitation now occurs in many places where N would have been expected to limit macroalgal growth (e.g. Venice lagoon), suggesting the need for improved P management (Pedersen *et al.*, 2010; Sfriso *et al.*, 2019). However, Fan *et al.* (2014) proposed a dual nutrient reduction strategy to mitigate eutrophication effects, denoting for the removal of both N and P from the ecosystem. According to them, management should avoid focussing on the reduction of a single nutrient if limitation by only one particular nutrient cannot be evidenced.

Generally, management can either be invasive (e.g. collecting the accumulating biomass), taking direct action against seaweed tides and their consequences, or non-invasive (e.g. monitoring of blooms via remote sensing; Fletcher, 1996; Smetacek and Zingone, 2013). Besides, bloom-forming, opportunistic macroalgae are successfully used as bioindicators with increasing regularity, allowing statements regarding changes in nutrient inputs into coastal waters and therefore facilitating management (Costanzo *et al.*, 2000; Lyngby *et al.*, 1999). Furthermore, sources of nutrients (e.g. agricultural run-off, sewage discharge) may be identified using isotopic signatures (∂^{15}N), which can further help to find solutions for the ongoing eutrophication problem (Cohen and Fong, 2006).

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Chapter 2: Manuscript

Seaweed tissue composition indicates no N limitation in major Irish seaweed tides.

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2.1. Abstract

Over the past decades, seaweed tides (mass accumulations of opportunistic bloom-forming macroalgae) have been in the focus of coastal management and environmental science due to their increasing severity and unforeseeable consequences for affected ecosystems and shoreline-based activities. Nutrient over-enrichment of coastal ecosystems, caused by anthropogenic activity, was considered a significant driver of macroalgal blooms, with N being considered the main nutrient limiting primary production in these systems. This study investigated nitrogen limitation in opportunistic macroalgal blooms in Ireland. The tissue N content of the main bloom-forming species (i.e. *Agarophyton vermiculophyllum*, *Ulva* spp., and *Pilayella littoralis*) was determined for four Irish estuaries severely affected by large blooms. From June 2016 to August 2017, biomass abundance and tissue N content were monitored over seven sampling occasions. Based on previous eco-physiological studies analysing the cell subsistence (Qs) and critical quota (Qc) of these or similar species, and the in this study observed tissue nitrogen contents, the nitrogen status of Irish macroalgal blooms was assessed. The highest tissue N content was found during winter, and the lowest during summer, coinciding with the peak of biomass (June-October). Overall, the obtained results indicated no N limitation for any species at any of the sampling locations, as tissue N content was higher than the Qc even during the peak of bloom. The assessment of the nutrient status of macroalgal blooms is crucial in order to predict the effects of different management and risk assessment strategies.

Keywords: Eutrophication; nutrient bioassessment; seaweed tides; nitrogen; phosphorus

2.2. Introduction

Blooms of fast-growing opportunistic macroalgae, also addressed as seaweed tides, are affecting coastlines around the world due to significant changes in our oceans related to human activities (Fletcher, 1996; Smetacek and Zingone, 2013; Wan *et al.*, 2017). Depending on the blooming species, seaweed tides are often named after the species' colour (i.e. green, golden and red tides; Bermejo *et al.*, 2019a; Smetacek and Zingone, 2013). Macroalgae of the genus *Ulva* (Chlorophyta), a cosmopolitan green alga, are responsible for the majority of blooms worldwide (Fletcher, 1996; Jiang *et al.*, 2020; Paumier *et al.*, 2018). One of the main factors explaining seaweed tide occurrence is the nutrient over-enrichment of coastal waters, although other influences such as global warming or the introduction of alien species are becoming more and more relevant (Smetacek and Zingone, 2013; Smith, 2003; Valiela *et al.*, 1997). Seaweed tides usually occur in estuaries since these environments are more susceptible to enhanced nutrient concentrations due to their hydrological and geomorphological characteristics (i.e. small bodies with relatively low rates of water renewal). In these shallow environments, light supply and temperatures are suitable for the massive development of blooming algae (Bermejo *et al.*, 2020; Hodgkin and Hamilton, 1993).

Macroalgal blooms were found to place coastal ecosystems and shore-based human activities under jeopardy by a variety of effects (Fletcher, 1996; Lyons *et al.*, 2014). Especially China, but also many European countries and the USA, have frequently been facing severe outbreaks of seaweed tides and the resulting threats over the past decades (Fletcher, 1996; Smetacek and Zingone, 2013). Although non-toxic to humans, seaweed tides alter benthic community structures and food webs, leading to changes in biogeochemical cycles and ecosystem functioning (Gonzalez *et al.*, 2013; Pedersen and Johnsen, 2017; Valiela *et al.*, 1997). They sometimes have a devastating impact on tourism and fisheries (Diaz and Rosenberg, 2008; Smetacek and Zingone, 2013), especially by causing unpleasant odours and through the formation of extensive piles of beached algal biomass along coastlines; and on fisheries as a consequence of mass mortalities of estuarine fauna due to the release of toxic compounds during the degradation of vast amounts of seaweed biomass, resulting in changes in species diversity and fish density (Fletcher, 1996; Lyons *et al*., 2014).

As mentioned above, macroalgal blooms are a symptom of nutrient over-enrichment of coastal and estuarine waters, mostly caused by anthropogenic action (Diaz and Rosenberg, 2008; Fort *et al*., 2020; Howarth *et al*., 2011). After the invention of synthetic nitrogen-based fertilisers by Fritz Haber and Carl Bosch at the beginning of the 20th century and the associated advancements of industrial agriculture, nutrient levels in marine systems experienced a significant uplift (Glibert *et al*., 2014). Apart from agricultural run-off, increasing urbanisation followed by enhanced sewage production, insufficient wastewater treatment and mariculture intensify the problem even further (Bouwman *et al*., 2013; Smetacek and Zingone, 2013; Valiela *et al*., 1997), as well as the continuous loss of saltmarshes and the deforestation in riverine areas, both of which lead to increased nitrogen loadings in the water (Valiela *et al*., 1997). Excess nutrients further caused hyper-dystrophic conditions in coastal waters with severe consequences for macroinvertebrate grazers (Sfriso *et al*., 2019). Moreover, over-harvesting of herbivores leads to a decreasing effect of grazing, too, allowing opportunistic macroalgae to prevail in coastal systems (Lyons *et al*., 2014; Teichberg *et al*., 2012).

Most of the opportunistic macroalgal species identified in blooms often possess several features allowing them to form massive seaweed tides, like the ability to tolerate highly variable environmental conditions (e.g. a broad range of salinities; Clark *et al*., 2018; Fong *et al*., 1996; Nelson *et al*., 2008), and they usually stand out through fast growth when no environmental constraints occur (Fletcher, 1996). Although species with slower growth often show more advanced mechanisms for nutrient incorporation and storage, at higher nutrient levels in the surrounding waters, blooming macroalgae usually take over due to their typical opportunistic behaviour (Lyngby *et al*., 1999; Pedersen and Johnsen, 2017). These bloom-forming species usually show simple morphology precluding accurate morphological identification of the different species since morphological traits seldomly provide enough information and species can easily be confused (Wan *et al*., 2017). Earlier, blooms have often been considered monospecific (Nelson *et al*., 2008) with little work being done on their composition, but now it becomes clear that seaweed tides frequently consist of more than one species, probably making them more persistent (Bermejo *et al*., 2019a; Wan *et al*., 2017). Different species within the same genus often show different ecological requirements (e.g. light, temperature, nutrients), which can lead to the extension of seaweed tides through varying optima of species during the blooming period if a bloom is multi-specific (Bermejo *et al*., 2019a; Nelson *et al*., 2008). This knowledge might have unforeseeable consequences for management. Still, there is a lack of studies concerning multi-specificity, and more research needs to be done to gain a better understanding of the implications of multi-specific blooms and the consequences of variable reactions of different species to predominant nutrient levels.

In pristine estuarine environments, nutrients (mainly nitrogen (N) and phosphorus (P)) usually constrain opportunistic macrophyte overgrowth, in periods where light and temperature are suitable for their development (i.e. spring and summer in cold-temperate estuaries; Duarte, 1992; Valiela *et al.*, 1997). Hence, the current nutrient over-enrichment of many estuaries across the world as a consequence of human activities (Fort *et al.*, 2020; Glibert *et al.*, 2014) alters the nutrient cycling and functioning of many coastal systems. These changes then favour the rise of macroalgal blooms by allowing enhanced biomass production, depending on the blooming species and its mechanisms for nutrient uptake and storage (Bjornsater and Wheeler, 1990; Pedersen and Johnsen, 2017). Differences regarding these mechanisms might arise through morphology and growth, competition for nutrients between species and nutrient supply, but also environmental factors like temperature (Fong *et al.*, 1996; Lee and Kang, 2020; Valiela *et al.*, 1997). Increased nutrient loadings might, therefore, allow the algae to store them until demand by providing nutrients at a time where there would usually be a lack of them (Fong *et al.*, 1998; Pedersen and Johnsen, 2017).

If a nutrient is limiting for a certain kind of seaweed depends on its requirements for each nutrient and its ability to incorporate and store nutrients, meaning that different species react differently to the same nutrient composition in the water (Fong *et al.*, 1998; Nelson *et al.*, 2008; Pedersen and Johnsen, 2017). Nutrient limitation is usually described using two relevant tissue nutrient contents. The critical quota (Qc) of a certain seaweed is the minimum tissue nutrient content necessary to maintain maximum growth rates, whereas the subsistence quota (Qs) stands for the minimum content of a nutrient allowing the plant to grow (Pedersen and Borum, 1997; Villares and Carballeira, 2004). Nitrogen was considered the main limiting nutrient in temperate waters in many previous studies (Jeffrey *et al.*, 1995; Paumier *et al.*, 2018; Pedersen and Johnsen, 2017; Villares and Carballeira, 2004). In more recent studies, it is more and more frequently discussed that in temperate estuaries, some blooms might also be P limited instead (Bermejo *et al.*, 2020; Duarte, 1992; Lapointe, 1985; Valiela *et al.*, 1997). Furthermore, Lapointe (1985) and Villares (2004) discovered that intense P limitation can induce physiological N limitation due to the importance of P-containing molecules for N uptake mechanisms, and vice versa (Lapointe, 1987). Since macroalgae entrained in muddy grounds use nutrients from the water column as well as from porewaters for their growth (Robertson and Savage, 2018), earlier approaches to determine nutrient limitation consisted of measurements of nutrient concentrations in estuarine waters. However, this turned out to give relatively transient results and that it is further necessary to investigate tissue nutrient concentrations for more representative information of the overall nutrient status (Lyngby *et al.*, 1999). Focusing on Ireland, successful management action in the past, undertaken to reduce estuarine nutrient levels, might have led to shifting N/P ratios in Irish coastal waters. However, alterations in nutrient concentrations were left undocumented until the 1990s, compounding a historical approach to changes in N and P loadings over time (O'Boyle *et al.*, 2016).

Considering the key role of nutrient over-enrichment in the development of macroalgal blooms (Smetacek and Zingone, 2013; Valiela *et al.*, 1996), identifying the limiting nutrient is a necessary first step in order to find effective management actions (Villares and Carballeira, 2004). Therefore, assessing the nutrient status of seaweed tides via tissue nutrient content analysis (as a measurement of nutrient limitation and growth) is essential for the management of agricultural fertilizers and sewage treatment. This study aimed to investigate the role of nitrogen as a limiting factor in five of the largest macroalgal blooms occurring in Ireland, and further to identify factors controlling biomass abundance in Irish estuaries. Samples of bloom-forming seaweeds (i.e. *Ulva compressa*, *U. prolifera*, *U. rigida*, *Agarophyton vermiculophyllum* and *Pilayella littoralis*) were collected during seven sampling occasions, following tissue N and P content determination (Bermejo *et al.*, 2020, 2019b).

2.3. Materials and Methods

Study sites

The four estuaries most affected by seaweed tides in Ireland (Fig. 2.1.; Killybegs on the northwestern coast (Fig. 2.4.), Argideen (Fig. 2.3.) and Clonakilty (Fig. 2.2.) in the Southwest, all at the Atlantic Ocean, and Tolka on the east coast, located at the Irish Sea (Fig. 2.5.)) were chosen for this study. In cold-temperate estuaries in the northeastern Atlantic region, the maximum extent of a seaweed tide is usually reached in June or July, with dropping biomass numbers in the colder seasons (Bermejo *et al.*, 2019b; Jeffrey *et al.*, 1995). The Clonakilty estuary is a shallow area prone to nutrient enrichment due to intensively used farmlands in the surrounding area and its vicinity to a wastewater treatment station. Next, the Argideen estuary lies within a close distance to the Clonakilty estuary and is marked by agriculture. The Tolka estuary, on the other hand, is located in the vicinity of Dublin, therefore receiving excess nutrients from sewage run-off and urban wastewaters. Last, the Killybegs estuary is surrounded by farmlands and the eponymous city Killybegs, making it prone to nutrients derived from agricultural and sewage run-off. A common feature of all the listed study sites is that they are sheltered and less susceptible to wave action, allowing higher nutrient concentrations within the estuaries (Bermejo *et al*., 2019b).

Previous molecular genetic analysis revealed that green tides in Ireland are multi-specific, consisting mainly of the aforementioned species, whereas golden and red tides are not. *Ulva* (Chlorophyta) is once again predominant here, especially in the Tolka, the Clonakilty and the Argideen estuary. Bermejo *et al*. (2019a) observed a temporal succession between different morphologies of *Ulva*, tubular species (e.g. *U. compressa* and *U. prolifera*) dominated the blooms in spring and early summer, whereas during late summer and autumn a co-dominance between tubular and laminar species (e.g. *U. rigida*) occurred. Furthermore, the recent appearance of massive red seaweed tides in areas of nutrient over-enriched estuaries, which were previously unaffected by macroalgal tides, was revealed to be produced by the invasive red seaweed *Agarophyton vermiculophyllum*, originating from the northwest Pacific (Rhodophyta; Bermejo *et al*., 2019b; Thomsen *et al*., 2006).

The Clonakilty estuary is affected by spatially segregated green and red macroalgal blooms, caused by *Ulva* spp. (*U. compressa*, *U. prolifera*, *U. rigida*) and *Agarophyton vermiculophyllum* respectively. Both the Argideen and the Tolka estuary are impacted by green tides (*Ulva* spp.). However, the brown alga *Pilayella littoralis* (Phaeophyta) was responsible for the largest golden tide ever recorded in Ireland, now dominating blooms in the Killybegs estuary (Bermejo *et al*., 2019b; Salovius and Bonsdorff, 2004). The positions of the sampling stations were chosen beforehand by looking at previous blooms using Google Earth images and located with Geographical Position System (GPS; Magellan Triton 400) once in the field.

Fig. 2.1.: Locations of the four studied estuaries along the Irish coast, Killybegs (A) in the Northwest, Tolka (B) in the East, and Conakilty (C) and Argideen (D) in the South

Fig. 2.2.: Map of Clonakilty with sampling points (green)

Fig. 2.3.: Map of Argideen with sampling points (green)

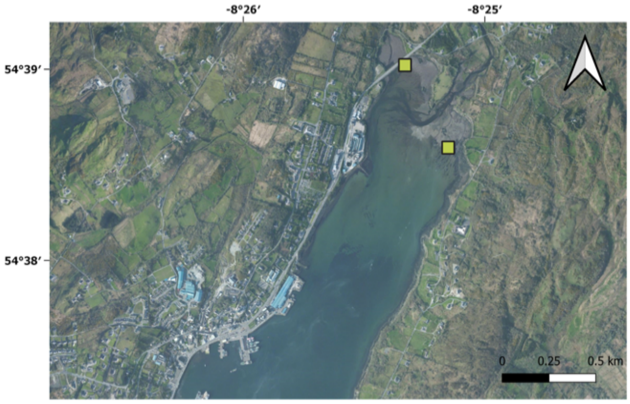

Fig. 2.4.: Map of Killybegs with sampling points (green)

Fig. 2.5.: Map of Tolka with sampling points (green)

Sample collection

The four estuaries along the Irish coast were studied over six or seven sampling occasions, between June 2016 and August 2017. Each estuary was divided into two sections, “Site 1” one kilometre upstream in the estuary and “Site 2” close to the open ocean, with three replicates carried out per site (Table 2.1.).

Table 2.1.: Locations of Site 1 and 2 in each studied estuary, displayed in coordinates

Killybegs	*P. littoralis*:	Site 1 [54°64’93.0”N 8°42’65.9”W]	Site 2 [54°64’54.4”N 8°42’29.7”W]
Tolka	*Ulva* spp.:	Site 1 [53°37’12.6”N 6°16’18.2”W]	Site 2 [53°36’83.1”N 6°16’67.5”W]
Argideen	*Ulva* spp.:	Site 1 [51°64’07.8”N 8°73’49.5”W]	Site 2 [51°63’72.5”N 8°72’87.3”W]
Clonakilty	*Ulva* spp.:	Site 1 [51°61’11.0”N 8°87’19.0”W]	Site 2 [51°60’59.2”N 8°87’22.1”W]
	A. vermiculophyllum:	Site 1 [51°61’90.3”N 8°87’85.3”W]	Site 2 [51°61’08.6”N 8°85’01.3”W]

Within each section, a transect containing three sampling points (10 m apart) was sampled, leading to a total of 18 samples per section. These three sampling stations were located in different parts of the intertidal (i.e. upper, middle, lower), which are usually covered during the maximum extension of a bloom. All nitrogen samples were collected from the middle part. The abundance of seaweed biomass from said species was measured using three quadrants (25 x 25 cm) per sampling station. The different specimens were transported to the laboratory in separate plastic bags.

Sample processing

After arrival at the laboratory, the seaweed biomass was rinsed with fresh water for the removal of epiphytes and other adherent materials. Subsequently, the different species were separated, and *Ulva* was divided with respect to morphotypes, based on macroscopic traits (e.g. laminar and tubular) or microscopic analysis, if necessary, using a Nikon Optiphot II Microscope (Nippon Kogakukk, Tokyo, Japan). A salad spinner was used to remove excess water before obtaining the overall mass of each specimen. Then, the standardization of the collected biomass to gram per square meter followed, multiplying weight per quadrant by 16. Three subsamples per section and sampling occasion were kept for tissue nutrient content analysis. As described below, the taxonomic identification especially of *Ulva* often requires genetic analysis, therefore further subsamples of morphologically representative specimens were collected to further perform a microscopic as well as a molecular identification.

Taxonomic identification

As already mentioned, the differentiation of the five species is rather difficult based only on comparing morphological traits, hence a molecular-genetic analysis of the samples was undertaken, using the larger subunit of the Ribulose Bisphosphate Carboxylase-Oxygenase (RuBisCO), commonly known as *rbcL*, as a genetic marker.

Tissue nutrient (N and P) analysis

For the subsequent tissue nutrient analysis, seaweed tissue was freeze-dried and afterwards ground into a homogeneous powder through the application of TissueLyser II (QIAGEN) and tungsten balls. Following the division of the homogenised material into two subsamples for N and P determination, tissue N content was determined using portions of the powder for the analysis, undertaken in duplicates, in an elemental analyser Vario ISOTOPE Cube (Elementar Analysensysteme GmbH, Hanau), which was connected to an isotope ratio mass spectrometer Isoprime 100 (Isoprime Ltd, Cheadle Hulm). The analysis followed an analytical precision of 0.15 %. Tissue P content analysis took place after conducting an oxidation step with boiling H_2SO_4 using said dried and ground algal tissue. Afterwards, a spectrophotometric analysis was carried out (Strickland and Parsons, 1972).

Statistical analysis

First, data for the algal biomass (in fresh weight) was extrapolated to g per m^2 from the 25 x 25 cm quadrants. Statistical data analysis was conducted using R (R Core Team, 2019), with the R-packages "car" (Fox and Weisberg, 2019) for linear modelling, and "ggplot2" (Wickham, 2016). The level of statistical significance (alpha) was set to 5%. Means were reported as mean ±standard deviation. The used boxplots represent the median (thick horizontal line), interquartile range (box), and the outer quartiles (whiskers). Additionally, outliers are defined as 1.5-times the interquartile range below the first or above the third quartile and represented as dots.

The assumptions for the linear models were verified with the Levene-Test, to assess homogeneity of variances, and using QQ-Plots (Quantile-Quantile-Plots), to assess normal distribution of error terms. To meet said assumptions, the biomass in fresh weight per m^2 was square-root-transformed. A three-way-ANOVA (analysis of variance) was conducted to evaluate the spatial and temporal variability of the algal biomass of *Ulva* spp. The differences between estuaries and seasons were of primary interest, additionally site, nested within estuary, was used as factor to estimate the magnitude of local differences. Subsequently, two-way ANOVAs were used to estimate the effects of season and site within each estuary, as well as for biomass of *P. littoralis* and *A. vermiculophyllum,* since they were only sampled in one estuary.

The spatial and temporal variability of the tissue nitrogen content [% of dry weight] was explored using a three-way-ANOVA in the case of *Ulva* spp., with the factors estuary, site and season, and by a two-way-ANOVA for each species and estuary with solely site and season as factors. For pairwise testing of the effect of season, a Tukey's HSD (honest significant difference) from the R-package "agricolae" (de Mendiburu, 2020) was conducted. In the case of *P. littoralis*, the factor site was omitted, due to the unbalanced distribution of measurements between season and site.

Tissue N and P contents, respectively, were then tested against the in other studies estimated critical quota (Qc) of each species (see Table 2.2.), applying a one-sided t-test with a subsequent Bonferroni-correction, to assess nutrient limitation. To calculate the relationship between nutrients and biomass, the means of nitrogen content, phosphorous content, as well as of biomass for each season and site were calculated and afterwards mapped on each other. Then, a Spearman correlation (r_s) was calculated by species between said variables; for sub-correlations in the case of *Ulva* spp., the p-value was adjusted (Field *et al.*, 2012).

Table 2.2.: Estimated values for the critical (Qc) and subsistence quotas (Qs) of all the species investigated in this study.

Species	**Nutrient**	**Qc [%]**	**Qs [%]**	**References**
Laminar *Ulva* spp.	Nitrogen	2.0-2.5	1.0-1.2	(reviewed by Villares and Carballeira, 2004)
Tubular *Ulva* spp.	Nitrogen	2.0-2.5	1.0	(reviewed by Villares and Carballeira, 2004)
Agarophyton vermiculophyllum	Nitrogen	2.14	0.71	(Pedersen and Johnsen, 2017)
	Phosphorus	0.14	0.045	(Pedersen and Johnsen, 2017)
Pilayella littoralis	Nitrogen	2.1	1.0	(Campbell, 2001)

For stable isotope analysis, a linear mixed-effects model (Kuznetsova *et al.*, 2017) was used to assess differences between estuaries in the case of *Ulva* spp., defining season and site as random effects, and nested within estuaries to make them inter-comparable. Then, the difference between the isotopic signatures of *A. vermiculophyllum* and *Ulva* spp. was estimated utilising the same method.

2.4. Results

Biomass abundance and tissue nutrient contents (N and P) varied between seasons in all investigated species. In the case of *Ulva* spp., tissue nutrient concentrations were affected by estuary and site, whereas site also led to variability in the tissue N content of *A. vermiculophyllum* and *P. littoralis*. Tissue N content was always higher than Qc in laminar and tubular *Ulva* spp., except for June 2016 in the Clonakilty estuary. Tissue N contents of *A. vermiculophyllum* and *P. littoralis* were always above Qc as well. Tissue P content of *A. vermiculophyllum*, however, was found to be below Qc during several months, namely during June 2016, April 2017, June 2017 and August 2017. In *A. vermiculophyllum*, the results indicated a negative correlation between biomass and tissue N content, whereas in neither of the other species such a correlation could be observed. Isotopic signatures (∂^{15}N) did not differ between laminar and tubular morphotypes of *Ulva* spp., but between the Clonakilty and the Tolka estuary in the case of *Ulva*. Argideen was intermediate between Tolka and Clonakilty. Furthermore, ∂^{15}N varied between *Ulva* spp. and *A. vermiculophyllum* in the Clonakilty estuary.

Taxonomic identification

The molecular analysis confirmed once more that green tides are often multi-specific in Ireland. The assessment of the species composition revealed the dominance of three species of *Ulva* (i.e. *U. compressa*, *U. prolifera and U. rigida*) during these seaweed tides. Furthermore, the remaining collected specimens were identified as *Agarophyton vermiculophyllum* and *Pilayella littoralis*.

Seasonal variability of biomass

Ulva spp.

In all the studied estuaries affected by green tides of *Ulva* spp. (i.e. the Argideen, Clonakilty and Tolka estuaries), biomass seasonality (F(5,278)=46.52, p=0.000) was observed in laminar (Fig. 2.6.) and tubular morphotypes (Fig. 2.7.) over the course of this study (Table 2.3.). The estuary of occurrence and different sites within estuaries influenced biomass numbers as well (F(2,278)=58.25, p=0.000 and F(3,278)=9.97, p=0.000, respectively). Season was the most important factor affecting biomass in both laminar (eta^2=0.29) and tubular (eta^2=0.30) morphotypes, followed by estuary in laminar *Ulva* (eta^2=0.15, compared to only 0.04 in tubular

Ulva). Mean biomass values for *Ulva* spp. (i.e. *Ulva* total, laminar and tubular) are displayed in Annex table 1.

In laminar *Ulva*, there was no difference in mean biomass between Tolka (75.1±140 g fresh weight per m^2) and Argideen (158±339 g fresh weight per m^2; Tukey-test, $p<0.94$), however, mean biomass in Clonakilty was higher than in both the other estuaries (292±462 g fresh weight per m^2; Tukey-test, $p=0.000$). In tubular *Ulva*, mean biomass did not differ between the specimens sampled in Clonakilty (986±1240 g fresh weight per m^2) and those collected in Argideen (1115±1310 g fresh weight per m^2; Tukey-test, $p = 0.88$). Here, the mean biomass of *Ulva* in the Tolka estuary was about twice as high than in the Argideen and Clonakilty estuary (1897±2266 g fresh weight per m^2; Tukey-test, $p=0.000$).

Table 2.3.: Three-Way-ANOVA on square-rooted biomass of Ulva spp., by the factors season, estuary and site

***Ulva* laminar**	**Source of Variation**	**SS**	**df**	**F**	**p-value**	**eta^2**
	Season	6803	5	46.52	0.000	0.29
	Estuary	3407.1	2	58.25	0.000	0.15
	Site (Estuary)	874.5	3	9.97	0.000	0.04
	Season x Estuary	1714	8	7.33	0.000	0.07
	Season x Site (Estuary)	2538	13	6.67	0.000	0.11
	Residuals	8131	278			
***Ulva* tubular**						
	Season	45310	5	38.9	0.000	0.30
	Estuary	5682	2	12.2	0.000	0.04
	Site (Estuary)	2053	3	2.94	0.034	0.01
	Season x Estuary	27781	8	14.91	0.000	0.18
	Season x Site (Estuary)	7527	13	2.49	0.003	0.05
	Residuals	64758	278			

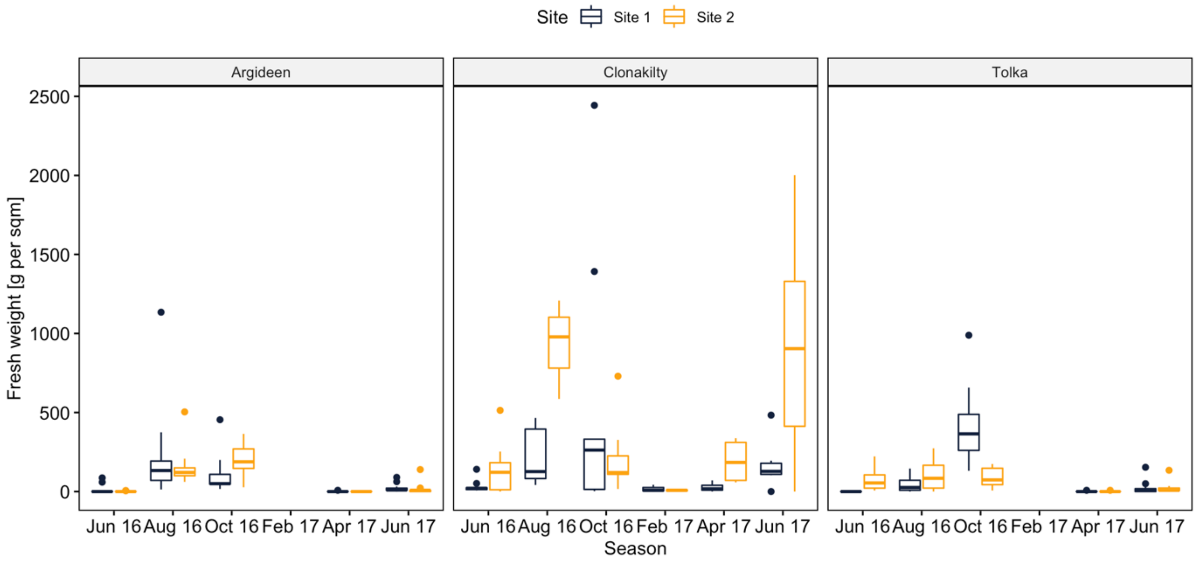

Fig. 2.6.: Biomass, in g fresh weight per m², of laminar Ulva, faceted by estuary, by season and site. During February 2017, biomass was absent in Argideen and Tolka.

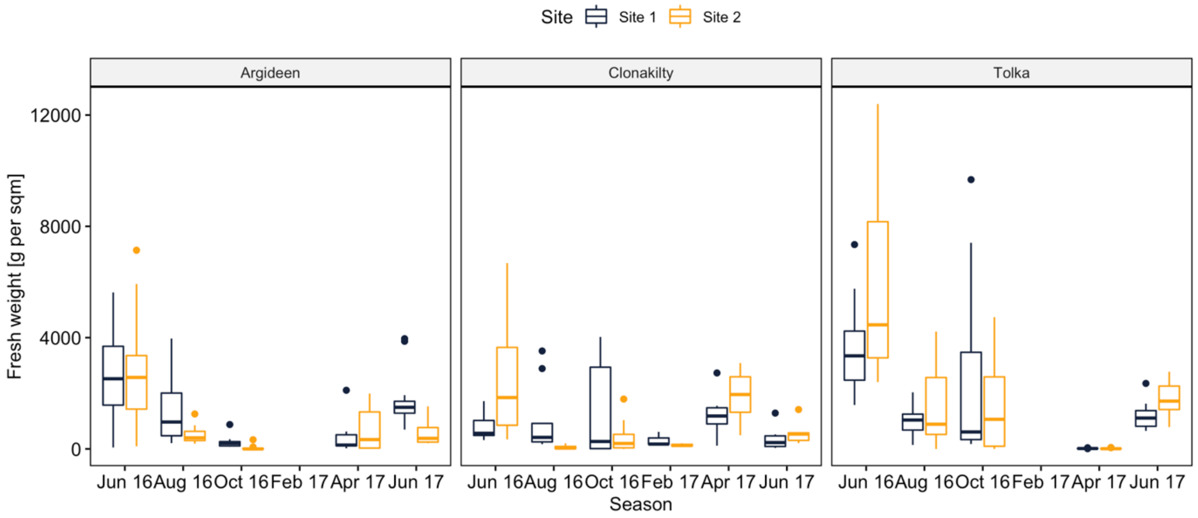

Fig. 2.7.: Biomass of tubular Ulva, in g fresh weight per m², by season and site, faceted by estuary. Here again, no biomass was found in Argideen and Tolka in February 2017.

A. vermiculophyllum

In the Clonakilty estuary, biomass of *A. vermiculophyllum* (1295 ±1099 g fresh weight per m^2) varied substantially between seasons (F(6,112)=33.82, p=0.000) and sites (F(1,112)=44.52, p=0.000; Table 2.4., Fig. 2.8.). Again, season was the factor with the highest impact on biomass (eta^2=0.52). Mean biomass values for *A. vermiculophyllum* can be found in Annex table 1.

Table 2.4.: Two-Way-ANOVA on square-rooted biomass of A. vermiculophyllum, by the factors season and site

Agarophyton vermiculophyllum	**Source of Variation**	**SS**	**df**	**F**	**p-value**	**eta^2**
	Season	16497	6	33.82	0.000	0.52
	Site	3619	1	44.52	0.000	0.11
	Season x Site	2742.9	6	5.62	0.000	0.09
	Residuals	9104.4	112			

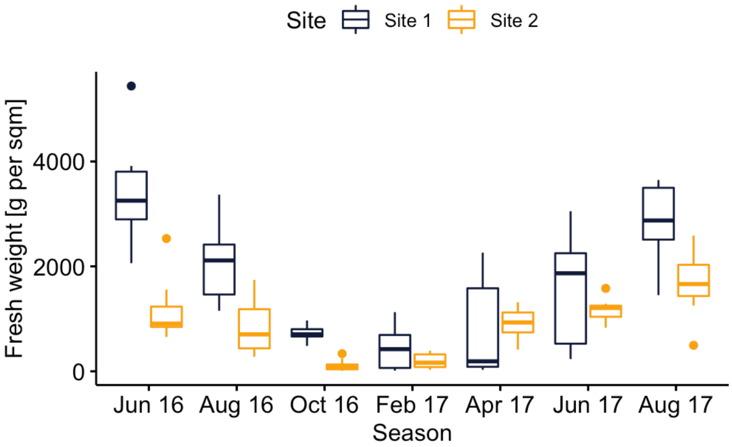

Fig. 2.8.: Biomass of Agarophyton vermiculophyllum, in g fresh weight per m^2, by season and site

P. littoralis

As for *Ulva* spp. and *A. vermiculophyllum*, clear biomass seasonality (F(5,90)=6.86, p=0.000) was also observed in *P. littoralis* in the Killybegs estuary (3650 ±3437 g fresh weight per m^2). Moreover, biomass varied between Site 1 and 2 within the estuary (F(1,90)=71.52, p=0.000, Table 2.5., Fig. 2.9.). The factor with the highest effect size was site (eta^2=0.31). Mean biomass numbers can be extracted from Annex table 1.

Table 2.5.: Two-Way-ANOVA on square-rooted biomass of P. littoralis, by the factors season and site

Pilayella littoralis	**Source of Variation**	**SS**	**df**	**F**	**p-value**	**eta^2**
	Season	12669	5	6.86	0.000	0.15
	Site	26400	1	71.52	0.000	0.31
	Season x Site	12830	5	6.95	0.000	0.15
	Residuals	33223	90			

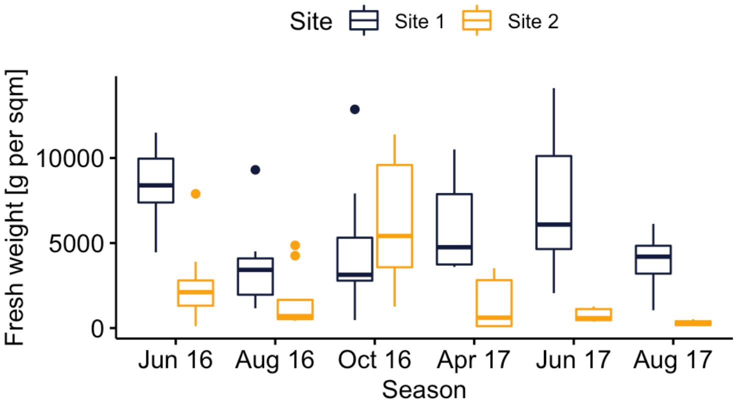

Fig. 2.9.: Biomass of Pilayella littoralis, in g fresh weight per m^2, by season and site. During February 2017, no biomass occurred in Killybegs, therefore, this month was excluded from the graph.

Spatial and temporal variability of tissue N content

Ulva spp.

The timing of the sampling occasions strongly impacted tissue N content in both morphotypes of *Ulva* spp. (laminar: [F(6,60)=49.36, p=0.000], tubular: [F(6,72)=13.68, p=0.000]) in all three estuaries (tubular *Ulva* spp. [Argideen: F(5,29)=3.93, p=0.008, Tolka: F(5,29)=3.52, p=0.013 and Clonakilty: F(5,29)=18.1, p=0.000], laminar *Ulva* spp. [Argideen: F(4,21)=7.1, p=0.001, Tolka: F(4,24)=18.78, p=0.000 and Clonakilty: F(25,26)=38.93, p=0.000]), with often remarkable differences between the investigated months, whereas the nutrient content did not vary between the different sites within an estuary in all cases (see Table 2.6., Figures 2.10. to 2.15.). Tissue N content was lowest in summer (i.e. June to August, usually starting to decrease in April), coinciding with the peak of macroalgal biomass in bloom areas, and reached its maximum during the colder months (i.e. October and February). Season was the factor with the strongest effect on tissue N content (tubular *Ulva*: [Argideen: eta^2=0.76, Tolka: eta^2=0.36, and Clonakilty: eta^2=0.75], laminar *Ulva*: [Argideen: eta^2=0.55, Tolka: eta^2=0.75, and Clonakilty: eta^2=0.88]. Mean tissue N contents for these months are displayed in Annex table 2 for the Tolka, Argideen and Clonakilty estuary.

Differences between estuaries were found to be significant for both laminar (F(2,60)=43.25, p=0.000) and tubular *Ulva* spp. (F(2,72)=33.76, p=0.000), after nesting section in estuary, as indicated by the ANOVA in Table 2.6.. In the case of tubular *Ulva* spp. all estuaries differed substantially from each other in their mean tissue nitrogen content (Tukey-test, adjusted p<0.05), with Tolka showing the highest (mean=4.57 ±1.10), Argideen the second highest (mean=3.84 ±0.76) and Clonakilty showing the lowest mean tissue N content (mean=3.35±1.27). In laminar *Ulva* spp., tissue N contents of the Argideen (mean=3.63±0.91) and the Tolka estuary (mean=3.67±1.13) did not vary (Tukey-test, adjusted p>0.05), whereas the one observed in Clonakilty (2.86±1.45) differed from both (Tukey-test, adjusted p<0.05).

In the laminar forms, mainly *U. rigida*, only in the Clonakilty estuary the mean of June 2016 (p-value=0.002) lies significantly below the Qc (see Table 2.2. Fig. 2.11.). However, in laminar *Ulva* from the Tolka and the Argideen estuary, no mean was observed to be significantly below the critical quota of laminar *Ulva* at any time during the study. A similar pattern was further noticed in tubular morphotypes, mainly *U. compressa* and *U. prolifera*, with all means being higher than the critical quota for tubular *Ulva* (Table 2.6.).

Table 2.6.: Three-Way-ANOVA including the factor estuary and Two-Way-ANOVA for each estuary on tissue N content of Ulva spp., by the factors season and site, separated by estuary.

***Ulva* tubular**	**Source of Variation**	**SS**	**df**	**F**	**p-value**	**eta^2**
	Estuary	38.51	2	33.76	0.000	0.25
	Season	46.82	6	13.68	0.000	0.30
	Site (Estuary)	3.30	3	1.93	0.133	0.02
	Estuary x Season	18.21	9	3.55	0.001	0.12
	Site (Estuary) x Season	8.82	15	1.03	0.435	0.06
	Residuals	41.06	72			
Argideen	Season	7.612	5	3.93	0.008	0.76
	Site	1.221	1	3.15	0.086	0.12
	Residuals	1.233	29			
Tolka	Season	15.26	5	3.52	0.013	0.36
	Site	1.664	1	1.92	0.176	0.04
	Residuals	25.131	29			
Clonakilty	Season	42.158	5	18.1	0.000	0.75
	Site	0.412	1	0.88	0.355	0.01
	Residuals	13.512	29			
***Ulva* laminar**						
	Estuary	25.04	2	43.25	0.000	0.17
	Season	85.69	6	49.36	0.000	0.57
	Site (Estuary)	1.66	3	1.91	0.137	0.01
	Estuary x Season	11.40	7	5.63	0.000	0.08
	Site (Estuary) x Season	7.97	11	2.51	0.012	0.05
	Residuals	19.36	60			
Argideen	Season	11.765	4	7.1	0.001	0.55
	Site	1.054	1	2.54	0.126	0.05
	Residuals	8.702	21			
Tolka	Season	28.161	4	18.78	0.000	0.75
	Site	0.167	1	0.45	0.511	0.00
	Residuals	8.995	24			
Clonakilty	Season	57.163	5	38.93	0.000	0.88
	Site	0.437	1	1.49	0.234	0.01
	Residuals	7.635	26			

Laminar Ulva spp.

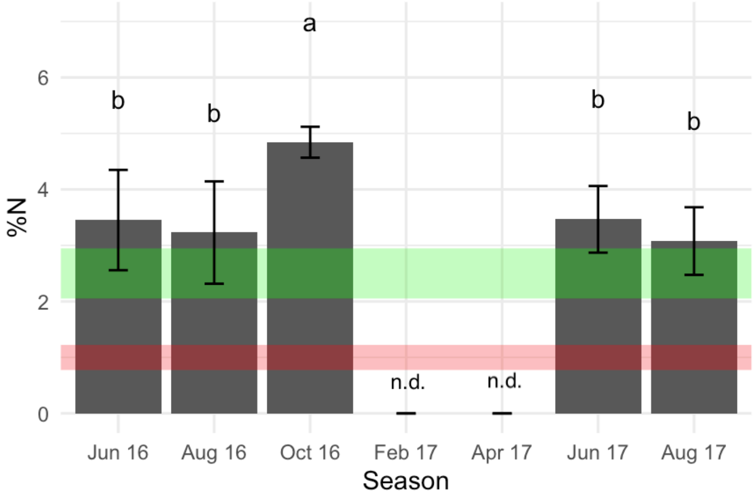

Fig. 2.10.: Mean tissue N content of laminar Ulva spp. in the Argideen estuary. Error bars represent standard deviation. Different sites (Site 1 and 2) are presented together as one single bar per sampling month due to a lack of data for some sampling occasions. Bars sharing the same letter(s) are not statistically different (t-test,p<0.05), and the green and the red line mark critical (Qc) and subsistence quota (Qs), respectively. "n.d." stands for "no data".

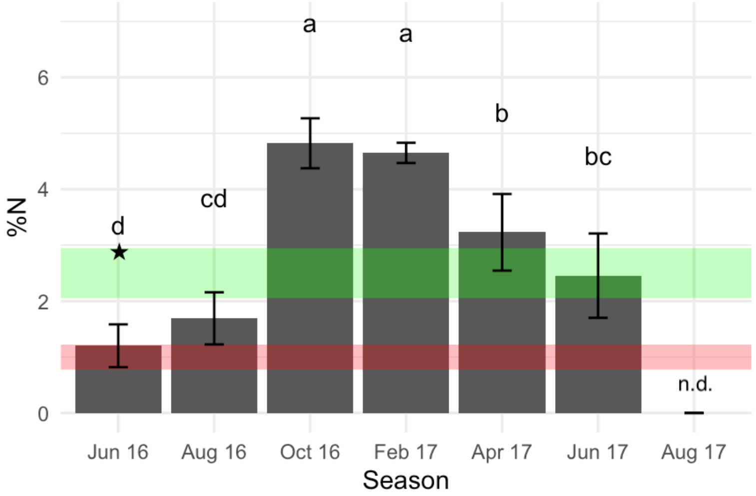

Fig. 2.11.: Mean tissue N content of laminar Ulva spp. in the Clonakilty estuary. Error bars represent standard deviation. Different sites (Site 1 and 2) are presented together as one single bar per sampling month due to a lack of data for some sampling occasions. Bars sharing the same letter(s) are not statistically different (t-test, p<0.05), and the green and the red line mark critical (Qc) and subsistence quota (Qs), respectively. Stars below letters were used for means significantly below Qc. "n.d." stands for "no data".

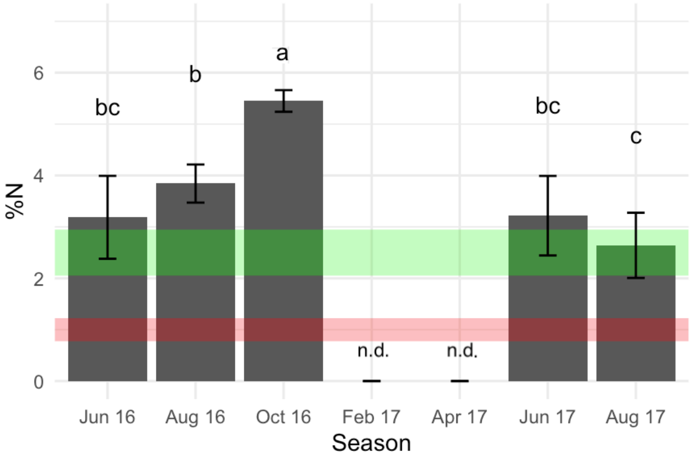

Fig. 2.12.: Mean tissue N content of laminar Ulva spp. in the Tolka estuary. Error bars represent standard deviation. Different sites (Site 1 and 2) are presented together as one single bar per sampling month due to a lack of data for some sampling occasions. Bars sharing the same letter(s) are not statistically different (t-test, $p<0.05$), and the green and the red line mark critical (Qc) and subsistence quota (Qs), respectively. "n.d." stands for "no data".

Tubular Ulva spp.

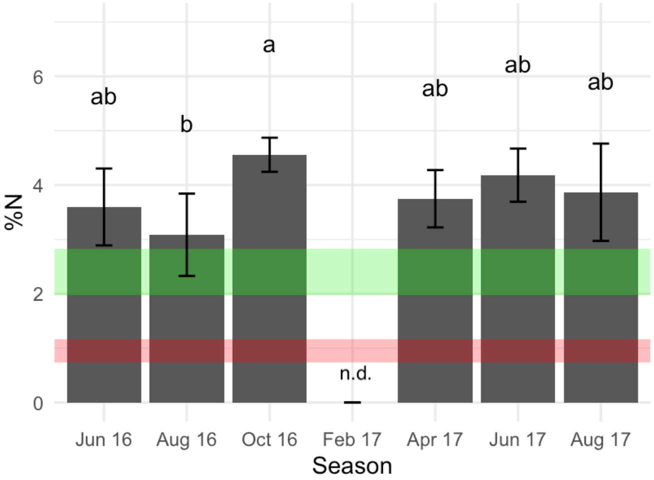

Fig. 2.13.: Mean tissue N content of tubular Ulva spp. in the Argideen estuary. Error bars represent standard deviation. Different sites (Site 1 and 2) are presented together as one single bar per sampling month due to a lack of data for some sampling occasions. Bars sharing the same letter(s) are not statistically different (t-test, $p<0.05$), and the green and the red line mark critical (Qc) and subsistence quota (Qs), respectively. "n.d." stands for "no data".

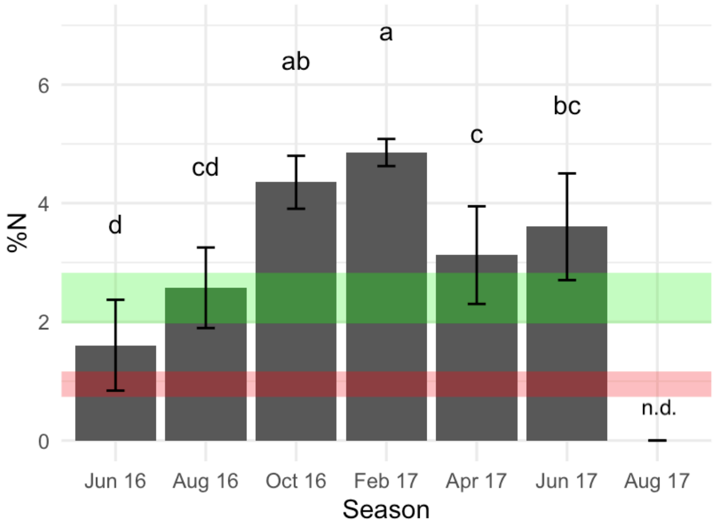

Fig. 2.14.: Mean tissue N content of tubular Ulva spp. in the Clonakilty estuary. Error bars represent standard deviation. Different sites (Site 1 and 2) are presented together as one single bar per sampling month due to a lack of data for some sampling occasions. Bars sharing the same letter(s) are not statistically different (t-test, p<0.05), and the green and the red line mark critical (Qc) and subsistence quota (Qs), respectively. "n.d." stands for "no data".

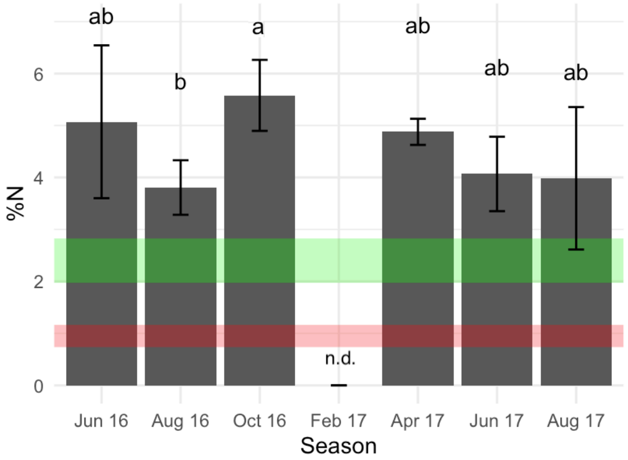

Fig. 2.15.: Mean tissue N content of tubular Ulva spp. in the Tolka estuary. Error bars represent standard deviation. Different sites (Site 1 and 2) are presented together as one single bar per sampling month due to a lack of data for some sampling occasions. Bars sharing the same letter(s) are not statistically different (t-test, p<0.05), and the green and the red line mark critical (Qc) and subsistence quota (Qs), respectively. "n.d." stands for "no data".

A. vermiculophyllum

Here again, the sampling occasion substantially impacted the tissue N content of *A. vermiculophyllum* (F(6,34)=25.43, p=0.000), collected in the Clonakilty estuary. Yet, it did not vary between Site 1 and 2 (Table 2.7.). Maximum values for %N were found in October and February, the minimum tissue content was reached during the summer months (i.e. June to August), starting to sink in April in this study (Fig. 2.16.). Season was the factor with the highest impact on tissue N content (eta^2=0.82). Mean tissue N contents per months can be extracted from Annex table 2. As illustrated below, tissue N content was always higher than the considered critical quota (see Table 2.2.) for *A. vermiculophyllum*.

Table 2.7.: Two-Way-ANOVA on square-rooted tissue N content of A. vermiculophyllum, by the factors season and site

Agarophyton vermiculophyllum	**Source of Variation**	**SS**	**df**	**F**	**p-value**	**eta^2**
	Season	27.861	6	25.43	0.000	0.82
	Site	0.025	1	0.14	0.712	0.00
	Residuals	6.21	34			

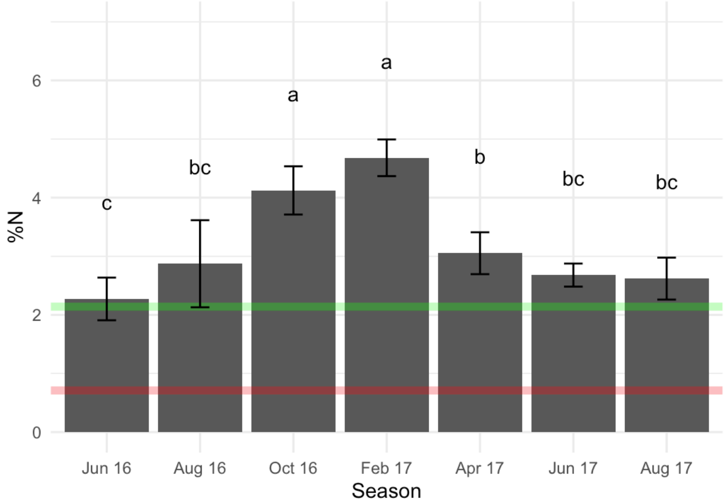

Fig. 2.16.: Mean tissue N content of A. vermiculophyllum in the Clonakilty estuary. Error bars represent standard deviation. Different sites (Site 1 and 2) are presented together as one single bar per sampling month due to a lack of data for some sampling occasions. Bars sharing the same letter(s) are not statistically different (t-test, p<0.05), and the green and the red line mark critical (Qc) and subsistence quota (Qs), respectively.

P. littoralis

As well as in all the other studied species, a change of season implicated variable %N of *P. littoralis* (F(6,32)=6.35, p=0.000, eta^2=0.54), but again, section did not (Table 2.8.), with tissue N content being lowest in summer (June to August) and highest in the colder period (October to April). Mean tissue N contents are displayed in Annex table 2. Furthermore, in Fig. 2.17., showing %N for *P. littoralis* from the Killybegs estuary, it can be seen that none of the means lies significantly below the considered Qc for this species (see Table 2.2.).

Table 2.8.: One-Way-ANOVA on square-rooted tissue N content of P. littoralis, by the factor season

Pilayella littoralis	**Source of Variation**	**SS**	**df**	**F**	**p-value**	**eta^2**
	Season	10.007	6	6.35	0.000	0.54
	Residuals	8.402	32			

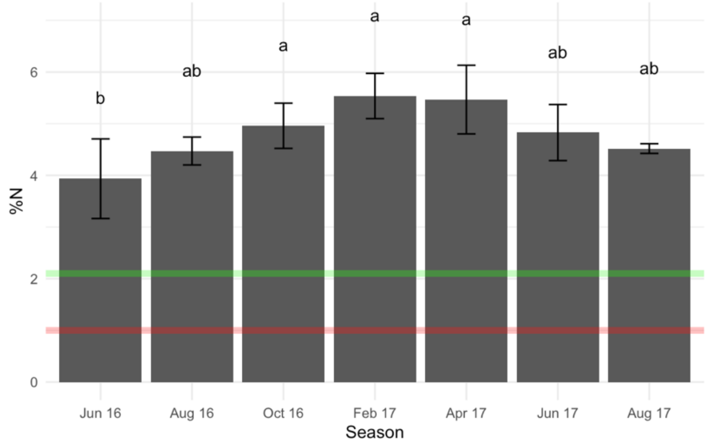

Fig. 2.17.: Mean tissue N content of P. littoralis in the Killybegs estuary. Error bars represent standard deviation. Different sites (Site 1 and 2) are presented together as one single bar per sampling month due to a lack of data for some sampling occasions. Bars sharing the same letter(s) are not statistically different (t-test, p<0.05), and the green and the red line mark critical (Qc) and subsistence quota (Qs), respectively.

Spatial and temporal variability of tissue P content

A. vermiculophyllum

P data was only available for *A. vermiculophyllum*. Tissue P content differed strongly between sampling occasions (F(6,34)=9.49, p=0.000), but not between Site 1 and 2 in the Clonakilty estuary, and was minimal during spring and summer (i.e. April to August), with peaks again in October and February (Table 2.9., Fig. 2.18.). Here, the means of June 2016 (p-value: 0.026), April 2017 (p-value: 0.000), June 2017 (p-value: 0.000) and August 2017 (p-value: 0.010) were significantly below the estimated Qc (see Table 2.2.) during the sampling period. Once again, season was the factor showing the highest effect size (eta^2=0.62). Mean tissue P contents are shown in Annex table 3.

Table 2.9.: Two-Way-ANOVA on square-rooted tissue P content of A. vermiculophyllum, by the factors season and site

Agarophyton vermiculophyllum	**Source of Variation**	**SS**	**df**	**F**	**p-value**	**eta^2**
	Season	0.052	6	9.49	0.000	0.62
	Site	0.0003	1	0.33	0.567	0.00
	Residuals	0.031	34			

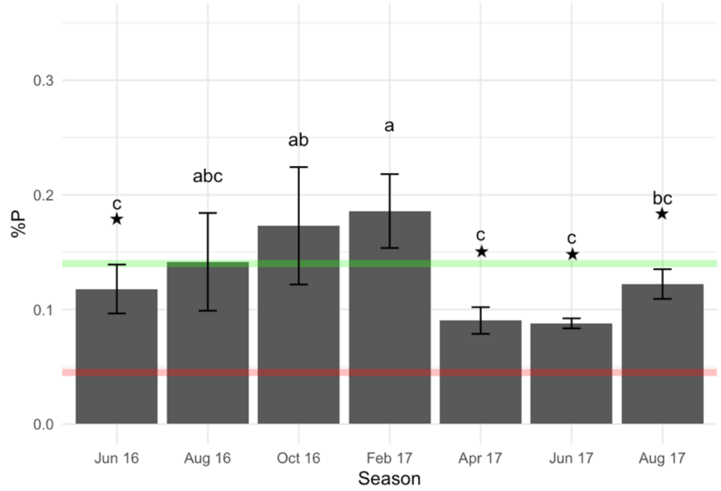

Fig. 2.18.: Mean tissue P content of A. vermiculophyllum in the Clonakilty estuary. Error bars represent standard deviation. Different sites (Site 1 and 2) are presented together as one single bar per sampling month due to a lack of data for some sampling occasions. Bars sharing the same letter(s) are not statistically different (t-test, p<0.05), and the green and the red line mark critical (Qc) and subsistence quota (Qs), respectively. Stars below letters were used for means significantly below Qc. "n.d." stands for "no data".

Biomass-nutrient-correlation

Since *A. vermiculophyllum* and *P. littoralis* were exclusively collected in the Clonakilty and the Killybegs estuary, respectively, no complete comparison regarding the correlation of biomass and tissue N content could be made between different estuaries for these two species. However, in *Ulva* spp., biomass abundance and tissue nitrogen content were not correlated in any kind in any of the studied estuaries, neither in *P. littoralis* in the Killybegs estuary (see Table 2.10., Fig. 2.19.). A negative correlation between tissue N content and accumulating biomass was observed in *A. vermiculophyllum* in the Clonakilty estuary (p-value: 0.000), but no correlation occurred between biomass and tissue P content (Fig. 2.20.).

Table 2.10.: Spearman correlation of biomass and nutrients, and adjusted p values for sub-correlations of Ulva spp.

Species	**Nutrient**	**Estuary**	**n**	**r_s**	**p-value**	**Adjusted p**
Ulva spp.	N	Argideen	21	-0.036	0.876	1.000
	N	Clonakilty	23	-0.16	0.478	1.000
	N	Tolka	22	0.44	0.040	0.120
	N	all	66	0.08	0.516	-
Agarophyton vermiculophyllum	N	Clonakilty	14	-0.84	0.000	-
	P	Clonakilty	14	-0.36	0.203	-
Pilayella littoralis	N	Killybegs	12	0.15	0.635	-

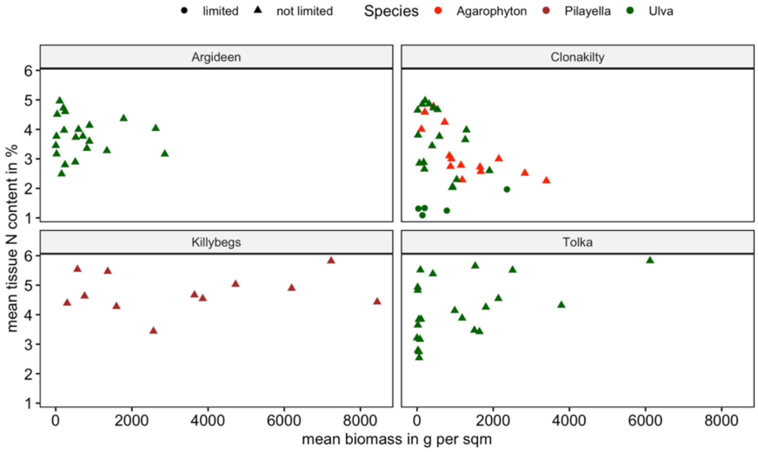

Fig. 2.19.: Tissue nitrogen content by biomass in g fresh weight per m^2. Faceted by estuary, colour by season, and shape indicating whether limited conditions occurred.

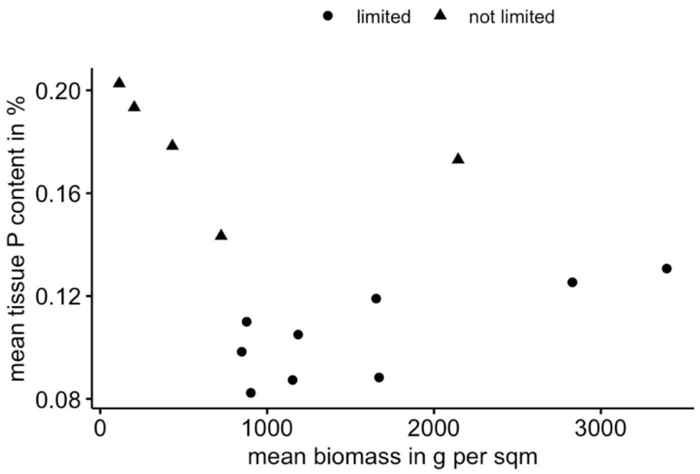

Fig. 2.20.: Tissue phosphorus content by biomass, in g fresh weight per m², of A. vermiculophyllum, shape indicates whether limited conditions occurred.

Stable isotope analysis ($\partial^{15}N$)

Stable isotope analysis revealed no difference between laminar and tubular morphotypes of *Ulva* spp. (Table 2.11., ANOVA, pairwise model comparison, p>0.05) and was therefore dropped as factor. First, isotopic signatures were compared for *Ulva* spp. between the Argideen, Clonakilty and Tolka estuary, and subsequently between *Ulva* spp. and *A. vermiculophyllum* for the Clonakilty estuary. ∂^{15}N differed between the Tolka and the Clonakilty estuary (p=0.000, Fig. 2.21.), but not between the Argideen estuary and either of the two others. Comparing isotopic signatures of *Ulva* spp. and *A. vermiculophyllum*, a significant difference could be found in the Clonakilty estuary (Fig. 2.22.).

Table 2.11.: Mixed-effects-ANOVA with $\partial^{15}N$ of Ulva spp. in estuaries, with site and season nested in estuary as random effects

***Ulva*, both morphotypes**	**Source of Variation**	**Chi²**	**df**	**p-value**	**Partial eta²**
	Estuary	22.752	2	0.000	0.41

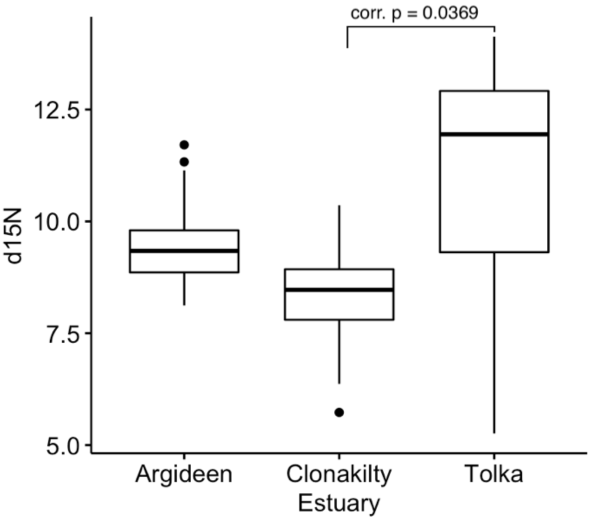

Fig. 2.21.: ∂^{15}N of Ulva spp., by estuaries, with pairwise comparison (emmeans, Tukey-test) annotated within the graph.

Table 2.12.: Mixed-effects-ANOVA with ∂^{15}N by species (A. vermiculophyllum and Ulva spp.), with site and season nested in estuary as random effects

Clonakilty	**Source of Variation**	**Chi^2**	**df**	**p-value**	**Partial eta^2**
	Species	8.8	1	0.003	0.816

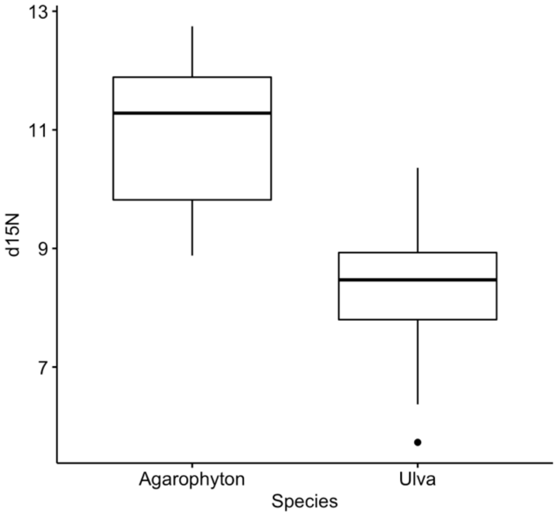

Fig. 2.22.: A. vermiculophyllum vs Ulva spp., ∂^{15}N by species in the Clonakilty estuary

2.5. Discussion

Overall, clear seasonal biomass dynamics were observed in green, golden and red tides alike in Ireland, with a peak of biomass during summer and minimum levels reached in winter, in accord with previous studies describing comparable seasonal patterns in other nutrient over-enriched, cold temperate estuaries (Pedersen and Borum, 1996; Teichberg *et al*., 2009). Similarly, tissue N content changed with estuaries (in *Ulva* spp.) and seasons, peaking in the colder period and dropping in spring and summer, in opposing trend to biomass. Considering the critical and subsistence quotas determined in other eco-physiological studies for these or similar species (Campbell, 2001; Pedersen and Borum, 1997; Villares and Carballeira, 2004), the obtained results suggest no N limitation at any time of the year in major Irish seaweed tides, contrasting to the findings of several previous studies in temperate coastal systems (Pedersen and Borum, 1996), but in accord with more recent ones (Pedersen *et al*., 2010). Neither in *Ulva* spp., except for laminar *Ulva* in the Clonakilty estuary during June 2016, nor in *P. littoralis* and in *A. vermiculophyllum*, N limitation occurred, as tissue N content was always higher than critical quota (Qc). In the case of *A. vermiculophyllum*, P limitation was observed during June 2016, April 2017, June 2017 and August 2017. The invasive rhodophyte was further the only species out of the five studied, where tissue N content decreased with increasing biomass, suggesting biomass dilution. Isotopic signatures indicate that sources of nutrients differed between specimens of *Ulva* spp. from the Tolka and the Clonakilty estuary, and between *Ulva* spp. and *A. vermiculophyllum* from Clonakilty.

In temperate estuaries exhibiting biomass seasonality, fluctuations of environmental parameters, among them light, temperature and photoperiod, co-impact biomass development together with nutrient availability (Pedersen and Borum, 1996; Teichberg *et al*., 2009). Ambient nutrient supply determines nutrient uptake and to which degree internal nutrient pools are filled and therefore the amount of algal biomass production (Teichberg *et al*., 2009): During periods of low light conditions but high exterior nutrient concentrations, seaweeds take up nutrients without utilizing them for growth and store them internally. However, in times of accelerated growth in spring and summer, when temperature and light conditions are optimal, storage is restricted since nutrients are immediately consumed for growth after uptake (Valiela *et al*., 1997), thus accounting for the observed seasonal changes of biomass of all five species in Irish estuaries. While the algal biomass is increasing and the general amount of N or P stays the same in the system, the tissue nitrogen or phosphorus decreases, as it distributes itself over the biomass (i.e. biomass dilution; Valiela *et al*., 1997). Differences in the amount of accumulating biomass between different estuaries, as in the case of green tides, or sites within the very same estuary, as in all studied species and morphotypes, might emerge due to site-specific fluctuations in nutrient or light availability (Fletcher, 1996; Valiela *et al.*, 1997).

The season in which the sampling took place explained the variability of tissue N (seasonal main effect according to eta^2: see Tables 2.6. (laminar and tubular *Ulva*), 2.7. (*A. vermiculophyllum*) and 2.8. (*P. littoralis*)) and P (eta^2: see Table 2.9. (*A. vermiculophyllum*)) content to a great part in all investigated species and estuaries in Ireland (Campbell, 2001; Pedersen and Borum, 1996). As in biomass, seasonal variability in environmental factors like temperature and light affect the development of blooms, (Bermejo *et al.*, 2020; Fan *et al.*, 2020), meaning that other reasons apart from altering nutrient availability might underlie the observed seasonal fluctuations of tissue nutrient content. Said limitations vary in their predominance throughout the year, here again, as with biomass, nutrient limitation usually occurs because of the depletion of the nutrient pool in summer. Less light and lower temperatures in the colder months allow bloom-forming seaweeds to store excess nutrients at a higher rate in winter (i.e. increasing tissue nutrient levels) than during the blooming period from late spring to summer when conditions are more favourable and macroalgal growth increases (Valiela *et al.*, 1997; Zhang *et al.*, 2019).

The fact that the sampling sites did not lead to variations of tissue N and P contents within any of the studied estuaries suggests that nutrient availability might not have differed substantially between different areas of a particular estuary and that nutrients were somewhat evenly distributed, which could be of relevance for monitoring actions.

When comparing tissue N contents of *Ulva* spp. between estuaries, calculated separately for each morphotype, N limitation occurred exclusively during June 2016 in the Clonakilty estuary during this study. Whilst, neither tubular nor laminar morphotypes of *Ulva* spp. were limited by N in the Tolka and the Argideen estuary at any time, according to tissue N levels above the critical quota (see Table 2.2.).

However, the rhodophyte *A. vermiculophyllum* showed clear P, but no N limitation in the Clonakilty estuary, indicated by tissue P contents significantly below the critical quota during June 2016, April 2017, June 2017 and August 2017, which coincides with the active growth season and therefore might have led to constrained bloom development.

It has generally been assumed that primary production is limited by N in temperate, marine ecosystems, whereas P has been considered the limiting nutrient in tropical and freshwater systems (Jeffrey *et al.*, 1995; Pedersen *et al.*, 2010; Teichberg *et al.*, 2009; Valiela *et al.*, 1997). However, this appears not to be the case in Irish estuaries, as also suggested by Lyngby (1999) and Longphuirt (2016) for other cold-temperate estuaries. Species-specific variations regarding the requirements and storing capacity for a certain nutrient were named as possible causes for variations in the duration of nutrient limitation in previous studies (Pedersen and Borum, 1996). On the other hand, differences between estuaries might arise due to their site-specific features, including hydrology, geomorphology and factors like freshwater inputs or water residence times within the estuary, leading to variable nutrient concentrations in the estuarine waters (Fletcher, 1996; Valiela *et al.*, 1997). They might also be the consequence of the variability of sources, responsible for the increased nutrient loadings, and therefore the quantity of nutrients reaching the estuary from its surroundings (Bermejo *et al.*, 2019b). Therefore, nutrient availability might have differed between estuaries during this study. Due to its vicinity to urban areas (Bermejo *et al.*, 2019a; Jeffrey *et al.*, 1995), the number of excess nutrients reaching the Tolka estuary might still exceed the demand of the blooming seaweed species there, even in periods of higher growth. The Clonakilty estuary receives the majority of its high nutrient loadings from agricultural run-off, so does the Argideen estuary, as stated by Bermejo *et al.* (2020), which might not be sufficient during the peak of a green tide and lead to inhibited growth due to temporary N limitation. Another reason for the exclusive occurrence of N limitation in the Clonakilty estuary might be the success of current management actions taken there (O'Boyle *et al.*, 2016), which would prove their suitability in this area. The aforementioned management actions undertaken in the last decades might have contributed to the in this study observed pattern of nutrient limitation in Ireland.

The following distribution patterns of green and red tides in the Clonakilty estuary were previously observed by Bermejo *et al.* (2020): *Ulva* spp. and *A. vermiculophyllum* bloomed in separate areas of the estuary with overlapping points in between (Fig. 2.23.).

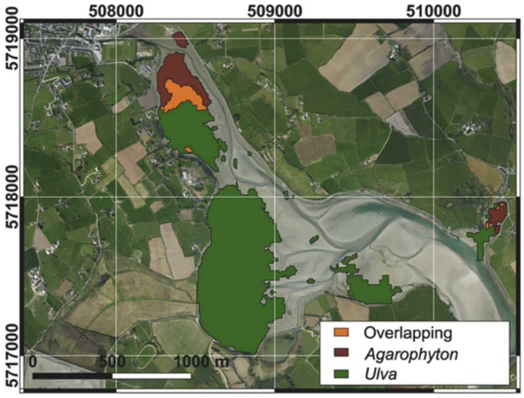

Fig. 2.23.: Potential extension and overlapping of A. vermiculophyllum and Ulva spp., satellite imagery (extracted from Bermejo et al., 2020).

While the N influx might have been sufficient for *A. vermiculophyllum* when a bloom started to arise in summer (Lyngby *et al*., 1999), it might not have met the requirements of *Ulva* spp. after biomass numbers started to increase (Nelson *et al*., 2008). Pedersen and Borum (1997) explained different responses of macroalgal species to low nutrient concentrations through the variable nutrient requirements among them, mainly caused by different growth rates, for example due to morphological differences, and to a lesser amount by different Nc concentrations (i.e. critical quotas for N). This means that at one and the same ambient nutrient concentration of nitrogen, for instance, one species might still be able to sustain maximum growth rates, whereas another might already be limited, which is followed by reduced biomass production, due to higher requirements for this particular nutrient or a lower storage capacity for the same (Duarte, 1995; Pedersen and Borum, 1996). This inter-specific variability might be the reason for the insufficiency of N for *Ulva* spp., but not for *A. vermiculophyllum* in the Clonakilty estuary.

Concerning P, a similar situation might have occurred, but tissue P content analysis will have to be conducted first for specimens of *Ulva* spp. to clarify the nutrient limitation of *Ulva* in this estuary. Duarte (1992) suggested that marine macroalgae might generally tend to be P limited during certain periods. Assuming that species of *Ulva* would turn out to be P limited during the warmer period in Ireland, the N limitation observed in June 2016 in Clonakilty might have been induced by said P limitation (Pedersen and Borum, 1996).

P. littoralis was not found to be limited by N in the Killybegs estuary at any time during this study, meaning that the N inputs were always sufficient for maximum growth during the study. Summing up, measuring nutrient concentrations directly in algal tissue indicated no N limitation in major Irish seaweed tides, contrasting to the results gained from studies only considering nutrient concentrations in seawater and suggesting N limitation in green tides (Longphuirt *et al*., 2016). This shows once more that this method is more suitable for assessing the nutrient status of macroalgal blooms (Costanzo *et al*., 2000; Lyngby *et al*., 1999). With P found to be the primary limiting nutrient in Irish estuaries, the results of this study differ from the ones obtained for the majority of other cold-temperate estuaries, where N is generally assumed to limit macroalgal growth (Fan *et al*., 2014; Lapointe, 1987; Valiela *et al*., 1997).

The data analysis further revealed that tissue N content is not only dependent on the sampling occasion, but, when looking at *A. vermiculophyllum*, also on the accumulating biomass. Tissue N content was not found to rise or fall with increasing biomass, when examining biomass-tissue N-interactions for species of *Ulva* spp. and *P. littoralis*, using mean tissue N content and mean biomass numbers. Nevertheless, biomass and tissue nutrient content can be described as interdependent, being interchangeably connected as explanatory and response variables. Enhanced nutrient availability gives rise to increasing biomass, but the accumulating biomass then again leads to decreasing exterior as well as tissue nutrient concentrations as consequence of higher uptake and biomass dilution, respectively (Jeffrey *et al*., 1995; Pedersen *et al*., 2010). Added to this, as soon as conditions improve in spring (i.e. temperatures rise and light availability increases), the excess nutrients released into estuarine waters allow marine macrophyte growth to exceed naturally occurring biomass accumulations by far, partially eliminating the biomass dilution issue.

Furthermore, stable isotope analysis ($\partial^{15}N$) of *Ulva* spp. suggested that nutrients were derived from different sources in the Tolka and Clonakilty estuary (i.e. agricultural run-off or urban wastewaters), whereas nutrient sources did not differ between the Argideen and the Clonakilty and Tolka estuary, respectively. However, both morphotypes of *Ulva* spp. were likely to have obtained nutrients from the same, as indicated by similar isotopic signatures. According to the obtained results, also *Ulva* spp. and *A. vermiculophyllum* did not share the same source of nutrients in the Clonakilty estuary, meaning that different sections within the estuary might receive nutrients from distinct sources (Teichberg *et al*., 2009).

Finally, the continuous problem of nutrient over-enrichment requires more suitable invasive management plans. Therefore, species-specific performances under distinct nutrient concentrations need to be taken into account in order to develop such adapted plans. Current plans might have to be revised or refocused on mitigating various nutrient sources (e.g. reducing agricultural run-off and urban sewages), or new approaches may need to be assessed (Jeffrey *et al*., 1995; Pedersen *et al*., 2010; Sfriso *et al*., 2019). According to Fan *et al.* (2014), a dual nutrient reduction strategy of N and P should generally be applied to avoid focusing on a single, possibly not primary limiting nutrient. Additionally, monitoring of blooms should be enhanced, also via the use of opportunistic macroalgae as bioindicators, and indices such as isotopic signatures used to determine sources of nutrients. However, apart from proposed regulations regarding nutrient sources, methods to use the beached algal matter were assessed as an optional alternative in previous studies: For instance, Allen *et al.* (2013) suggested that the accumulating biomass of blooming seaweeds might potentially be used as a source of renewable gaseous fuel in the form of biomethane.

Nitrogen was discovered to be harder to remove from wastewaters than phosphorus, implicating that this fact together with generally more considerable variability in available P, according to Duarte (1992), could lead to the observed P limitation in Irish estuarine waters. For this reason, a site- and species-dependent shift from N management to P management might be necessary for Ireland and possibly also in other affected countries. Since P data was only available for *A. vermiculophyllum*, the nutrient status could not be fully assessed for the remaining species. Further research containing tissue P content analysis will be needed to fill these gaps and to answer the question if green and golden tides in Ireland might experience seasonal P limitation.

Primary findings of this study:

1. N was not found to be the primary limiting nutrient for bloom-forming macroalgae in Irish estuaries.
2. Biomass abundance as well as tissue N content are mainly controlled by season.
3. Comparing the nutrient status of estuaries in Ireland to that of other cold-temperate estuaries, a distinct pattern was observed., since other European coastal systems are usually considered N limited.
4. If N is not limiting macroalgal growth and P is constraining seaweed tide development in Ireland instead, current management and regulations need to be altered towards P management in order to prevent future blooms. Special care needs to be taken since only slight increases or decreases in P loadings might have a substantial effect on bloom development.

2.6. Conclusion

In conclusion, seaweed tides in Ireland were not found to be N limited, when comparing tissue N content to the in previous studies extracted critical quotas (Qc) of the investigated species, contrasting to the predominant conception considering N the primary limiting nutrient in temperate estuaries. This information shall further be used to develop improved management options, for instance for agricultural fertilisation and sewage treatment. Increased P management needs to be taken into consideration, or the combined reduction of N and P in Irish coastal waters. In light of the worldwide worsening situation with habitat and biodiversity loss induced by climate change and other anthropogenic stressors, it is of utmost importance to approximate a pristine state in estuaries once again.

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ANNEX

Annex table 1: Algal biomass by species, estuary, season and site.

Species	**Estuary**	**Season**	**Site**	**Biomass in g (mean±SD)**	**Laminar biomass in g (mean±SD)**	**Tubular biomass in g (mean±SD)**
Agarophyton vermiculophyllum	Clonakilty	Jun-16	Site 1	3395.09 (±973.70)		
Agarophyton vermiculophyllum	Clonakilty	Jun-16	Site 2	1185.71 (±574.18)		
Agarophyton vermiculophyllum	Clonakilty	Aug-16	Site 1	2144.91 (±797.22)		
Agarophyton vermiculophyllum	Clonakilty	Aug-16	Site 2	877.28 (±528.50)		
Agarophyton vermiculophyllum	Clonakilty	Oct-16	Site 1	724.27 (±153.76)		
Agarophyton vermiculophyllum	Clonakilty	Oct-16	Site 2	113.60 (±108.48)		
Agarophyton vermiculophyllum	Clonakilty	Feb-17	Site 1	432.00 (±376.98)		
Agarophyton vermiculophyllum	Clonakilty	Feb-17	Site 2	203.38 (±140.19)		
Agarophyton vermiculophyllum	Clonakilty	Apr-17	Site 1	847.82 (±949.17)		
Agarophyton vermiculophyllum	Clonakilty	Apr-17	Site 2	902.22 (±309.78)		
Agarophyton vermiculophyllum	Clonakilty	Jun-17	Site 1	1670.76 (±1049.07)		
Agarophyton vermiculophyllum	Clonakilty	Jun-17	Site 2	1152.53 (±231.05)		
Agarophyton vermiculophyllum	Clonakilty	Aug-17	Site 1	2829.33 (±705.10)		
Agarophyton vermiculophyllum	Clonakilty	Aug-17	Site 2	1653.33 (±592.92)		
Pilayella littoralis	Killybegs	Jun-16	Site 1	8440.80 (±2214.61)		
Pilayella littoralis	Killybegs	Jun-16	Site 2	2565.94 (±2353.73)		
Pilayella littoralis	Killybegs	Aug-16	Site 1	3641.42 (±2398.19)		
Pilayella littoralis	Killybegs	Aug-16	Site 2	1592.71 (±1727.65)		
Pilayella littoralis	Killybegs	Oct-16	Site 1	4721.42 (±3712.97)		
Pilayella littoralis	Killybegs	Oct-16	Site 2	6194.13 (±3566.04)		
Pilayella littoralis	Killybegs	Feb-17	Site 2	569.96 (±338.92)		
Pilayella littoralis	Killybegs	Apr-17	Site 1	5924.62 (±2524.33)		
Pilayella littoralis	Killybegs	Apr-17	Site 2	1365.40 (±1497.95)		
Pilayella littoralis	Killybegs	Jun-17	Site 1	7231.20 (±4173.60)		
Pilayella littoralis	Killybegs	Jun-17	Site 2	756.27 (±406.39)		
Pilayella littoralis	Killybegs	Aug-17	Site 1	3859.20 (±1570.49)		
Pilayella littoralis	Killybegs	Aug-17	Site 2	298.84 (±143.74)		
Ulva spp.	Argideen	Jun-16	Site 1	2636.40 (±1650.66)	12.13 (±28.92)	2624.27 (±1638.96)
Ulva spp.	Argideen	Jun-16	Site 2	2863.60 (±2017.59)	0.93 (±2.21)	2862.67 (±2018.67)
Ulva spp.	Argideen	Aug-16	Site 1	1561.47 (±1223.89)	218.13 (±306.83)	1343.33 (±1148.40)
Ulva spp.	Argideen	Aug-16	Site 2	664.93 (±367.96)	153.07 (±117.83)	511.87 (±308.52)
Ulva spp.	Argideen	Oct-16	Site 1	351.87 (±253.37)	104.13 (±120.89)	247.73 (±217.79)

Ulva spp.	Argideen	Oct-16	Site 2	240.80 (±151.89)	203.47 (±101.25)	37.33 (±95.06)
Ulva spp.	Argideen	Apr-17	Site 1	525.60 (±808.50)	1.33 (±3.27)	524.27 (±805.35)
Ulva spp.	Argideen	Apr-17	Site 2	711.42 (±783.63)	0.00 (±0.00)	711.42 (±783.63)
Ulva spp.	Argideen	Jun-17	Site 1	1804.27 (±1042.76)	21.33 (±27.47)	1782.93 (±1052.23)
Ulva spp.	Argideen	Jun-17	Site 2	615.56 (±497.39)	18.33 (±40.62)	597.24 (±473.56)
Ulva spp.	Argideen	Aug-17	Site 1	1704.27 (±1256.52)	818.00 (±788.22)	886.27 (±731.74)
Ulva spp.	Argideen	Aug-17	Site 2	1125.47 (±809.88)	242.80 (±182.24)	882.67 (±744.59)
Ulva spp.	Clonakilty	Jun-16	Site 1	814.67 (±482.15)	33.78 (±42.23)	780.89 (±477.76)
Ulva spp.	Clonakilty	Jun-16	Site 2	2501.87 (±2135.26)	141.69 (±165.71)	2360.18 (±2114.41)
Ulva spp.	Clonakilty	Aug-16	Site 1	1240.22 (±1400.22)	198.80 (±172.24)	1041.42 (±1258.31)
Ulva spp.	Clonakilty	Aug-16	Site 2	997.60 (±238.58)	934.93 (±238.91)	62.67 (±78.83)
Ulva spp.	Clonakilty	Oct-16	Site 1	1835.38 (±2031.65)	539.78 (±833.54)	1295.60 (±1609.90)
Ulva spp.	Clonakilty	Oct-16	Site 2	646.76 (±532.41)	211.38 (±215.83)	435.38 (±606.81)
Ulva spp.	Clonakilty	Feb-17	Site 1	332.80 (±251.26)	17.07 (±22.98)	315.73 (±258.73)
Ulva spp.	Clonakilty	Feb-17	Site 2	144.00 (±65.01)	6.93 (±6.47)	137.07 (±61.50)
Ulva spp.	Clonakilty	Apr-17	Site 1	1291.73 (±870.06)	26.40 (±26.63)	1265.33 (±867.84)
Ulva spp.	Clonakilty	Apr-17	Site 2	2091.73 (±1051.96)	191.47 (±136.07)	1900.27 (±983.23)
Ulva spp.	Clonakilty	Jun-17	Site 1	568.27 (±526.83)	172.80 (±164.73)	395.47 (±472.61)
Ulva spp.	Clonakilty	Jun-17	Site 2	1507.73 (±1094.13)	921.60 (±747.48)	586.13 (±436.78)
Ulva spp.	Tolka	Jun-16	Site 1	3789.33 (±1798.16)	0.00 (±0.00)	3789.33 (±1798.16)
Ulva spp.	Tolka	Jun-16	Site 2	6194.80 (±3578.34)	76.93 (±70.90)	6117.87 (±3551.33)
Ulva spp.	Tolka	Aug-16	Site 1	1035.07 (±498.82)	46.27 (±48.02)	988.80 (±529.12)
Ulva spp.	Tolka	Aug-16	Site 2	1608.40 (±1473.95)	103.60 (±88.19)	1504.80 (±1445.75)
Ulva spp.	Tolka	Oct-16	Site 1	2923.60 (±3303.24)	415.20 (±244.13)	2508.40 (±3376.36)
Ulva spp.	Tolka	Oct-16	Site 2	1614.80 (±1582.39)	87.73 (±61.17)	1527.07 (±1573.84)
Ulva spp.	Tolka	Apr-17	Site 1	18.93 (±8.46)	0.67 (±2.31)	18.27 (±8.62)
Ulva spp.	Tolka	Apr-17	Site 2	16.67 (±15.20)	0.67 (±2.31)	16.00 (±15.33)
Ulva spp.	Tolka	Jun-17	Site 1	1206.40 (±498.31)	22.80 (±44.00)	1183.60 (±484.68)
Ulva spp.	Tolka	Jun-17	Site 2	1827.47 (±585.24)	21.33 (±37.23)	1806.13 (±583.56)
Ulva spp.	Tolka	Aug-17	Site 1	1689.87 (±785.70)	51.33 (±37.98)	1638.53 (±783.99)
Ulva spp.	Tolka	Aug-17	Site 2	2190.40 (±733.73)	55.60 (±125.58)	2134.80 (±758.64)

Annex table 2: Tissue N content by species, estuary, season and site.

Species	Morphology	Estuary	Season	Site	Tissue nitrogen content % (mean (±SD))
Agarophyton vermiculophyllum		Clonakilty	Jun-16	Site 1	2.26 (±0.27)
Agarophyton vermiculophyllum		Clonakilty	Jun-16	Site 2	2.29 (±0.51)
Agarophyton vermiculophyllum		Clonakilty	Aug-16	Site 1	3.00 (±0.51)
Agarophyton vermiculophyllum		Clonakilty	Aug-16	Site 2	2.74 (±1.03)
Agarophyton vermiculophyllum		Clonakilty	Oct-16	Site 1	4.24 (±0.20)
Agarophyton vermiculophyllum		Clonakilty	Oct-16	Site 2	4.00 (±0.59)
Agarophyton vermiculophyllum		Clonakilty	Feb-17	Site 1	4.78 (±0.44)
Agarophyton vermiculophyllum		Clonakilty	Feb-17	Site 2	4.58 (±0.15)
Agarophyton vermiculophyllum		Clonakilty	Apr-17	Site 1	3.10 (±0.31)
Agarophyton vermiculophyllum		Clonakilty	Apr-17	Site 2	3.00 (±0.47)
Agarophyton vermiculophyllum		Clonakilty	Jun-17	Site 1	2.57 (±0.25)
Agarophyton vermiculophyllum		Clonakilty	Jun-17	Site 2	2.78 (±0.05)
Agarophyton vermiculophyllum		Clonakilty	Aug-17	Site 1	2.51 (±0.05)
Agarophyton vermiculophyllum		Clonakilty	Aug-17	Site 2	2.72 (±0.53)
Pilayella littoralis		Killybegs	Jun-16	Site 1	4.43 (±0.30)
Pilayella littoralis		Killybegs	Jun-16	Site 2	3.44 (±0.81)
Pilayella littoralis		Killybegs	Aug-16	Site 1	4.67 (±0.14)
Pilayella littoralis		Killybegs	Aug-16	Site 2	4.28 (±0.22)
Pilayella littoralis		Killybegs	Oct-16	Site 1	5.03 (±0.46)
Pilayella littoralis		Killybegs	Oct-16	Site 2	4.89 (±0.51)
Pilayella littoralis		Killybegs	Feb-17	Site 2	5.54 (±0.44)
Pilayella littoralis		Killybegs	Apr-17	Site 2	5.47 (±0.66)
Pilayella littoralis		Killybegs	Jun-17	Site 2	4.63 (±0.27)
Pilayella littoralis		Killybegs	Aug-17	Site 1	4.54 (±0.08)
Ulva spp.	laminar	Argideen	Jun-16	Site 2	3.45 (±0.90)
Ulva spp.	laminar	Argideen	Aug-16	Site 1	3.97 (±0.58)
Ulva spp.	laminar	Argideen	Aug-16	Site 2	2.49 (±0.33)
Ulva spp.	laminar	Argideen	Oct-16	Site 1	4.96 (±0.26)
Ulva spp.	laminar	Argideen	Oct-16	Site 2	4.72 (±0.28)
Ulva spp.	laminar	Argideen	Jun-17	Site 1	3.17 (±0.11)
Ulva spp.	laminar	Argideen	Jun-17	Site 2	3.77 (±0.78)
Ulva spp.	laminar	Argideen	Aug-17	Site 1	3.36 (±0.79)
Ulva spp.	laminar	Argideen	Aug-17	Site 2	2.80 (±0.25)
Ulva spp.	laminar	Clonakilty	Jun-16	Site 1	1.31 (±0.50)
Ulva spp.	laminar	Clonakilty	Jun-16	Site 2	1.09 (±0.28)
Ulva spp.	laminar	Clonakilty	Aug-16	Site 1	1.33 (±0.36)
Ulva spp.	laminar	Clonakilty	Aug-16	Site 2	2.05 (±0.16)
Ulva spp.	laminar	Clonakilty	Oct-16	Site 1	4.67 (±0.37)
Ulva spp.	laminar	Clonakilty	Oct-16	Site 2	4.97 (±0.54)
Ulva spp.	laminar	Clonakilty	Feb-17	Site 1	4.65 (±0.18)

Ulva spp.	laminar	Clonakilty	Apr-17	Site 1	3.81 (±0.14)
Ulva spp.	laminar	Clonakilty	Apr-17	Site 2	2.65 (±0.40)
Ulva spp.	laminar	Clonakilty	Jun-17	Site 1	2.88 (±0.78)
Ulva spp.	laminar	Clonakilty	Jun-17	Site 2	2.03 (±0.52)
Ulva spp.	laminar	Tolka	Jun-16	Site 1	3.21 (±1.27)
Ulva spp.	laminar	Tolka	Jun-16	Site 2	3.16 (±0.13)
Ulva spp.	laminar	Tolka	Aug-16	Site 1	3.84 (±0.42)
Ulva spp.	laminar	Tolka	Aug-16	Site 2	3.85 (±0.41)
Ulva spp.	laminar	Tolka	Oct-16	Site 1	5.38 (±0.23)
Ulva spp.	laminar	Tolka	Oct-16	Site 2	5.52 (±0.21)
Ulva spp.	laminar	Tolka	Jun-17	Site 1	2.79 (±0.18)
Ulva spp.	laminar	Tolka	Jun-17	Site 2	3.65 (±0.95)
Ulva spp.	laminar	Tolka	Aug-17	Site 1	2.74 (±0.95)
Ulva spp.	laminar	Tolka	Aug-17	Site 2	2.54 (±0.25)
Ulva spp.	tubular	Argideen	Jun-16	Site 1	4.03 (±0.82)
Ulva spp.	tubular	Argideen	Jun-16	Site 2	3.16 (±0.14)
Ulva spp.	tubular	Argideen	Aug-16	Site 1	3.28 (±1.09)
Ulva spp.	tubular	Argideen	Aug-16	Site 2	2.89 (±0.37)
Ulva spp.	tubular	Argideen	Oct-16	Site 1	4.60 (±0.37)
Ulva spp.	tubular	Argideen	Oct-16	Site 2	4.51 (±0.32)
Ulva spp.	tubular	Argideen	Apr-17	Site 1	3.73 (±0.56)
Ulva spp.	tubular	Argideen	Apr-17	Site 2	3.77 (±0.61)
Ulva spp.	tubular	Argideen	Jun-17	Site 1	4.36 (±0.70)
Ulva spp.	tubular	Argideen	Jun-17	Site 2	4.00 (±0.12)
Ulva spp.	tubular	Argideen	Aug-17	Site 1	4.14 (±0.44)
Ulva spp.	tubular	Argideen	Aug-17	Site 2	3.60 (±1.26)
Ulva spp.	tubular	Clonakilty	Jun-16	Site 1	1.25 (±0.24)
Ulva spp.	tubular	Clonakilty	Jun-16	Site 2	1.97 (±1.01)
Ulva spp.	tubular	Clonakilty	Aug-16	Site 1	2.30 (±0.84)
Ulva spp.	tubular	Clonakilty	Aug-16	Site 2	2.85 (±0.47)
Ulva spp.	tubular	Clonakilty	Oct-16	Site 1	3.98 (±0.15)
Ulva spp.	tubular	Clonakilty	Oct-16	Site 2	4.73 (±0.24)
Ulva spp.	tubular	Clonakilty	Feb-17	Site 1	4.86 (±0.22)
Ulva spp.	tubular	Clonakilty	Feb-17	Site 2	4.85 (±0.29)
Ulva spp.	tubular	Clonakilty	Apr-17	Site 1	3.65 (±0.84)
Ulva spp.	tubular	Clonakilty	Apr-17	Site 2	2.60 (±0.41)
Ulva spp.	tubular	Clonakilty	Jun-17	Site 1	3.44 (±1.37)
Ulva spp.	tubular	Clonakilty	Jun-17	Site 2	3.76 (±0.26)
Ulva spp.	tubular	Tolka	Jun-16	Site 1	4.32 (±1.92)
Ulva spp.	tubular	Tolka	Jun-16	Site 2	5.83 (±0.16)
Ulva spp.	tubular	Tolka	Aug-16	Site 1	4.14 (±0.58)
Ulva spp.	tubular	Tolka	Aug-16	Site 2	3.47 (±0.12)
Ulva spp.	tubular	Tolka	Oct-16	Site 1	5.51 (±0.64)
Ulva spp.	tubular	Tolka	Oct-16	Site 2	5.65 (±0.86)

Ulva spp.	tubular	Tolka	Apr-17	Site 1	4.83 (±0.23)
Ulva spp.	tubular	Tolka	Apr-17	Site 2	4.93 (±0.31)
Ulva spp.	tubular	Tolka	Jun-17	Site 1	3.88 (±0.86)
Ulva spp.	tubular	Tolka	Jun-17	Site 2	4.25 (±0.66)
Ulva spp.	tubular	Tolka	Aug-17	Site 1	3.42 (±0.88)
Ulva spp.	tubular	Tolka	Aug-17	Site 2	4.55 (±1.72)

Annex table 3: Tissue P content by species, estuary, season and site.

Species	**Estuary**	**Season**	**Site**	**Tissue P content %**
MEAN (±SD)				
Agarophyton vermiculophyllum	Clonakilty	Jun-16	Site 1	0.131 (±0.009)
Agarophyton vermiculophyllum	Clonakilty	Jun-16	Site 2	0.105 (±0.024)
Agarophyton vermiculophyllum	Clonakilty	Aug-16	Site 1	0.173 (±0.037)
Agarophyton vermiculophyllum	Clonakilty	Aug-16	Site 2	0.110 (±0.014)
Agarophyton vermiculophyllum	Clonakilty	Oct-16	Site 1	0.143 (±0.049)
Agarophyton vermiculophyllum	Clonakilty	Oct-16	Site 2	0.203 (±0.039)
Agarophyton vermiculophyllum	Clonakilty	Feb-17	Site 1	0.178 (±0.042)
Agarophyton vermiculophyllum	Clonakilty	Feb-17	Site 2	0.193 (±0.026)
Agarophyton vermiculophyllum	Clonakilty	Apr-17	Site 1	0.098 (±0.011)
Agarophyton vermiculophyllum	Clonakilty	Apr-17	Site 2	0.082 (±0.006)
Agarophyton vermiculophyllum	Clonakilty	Jun-17	Site 1	0.088 (±0.002)
Agarophyton vermiculophyllum	Clonakilty	Jun-17	Site 2	0.087 (±0.006)
Agarophyton vermiculophyllum	Clonakilty	Aug-17	Site 1	0.125 (±0.016)
Agarophyton vermiculophyllum	Clonakilty	Aug-17	Site 2	0.119 (±0.011)

Other planned experiments (unfinished)

Except for one sampling trip to the Clonakilty estuary with subsequent sample processing at the Martin Ryan Institute, the scheduled sampling of algal biomass could not take place and experiments could not be conducted as planned due to the outbreak of SARS-CoV-2. Between mid-March and July 2020, access to the laboratory and unnecessary travels within Ireland were no longer permitted. Therefore, nutrient enrichment and temperature experiments could not be finished or even started timely before the deadline of the master thesis but will hopefully be continued when restrictions are eased again.

Determination of nutrient requirements – unfinished

Sampling in the Clonakilty estuary, 9 March 2020

Specimens of *A. vermiculophyllum*, *U. compressa*, *U. prolifera* and *U. rigida* were randomly collected at low tide in the Clonakilty estuary (red and green tides). Samples were kept separately (i.e. *A. vermiculophyllum*, laminar and tubular *Ulva*) in plastic bags during the four-hour-transport to the NUI Galway.

Sample cultivation

In the laboratory, the different seaweeds were thoroughly washed with seawater, subsequently cleaned and blotted dry with tissue paper. As in the above-described experiments (see Chapter 2: Manuscript, **Materials and Methods**), laminar and tubular *Ulva* were split. Afterwards, the specimens were kept in 500 mL and 1 L flasks, respectively, for a 2-week-acclimatisation, depending on the amount of biomass, using enriched artificial seawater with f/2 medium (Guillard and Ryther, 1962; Guillard, 1975). During the cultivation, containers were placed on orbital shakers at 100 rpm, to ensure water movement and a reduction of the boundary layers, at a constant temperature of 15.5 °C in the culture chamber and a salinity of 35 PSU. The specimens were cultivated at an average irradiance of 90 µmol photons m^{-2} s^{-1} with a 16:8 hours light-dark cycle. Every Monday and Friday, the water of the samples was exchanged to avoid nutrient depletion and contamination. Hence, the algae were rinsed with seawater, using a sieve for retaining them. Artificial seawater and medium in the flasks were replaced, after washing the containers with tap water and cleaning them with a brush, if necessary. After usage, the flasks were washed in acid to reduce contamination.

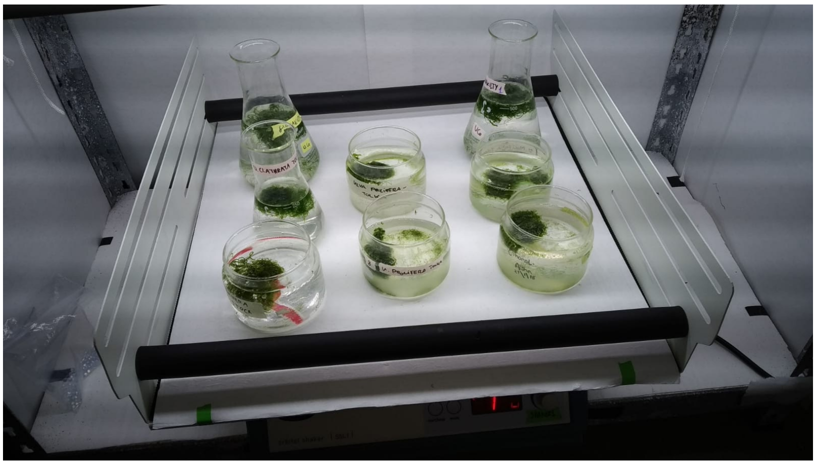
Nutrient treatments

To mimic various nutrient management scenarios, the biological performance of the different species was studied during several nutrient treatments. The four specimens were cultivated in a nitrogen-limited, a phosphorus-limited medium and a medium without a nutrient limitation, respectively, for at least four weeks. In the culture chamber, the cultivation in 250 mL wide-mouth conical flasks with approximately 0.4 g biomass each took place in fully artificial seawater without nitrogen and phosphorus, respectively, to avoid a pre-contamination of the samples, and under the following conditions: Per species, 20 different nutrient conditions (e.g. ten different nutrient concentrations per nutrient (N and P)) were tested. Placed on an orbital shaker at 100 rpm, the samples were cultivated under a light intensity of 40 µmol photons m^{-2} s^{-1}, while salinity and temperature stayed the same as before. In order to prepare the artificial seawater, 371 g of salt were dissolved in 10 litres of distilled water, using a magnetic stick for stirring, whereas the f/2 medium was mixed after Guillard and Ryther (1962) and Guillard (1975), always adapting the required components (only P or only N). Water and medium of the samples were replaced once per week.

Tissue nutrient (N and P) analysis

The assessment of the nutrient status of the red and green algae from the Clonakilty estuary unfortunately could not take place yet, therefore also preliminary results are not available at the moment.

Annex Figure 1: Sampling of Ulva spp. and A. vermiculophyllum specimens in the Clonakilty estuary, 9 March 2020.



Annex Figure 2: Samples of Ulva spp. on an orbital shaker (culture chamber).