Spatial and temporal variability of biomass and composition of green tides in Ireland

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ABSTRACT

Although nutrient enrichment of estuarine and coastal waters is considered a key factor for the development of green tides, the extent, distribution, and species composition of blooms vary among systems of similar nutrient loading, which compromises our ability to predict these events based on information about nutrient status alone. Additional factors may play a role in the control and development of macroalgal blooms. The identification of relevant scales of variation is a necessary prerequisite before explanatory models can be proposed and tested. In this study spatial and temporal patterns of biomass distribution were assessed for two *Ulva* morphologies in two Irish estuaries heavily affected by green tides (wet biomass >1kg m^{-2} during the peak bloom). Moreover, using genetic markers, the species composition of these green tides was assessed. Results revealed that these blooms were multi-specific, with *Ulva prolifera, U. compressa* and *U.rigida* the most frequent species. The species *U. prolifera* and *U. compressa* usually showed a tubular morphology, while *U. rigida* was mainly laminar. A seasonal succession common to both estuaries was also identified, with the bloom dominated by tubular species during spring and early summer, and co-dominated by tubular and laminar morphologies during late summer and autumn. Moreover, tubular and laminar morphologies exhibited different distribution patterns, with tubular morphologies varying at bigger spatial scales and higher biomass than the laminar. As tubular and laminar morphologies exhibited different distribution patterns, varying tubular morphologies along bigger spatial scales with higher biomass levels than the laminar. Considering that tubular morphologies were usually anchored to the sediment, while laminar *Ulva* were usually observed free-floating, these differences could explain a differential influence by water motion. An important annual and decadal variability in biomass levels of *Ulva* was observed, in the case of the Tolka estuary a noticeable increase over the last two decades. These findings should be considered for the development of management and monitoring strategies since the different habitat of laminar and tubular morphologies (anchored vs. free-floating) may play an important role in the balance of nutrients and biomass in the estuary, or determine the response to pollutant exposure. Furthermore, the presence of different species with different ecological requirements could favour the duration and extension of the bloom though temporal and spatial successions.

Keywords: *Ulva*; Green tide; Macroalgal bloom; Eutrophication; Temporal succession; Biodiversity.

1. Introduction

Nutrient enrichment of marine and transitional waters has increased worldwide as consequence of the growing human population, especially in the coastal zone (Nixon et al., 1986; Teichberg et al., 2010). In European coastal waters eutrophication has been identified as a key pressure, and the reduction of nutrient loads as the main restoration measure (Hering et al., 2010). In transitional water bodies such as coastal lagoons or estuaries, one of the most evident signs of eutrophication is the accumulation of fast-growing opportunistic macroalgal species, mainly Ulvoid species (Teichberg et al., 2010). These accumulations of macroalgae, or so-called “seaweed tides” or “seaweed blooms”, are named according to the colour of the main species present, either green tides (e.g. *Ulva*, *Cladophora*, *Chaetomorpha* or *Ulvaria*) or golden tides (e.g. *Sargassum* or *Ectocarpaceae*), and in the case of red macroalgae, red seaweed tides (e.g. *Gracilaria*) (Smetacek and Zingone, 2013; Thornber et al., 2017). Although macroalgae causing seaweed tides are not themselves toxic to humans, the blooms can affect shore-based activities by virtue of their sheer physical mass (e.g. navigation, tourism, fisheries; Smetacek and Zingone, 2013). More importantly, biomass degradation can cause serious amenity and public health impacts as a consequence of oxygen depletion and the release of sulphuric compounds, which can be harmful for humans and wildlife (Valiela et al., 1997; Sfriso et al., 2003).

Although nutrient enrichment of estuarine and coastal waters seems to be a key factor explaining the development of macroalgal blooms (Valiela et al., 1997; Teichberg et al., 2010; Smetacek and Zingone, 2013), the extent, distribution, and species composition of blooms vary strongly among systems of similar nutrient load, which compromises our ability to predict such events based on information about nutrient status alone (Bonsdorff, 1992; Malta and Verschuure, 1997; Lotze et al., 2000). Additional factors may play a role in the development of macroalgal blooms, such as local coastal geomorphology, hydrodynamic conditions, irradiance, water temperature, grazing or propagule bank size (Lotze et al., 2000; Nelson et al., 2008; Thornber et al., 2017). In addition, the local pool of species capable of producing a bloom can also stimulate or prolong the intensity and duration of the bloom, since a temporal and spatial succession can occur (Lavery et al., 1991; Nelson et al., 2003; Guidone and Thornber, 2013). In this sense, the arrival of cryptic non-native species could account for the occurrence of macroalgal tides in places where nutrients conditions have remained more or less constant (e.g. Yabe et al., 2009; Yoshida et al., 2015). Therefore an understanding of the factors that control the occurrence and development of seaweeds tides is key for the implementation of effective management strategies.

The genus *Ulva* is notorious for producing green tides (Valiela et al., 1997; Wang et al., 2015; Thornber et al., 2017). Species belonging to *Ulva* genus have a simple morphology, consisting either of a monostromatic tubular thallus (formerly *Enteromorpha* genus; Hayden et al., 2003) or a distromatic laminar thallus. Although in some cases microscopic traits can be useful for taxonomic identification, the high plasticity and the lack of reliable traits especially in

detached specimens forming *Ulva* blooms (e.g. lack of the basal part, intermediate size of cells or numbers of pyrenoids, etc) confounds accurate identification (Malta et al., 1999; Guidone and Thornber, 2013). Molecular genetic information is a powerful tool to overcome these challenges. In this sense, the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L) has been shown to reliably amplify with published primers and to provide enough variation to distinguish species within the genus *Ulva* (e.g. Hayden et al., 2003; Heesch et al., 2009; Wan et al., 2017).

The study of spatial and temporal patterns of variation in natural assemblages can be helpful to identify or infer the processes or factors affecting benthic assemblages (e.g. Benedetti-Cecchi et al., 2001; Terlizzi et al., 2007; Bermejo et al., 2015). The identification of relevant scales of spatial and temporal variation is a necessary prerequisite, before explanatory models can be proposed and tested (Andrew and Mapstone, 1987). Estuaries are highly dynamic and complex ecosystems subjected to significant fluctuations in physico-chemical conditions due to natural and anthropogenic processes, which produce an important variability in the structure and composition of natural assemblages (Nelson et al., 2003; Carvalho et al., 2011). In the case of green tides formed by *Ulva* spp., significant variations in biomass distribution and abundance have been observed at different temporal and spatial scales. This has been explained by diverse biotic and abiotic processes, such as desiccation, time availability for nutrient uptake and photosynthesis, grazing, salinity gradients, or geomorphological characteristics in relation to prevailing winds, currents, and tidal dynamics, which favour or preclude the accumulation of drift seaweeds (Aldridge and Trimmer, 2009; Park et al., 2014; Lanari and Copertino, 2016).

The objectives of this study were: i) to infer the most important factors controlling the development and persistence of green tides, by assessing spatial and temporal patterns of biomass distribution in two estuaries; and ii) by combining morphological characters and genetic markers, to assess the species composition of these tides.

2. Materials and methods

2.1. Study sites, experimental design and sample collection

Two estuaries in Ireland (Tolka on the east coast – Irish Sea; and Argideen along the south coast – Atlantic Ocean; Fig. 1) affected by large green tides were studied over seven sampling occasions, in order to assess the spatial and temporal patterns of variation in *Ulva* biomass between June 2016 and August 2017. Both study areas are shallow, nutrient enriched estuaries with broadly similar physical characteristics. The Argideen is the larger estuary with a surface area of 9.3 km^2 and a length of 6.49 km vs 3.6 km^2 and 4.58 km for the Tolka. The residence time in the Argideen is 4 days (Ní Longphuirt *et al.*, 2015) compared to 5.4 in the Tolka estuary (calculated as per Hartnett et al., 2011). The mean depth was 3.7 m for the Argideen and 2.1 m for the Tolka. Both estuaries showed a similar tidal range (4.4 m for the Tolka, and 4.3 m for the Argideen).

The Tolka estuary is located in a large urban catchment in the greater Dublin area. Maximum DIN concentrations are found during winter (28.43 µM in February 2017). In the case of SRP, the highest concentrations are measured during summer (1.98 µM in June 2017). The Argideen estuary is dominated by an agricultural landscape with diffuse nutrient inputs. The highest DIN concentrations are observed during winter (18.13 µM in February 2017). The SRP do not follow a clear seasonal pattern (Table 1) in this estuary, ranging from 0.20 µM (April 2017) to 0.71 (August 2017). The Tolka was classified as eutrophic and the Argideen as potentially eutrophic in a recent assessment of water quality (O'Boyle and Tierney, 2018).

Both estuaries were sampled within a maximum period of one week on each sampling occasion, so as to avoid temporal effects that may confound any spatial variation. For logistical reasons, the first sampling occasion was performed within a period of three weeks. On each sampling occasion, a hierarchical design was applied to identify relevant scales of spatial variation, both parallel and perpendicular to the coast (Fig. 1 a and b). The estuaries were divided into two sections, one close to the open sea ("outer section") and another one kilometre upstream towards the freshwater-tidal limit ("inner section"). In each section, two sampling sites separated by one hundred meters were selected. Two random transects perpendicular to the main channel and separated by 10 meters were sampled per site. The only exception was the first sampling occasion in Tolka, where only one transect per site was collected in the inner section of the estuary. Along each transect, three sampling station were positioned in the upper, middle and lower part of the intertidal covered by the bloom during their maximum extension, which usually occurs in June or July in cold-temperate North Eastern Atlantic estuaries (e.g. Jeffrey et al., 1995; Malta and Verschuure, 1997; Lyngby et al., 1999; Scanlan et al., 2007). The sampling stations were pre-determined using Google Earth images of blooms from previous years. The pre-defined sampling stations were located in the field using a Geographical Position System (GPS; Magellan Triton 400). At each sampling station, three quadrats (25 x 25 cm) were used to assess the abundance of seaweed. All living material present in each quadrat was collected, placed in a labelled plastic bag and transported to the laboratory.

2.2. Sample processing

Seaweed biomass was rinsed with freshwater to remove adherent sedimentary and particulate material, debris and other organisms. Laminar and tubular morphologies of *Ulva* were sorted, and their mass was recorded after the removal of the excess water using a manually operated low-speed centrifuge (i.e. salad spinner). Subsequently, biomass was standardised to gram per square meter, by multiplying the obtained weight in the 25 x 25 cm quadrat by 16. Finally, a subsample of morphologically representative specimens of *Ulva* was collected for further microscopic and molecular identification.

2.3. Taxonomic identification

Based on macroscopic and microscopic traits, representative specimens of *Ulva* were collected on five sampling occasions (June16; August16; October16; April 17; and June 17). A part of each specimen was then stored in dry silica gel for genetic analysis, and for specimens of sufficient size, the remainder was prepared as a herbarium voucher.

A plastid-encoded marker was employed to genetically identify the samples, the large subunit of the Ribulose Bisphosphate Carboxylase-Oxygenase (RuBisCO) (*rbc*L). Whole genomic DNA was extracted from ~5-20 mg of preserved *Ulva* tissue using a commercial kit [NucleoSpin® Plant II, Macherey-Nagel, Düren, Germany]. The *rbc*L gene region was amplified in polymerase chain reactions (PCRs), using primers SHF1 and SHR4 at an annealing temperature of 50ºC (Heesch et al., 2009). Protocols for PCR amplification, purification of the products and sequencing are given in Heesch et al. (2016).

Representative sequences from both estuaries were included in an alignment containing 62 sequences in total: 35 published and 19 newly generated in this study of the genus *Ulva*, and an outgroup comprised of eight sequences, six of the related genus *Umbraulva* and one each of the genera *Gemina* and *Ulvaria* (see Fig. 2 for GenBank accession numbers and references). Sequence treatment (quality control and alignment) as well as data analysis under the Maximum Likelihood (ML) criterion followed methods published in Heesch et al. (2016). The taxonomical algal nomenclature followed AlgaeBase (Guiry et al., 2014).

2.4. Statistical analyses

2.4.1. Spatial and temporal patterns of variation

Statistical analyses were performed using the Vegan package (Oksanen et al., 2012) for R and PERMANOVA+ add-on PRIMER 6 (Plymouth Routines in Multivariate Ecological Research) software. In all statistical analyses significance was set at $p < 0.05$ probability, and when necessary were based on 1999 permutations. When comparisons with previous studies were made, dry weights were transformed to fresh weights assuming a 0.12 fresh:dry biomass ratio following Merceron et al. (2004).

In order to identify relevant spatial and temporal scales of distribution in the morphological composition of the green tides, a multivariate six-way permutational analysis of variance (PERMANOVA; Anderson et al., 2008) was performed based on the Euclidean distances between quadrats considering the standardized biomass of both tubular and laminar morphologies. The six factors considered (three fixed and three random) were: Sampling occasion (fixed; seven levels: "June 16", "August 16", "October 16", "February 17", "April 17", "June 17", and "August 17"), Position in the bloom (fixed; three levels: "upper", "middle" and "lower"), Estuary (fixed; two levels: "Tolka" and "Argideen"), Section (random; two levels nested in Estuary: "Inner" and "Outer"), Site (random; two levels nested in the interaction between Section and Sampling occasion), and Sampling station (random; two levels nested in the interaction between Site and Position). In case of significant effects for a factor, a distance-based test for homogeneity of multivariate dispersion (PERMDISP; Anderson et al., 2008) and a

pairwise PERMANOVA test (Anderson et al., 2008) were performed in order to interpret patterns.

Furthermore, to identify the most relevant scales of variation in biomass for each of the morphologies in the two estuaries, two univariate PERMANOVA analyses based on Euclidean distances between quadrats were performed following the six-way model previously described.

2.4.2. Correlations between biomass and environmental variables

In order to identify the factors that better explain bloom development, Spearman's correlations among the biomass of both tubular and laminar morphologies, and environmental variables were measured. To perform these correlations, the median biomass of both tubular and laminar morphologies for each estuary and sampling occasion were considered. Daily meteorological information (i.e. rainfall, solar radiation, maximum air temperature and minimum air temperature) was obtained from the Irish meteorological service (Met Éireann; http://www.met.ie/), or provided by the Environmental Protection Agency from *in-situ* measurements (i.e. Salinity, DIN and SRP). In the case of the Tolka estuary all the climatological information was obtained from the meteorological station at Dublin Airport, located less than 10 km from this estuary. In the case of the Argideen estuary, rainfall data were taken from the closest pluviometric station (i.e. Ballinspittle, ~11km away). The data for solar radiation, maximum and minimum air temperature were linearly interpolated considering the distance from the sampling site to the two closest meteorological stations: Sherkin Island (52 kilometres) and Roche's Point (38 kilometres). In the case of water physico-chemical attributes, only six of the seven sampling occasions were considered, since data from June 2016 were missed.

3. Results

3.1. Species composition

Seventy-eight *rbc*L sequences were obtained from *Ulva* specimens collected from the Tolka (42) and the Argideen (36) estuaries, which were between 569 and 1050 bases long. Sequence comparisons showed that the two blooms studied contained at least seven *Ulva* species (Fig. 2). Four species were found in the Tolka and seven in the Argideen: four were common to both estuaries (*Ulva compressa* L.; *Ulva procera* (K. Ahlner) Hayden et al.; *Ulva prolifera* O.F. Muller; and *Ulva rigida* C. Agardh), and three occurred exclusively in the Argideen estuary (*Ulva intestinalis* Linnaeus; *Ulva gigantea* (Kützing) Bliding; and *Ulva lactuca* L.). The most frequently encountered species were *U. prolifera* (33 sequences; 32 tubular and one laminar specimens), *U. compressa* (13 sequences; 13 tubular and no laminar specimens) and *U. rigida* (13 sequences; one tubular and 12 laminar specimens), while the remaining species identified were less common. These three species were present on all of the sampling occasions assessed.

Overall, *U. rigida* exhibited laminar morphology, while *U. prolifera* and *U. compressa* were tubular.

Other species such as *Chaetomorpha linum* (O.F.Müller) Kützing, *Chaetomorpha ligustica* (Kützing) Kützing, *Gracilaria vermiculophylla* (Ohmi) Papenfuss or different Ectocarpales were also encountered in the blooms, identified based on morphological traits. Specimens of *Chaetomorpha* spp. were usually present in samples, reaching measurable biomass densities in October 2016 in both estuaries.

3.2. Spatial and temporal patterns of variation

The PERMANOVA analysis regarding the morphological composition of *Ulva* blooms from the Argideen and Tolka estuaries revealed significant differences between the Estuaries, Sampling occasions and Positions (Table 2a). Most of the significant interactions included Sampling occasion (i.e. Estuary x Sampling occasion, Sampling occasion x Section), which indicated that the distribution and morphological composition of *Ulva* blooms showed an important seasonal dynamic, with a change in maximum biomass from one section of the estuary to another (Fig. S1).

As evident from Figure 3, Sampling occasion appears to be the factor that best explained this variance, while differences between Positions were less obvious. The results suggested a temporal shift from tubular to laminar morphologies throughout the bloom season, which was common to both estuaries. In February, when no bloom occurred, the biomass for both morphologies was measured as lower than 0.1 g/m^2. Subsequently, in April, tubular *Ulva* exhibited a low biomass, while laminar *Ulva* was either absent or present in only negligible amounts - most of the variability occurred on the "x" axis representing the biomass of the tubular *Ulva* (Fig. 3). The peak of biomass for tubular *Ulva* occurs in June, with some variability evident on the "y" axis during this month as a consequence of the presence of laminar *Ulva* in measurable biomasses. In the Tolka Estuary from August to October the point cloud is moved from the bottom-right part of the "xy" coordinate system to the upper-left, showing a decrease in the biomass of tubular *Ulva* and an increase in the laminar form. In the Argideen Estuary, an increase in laminar *Ulva* from June to August was observed, with a subsequent decrease from August to October, while no biomass was present in February.

In the case of the position in the bloom, the pairwise PERMANOVA found significant differences between Middle and Upper positions in both estuaries (Table 2b), existing also significant differences between middle and lower positions in the Tolka. In the Argideen, the Upper position showed the lowest data dispersion (PERMDISP test and pairwise comparisons; p-values <0.01) while in the Tolka, no differences in data dispersion were found among the different positions (PERMDISP, p-values >0.05), although a similar trend in data dispersion was observed with minimum dispersion in the Upper position. The lower dispersion at the Upper positions of the green tide is a consequence of lesser biomass of both morphologies at this

shore height, where maximum biomass was lower than that observed at the Middle and Lower shore positions. These differences were more obvious in the Argideen than in the Tolka (Fig. 3).

On the other hand, the significant differences observed between sampling stations, and the significant interaction between Position and Site, revealed a noticeable variability in the morphological composition and biomass distribution at spatial scales of meters or tens of meters. In general terms, the biomass of tubular *Ulva* was higher than the laminar form, even when the latter reaches peak biomass in late summer or early autumn.

3.2.1. Biomass of the tubular morphology

The PERMANOVA results for the biomass of tubular *Ulva* revealed significant differences between estuaries, sampling occasions and positions (Table 3). Significant interactions were observed between sampling occasion and most of the spatial scales of variation assessed for temporal interactions (i.e. Estuary x Sampling occasion, Sampling occasion x Section). The annual peaks of biomass were detected in June for both estuaries, with significant differences between June of 2016 and June of 2017 for both estuaries (Fig. 4). In June 2016 the median values of biomass of tubular *Ulva* were 1193.6 g m^{-2} and 3345.6 g m^{-2} for the Argideen and the Tolka respectively. In June 2017 the median values of biomass of tubular *Ulva* were 676.8 g m^{-2} for the Argideen and 1558 g m^{-2} for the Tolka. The maximum values for biomass were measured in June 2016 for both estuaries, reaching 12398.4 g m^{-2} in the Tolka estuary, and 7140.8 g m^{-2} in the Argideen. Overall, the Tolka has a greater biomass of *Ulva* than the Argideen, with the exception of April 2017 when the opposite trend was observed. In relation to shore position of the green tide, significant differences occurred for this morphology between the upper and middle positions. Overall, a higher biomass was found on the middle shore as opposed to the upper reaches of the bloom.

At smaller spatial scales of variation, significant differences were observed between Sites (Table 3). Moreover, a significant interaction between Site and Position was identified. No relevant scales of variation were found at scales of variation smaller than one hundred meters (i.e. Sampling Stations).

3.2.2. Biomass of the laminar morphology

The PERMANOVA results regarding biomass of laminar *Ulva* indicated significant differences between Sampling occasions, Sections and Sampling stations. Significant interactions between Sampling occasion and Estuary, and Sampling occasion and Section, and Position and Site were also identified (Table 3).

The annual peaks of biomass for the laminar morphology were observed during August in the Argideen, and during October in the Tolka (Fig. 5), with median values of 180.0 g m^{-2} and 322.4 g m^{-2} respectively. No significant differences in the biomass abundance during the peak bloom were found between both estuaries. The maximum values of biomass abundance were

measured in the Tolka during October 2016 (1652.8 g m^{-2}) and in the Argideen in August 2017 (2369.6 g m^{-2}). During February and April the biomass of laminar *Ulva* was negligible for both estuaries.

On the other hand, the differences between Sections varied through the bloom season. Before and after the peak bloom of laminar *Ulva*, no differences in biomass densities between Sections (Tolka: June 16, June 17, and August 17; Argideen: June 16 and June 17), or higher levels of biomass in the outer part of the estuary (Tolka: August 17; Argideen: October 16) were observed (Fig. S1). During peak bloom conditions the biomass was significantly higher in the inner section in both estuaries.

3.3 Correlations between biomass and environmental variables

Significant and direct Spearman correlations were found between maximum (rho = 0.61) and minimum (rho =0.54) air temperature, and the biomass of laminar morphologies of *Ulva* (Table 4). In the case, of the tubular *Ulva*, significant and direct correlations were observed with maximum air temperature (rho = 0.84), and solar radiation (rho = 0.70). Significant and indirect correlation was observed between the biomass of tubular *Ulva* and DIN (rho = - 0.60), suggesting an important bio-filtration potential of the green tide.

4. Discussion

This study indicated that tubular morphologies of *Ulva* dominated two of the largest macroalgal blooms in Ireland. Contrary to what was previously thought (Wan et al., 2017), the results revealed that the blooms in both these estuaries were multi-specific. Our observations revealed the presence of at least three species of *Ulva* (*U. prolifera, U. compressa* and *U.rigida*) and members of the genus *Chaetomorpha* in noticeable biomasses at certain times of the year. A significant spatial and temporal variability of *Ulva* biomass was observed. Regarding temporal variability, a seasonal succession was identified, with the bloom dominated by tubular species during spring and early summer, and co-dominated by tubular and laminar morphologies during late summer and autumn. Although long-term changes in species composition of macroalgal blooms (Lavery et al., 1991), or differences in spatial distribution between different species has been previously reported (Hernandez et al., 1997; Nelson et al., 2003; Guidone and Thornber, 2013), this seasonal pattern that is common to both estuaries and both years has not been previously described in other European estuaries - although in California a relatively similar temporal succession was observed between *U. intestinalis* and *U. expansa* (Fong et al., 1996). In addition, the spatial variability of tubular and laminar morphologies exhibited different distribution patterns suggesting that the mechanism determining the distribution of laminar and tubular morphologies were different. Considering that tubular morphologies were usually anchored in the sedimentary substrate due to the burial of their basal part (Schories and Reise,

1993; this study), while laminar *Ulva* were usually observed free-floating or tangled in the tubular canopy, these differences could explain a differential influence by wind and wave action.

4.1. Species composition

Similar to other recent studies concerning the species composition of problematic macroalgal blooms (e.g. Nelson et al., 2003; Guidone and Thornber, 2013), our results revealed that the green tides contained multiple species of *Ulva* (Fig. 2), suggesting *U. prolifera*, *U. compressa* and *U. rigida* as the most frequent and abundant entities in both estuaries. Wan et al. (2017) stated that *Ulva* blooms in seven estuaries in Ireland (from one sampling occasion, including the Tolka and the Argideen) were primarily comprised of *U. rigida*. Although *U. rigida* appears to be the dominant laminar species of *Ulva* in Irish green tides, reaching important biomass densities in late summer and autumn (33% and 24% of total biomass during August and October in the Argideen and Tolka, respectively), the results obtained in the present study showed that the tubular *U. prolifera* and *U. compressa* are more abundant and comprise the main species forming green tides in the Argideen and Tolka Estuaries. This finding is in accordance with reports by Jeffrey et al. (1995), who identified two tubular species, *U. prolifera* and *U. flexuosa,* as the main components of the macroalgal blooms occurring in the Tolka Estuary between June 1989 and September 1990. The differences between the present study and the results obtained by Wan et al. (2017) were due to a sampling bias as that study only collected laminar specimens of *Ulva* for molecular identification on a single sampling occasion.

4.2. Temporal variability

Green tides in both estuaries (Figs. 4 and 5; Jeffrey et al., 1995) started in late March or early April, and persisted until the end of November or early December, showing a slight mismatch in the occurrence of blooms. Algal growth in the southern site (i.e. Argideen) appear to start, peak and finish before the bloom on the east coast (i.e. Tolka), as can be observed in Figures 4 and 5 for both *Ulva* morphologies, suggesting a key role of temperature and light as triggers of bloom development (e.g. Malta and Verschuure, 1997; Gao et al., 2016; Wang et al., 2018). These results also revealed a mismatch between the peaks of biomass of tubular and of laminar morphologies, indicating a temporal succession between species that was common to both estuaries. The studied *Ulva* blooms were dominated by tubular morphologies (i.e. *U. prolifera* and *U. compressa*) in early summer, and co-dominated by laminar (i.e. *U. rigida*) and tubular morphologies (i.e. *U. prolifera* and *U. compressa*) in late summer or early autumn. Additionally, during late summer and autumn, measurable biomasses of *Chaetomorpha* spp. were observed (pers. obs.). The presence of different species of *Ulva* in Irish green tides and the observed sequence of occurrence suggest that the duration and extension of macroalgal blooms in Ireland could be stimulated through spatial and temporal successions between species with different ecological requirements if the biotic interactions between them are weak

or negligible (i.e. diversity-stability hypothesis (McCann, 2000)). This hypothesis is also supported by the occurrence of macroalgal tides as a consequence of the arrival of alien species in estuaries previously unaffected by such phenomena (Yabe et al., 2009; Yoshida et al., 2015), or by the changes in dominant species forming green tides due to alterations in environmental conditions (Lavery et al., 1991), highlighting the importance of knowledge about the species composition of seaweed blooms and their eco-physiological performance. Spearman correlations results (Table 4) suggested different eco-physiological responses of the laminar and tubular morphologies to environmental conditions. Temperature seem to play an important role in the development of the laminar *Ulva*, while the biomass of the tubular *Ulva* seems to be explained by temperature and solar radiation. Further studies will be necessary for a better understanding of the environmental factors controlling bloom development. The biomass of tubular morphologies and DIN concentration showed a significant and indirect correlation (rho = -0.60), supporting the idea that these macroalgal blooms and other estuarine primary producers (e.g. phytoplankton, microbenthos, epiphytes, salt marshes plants, seagrasses) could accumulate dissolved inorganic nutrients in such quantities that can reduce nutrient concentration in surrounding seawaters at medium scales (Viaroli *et al.*, 1996; Valiela *et al.*, 1997).

4.3. Spatial variability

Our findings support the existence of an important spatial variability in the studied blooms, suggesting a patchy distribution similar to those observed in other macroalgal blooms dominated by Ulvoids (e.g. Malta and Verschuure, 1997; Nelson et al., 2003; Thornber et al., 2017). Marked differences in spatial variability were found between laminar and tubular morphologies (Table 2), suggesting important differences in the main factors determining biomass distribution patterns for both morphologies. Interestingly, while tubular morphologies were usually buried with their basal parts and thus anchored to the sediment (Schories and Reise, 1993; this study), laminar *Ulva* usually occurred free-floating or tangled in the tubular canopy. As a consequence, it is expected that wind and wave action are more likely to influence the transport or movement laminar *Ulva* biomass. In contrast, the tubular species anchored to the substrate may be less affected by these factors and more susceptible to the effects of local environmental conditions on their biological performance. Considering that the studied green tides were thriving in the sedimentary tidal flats of two different estuaries, where the habitat and environmental heterogeneity was relatively low at small spatial scales, and an important biomass transport to the open sea is expected due to tidal currents, the observed patterns partially support this hypothesis. In this case, the biomass of tubular morphologies varies along bigger spatial scales (i.e. Sites; 100 meters) than the laminar ones (i.e. Sampling Stations; 10 meters), and this morphology was more abundant than the laminar. Our results showed significant differences between estuaries and the position of the green tide for tubular

morphologies. With respect to laminar morphologies, significant differences were observed between sampling stations and sections, but no significant differences were observed between shore positions or between estuaries. This suggests that the spatial patterns of tubular morphologies may be determined by factors affecting the biological performance of the species such as climatic conditions, desiccation, light and nutrient availability (i.e. factors generating spatial patterns at scales of estuaries or between positions in the green tide). On the other hand, the spatial distribution of laminar morphologies might be determined by hydrodynamic conditions as well as interactions with the substrate morphology and other canopy forming species, rather than local conditions maximising the biological performance of the species. In this sense, the anchorage of tubular morphologies to the substrate could favour this morphological form developing into green tides in areas where the biomass transport due to tidal currents favours the export of biomass. Moreover, the burial of the basal part of the tubular morphology could provide access to nutrients from porewaters, which would not be available to the laminar morphologies (Robertson and Savage, 2018).

4.4. Annual and decadal variability in biomass density

Our results suggest important between-years differences in the levels of biomass present. These were especially relevant between June 2016 (3912.9±2473.8 g m^{-2} and 1193.6±1665.3 g m^{-2} for the Tolka -n=54- and the Argideen -n=72- respectively) and June 2017 (1851.5±1208.1 g m^{-2} and 889.3±846.4 g m^{-2} for the Tolka and the Argideen respectively; n=72), when these blooms seemed to reach maximum biomass. In both cases, higher biomasses were observed in June 2016 than in June 2017, which could be related to climate factors. It is worth noting that from January to May the total rainfall in meteorological stations close to both estuaries was noticeably lower in 2017 than in 2016 (www.met.ie), which may have resulted in suboptimal conditions during the first stages of bloom development in 2017 (i.e. lower nutrient input and higher salinity). Significant differences in between-year-variation in macroalgal biomass have been observed in other green tides (Malta and Verschuure, 1997; Lyngby et al., 1999), leading to suggestions of a critical role of environmental conditions during the early bloom development which subsequently determine the total biomass accumulated at peak bloom (Malta and Verschuure, 1997; Gao et al., 2018). When the mean values of biomass observed in the Tolka estuary in 2016 and 2017 during the peak of biomass were compared with those obtained by Jeffrey et al. (1995) in 1989 (666-750 g m^2 of fresh weight considering a 0.12 ratio dry:fresh weight) and 1990 (790-885 g m^{-2}), a noticeable increase in biomass was observed.

4.5. Relevance for environmental management

Maximum values of biomass in the Tolka and Argideen were lower than reported in the 1980's and 1990's for the Venice lagoon and the French coast of Brittany, but similar to other European estuaries (Table 5). Although the effect of seaweed tides are very much dependent

on local environmental conditions, different studies suggest that *Ulva* biomass concentrations higher than 1000 g FW m^{-2} have significant harmful effects on associated biota (Lowthion et al., 1985; Hull, 1987; Wither, 2003). In the context of the European Water Framework Directive (WFD), Scanlan et al. (2007) therefore proposed biomass levels as one of the sub metrics to assess the Ecological Status in transitional water bodies, with 1 kg of wet weight biomass per square meter during the peak bloom being the threshold between a Good and Moderate Ecological Status. In the present study the mean values of biomass exceeded this threshold by five times in the Tolka estuary (June 2016, August 2016, October 2016, June 2017 and August 2017) and three times in the Argideen (June 2016, August 2016, August 2017) (Fig. 6). This becomes particularly important considering legal implications when a good Ecological Status is not reached (European Commission 2000), as it necessitates the development of management actions aimed at reducing the total seaweed biomass in order to reach a good ecological status complying with obligations under the requirements of the WFD. The different habitats of tubular and laminar morphologies of *Ulva* (anchored vs. free-floating) may lead to important differences in the spatial integration of environmental conditions or exposure to pollutants that need to be considered for the design of monitoring programs using *Ulva* species as bioindicators of environmental quality and ecological status. Furthermore, as the tubular morphologies might be more susceptible to biomass burial rather than exportation to sea, this could play an important role in the balance of nutrients in the estuary, favouring accumulation in pore-waters and sediments (Corzo et al., 2009). This accumulation of nutrients in sediments could act a reservoir for further blooms, limiting the impact of management actions such as the reduction in nutrient loadings.

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REFERENCES

Aldridge, J.N., Trimmer, M., 2009. Modelling the distribution and growth of “problem” green seaweed in the Medway estuary, UK. Hydrobiologia 629, 107–122.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK.

Benedetti-Cecchi, L., Rindi, F., Bertocci, I., Bulleri, F., Cinelli, F., 2001. Spatial Variation in Development of Epibenthic Assemblages in a Coastal Lagoon. Estuar. Coast. Shelf Sci. 52, 659–668.

Bermejo, R., Ramírez-Romero, E., Vergara, J.J., Hernández, I., 2015. Spatial patterns of

1 macrophyte composition and landscape along the rocky shores of northern coasts of the
2 Alboran Sea. Estuar. Coast. Shelf Sci. 155, 17–28.
3 Bonsdorff, E., 1992. Drifting algae and zoobenthos - effects on settling and community
4 structure. J. Sea Res. 62, 57–62.
5 Carvalho, S., Pereira, P., Pereira, F., de Pablo, H., Vale, C., Gaspar, M.B., 2011. Factors
6 structuring temporal and spatial dynamics of macrobenthic communities in a eutrophic
7 coastal lagoon (Óbidos lagoon, Portugal). Mar. Environ. Res. 71, 97–110.
8 Corzo, A., Van Bergeijk, S.A., García-Robledo, E., 2009. Effects of green macroalgal blooms on
9 intertidal sediments: net metabolism and carbon and nitrogen contents. Mar. Ecol. Prog.
10 Ser. 380, 81–93.
11 Fong, P., Boyer, K.E., Desmond, J.S., Zelder, J.B., 1996. Salinity stress, nitrogen competition
12 and facilitation: what contrals seasonal succession of 2 opportunistic green macroalgae?
13 J. Exp. Mar. Bio. Ecol. 206, 203–221.
14 Gao, G., Beardall, J., Bao, M., Wang, C., Ren, W., Xu, J., 2018. Ocean acidification and nutrient
15 limitation synergistically reduce growth and photosynthetic performances of a green tide
16 alga *Ulva linza*. Biogeosciences Discuss. 86, 1–43.
17 Gao, G., Clare, A.S., Rose, C., Caldwell, G.S., 2016. Eutrophication and warming-driven green
18 tides (*Ulva rigida*) are predicted to increase under future climate change scenarios. Mar.
19 Pollut. Bull. 114, 439–447.
20 Guidone, M., Thornber, C.S., 2013. Examination of *Ulva* bloom species richness and relative
21 abundance reveals two cryptically co-occurring bloom species in Narragansett Bay, Rhode
22 Island. Harmful Algae 24, 1–9.
23 Guiry, M.D., Guiry, G.M., Morrison, L., Miranda, S.V., Mathieson, A.C., Parker, B.C.,
24 Langangen, A., John, D.M., Bárbara, I., Carter, C.F., Garbary, D.J., 2014. AlgaeBase: an
25 on-line resource for Algae. Cryptogam. Algol. 35, 105–115.
26 Hartnett, M., Wilson, J.G., Nash, S., 2011. Irish estuaries: Water quality status and monitoring
27 implications under the water framework directive. Mar. Policy 35, 810–818.
28 Hayden, H.S., Blomster, J., Maggs, C.A., Silva, P.C., Stanhope, M.J., Waaland, J.R., 2003.
29 Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. Eur. J.
30 Phycol. 38, 277–294.
31 Hayden, H.S., Waaland, J.R., 2002. Phylogenetic systematics of the Ulvaceae (Ulvales,
32 Ulvophyceae) using chloroplast and nuclear sequences. J. Phycol. 38, 1200–1212.
33 Hayden, H.S., Waaland, J.R., 2004. A molecular systematic study of *Ulva* (Ulvaceae, Ulvales)
34 from the northeast Pacific. Phycologia 43, 364–382.
35 Heesch, S., Broom, J.E.S., Neill, K.F., Farr, T.J., Dalen, J.L., Nelson, W.A., 2009. *Ulva,*
36 *Umbraulva* and *Gemina*: Genetic survey of New Zealand taxa reveals diversity and
37 introduced species. Eur. J. Phycol. 44, 143–154.
38 Heesch, S., Pažoutová, M., Moniz, M.B.J., Rindi, F., 2016. Prasiolales (Trebouxiophyceae,
39 Chlorophyta) of the Svalbard Archipelago: diversity, biogeography and description of the
40 new genera Prasionella and Prasionema. Eur. J. Phycol. 51, 171–187.

Hering, D., Borja, A., Carstensen, J., Carvalho, L., Elliott, M., Feld, C.K., Heiskanen, A.-S., Johnson, R.K., Moe, J., Pont, D., Solheim, A.L., de Bund, W. Van, 2010. The European Water Framework Directive at the age of 10: a critical review of the achievements with recommendations for the future. Sci. Total Environ. 408, 4007–4019.

Hernandez, I., Peralta, G., Perez-Llorens, J.L., Vergara, J.J., Niell, F.X., 1997. Biomass and growth dynamics of *Ulva* species in Palmones River estuary. J. Phycol. 33, 764–772.

Hiraoka, M., Shimada, S., Uenosono, M., Masuda, M., 2003. A new green-tide-forming alga, *Ulva ohnoi* Hiraoka et Shimada sp. nov. (Ulvales, Ulvophyceae) from Japan. Phycol. Res. 51, 17–29.

Jeffrey, D.W., Brennan, M.T., Jennings, E., Madden, B., Wilson, J.G., 1995. Nutrient sources for in-shore nuisance macroalgae: The dublin bay case. Ophelia 42, 147–161.

Lanari, M., Copertino, M., 2017. Drift macroalgae in the Patos Lagoon Estuary (southern Brazil): effects of climate, hydrology and wind action on the onset and magnitude of blooms. Mar. Biol. Res. 13, 36-47.

Lavery, P.S., Lukatelich, R.J., McComb, A.J., 1991. Changes in the biomass and species composition of macroalgae in a eutrophic estuary. Estuar. Coast. Shelf Sci. 33, 1–22.

Lotze, H.K., Worm, B., Sommer, U., 2000. Propagule banks, herbivory and nutrient supply control population development and dominance patterns in macroalgal blooms. Oikos 89, 46–58.

Loughnane, C.J.C., McIvor, L.L.M., Rindi, F., Stengel, D.B., Guiry, M.D., 2008. Morphology, *rbc*L phylogeny and distribution of distromatic *Ulva* (Ulvophyceae, Chlorophyta) in Ireland and southern Britain. Phycologia 47, 416–429.

Lyngby, J.E., Mortensen, S., Ahrensberg, N., 1999. Bioassessment techniques for monitoring of eutrophication and nutrient limitation in coastal ecosystems. Mar. Pollut. Bull. 39, 212–223.

Malta, E.-J., Draisma, S.G.A., Kamermans, P., 1999. Free-floating *Ulva* in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison. Eur. J. Phycol. 34, 443–454.

Malta, E.-J., Verschuure, J.M., 1997. Effects of environmental variables on between-year variation of *Ulva* growth and biomass in a euthropic brackish lake. J. Sea Res. 38, 71–84.

McCann, K.S., 2000. The diversity–stability debate. Nature 405, 228–233.

Nelson, T.A., Haberlin, K., Nelson, A. V., Ribarich, H., Hotchkiss, R., Alstyne, K.L. Van, Buckingham, L., Simunds, D.J., Fredrickson, K., 2008. Ecological and physiological controls of species composition in green macroalgal blooms. Ecology 89, 1287–1298.

Nelson, T.A., Nelson, A. V, Tjoelker, M., 2003. Seasonal and Spatial Patterns of “Green Tides” (Ulvoid Algal Blooms) and Related Water Quality Parameters in the Coastal Waters of Washington State, USA 1. Bot. Mar. 46, 263–275.

Ní Longphuirt, S., Boyle, S.O., Wilkes, R., Dabrowski, T., Stengel, D.B., 2016. Influence of Hydrological Regime in Determining the Response of Macroalgal Blooms to Nutrient Loading in Two Irish Estuaries. Estuaries and Coasts. 39, 478–494

Nixon, S.W., Oviatt, C.A., Frithsen, J., Sullivan, B., 1986. Nutrients and the productivity of

estuarine and coastal marine ecosystems. J. Limnol. Soc. South. Africa 12, 43–71.
O'Boyle, S., Tierney, D., 2018. WATER QUALITY IN 2016: An Indicators Report. Environmental Protection Agency. Wexford.
Oksanen, J., Guillaume-Blanchet, F., Roeland-Kindt, P., Legendre, P., Minchin, R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2012. Package "Vegan." R-project.
Park, S.R., Kang, Y.H., Lee, H.J., Ko, Y.W., Kim, J.H., 2014. The importance of substratum and elevation in recruitment and persistence of ulvoid algal blooms on rocky intertidal shores of the southern Korean coast. Bot. Mar. 57, 55–66.
Robertson, B.P., Savage, C., 2018. Mud-entrained macroalgae utilise porewater and overlying water column nutrients to grow in a eutrophic intertidal estuary. Biogeochemistry 139, 1–16.
Saunders, G.W., Kucera, H., 2010. An evaluation of *rbc*L, *tuf*A, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. Cryptogam. Algol. 31, 487–528.
Scanlan, C.M., Foden, J., Wells, E., Best, M.A., 2007. The monitoring of opportunistic macroalgal blooms for the water framework directive. Mar. Pollut. Bull. 55, 162–171.
Schories, D., Reise, K., 1993. Germination and anchorage of *Enteromorpha* spp. in sediments of the Wadden Sea. Helgoländer Meeresuntersuchungen 47, 275–285.
Sfriso, A., Facca, C., Ghetti, P.F., 2003. Temporal and spatial changes of macroalgae and phytoplankton in a Mediterranean coastal area: The Venice lagoon as a case study. Mar. Environ. Res. 56, 617–636.
Sherwood, A.R., Garbary, D.J., Sheath, R.G., 2000. Assessing the phylogenetic position of the Prasiolales (Chlorophyta) using *rbc*L and 18S rRNA gene sequence data. Phycologia 39, 139–146.
Shimada, S., Hiraoka, M., Nabata, S., Lima, M., Masuda, M., 2003. Molecular phylogenetic analyses of the Japanese *Ulva* and *Enteromorpha* (Ulvales, Ulvophyceae), with special reference to the free-floating *Ulva*. Phycol. Res. 51, 99–108.
Smetacek, V., Zingone, A., 2013. Green and golden seaweed tides on the rise. Nature 504, 84–88.
Spalding, H.L., Conklin, K.Y., Smith, C.M., Kelly, C.J.O., Sherwood, A.R., 2016. New Ulvaceae (Ulvophyceae, Chlorophyta) from mesophotic ecosystems across the Hawaiian Archipelago. J. Phycol. 53, 40–53.
Sverdrup, H. U. (1953) On Conditions for the Vernal Blooming of Phytoplankton. ICES J. 18, 287–295.
Teichberg, M., Fox, S.E., Olsen, Y.S., Valiela, I., Martinetto, P., Iribarne, O., Muto, E.Y., Petti, M.A. V, Corbisier, T.N., Soto-Jiménez, M., Páez-Osuna, F., Castro, P., Freitas, H., Zitelli, A., Cardinaletti, M., Tagliapietra, D., 2010. Eutrophication and macroalgal blooms in temperate and tropical coastal waters: Nutrient enrichment experiments with *Ulva* spp. Glob. Chang. Biol. 16, 2624–2637.
Terlizzi, A., Anderson, M.J., Fraschetti, S., Benedetti-Cecchi, L., 2007. Scales of spatial

variation in Mediterranean subtidal sessile assemblages at different depths. Mar. Ecol. Prog. Ser. 332, 25–39.

Thornber, C.S., Guidone, M., Deacutis, C., Green, L., Ramsay, C.N., Palmisciano, M., 2017. Spatial and temporal variability in macroalgal blooms in a eutrophied coastal estuary. Harmful Algae 68, 82–96.

Valiela, I., Mcclelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K., 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnol. Oceanogr. 42, 1105–1118.

Viaroli, P., Naldi, M., Bondavalli, C. and Bencivelli, S. (1996) Growth of the seaweed *Ulva rigida* C. Agardh in relation to biomass densities, internal nutrient pools and external nutrient supply in the Sacca di Goro lagoon (Northern Italy). Hydrobiologia, 329(1–3), 93–103.

Wan, A.H.L., Wilkes, R.J., Heesch, S., Bermejo, R., Johnson, M.P., Morrison, L., 2017. Assessment and characterisation of Ireland's green tides (*Ulva* species). PLoS One 12, e0169049.

Wang, S., Huo, Y., Zhang, J., Cui, J., Wang, Y., Yang, L., Zhou, Q., Lu, Y., Yu, K., He, P., 2018. Variations of dominant free-floating *Ulva* species in the source area for the world's largest macroalgal blooms, China: Differences of ecological tolerance. Harmful Algae 74, 58–66.

Wang, Z., Xiao, J., Fan, S., Li, Y., Liu, X., Liu, D., 2015. Who made the world's largest green tide in china?—an integrated study on the initiation and early development of the green tide in yellow sea. Limnol. Oceanogr. 60, 1105–1117.

Yabe, T., Ishii, Æ.Y., Amano, Æ.Y., Koga, T., Hayashi, Æ.S., Nohara, Æ.S., 2009. Green tide formed by free-floating *Ulva* spp . at Yatsu tidal flat , Japan. Limnology 10, 239–245.

Yoshida, G., Uchimura, M., Hiraoka, M., 2015. Persistent occurrence of floating *Ulva* green tide in Hiroshima Bay, Japan: seasonal succession and growth patterns of Ulva pertusa and Ulva spp. (Chlorophyta, Ulvales). Hydrobiologia 758, 223–233.

Fig. 1.- Geographical location of the two estuaries monitored in Ireland. Detailed map of the Tolka (A) and the Argideen (B) estuaries showing a schematic representation of the spatial sampling design.

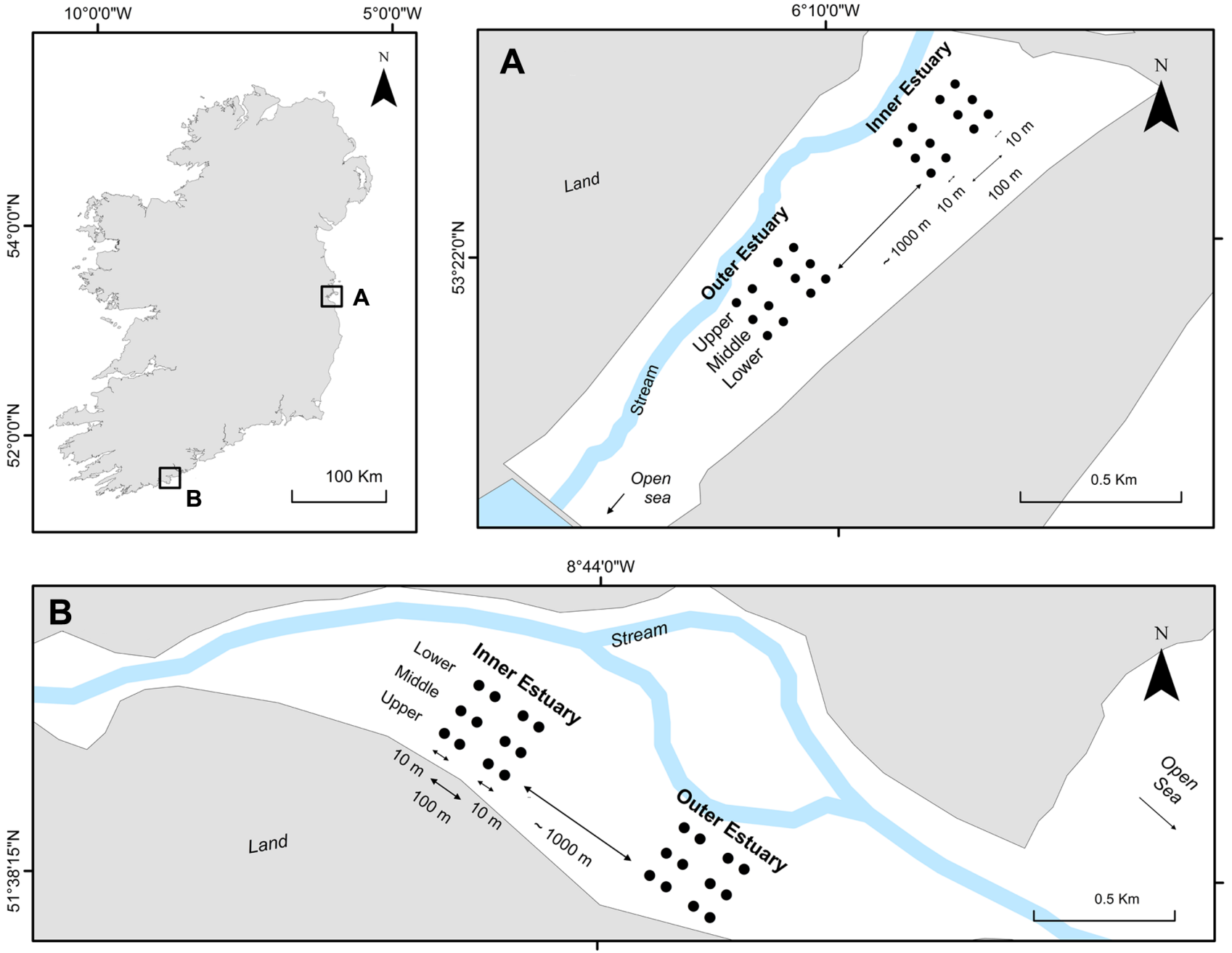
Fig. 2. Phylogenetic tree inferred by MaximumLikelihood analysis from partial rbcL sequences of Ulva species. Numbers above lines indicate bootstrap support values (branches without number received less than 60% support). GenBank/ENA accession numbers are followed by the species name (reflecting current nomenclature; Guiry & Guiry 2018), origin of the sample, and a number indicating the reference: 1- Hayden and Waaland (2002); 2- Hayden and Waaland (2004); 3- Hayden et al. (2003); 4- Heesch et al. (2009); 5- Hiraoka et al. (2003); 6- Loughnane et al. (2008); 7- Shimada et al. (2003); 8- Saunders and Kucera (2010); 9- Spalding et al. (2016); 10- Sherwood et al. (2000); 11- this study (sequences set in bold are from the studied estuaries).

Fig. 3.- Median values of biomass (wet wt.) (n=3) of laminar (y-axis) and tubular (x-axis) morphologies of *Ulva* for each sampling station in the Tolka and the Argideen over seven different sampling occasions. The dotted diagonal line represents a 1:1 biomass equivalence of laminar and tubular *Ulva*.

Fig. 4.- Median values of biomass (wet wt.) of tubular *Ulva* according to Estuary and Sampling occasion. Lower and Upper error bars represent the 25^{th} and 75^{th} percentile respectively; n = 72, except for Tolka June 2016 when n = 54. Letters over the bars represent significant differences.

Fig. 5.- Median values of biomass (wet wt.) of laminar *Ulva* according to Estuary and Sampling occasion. Lower and Upper error bars represent the 25^{th} and 75^{th} percentile respectively; n = 72, except for Tolka June 2016 when n = 54. Letters over the bars represent significant differences.

Fig. 6.- Biomass (wet wt.) of *Ulva* according to Estuary and Sampling occasion. Mean ± standard deviation; n = 72, except for Tolka June 2016 when n = 54. The red line represent the biomass level of 1 kg m^{-2} established as the threshold between good and moderate status in the context of the Water Framework Directive (Scanlan et al., 2007).


10°0'0"W
5°0'0"W
54°0'0"N
52°0'0"N
N
A
B
100 Km
6°10'0"W
53°22'0"N
Land
Inner Estuary
Outer Estuary
Upper
Middle
Lower
Stream
Open sea
10 m
100 m
~ 1000 m
0.5 Km
8°44'0"W
51°38'15"N
Open Sea

SRN 91 Ulva rigida Tolka Ireland[11]
SRN 90 Ulva rigida Tolka Ireland[11]
RBU 29 Ulva rigida Argideen Ireland[11]
EU484416 Ulva rigida Ireland[6]
AY255870 Ulva rigida UK[3]
EF110302 Ulva rigida New Zealand[4]
AY422564 Ulva rigida Chile[2]
AY422567 Ulva taeniata USA[2]
AB097636 Ulva spinulosa Japan[7]
AB097635 Ulva reticulata Philippines[7]
AY422565 Ulva fasciata Hawaii[2]
AY255871 Ulva sp. Chile[3]
EF109979 Ulva sp. 1 New Zealand[4]
AB116040 Ulva ohnoi Japan[5]
SBDN 267 Ulva clathrata Ireland[11]
AY422563 Ulva clathrata Spain[2]
SRN 101 Ulva gigantea Argideen Ireland[11]
EU484402 Ulva gigantea Ireland[6]
AY422562 Ulva procera Japan[2]
SRN 111 Ulva procera Argideen Ireland[11]
SRN 92 Ulva procera Tolka Ireland[11]
SRN 94 Ulva procera Tolka Ireland[11]
AY255861 Ulva linza USA[3]
SRN 93 Ulva prolifera Tolka Ireland[11]
SRN 106 Ulva prolifera Argideen Ireland[11]
AY255864 Ulva prolifera UK[3]
RBU 14 Ulva prolifera Tolka Ireland[11]
RBU 67 Ulva prolifera Tolka Ireland[11]
SRN 105 Ulva prolifera Argideen Ireland[11]
RBU 21 Ulva prolifera Argideen Ireland[11]
EU484415 Ulva californica Ireland[6]
AF189071 Ulva curvata USA[10]
AY255866 Ulva californica USA[3]
AB097619 Ulva flexuosa Japan[7]
AY255858 Chloropelta caespitosa Japan[3]
AY255874 Ulva stenophylla USA[3]
EF110466 Ulva sp. 9 New Zealand[4]
AY255869 Ulva compressa UK[3]
AY255859 Ulva compressaIreland[3]
SRN 107 Ulva compressa Argideen Ireland[11]
EU484397 Ulva compressaIreland[6]
RBU 22 Ulva compressa Argideen Ireland[11]
RBU 10 Ulva compressa Tolka Ireland[11]
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AY255860 Ulva intestinalis UK[3]
SRN 109 Ulva lactuca Argideen Ireland[11]
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AY255873 Ulva pertusa USA[3]
AY422549 Ulva pertusa Japan[2]
EU484406 Ulva rotundata Ireland[6]
EF109945 Ulva sp. 4 New Zealand[4]
AY255876 Umbraulva olivascens Ireland[3]
RBU 90 Umbraulva olivascens Coral Beach Ireland[11]
KT932987 Umbraulva kuaweuweu Hawaii[9]
KT932990 Umbraulva kaloakulau Hawaii[9]
AB097614 Umbraulva amamiensis Japan[7]
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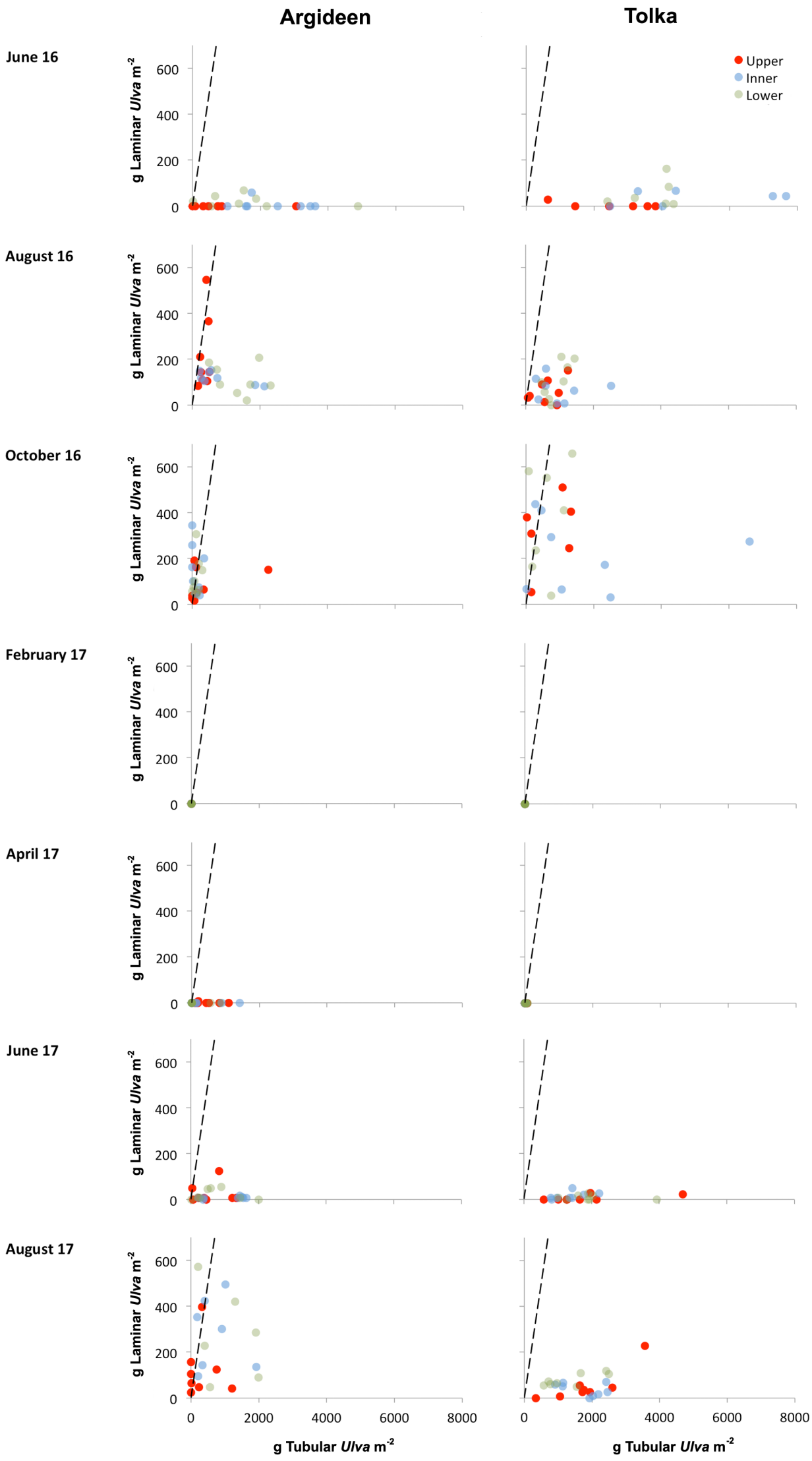
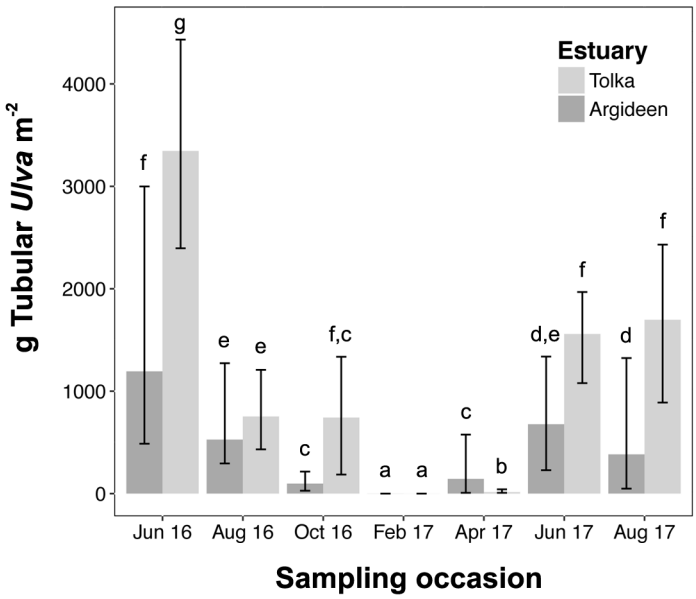

Argideen
Tolka
June 16
August 16
October 16
February 17
April 17
June 17
August 17
Upper
Inner
Lower
g Laminar Ulva m-2
g Tubular Ulva m-2
0
200
400
600
2000
4000
6000
8000


Estuary
Tolka
Argideen
g Tubular Ulva m-2
4000
3000
2000
1000
0
f
g
e
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c
f,c
a
a
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d,e
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Jun 16
Aug 16
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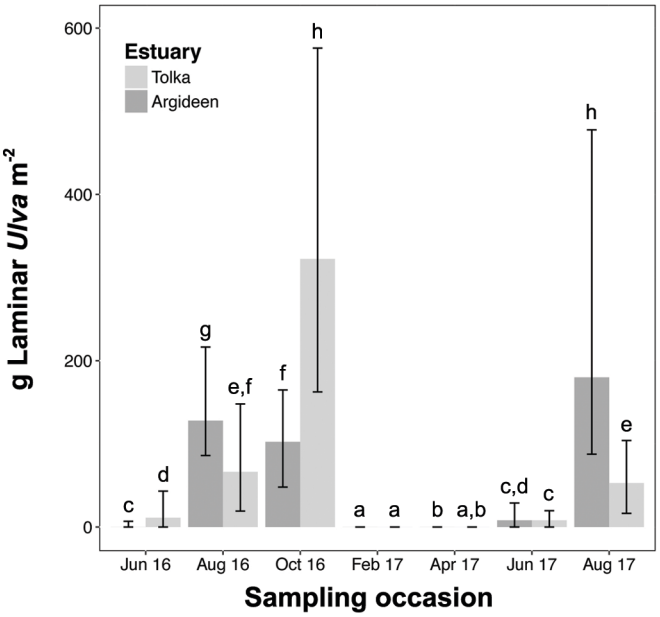
Jun 17
Aug 17
Sampling occasion


Estuary
Tolka
Argideen
g Laminar Ulva m-2
600
400
200
0
c
d
g
e,f
f
h
a
a
b
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c,d
c
h
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Jun 16
Aug 16
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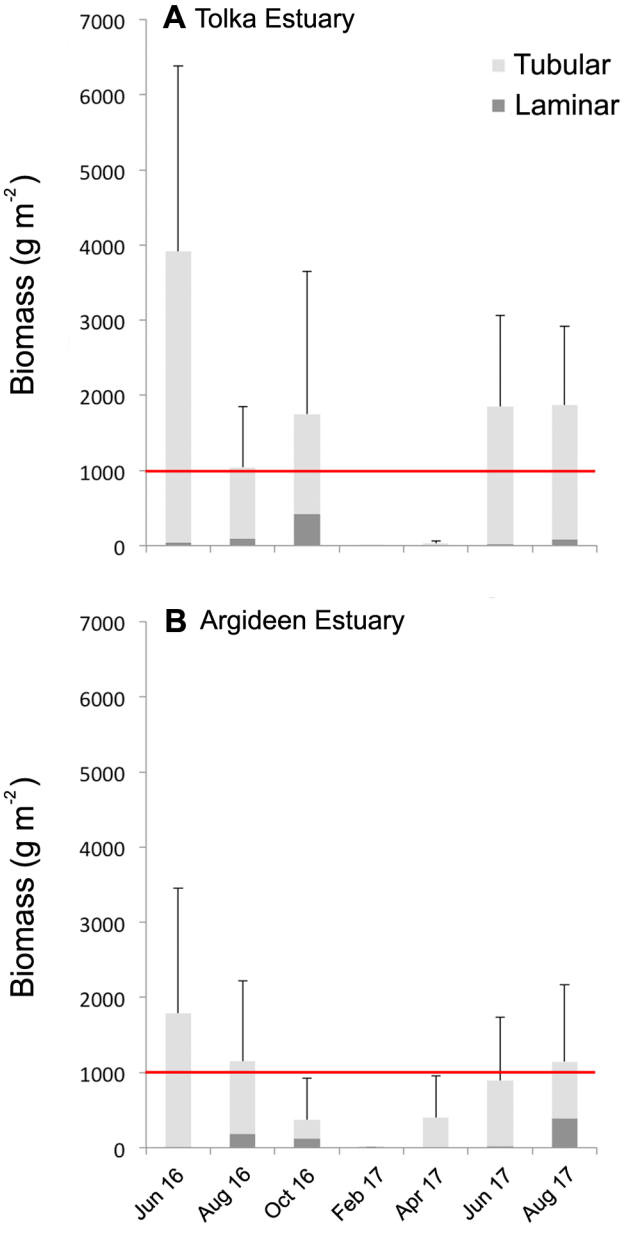
Jun 17
Aug 17
Sampling occasion


A Tolka Estuary
Tubular
Laminar
Biomass (g m-2)
7000
6000
5000
4000
3000
2000
1000
0
B Argideen Estuary
Biomass (g m-2)
7000
6000
5000
4000
3000
2000
1000
0
Jun 16
Aug 16
Oct 16
Feb 17
Apr 17
Jun 17
Aug 17