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Stable isotope analysis of Dublin Bay food web

Thesis submitted in fulfilment for the degree of Doctor of
Philosophy to the University of Dublin

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2017

School of Natural Sciences, Zoology



Trinity College Dublin

Coláiste na Tríonóide, Baile Átha Cliath

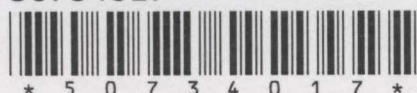
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SUMMARY

Coastal ecosystems are being increasingly impacted by human activities. Among the main threats, eutrophication is a major issue worldwide which can lead to major dysfunctions such as the excessive development of opportunistic macroalgae that affect benthic communities. Impacts of such macroalgal blooms on the food webs are however poorly understood. Dublin Bay has had a long history of organic pollution and eutrophication through urban sewage which induces, among other symptoms, severe green macroalgal blooms. The bay yet sustains valuable habitats and high ecological functions but its trophic functioning remains relatively unknown. This work characterises the benthic food web of Dublin Bay, identifying the basal sources supporting it and the major pathways of carbon transfer, and explores the impacts of macroalgal blooms and the influence of a changing regime of sewage particulates inputs on the food web functioning. For this, a carbon and nitrogen stable isotope analysis was applied seasonally (4 surveys), in combination with hydrobiological measurements of the particulate organic matter (POM), through an extensive field study of five sites featuring contrasted oceanic influence and exposition to the watershed.

The role of anthropogenic effluents in sustaining opportunistic blooms of green macroalgae (*Enteromorpha* spp.) occurring annually in Bull Island South Lagoon (BISL), a semi-enclosed mudflat directly submitted to the estuarine waters where sewage effluents are discharged, was demonstrated through the high $\delta^{15}\text{N}$ of the green algae at this affected site. Sewage had a moderate trophic role for benthic consumers through particulates and major impacts on the food web were indirect, via the macroalgal blooms it sustains (Chapter 3). The high biomass of *Enteromorpha* spp. affected the trophic interactions at different levels. It influenced species-specific diets of most consumers, from primary consumers (all feeding groups: suspension-feeders, deposit-feeders, grazers), including the meiofauna compartment, to omnivorous species, and was channeled to upper trophic levels (resident fish) of the food web, via the deposit-feeder guild mainly. This macroalgal biomass appeared to be incorporated in the local food web mainly through the detrital pathway during the decomposition and burial of the mats, as revealed by the $\delta^{15}\text{N}$ shift of the consumers' tissues recorded at the end of autumn. It was particularly assimilated by deposit-feeders, which derived up to 66% of their food from it. This study also revealed a simplification of the food web structure with a main trophic pathway, based on detrital *Enteromorpha* spp., emerging following the collapse of the

macroalgal mat and ill in sediments. This was reflected in a narrowed base of the food web and $\delta^{15}\text{N}$ shifts at different trophic levels, and depicted through dietary mixing models of consumers. These findings bring new insights on the response of food webs to macroalgal blooms in sheltered tidal systems.

Sewage was found to affect (directly or indirectly) the intertidal benthic system from the south of the bay. A ratio-temporal analysis of the isotopic compositions of basal sources and intertidal consumers (Chapter 4), benthic sources were found to display large spatial variations across the bay, which were mirrored in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of deposit-feeders. Yet, primary consumers displayed, in the absence of major disturbance, a higher temporal stability of their diets than in BSL. Excluding the winter signal of disturbance isotopic signatures of consumers from BSL, the deposit-feeder *A. marina* and suspension-feeder *C. edule* (two dominant species at the bay scale) had relatively constant diets across the bay, relying mostly on marine POM and local primary producers, proportions varying with the sources abundance and quality.

The subtidal food web was stable both in space and time and did not reveal any major influence from sewage (Chapter 5). It was composed of ~4 trophic levels and exhibited one main trophic pathway based on marine planktonic matter. The detrital pathway seemed to play an important role for the food web over the year, through a strong benthic-pelagic coupling enhanced by resuspension events in winter. Deposit-feeders exhibited some strong niche partitioning based on the sedimentary organic matter and the bay was characterized by a strong degree of omnivory directed toward predation, which is considered a predictor of stability.

This first investigation of the benthic food web of Dublin Bay draws the overall structure of trophic connections between food sources, meiofauna and macrofauna and the main pathways of organic matter transfer up to large predatory fish. The seasonal approach implemented proved to be relevant to depict the role of macroalgal blooms (*Enteromorpha* spp.) in the food web functioning of an impacted mudflat. This study evidenced the low role of sewage particulates at the bay scale, as expected since the upgrade of the STW Treatment Plant, nonetheless showing a moderate and localized trophic influence at sites under estuarine influence (e.g. BSL). Interestingly, this study revealed impacts of sewage effluents on the trophic structure and functioning of the system mostly indirectly, via the macroalgal blooms it sustains. This must be an important consideration for future management of Dublin Bay ecosystem.

« Se donner du mal pour les petites choses c'est parvix grandes, avec
le temps »

« Striving for small things, will enable you, with time each bigger »

Samuel Becket

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When I applied for this PhD at Trinity College, I did not know to what extent it would have changed my life, from both a personal and professional point of view. I did not know either that it would have taken such a long time. However, I sincerely believe that I made a good choice to relocate to Ireland for 4 years. That was a good move, a very rich experience. I should even say that the entire PhD was an adventure. Let's pass through it briefly.

I remember when I first landed in Dublin. I was excited by the new challenges: to take control of my scientific research, to get used to a different language, to a different culture and to a new environment. It did not take me too long to feel good in Dublin owing to many people. I would like to thank all of them warmly today, as their support has been a permanent driving force. We shared a lot together, at the lab, on the field, in the mud, by rivers, at sea, and at the pub of course. That was rich and intense. I think that studying at the Zoology Department was a real chance. I met with a lot of scientists, working on bees, birds and mammals, genetics, lakes, ... and that was a real benefit.

When I left Dublin, after 4 intense years of field/lab/office work and much more, I did not leave some colleagues but some great friends who made these Irish years so special and worthwhile. I am thinking to Elaine, Lynsey, Barry, Marjolein, Gwen, Blaithin, Fionnuala, Paddy F, Paddy K, Louise, Mike, Vesela, Jorge, Karen, Emma, and Yvonne you smart Fishhead. Ireland is beautiful that's for sure. Its landscapes are amazing, I agree. But above all, my dear friends, you make this country even better and enjoyable.

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Table 3.7: Results of the 2-way ANOVA performed on $\delta^{15}\text{N}$ of nematodes and primary consumers collected during the four surveys, using a 0.01 alpha level of significance. df: degrees of freedom, MS: Mean Square, F: statistic test, Sig: significance level.

Table 3.8: Temporal variability of the $\delta^{15}\text{N}$ values of nematodes and primary consumers in BISL. Results of the analysis of variance (ANOVA and Kruskal-Wallis test) among sampling surveys (A: June 2005; B: December 2005; C: April 2006; D: July 2006). Post hoc tests were LSD for ANOVAs and Dunn's Post hoc for Kruskal-Wallis tests (significance level : 0.05).

Table 3.9: Trophic levels estimations for all secondary consumers sampled in BISL from June 2005 to July 2006.

Table 4.1: Characteristics of the seston (mean ± standard deviation) collected in Sandymount (SM) and Blackrock (BR). Results measured in the Bull Island South Lagoon (BISL), in the Liffey river and offshore (Open Sea) and previously described in Chapter 3 are reminded here to facilitate comparisons. POC: Particulate Organic Carbon, Chl *a*: Chlorophyll *a*. In brackets: number of replicates.

Table 4.2: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (overall mean $\pm$ sd) of the sources collected at the three intertidal sites of Bull Island South Lagoon (BISL), Sandymount (SM) and Blackrock (BR). n: number of samples, sd: standard deviation.

Table 4.3: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the primary consumers sampled from June 2005 to July 2006 in Sandymount. Elements of taxonomic classification are indicated in brackets: Amph.: Amphipoda, Biv.: Bivalvia, Gastr.: Gastropoda, Pol.: Polychaeta. FS: Feeding Strategy; SF: suspension-feeder, G: Grazer, IF: Interface-feeder, SDF: Surface Deposit-feeder, SSDF: Sub-Surface Deposit-feeder. sd: standard deviation; n: sample size; * indicates that several individuals were pooled.

Table 4.4: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the primary consumers sampled from June 2005 to July 2006 in Blackrock. Elements of taxonomic classification are indicated in brackets: Biv.: Bivalvia, Pol.: Polychaeta. FS: Feeding Strategy; SF: suspension-feeder, G: Grazer, IF: Interface-feeder, SDF: Surface Deposit-feeder, SSDF: Sub-Surface Deposit-feeder. sd: standard deviation; n: sample size; * indicates that several individuals were pooled.

Table 4.5: Results of the non-parametric ANOVA (Kruskal-Wallis tests) performed on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of primary consumers collected during the four surveys at the three intertidal sites. Sites are: 1: Bull Island South Lagoon, 2: Sandymount and 3: Blackrock. Surveys are 1: June 2005, 2: December 2005, 3: April 2006, 4: July 2006.

Table 5.1: Biochemical characteristics (C:N ratio, Chlorophyll *a* (Chl *a*) concentration and Particulate Organic Carbon (POC) to Chl *a* ratio) of the potential food sources for the subtidal food web. n: sample size, POM: Particulate Organic Matter, SOM: Sedimentary Organic Matter. Results relative to the general POM end-members (river, sewage and open sea) have been previously described in Chapter 3 and are reminded here to facilitate comparisons.

Table 5.2: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean values) of the consumers sampled from May 2005 to June 2006 in the subtidal area of Dublin Bay (north and south sites). FS: Feeding Strategy; aSF: active suspension-feeder, pSF: passive suspension-feeder, DF: deposit-feeder, SSDF: sub-surface deposit-feeder, IF: interface-feeder, O: omnivorous, P: Predator; FS of fish: Pi: benthivorous (invertebrates), Pp: piscivorous; n: sample size.

Tables 5.3a and b: Trophic levels (TL) calculated for species sampled in May 2005, November 2005, March 2006 and June 2006.

Acronyms

ANOVA: Analysis of variance
BISL: Bull Island South Lagoon
BR: Blackrock
C: Carbon
Chl *a*: Chlorophyll *a*
DF: Deposit-Feeder
DIC: Dissolved Inorganic Carbon
DOM: Dissolved Organic Matter
DW: Dry Weight
ESB: Electricity Supply Board
EU: European Union
FS: Feeding Strategy
IF: Interface-feeder
KW: Kruskal-Wallis
LSD test: Least Significance Difference test
MBP: Microphytobenthos
MW: Mann-Whitney
N: Nitrogen
OM: Organic Matter
P: Predator
POC: Particulate Organic Carbon
POM: Particulate Organic Matter
SD: Standard Deviation
SDF: Surface Deposit-Feeder
SF: Suspension-feeder
SIA: Stable Isotope Analysis
SIAR: Stable Isotope Analysis in R
SM: Sandymount
SOM: Sedimentary Organic Matter
SSDF: Sub-Surface Deposit-Feeder
STP: Sewage Treatment Plant
TL: Trophic level
TSM: Total Suspended Matter

CHAPTER 1

General Introduction

1.1. Ecological importance of coastal and estuarine ecosystems

Although they represent less than 10% of the surface of the oceans, coastal ecosystems are among the most productive on Earth (Mann, 2000) regarding primary production (Valiela, 1995) as well as secondary production (Knox, 1986). Thus they concentrate 90% of the marine species commercially exploited (Pauly & Christensen, 1995). Coastal ecosystems are also characterised by a very high biological diversity on the global scale (Gray, 1997) and a large diversity of benthic habitats.

Coastal ecosystems support important ecological functions and through these provide considerable goods and services to human populations (Costanza *et al.*, 1997; Costanza *et al.*, 2014), which in turn expose them to high anthropogenically-induced stress (see Crowe & Frid (2015) for a review). Coastal and estuarine systems act as nurseries for many fish species and represent important feeding grounds for fish, sedentary and migratory birds. Through biological processes such as photosynthetic activity, heterotrophic production, decomposition, mineralization and flows of energy through food webs, coastal ecosystems play a fundamental role in the fluxes and biogeochemical cycles of elements such as carbon and nitrogen (Kennish, 1990). These processes encompass functions such as nutrient and organic matter recycling, or stabilization and clearance of particles from the water column (Crowe *et al.*, 2015). Benthic habitats and their inhabitants play a fundamental role in these processes (Giller *et al.*, 2004; Norling *et al.*, 2007). Whether estuaries and embayments act as a sink of organic carbon or as exporters is not always clear and this may depend on the geomorphology, the level of freshwater inflow, the tidal range and the local hydrodynamism (Kennish, 1990).

Humans derive many benefits from marine and particularly coastal ecosystems, in line with the ecological functions and services these systems deliver, as outlined above. These benefits can be categorised as provision for food and biotic and abiotic raw materials, regulating services (e.g. climate regulation, water and pollution treatment and assimilation), and cultural services including recreational grounds (Crowe & Frid, 2015)

1.2. Trophic functioning of coastal bays and estuarine ecosystems

1.2.1 The food web concept

A food web refers to the set of feeding connections linking the different species and biological compartments of an ecosystem. It depicts the interactions among species through their prey-predator relationships. It also implies to understand the fluxes of energy and pathways of transfer of organic matter within (and among) the ecosystem(s), aspects which are fundamental in ecology to understand the structuring and functioning of ecosystems. A food web can be described as a combination of food chains, tightly interacting with each other at one or different levels. Primary producers are the basal components and considered as the first trophic level. They supply energy to a suite of consumers (herbivores then one or several levels of carnivores) up to top-predators (Fig. 1.1). From each level, dead organic matter can be recycled by decomposers and through the microbial loop (Mann, 1988), and the nutrients liberated may be reused for photosynthesis by primary producers. Marine primary consumers can be categorized in different feeding groups (Bremner *et al.*, 2003) according to the type of resource they feed from (e.g. detritivores, herbivores) and/or according to their mode of food acquisition linked with the provenance of their food (e.g. suspension-feeders, deposit-feeders, grazers). However, many species are opportunistic and have the ability to feed on different types of food or have developed different manners to capture their food such that they are not restricted to one group only.

The balance of the interactions between the different compartments of the food web, and therefore the stability of the food web and the ecosystem, is maintained into a dynamic equilibrium by two chains of retro-control, regulated by consumers (top-down control) or controlled by the resources (bottom-up control). This theory (e.g. Polis & Strong, 1996 for bottom-up control) implies that a disturbance or increased pressure at one end (predator or source) can have cascading effects on the other compartments. This is an important consideration in food web ecology for the understanding of ecosystem functioning in response to human disturbances.

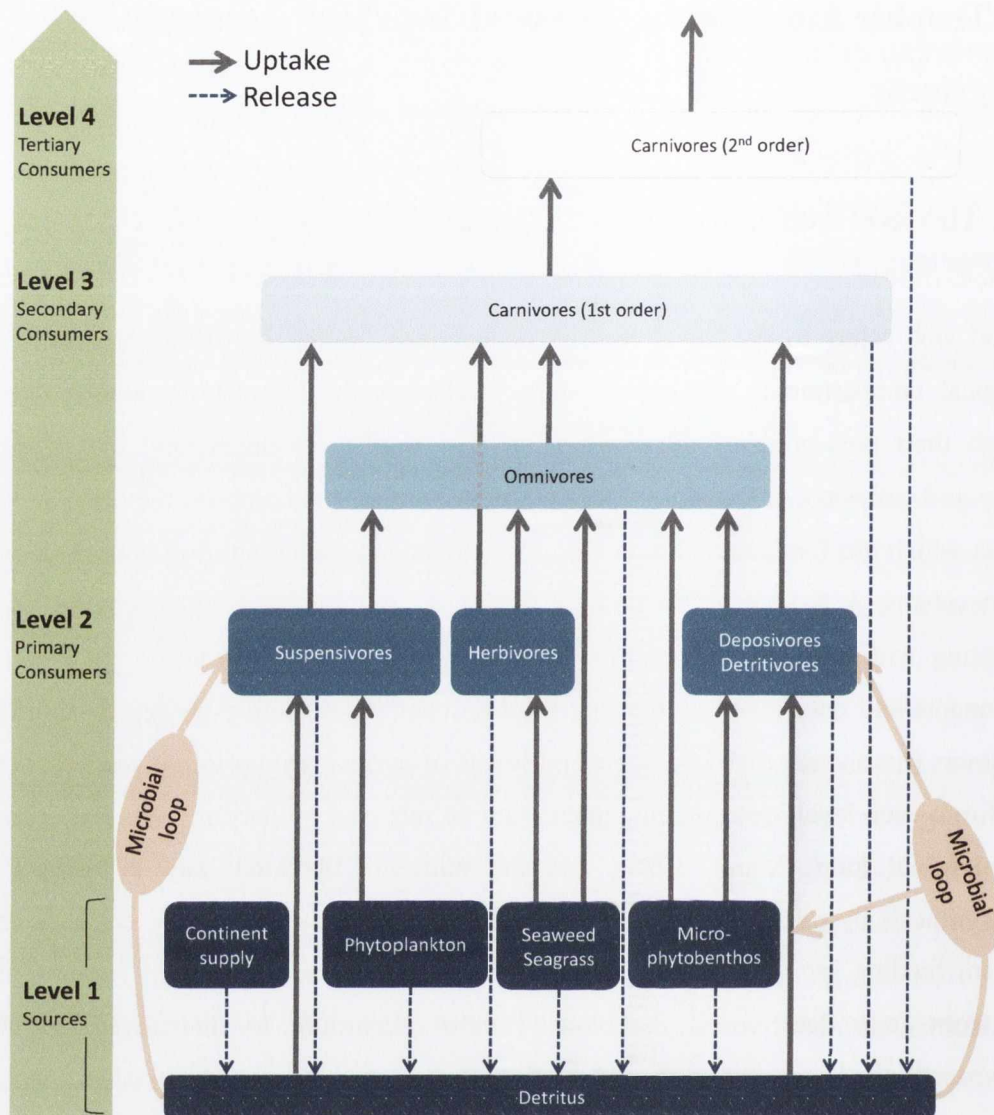


Figure 1.1: Theoretical flow diagram of a benthic food web in coastal ecosystems.

1.2.2. Diversity of food sources and their spatio-temporal variability

In contrast with the deep sea where organic matter supplying benthic consumers mostly derives from detrital plankton sedimenting from the euphotic zone of the overlying water column (Gray & Elliott, 2009), shallow coastal zones perceive the light enabling primary producers to develop and are therefore characterised by a multitude of organic matter sources, produced *in situ*, such as planktonic and benthic algae (phytoplankton, microphytobenthos, macroalgae) and marine and terrestrial phanerogams (e.g. seagrasses, saltmarsh plants), or conveyed from allochthonous sources by the sea and rivers.

Net primary production in estuaries reaches high levels of about $2000 \text{ gC m}^{-2} \text{ yr}^{-1}$ (Knox, 1986), which are lower than tropical seas, but much higher than most terrestrial ecosystems ($750 \text{ gC m}^{-2} \text{ yr}^{-1}$). A large part of this primary production comes from saltmarsh plants and macroalgae, but the microphytobenthos can also make a very important contribution to the organic carbon budget when intertidal flats are extensive (Valiela, 1995).

Coastal areas under estuarine influence are highly dynamic environments where organic matter sources are characterised by a high spatio-temporal variability. Apart from the tidal gradient of distribution of benthic species (e.g. saltmarsh plants on upper shore), this spatial-temporal variability of the sources is the resultant of different factors including climate-induced seasonal variations, river discharge rate, and local hydrodynamism (Knox, 1986), which control light conditions and nutrients (Underwood & Kromkamp, 1999).

1.2.3. Trophic transfer pathways in coastal and estuarine systems

Trophic transfers and the fate of primary production in coastal and estuarine ecosystems have been increasingly studied in the last 30 years with the development of tracing methodologies. These studies largely contributed to identify some major trophic pathways in these systems. Yet they highlighted the effect of local conditions on the variability of trophic pathways linked with the spatio-temporal variability of the food sources.

In marine open areas, phytoplankton usually represents the major food source for benthic consumers together with detrital particulate organic matter (Cloern *et al.*, 2002). Phytoplankton has also often shown to be the main source of food on intertidal sandflats (Yokoyama *et al.*, 2005b; Bode *et al.*, 2006; Lefebvre *et al.*, 2009).

In estuarine and coastal systems, food sources are much more diverse as seen in the previous section, and many of them can significantly sustain the food web, depending on their abundance and quality. Phytoplankton and microphytobenthos have been widely shown to be important trophic sources for estuarine and coastal macroinvertebrates. Microphytobenthos constitutes a highly assimilable source of food for primary consumers (Miller *et al.*, 1996). In estuarine and sheltered environments, benthic microalgae can

generate a high productivity in cohesive sediments left uncovered at low tide (Valiela, 1995). The productivity of microphytobenthos was for example reported by Jesus *et al.* (2009) to represent up to 50% of estuarine primary productivity, and it is largely demonstrated that these benthic microalgae constitute an important trophic resource for macro-invertebrates in these environments (e.g. Currin *et al.*, 1995; Riera & Richard, 1996; Herman *et al.*, 2000; Middelburg *et al.*, 2000; Sauriau & Kang, 2000; Kang *et al.*, 2006). Benthic microalgae are thus consumed by deposit-feeders or grazers as well as suspension-feeders following their resuspension by tidal currents or wind-driven wave action (Ubertini *et al.*, 2012). Within some semi-enclosed estuaries, they have also been shown to be exported to subtidal areas where they are consumed by suspension-feeding bivalves together with phytoplankton (Kang *et al.*, 2003; Yokoyama *et al.*, 2009). The relative proportions of phytoplankton and benthic microalgae in the diet of invertebrates vary depending on seasonal availability (e.g. Page & Lastra, 2003).

The trophic role of saltmarsh plants as detritus has been particularly evidenced in estuarine systems from the east coast of the United States where they form extensive marshes (Peterson *et al.*, 1985; Currin *et al.*, 1995). Both C₃ plants (*Juncus*, *Phragmites*) and C₄ plants (*Spartina*) were shown to represent a source of food to consumers according to their dominance on the site studied (Currin *et al.*, 1995; Wainright *et al.*, 2000; Currin *et al.*, 2003). The trophic importance of saltmarsh plants is much less documented in Europe (Decottignies *et al.*, 2007).

Terrestrial organic matter conveyed with rivers has been shown in different European ecosystems to supply food to marine or estuarine organisms, particularly toward the river mouth. Hence, Riera & Richard (1997) showed that oysters may derive a significant part of their food from material carried in by the Charente river in winter during higher discharge. At the mouth of the Rhone river in the Mediterranean sea, Darnaude *et al.* (2004b) demonstrated the existence of a trophic pathway based on terrestrial organic matter supporting demersal fisheries (flatfish such as the common sole *Solea solea*) through depositivores polychaetes, while another trophic pathway was based on phytoplankton (Darnaude *et al.*, 2004a; Darnaude *et al.*, 2004b). On the other hand, the food web at the mouth of the Po river (main river in Italy) was shown to rely mostly on marine phytoplankton (Camusso *et al.*, 1998).

Although macroalgae and phanerogams often account for most of the primary production in estuarine systems, only a minor fraction is grazed by herbivorous species (Duarte & Cebrian, 1996) and it is considered that 90% of the macrophytes production incorporates the detrital chain (Mann, 2000). Many other sources can in fact enter the detrital pool which thus constitutes a heterogeneous mixture (Hedges, 1992), whose composition varies depending on the ecosystem characteristics. Detrital matter may thus originate from macrophytes material produced *in situ* (C_3 and C_4 plants, seagrasses, macroalgae), detritus carried in by rivers which are themselves issued from terrestrial plants from the catchment and primary producers from the river (Cai *et al.*, 1988), feces and dead animal organic matter, fungi, protists, etc. The importance of trophic fluxes based on detrital matter has been acknowledged in estuaries (Tenore, 1983; Peterson *et al.*, 1985; Mann, 1988; Currin *et al.*, 1995). Most detritus are however refractory as essentially constituted of lignocellulosic compounds (i.e. with a high C:N ratio), and benthic invertebrates cannot assimilate them as such, but through the bacteria that are associated to this detritus substrate (Findlay & Tenore, 1982; Tenore, 1983). Bacteria are also an important component of the organic matter in coastal and estuarine sediments, but according to Van Oevelen *et al.* (2006), this bacterial carbon would only represent about 10% of the meiobenthos and macrobenthos carbon requirements.

While the role of detritus in trophic fluxes is acknowledged, a large number of recent studies on the diet of marine primary consumers have in fact evidenced the dominant trophic role of the marine autochthonous production pathway, either from phytoplankton or microphytobenthos (e.g. Kang *et al.*, 1999; Sauriau & Kang, 2000; Riera *et al.*, 2002; Kang *et al.*, 2003; Page & Lastra, 2003; Yokoyama *et al.*, 2005b; Kang *et al.*, 2006; Decottignies *et al.*, 2007; Riera, 2007; Yokoyama *et al.*, 2009; Riera, 2010).

1.3. Stable isotopes: a privileged tool for food web studies

1.3.1. Limits of traditional methods used in trophic ecology

Several methods are used to qualitatively describe marine trophic interactions. The classical method reverts to observing and analysing the stomach content, or the feces content, of some target organisms. Analysing stomach contents is largely used in ecology

for studies involving large organisms such as fish or birds. Although this method brings considerable information, it is often described as time-consuming and needs considerable manpower to undertake. Moreover such analysis can be hardly used when studying small and/or partially decomposed organisms, and is inefficient when studying species feeding on detritus for example. Other limits can be underlined for this method. For example, stomach analysis only informs on the ingested fraction of food and not on the assimilated fraction and so only gives a snapshot of the studied organism's diet.

For these different points, choosing stomach content analysis for food web studies can be inappropriate especially when studying benthic communities where microphage organisms (detritivorous and depositivorous) are mainly represented (Lopez & Levinton, 1987). To bypass such limits, a time series study must be deployed, thereby increasing the time, manpower and cost constraints.

Since the 1970s, the use of isotopes in environmental sciences has considerably developed. Analysis of natural stable isotopes (^{13}C , ^{15}N and ^{34}S) is currently the most used method for food web studies. The success of stable isotopes in food web analysis is partly explained by the fact that it overcomes the limits described for stomach content analysis. Thus it provides measures integrated over time of which the food was assimilated by the organisms which can vary depending on the type of studied tissue and on its turnover time. It also requires small amounts of biological sample (less than 1mg DW), which enables to study the diet of small organisms and hence to have a detailed view of each compartment of the food web.

1.3.2. Reminders about isotopes

Isotopes are atoms which have the same number of protons but differ by their number of neutrons. These differences lead to different mass number of the nucleus, giving heavier or lighter isotopes. Whereas radioactive isotopes (e.g. ^{14}C) disintegrate over time (lose a neutron), stable isotopes are lighter atoms, abundantly found in the environment, and that persist as such. This is why they are largely used as tracers. The lighter stable isotopes (for instance ^{12}C and ^{14}N) are far more abundant in the environment compared to the heavier equivalent isotopes (^{13}C and ^{15}N , Table 1.1). In food web ecology, the most used elements are carbon and nitrogen.

Table 1.1: Natural abundances (%) in the environment of the main stable isotopes used in ecology

Element	Light Isotope		Heavy Isotope	
Hydrogen	¹ H	99.984	² H	0.016
Carbon	¹² C	98.89	¹³ C	1.11
Nitrogen	¹⁴ N	99.64	¹⁵ N	0.36
Oxygen	¹⁶ O	99.76	¹⁷ O	0.2
Sulphur	³² S	95.00	³⁴ S	4.22

The isotopic ratio “heavy isotope/light isotope” is used to distinguish different compartments in a system. Regarding the abundance of light isotopes in the environment, ratio values are hence extremely low and differences between compartments are small. However, these differences can be measured by using high precision analytical techniques such as mass spectrometry. The isotopic composition of a sample ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) is expressed as the relative difference between isotopic ratios in the sample and in conventional standards. For carbon isotope ratios, the carboniferous fossil stone Pee Dee Belemnite is the original standard, whilst atmospheric N_2 is the standard for nitrogen. Such ratios are expressed in δ units (‰).

$$\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000, \text{ with } R = {}^{13}\text{C}/{}^{12}\text{C}$$
$$\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000, \text{ with } R = {}^{15}\text{N}/{}^{14}\text{N}$$

1.3.3. Natural isotopic tracing in food web ecology

Stable isotopes have proved to be powerful tools to delineate food web interactions and the fate of organic matter in ecosystems. This leans on the existence of characteristic carbon isotopic signatures of primary producers and on the preservation of this signature during transfer through the food chain, with a small and predictable increase of the isotopic signature due to isotopic fractionation (De Niro & Epstein, 1978, 1981; Rau *et al.*, 1983; Minagawa & Wada, 1984). The isotopic fractionation process is defined by the fact that the relative abundance of different isotopes of an element changes during

nutrient assimilation and during the downstream metabolic reactions (respiration, excretion). Such reactions tend to eliminate the lighter isotopes. At each trophic-step, consumers thus become enriched with the heavier isotope (^{15}N , ^{13}C) compared to their food source by a relatively constant factor called fractionation factor (Martinez del Rio *et al.*, 2009).

Primary producers display a large range of carbon isotopic signatures in the environment (Fry & Sherr, 1984). These various isotopic compositions depend essentially on 1) the type of metabolism used by the primary producer, such as C_3 , C_4 or CAM plants (Maberly *et al.*, 1992; Raven *et al.*, 2002); and 2) the source of inorganic carbon used for photosynthesis (atmospheric CO_2 , freshwater or seawater dissolved CO_2 , HCO_3^-), these having specific isotopic compositions (Fry, 1996). These fundamental properties are particularly useful for the study of estuarine and coastal ecosystems where there is a large diversity of food sources (e.g. saltmarsh plants, phytoplankton, microphytobenthos, riverine organic matter, etc) which can thus be distinguished by their $\delta^{13}\text{C}$ (Fig. 1.2). Environmental factors such as temperature, pH, salinity and the level of irradiance can also affect the primary producers $\delta^{13}\text{C}$ (by influencing the rate of photosynthesis and in turn the rate or the form of inorganic carbon assimilated) and $\delta^{15}\text{N}$ (Thompson & Clavert, 1994; Cornelisen *et al.*, 2007; Brutemark *et al.*, 2009). Reviews and experimental studies carried out on the carbon fractionation factors have shown that it varies only slightly (+0.4 to +1.5‰) during the assimilation of a plant by an organism (Gearing *et al.*, 1984; Minagawa & Wada, 1984; Vander Zanden & Rasmussen, 2001; McCutchan *et al.*, 2003; Yokoyama *et al.*, 2005a). If the sources of a system are sufficiently distinct regarding their $\delta^{13}\text{C}$, then the low $\delta^{13}\text{C}$ enrichment can inform different trophic pathways (*i.e.* based on different food sources) of consumers even up to high trophic levels.

Nitrogen isotopes give additional information about the trophic level of a consumer owing to a higher fractionation factor (Cabana & Rasmussen, 1996; Post, 2002a; Pinnegar *et al.*, 2003). The nitrogen isotopic fractionation between a food source and its consumer is however more variable than for $\delta^{13}\text{C}$. Experimental studies have reported fractionation values between 2.5 and 4‰ (De Niro & Epstein, 1981; Minagawa & Wada, 1984; Vander Zanden & Rasmussen, 2001; Post, 2002a; McCutchan *et al.*, 2003; Vanderkluft & Ponsard, 2003; Yokoyama *et al.*, 2005a; Dubois *et al.*, 2007). The trophic fractionation can vary according to the taxonomic class, the methods of sample

preparation (e.g. acidification, starvation), the type of nitrogen excretion (ammonia, urea, etc), the type of tissue analysed, the trophic level or the diet (Vanderklift & Ponsard, 2003; Martinez del Rio *et al.*, 2009). Vanderklift & Ponsard (2003) advised considering these sources of variation when choosing trophic fractionation factors from the literature.

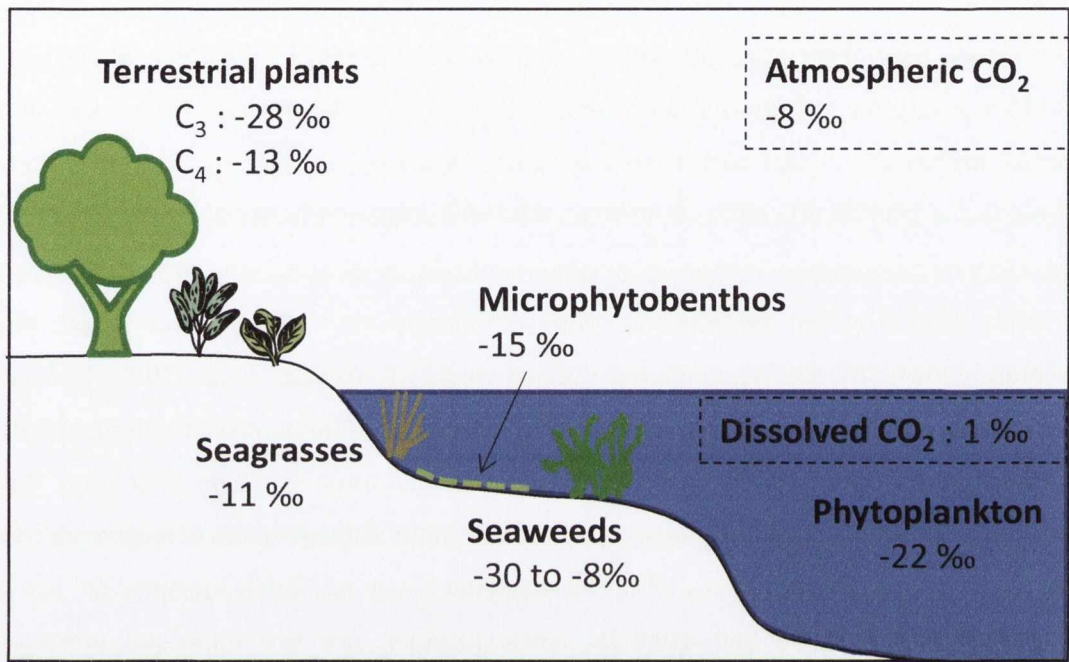


Figure 1.2: Distribution of $\delta^{13}\text{C}$ values of primary producers in coastal ecosystems. Adapted from Carlier (2007).

Fractionation processes and the resulting level of enrichment in organisms are thus fundamental in trophic ecology studies. Low $\delta^{13}\text{C}$ enrichment can inform different trophic pathways of consumers even up to high trophic levels, whilst high $\delta^{15}\text{N}$ enrichment can define the trophic levels of studied organisms as well as the length of the food web.

At intraspecific level, isotopic signatures inform on the size of the trophic niche of a species (Bearhop *et al.*, 2004). For instance, the $\delta^{13}\text{C}$ variability for one same species gives indication on its trophic plasticity. A large variability would underline an opportunistic character, meaning that the species is able to feed on several types of food sources depending on their availability. On the other hand, low variation of $\delta^{13}\text{C}$ for one same species (including through time) can highlights its selectiveness in the choice of

food. A large variability of $\delta^{15}\text{N}$ is often associated to an omnivorous regime, i.e. feeding on preys belonging to different trophic levels (Sweeting *et al.*, 2005).

Knowledge of the time window over which food resources are integrated and isotopically reflected in the consumer's tissues is another fundamental aspect in trophic studies for an accurate interpretation of isotopic data (Martinez del Rio *et al.*, 2009). Isotopic turnover rates vary widely, from days to years, across taxa and tissue types (Tieszen *et al.*, 1983; Boecklen *et al.*, 2011; Vander Zanden *et al.*, 2015). Metabolically active tissues such as internal organs (ex: liver) and plasma typically turn over in a very short time frame (days), faster than blood cells and muscles, whose incorporation rate can vary from days to weeks or even months, depending on species. On the other hand, structural tissues such as bone collagen or fish otoliths incorporates elements very slowly (over years), thus revealing dietary information spanning over an entire life (Radtke *et al.*, 1996; Dalerum & Angerbjorn, 2005). While studies assessing the rate of isotopic incorporation (usually expressed as isotopic half-life) and factors governing it were scarce 15 years ago, they have considerably increased in numbers in the last years following the extensive call for more experiments (Gannes *et al.*, 1997; Martinez del Rio *et al.*, 2009; Martinez del Rio & Carleton, 2012). Growth and catabolic tissue turnover are two important processes inducing changes in tissues isotopic values (Fry & Arnold, 1982; Hesslein *et al.*, 1993). Growth would be the main factor affecting nitrogen turnover while carbon isotopic turnover is mainly driven by catabolism (Mont'Alverne *et al.*, 2016). Differential incorporation rates between these two elements (C and N) can therefore be expected but results on this topic are rather inconsistent. Some studies found a higher half-life for nitrogen than carbon (Carleton & Martinez del Rio, 2005; Sacramento *et al.*, 2016) while the opposite trend was also reported (Church *et al.*, 2009). More recently, Hamaoka (2016) found no differences between the half-life of carbon and nitrogen, as Vander Zanden *et al.* (2015) from their extensive literature analysis. Recent synthesis of published data highlighted that the isotopic turnover rates of carbon and nitrogen in muscle tissues and in whole organisms (as often analysed for small species) increase allometrically with body mass (Thomas & Crowther, 2015; Vander Zanden *et al.*, 2015), an effect modulated by temperature according to Thomas & Crowther (2015). Vander Zanden *et al.* (2015) found that this relationship between turnover rates and body mass particularly applies for ectotherms, but not for endotherm species.

Turnover rates for carbon and nitrogen have also been shown to vary according to salinity, being considerably slower in freshwaters than in estuarine and marine waters (Mont'Alverne *et al.*, 2016).

1.4. Influence of anthropogenic pressures on coastal food webs: organic pollution and eutrophication

With more than 60% of the world population living on the coastline and the constant increase of coastal urbanisation (Kay & Alder, 2005), littoral ecosystems are being increasingly impacted by human activities and their integrity and equilibrium can be profoundly affected (Gray, 1997; Halpern *et al.*, 2008). Among the main threats on coastal ecosystems, organic pollution and eutrophication are major issues for coastal embayments and estuaries (Cloern, 2001; Millennium Ecosystem Assessment, 2005) which are like collectors where continental inputs may concentrate. Eutrophication is the process of enriching systems with nutrients, mainly nitrogen and phosphorus, thereby inducing changes in nutrient ratios which support the overgrowth of plants or algae and result in the increase of organic matter inputs to the seabed (Nixon, 1995; Korpinen & Bonsdorff, 2015). This alteration of water quality can be a natural phenomenon but it has dramatically increased during the 20th century due to the rising use of agricultural fertilisers, to urban runoff and wastewater treatment plants effluents (Bricker *et al.*, 2008; Korpinen & Bonsdorff, 2015). The major symptoms of coastal eutrophication, that do not always co-occur, are phytoplanktonic blooms (notably from harmful microalgae) and the excessive proliferation of fast-growing and short-lived macroalgae (Valiela *et al.*, 1997; Cloern, 2001). The occurrence of macroalgal blooms, which are mainly composed by opportunistic Chlorophyceae, is a spreading phenomenon which is increasing in frequency and intensity worldwide, especially on coastlines of developed countries (Ye *et al.*, 2011). In the first stages of the bloom, the increase of algal biomass can foster primary consumers which are able to respond quickly to the new level of productivity (Lotze & Worm, 2000). However, as the development of ephemeral macroalgae progresses and biomass accumulates, major ecological dysfunctions appear. High biomasses of opportunistic algae modify the biogeochemistry of the water column and the sediments (Morand & Briand, 1996; Valiela *et al.*, 1997), and induce a strong

increase of the oxygen demand at night (Sundback *et al.*, 1990). As the excess of organic matter decomposes, hypoxia or even anoxia events may appear (Wu, 2002; Diaz & Rosenberg, 2008). Macroalgal blooms can also reduce light penetration, with negative impacts for other primary producers such as perennial macrophytes, eelgrass and saltmarsh plants (Cloern, 2001; Deegan *et al.*, 2002; Hauxwell *et al.*, 2003; Newton & Thornber, 2013; Watson *et al.*, 2015). Ephemeral macroalgae proliferation can have significant impacts on benthic communities associated with soft-sediments (see Raffaelli *et al.*, 1998; Grall et Chauvaud, 2002; Lyons *et al.*, 2014 for reviews) and on birds and fish populations (Raffaelli, 1999; Nordström & Booth, 2007; Wennhage & Pihl, 2007). It can alter the recruitment, modify the species richness and diversity, their abundance and biomass (Bolam *et al.*, 2000; Raffaelli, 2000; Jones & Pinn, 2006; Quillien *et al.*, 2015a), lead to changes in the composition and the structure of benthic communities through antagonistic effects on species and feeding guilds, and may ultimately induce a shift of the faunal assemblage compared to undisturbed conditions (Bonsdorff, 1992; Norkko & Bonsdorff, 1996; McClelland & Valiela, 1998; Raffaelli *et al.*, 1998; Norkko *et al.*, 2000; Cardoso *et al.*, 2004). These effects can greatly alter the functioning of the ecosystem exposed to this pressure. Eutrophication may thus induce radical changes of the food web structure by modifying trophic pathways. In estuarine and sheltered systems (including lagoons), eutrophication has been shown to favor the detritivorous pathway, deposit-feeders becoming the dominant trophic group to the detriment of suspension-feeders and grazers (Bachelet *et al.*, 2000; Österling & Pihl, 2001; Marques *et al.*, 2003; Keats *et al.*, 2004). Higher trophic level groups can be disadvantaged and the fractions of omnivorous and carnivorous consumers decrease (Marques *et al.*, 2003; Coll *et al.*, 2011). These effects globally agree with the ones depicted by the well-known empirical model of Pearson & Rosenberg (1978) describing the spatial and temporal successions of macrofauna in relation to organic loading to the sediments and the accompanying changes in trophic characteristics. Some other studies report different results from the ones described above. For example, from an extensive survey investigating large-scale effects of green tides (i.e. the accumulation of unattached green macroalgae) on exposed sandy beaches, Quillien *et al.* (2015b) found that herbivorous and suspension-feeding invertebrates were favored, while sub-surface deposit-feeders and larger species were negatively impacted. While these impacts can be specific to this type of environment and its harsh hydrodynamics and physical conditions, they may also reflect an intermediate disturbance state, corresponding to the successional (transitory) increase in biomass and

secondary production due to the increase in food supply (Pearson & Rosenberg, 1978). Tewfik *et al.* (2007) consistently showed that distinct functional groups of primary consumers predominate at the various stages of anthropogenic nutrient enrichment of seagrass beds. The few studies which have considered the impacts of eutrophication on community-wide food web structure highlight a simplification of the food web with the reduction of the number of trophic groups, the reduction of the number of trophic pathways and eventually a reduced food web length (Tewfik *et al.*, 2007; Carlier *et al.*, 2008; Coll *et al.*, 2008; Coll *et al.*, 2011). The impact level can however differ among environments (Lyons *et al.*, 2014), depending on the type of habitat, the local hydrodynamic conditions and/or the pressure level. Responses of communities may also vary if they are affected by other stressors (such as overfishing, chemical pollution, etc) even for one same habitat (Coll *et al.*, 2011). Considering this, investigating food web structure and trophic transfer pathways by integrating site-specific characteristics appear essential to understand the ecological state of an ecosystem and its response to anthropogenic pressures according to its own biotic and abiotic features, especially if conservation or management measures are to be taken (Lyons *et al.*, 2014).

Nitrogen stable isotopes are a good indicator of anthropogenic sources of inorganic nitrogen which typically exhibit higher $\delta^{15}\text{N}$. $\delta^{15}\text{N}$ has thus been largely and successfully used as an indicator of anthropogenic nutrient enrichment (notably from sewage) in aquatic systems especially since the fate of this nitrogen can be traced in primary producers and food webs (McClelland *et al.*, 1997; Costanzo *et al.*, 2001; Cole *et al.*, 2005). This makes it a valuable tool for the study of eutrophicated coastal areas, notably to differentiate them from pristine areas.

1.5. The case of Dublin Bay

Dublin Bay is a small embayment under estuarine influence surrounded on its three sides by Dublin conurbation with over 1 million inhabitants. This ecosystem has had a long eutrophication history, receiving substantial sewage particulates and nutrients (P and N, mostly as ammonia NH_4) released in the lower estuary. These inputs have been related to the occurrence of excessive macroalgal growth in the inner subtidal and especially in the intertidal area of Bull Island South Lagoon (BISL), these algal blooms being fueled

directly by nutrients or through sediment remineralisation of the sewage particulate organic matter (Brennan *et al.*, 1994; Jeffrey *et al.*, 1995; Khan, 1998). The first descriptions of the macroalgal bloom in BISL, which is due to the proliferation of the filamentous mat-forming green algae *Enteromorpha* spp., date back to Baily (1887) and to Adeney (1908). The intensity of the bloom, in term of biomass, had greatly increased in 1990 compared to the 70's (Jeffrey *et al.*, 1992) and was found by Khan (1998) to be similar to other sites highly impacted in Europe. The impacts of sewage effluents on benthic habitats, through the high loads of particulates or as stimulator of planktonic and benthic algal growth, seemed to be confined to the estuary and the northern part of the bay (BISL, inner subtidal) because of the hydrographic conditions (Wilson *et al.*, 1986; ERU, 1992; Jeffrey *et al.*, 1992; McBreen & Wilson, 2005; O'Higgins & Wilson, 2005). Similarly, the high concentrations of estuarine nutrients were not traced in the south of the bay, suggesting it to be much less impacted by anthropogenic inputs (O'Higgins, 2005).

While benthic communities are considered as fairly rich in the outer bay (Walker & Rees, 1980), population densities of infaunal invertebrates are rather low inshore (Wilson, 1982). Yet the bay sustains high numbers of wintering waders and migratory birds (including the site suffering from green macroalgal blooms) such that parts of it are protected under several national, European and international designations (e.g. UNESCO World Biosphere Reserve, RAMSAR, see McCorry & Ryle (2009) for details). The relative roles of the various organic matter sources in supporting the food web of sites of such high ecological value but though undergoing major disturbances remains however poorly known. A first analysis of energy flow through the system by Ecological Network Analysis (ENA) highlighted the anthropogenic stress on the system. It suggested that sewage particulate matter played a major role in sustaining a high degree of detritivory in the bay and, not surprisingly, that benthic invertebrates had a key role for the transfer of energy to higher trophic levels and the recycling of organic matter (Wilson & Parkes, 1998). Spatial variations of anthropogenic influence and habitat-specific characteristics (including the role of macroalgal mats) across the bay were however not considered in the model and many unknowns remained, such that the authors acknowledged the crucial need for a better understanding of the system functioning. In 2003, the sewage treatment plant (STP) was upgraded inducing a strong modification of the effluent composition. The levels of suspended solids and ammonia were much reduced (by 83% and 81%

respectively between 2001 and 2004). But on the other hand, oxidised forms of nitrogen increased substantially, such that the amount of inorganic nitrogen released by the effluent was thus only reduced by 20% between 2001 and 2004 (O'Higgins, 2005). Considering the role that urban effluents from sewage were considered to play on Dublin Bay ecosystem, questions arose then on the impact this changing discharge regime would have on the organic matter fluxes through the system and on the ecosystem functioning. The role of sewage particulates as a food resource can reasonably be expected to be much reduced at the bay scale, as considered by Wilson *et al.* (2007) is their updated ENA of Dublin Bay in which sewage POM was just totally removed. But one may still question the trophic role of these particulates at small spatial scale, especially for nearby sites of high ecological value such as the mudflat of Bull Island South Lagoon. Moreover, considering that nutrient released with sewage effluents have been only slightly reduced, eutrophication symptoms may persist. This more generally poses the question of the direct and indirect impact of sewage on Dublin bay ecosystem, and particularly on its trophic functioning.

1.6. Aims and structure of the thesis

The above review of eutrophication (and more generally of organic enrichment) impacts on the functioning of benthic communities reveal some tendencies such as a simplification of the food web and the fostering of the deposit-feeders trophic guild although effects varied among habitats. The intensity of these impacts may also vary non-linearly depending on the pressure level, such that the benthic community and other feeding guilds can be stimulated at lower levels. The literature review additionally highlights the influence that local biotic and abiotic conditions can have in modulating these impacts on benthic systems.

In Dublin Bay, despite a long history of anthropogenic stress from the urban centre through sewage loadings and the resultant eutrophication symptoms, little is known about the trophic functioning of the benthic system and how it copes with these anthropogenic pressures, even more since the modification of the sewage discharge regime in 2003, as detailed in the previous section.

In this context, the aim of this thesis was to characterise the food web structure and functioning in Dublin Bay, to identify the basal sources supporting the food web and the trophic pathways and to investigate the effects of anthropogenic sources of stress from the urban centre on the functioning of benthic communities. More specifically, this thesis explore the direct (POM) and indirect (macroalgal bloom) effects of sewage inputs on the food web across the bay. Based on previous work and considering the evolution of sewage inputs since 2003, it was expected that the impact of anthropogenic loadings on the bay food web is rather localised inshore and now occurs mostly indirectly, via the macroalgal bloom it sustains. In other words, the over-arching hypotheses of this thesis work were that:

- anthropogenic POM released with sewage has a minor impact on benthic consumers (i.e. constitutes a minor food source) at the bay scale, and that its influence is very localised inside the bay, to the northern inshore area.
- the presence of the green macroalgal bloom of *Enteromorpha* spp., whose proliferation is stimulated by sewage-derived nutrients, influences the food web locally.

To test these hypotheses, a carbon and nitrogen stable isotope approach ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) has been implemented as the main tool to identify the organic matter sources supporting the food web and the major trophic pathways and to determine how they vary with time and space across the bay. For this, I focused my study on five sites featuring contrasted exposition to the watershed (from inshore and estuarine conditions to offshore and northern vs southern parts of the bay).

After this presentation of the context and aims of the study, the manuscript has been organised as follows:

- Chapter 2 describes the studied sites and their biological and environmental characteristics and then details the methodology applied.

The following chapters (3, 4 and 5) present the results of the work undertaken:

- Chapter 3 assesses the direct and indirect influence of urban sewage on the food web of a stressed estuarine mudflat (Bull Island South Lagoon) located close to the sewage discharge point and experiencing green macroalgal blooms. Through the characterisation

of basal resources and the whole benthic food web structure over time (4 surveys), this chapter quantifies the importance of sewage particulate organic matter for benthic consumers of this exposed site and investigates the effects of macroalgal blooms on species-specific diets and on the food web structure. All the basal sources and the main faunistic compartments (meiofauna, macrofauna, and fish) of the benthic food web were considered.

- Chapter 4 examines the differential influence of sewage effluents across the study area and its potential effects on the base of the food web, especially on the primary consumers diets. This was undertaken through a spatio-temporal analysis of the stable isotopic composition of basal sources and primary consumers at three intertidal sites from the north and south intertidal areas featuring different exposure to the watershed. Spatio-temporal changes of the primary consumers isotopic signatures were assessed in relation with the food sources at each site.

- Chapter 5 explores the structure of the benthic food web of two subtidal areas of the outer bay over four surveys. This chapter aims to identify the sources of organic matter supporting the food web and to characterise the trophic structure through an assessment of the consumers' trophic levels and the food web length. The role of sewage organic matter as a potential food source for subtidal benthic communities was investigated.

CHAPTER 2

General Methodology

2.1. Study area

Dublin Bay is a semi-circular sandy embayment of 70 km². It is surrounded on its northern, western and southern sides by the conurbation of Dublin and opens on its eastern side on the Irish Sea (Fig. 2.1). The entrance is 10 km extent north to south and delimited by the rocky headlands of Howth to the north and Dalkey to the south, while the rest of the coast is low and sandy. Dublin Bay is a shallow basin (< 15 m depth) whose intertidal areas occupy the inner third of the bay. Waters in the bay are well-mixed by strong tidal currents (Crisp *et al.*, 1976) flowing clockwise through the bay from south to north and exiting in the north near Howth head at velocities up to 0.5 m s⁻¹ (ERU, 1992). The bay receives sewage effluents released from Ringsend Sewage Treatment Plant (STP) in the lower estuary and freshwaters from the Liffey river, which is walled on either sides and runs through Dublin city. There are several tributaries to the Liffey estuary but the dominant freshwater inflow comes from the Liffey river (catchment area: 1256 km², mean flow: 12.2 m³ s⁻¹, max flow: 132.6 m³ s⁻¹), which accounts for 75% of the freshwater inputs to Dublin Bay (Wilson, 2005). The Liffey plume mostly tends to extend north and eastwards toward Howth head. According to the current studies undertaken by ERU (1992), the Liffey plume does not reach the shoreline waters of the south bay.

In the northern part of the bay lies the dune-covered sand spit of Bull Island which encloses the north and south “lagoons” on its western side. These two semi-enclosed intertidal areas are composed of mudflats and muddy sandflats separated by a causeway preventing any water exchange. There are abusively called “lagoons” locally. Although this designation was kept in this study, it must be stressed that such intertidal areas cannot be classified as this habitat (McCorry & Ryle, 2009), which refers to an isolated or partially enclosed body of brackish water with a restricted tidal range (Healy, 2003). Extensive sand flats cover the eastern side of Bull Island and the southern half of the inner bay, hence representing 70% of the bay intertidal area.

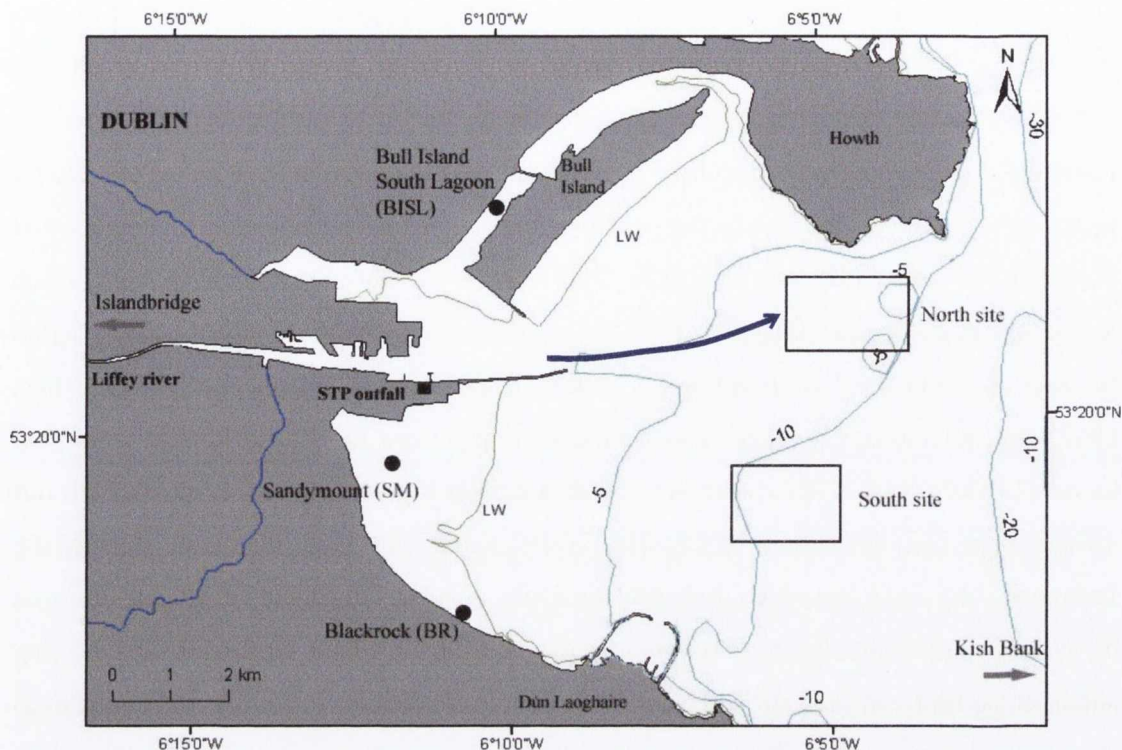


Figure 2.1: Dublin Bay and sampling sites in the intertidal (black dots) and in the subtidal (black rectangles) areas. STP: Sewage Treatment Plant, LW: Low Water mark. The blue arrow indicates the dominant flowing direction of the Liffey plume.

2.2. Presentation of the habitats studied

2.2.1. Intertidal sites

The three sites studied are representative of the different intertidal habitats of the bay and exhibit contrasted exposition to the watershed and to the Irish Sea influence:

- The site of **Bull Island South Lagoon (BISL)** is located on the northern part of the bay (Fig. 2.1). It is a shallow sandy mudflat of 72ha quite exposed to the urbanised watershed, due on one hand to its inshore position within Dublin Bay inducing to be tidally ventilated only through the Liffey estuarine waters, and on the other hand to its sheltered and depositional nature. BISL suffers from nutrient enrichment through tidal waters entering the site (Jeffrey *et al.*, 1992; Jeffrey *et al.*, 1995), and this results in the annual occurrence of green macroalgal blooms dominated by filamentous *Enteromorpha*

spp.. These blooms represent about 57% of the green macroalgal biomass of all the bay intertidal areas, all sub-environments considered (Jeffrey *et al.*, 1992 and references therein), although the surface area of the present study site only covers 3.6% of the intertidal area of the bay. In summer 1989, algal mats were reported to cover up to 64% of the site sandflat, with an average standing biomass of 84.5g DW m⁻² (averaged over the whole flat area, Jeffrey *et al.*, 1992). Such blooms usually start to form in April, reach a biomass peak (up to 500g DW m⁻²) between June and mid-September depending on weather conditions and are progressively buried from October (Jeffrey *et al.*, 1992; Khan, 1998). The location of the algal mats on the flat was shown to be quite similar from year to year (Jeffrey *et al.*, 1992). Although there is neither biomass nor coverage data for our sampling period, the green macroalgae were again observed abundant during the summers 2005 and 2006 when this study took place. BISL is bordered on its eastern side by saltmarshes dominated by *Halimione portulacoides*. A minor freshwater stream (the Naniken, mean flow: 0.03 m³ s⁻¹) enters the site on its northern extremity and contributes in maintaining a narrow channel at low tide but the most significant freshwater input enters during the flood with estuarine waters.

This site is characterised by a *Hediste diversicolor*, *Macoma balthica* and *Arenicola marina* sandy mud community.

- The sites of **Sandymount (SM)** and **Blackrock (BR)** are located in the south part of the bay and considered not to be affected by estuarine waters owing to the bay hydrodynamical conditions (ERU, 1992).

The site of **Sandymount** is more sheltered than the site of Blackrock. It is located mid-shore on an extensive muddy sand flat with a *Macoma balthica* and *Arenicola marina* community (Wilson & Emblow, 2002).

The site of **Blackrock** is located lower on a sand shore characterised by clean sands with burrowing amphipods and polychaetes. *Lanice conchylega* and *Angulus tenuis* are characteristic species of this site (Wilson & Emblow, 2002).

The sites of Sandymount and Blackrock have not been characterised as much as BISL in terms of autochthonous macroalgal primary productivity and biomass, but these parameters differ sharply between them and BISL. Macroalgae such as *Enteromorpha* spp. and *Ulva* sp. can develop in Sandymount in spring/summer but reach very limited biomass in comparison to BISL. In Blackrock, the instability of the sediment does not permit macroalgae to develop. Stranded algae are however common on this site, maybe

originating from adjacent rocky shores of Blackrock and Dun Laoghaire. Qualitative information presented in the Table 2.1 gives indications about macrophytes occurrence in each of these three intertidal sites. It has been compiled based on field observations made during the course of this study.

Table 2.1: Field work observations on the types of macrophytes present on the three intertidal sites studied during this study. +++++: extremely abundant, ++: slightly abundant, X: present, o: observed but negligible quantities. Grey shading indicates that these observations were only made on detrital stranded algae.

Sources	BISL	Sandymount	Blackrock
Chlorophyta	X (+++++)	X (++)	X
Ochrophyta	o	X	X
Fucales			X
Kelps			X
<i>Ectocarpus</i>		X	X
Rhodophyta			X
Saltmarsh plants	X		

2.2.2. Subtidal sites

The subtidal seabed of Dublin Bay (<20m depth) is dominated by well-sorted fine sands with relatively low silt-clay levels (<10%) and low organic carbon contents (Harris, 1980; ERU, 1992). Most of the bay (including the sampling sites) hosts a benthic community intermediate between the shallow *Venus* and *Abra* community and similar to the *Acrocnida brachiata*/*Clymene oesterdi* community of Glémarec (1969), as thoroughly described by Walker and Rees (1980). These authors reported the benthic fauna as fairly rich, both in terms of abundance and species richness. Two sites were studied in the subtidal area (Fig. 2.1). The north site is likely to be influenced by organic matter inputs conveyed with the Liffey plume, while the south site is directly exposed to the Irish Sea waters entering the bay from the south-east.

2.3 Temporal evolution of the Liffey river flow

The evolution of the Liffey river flow from October 2004 to September 2006 is represented on Figure 2.2. These data were recorded at Leixlip dam outflow by the Electricity Supply Board (ESB).

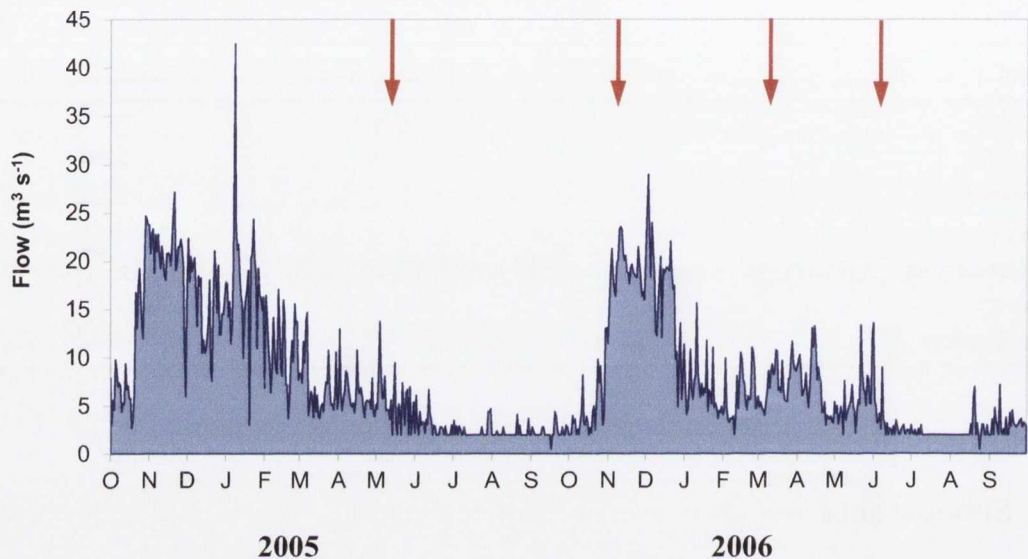


Figure 2.2: Temporal variations of the Liffey river daily mean flow between October 2004 and 2006 (Data recorded at Leixlip by ESB). Red arrows indicate sampling dates.

During the time of the study, the Liffey flow was characterised by normal seasonal variations with a higher discharge in winter and a low flow in summer. Winter discharges were moderate compared to some previous years (e.g. 2000/2001, 2002/2003) with a mean flow of $16.5 \text{ m}^3 \text{ s}^{-1}$ from November 2004 to March 2005, and $12.3 \text{ m}^3 \text{ s}^{-1}$ from November 2005 to the 1st March 2006. During the winter 2005/2006, higher discharge only occurred in November and December (from 10 to $29 \text{ m}^3 \text{ s}^{-1}$), before early falling down to low levels in January ($<15 \text{ m}^3 \text{ s}^{-1}$). These winter periods both differ from previous years (e.g. in 2000: max flow of $88 \text{ m}^3 \text{ s}^{-1}$, 2002: max flow of $68 \text{ m}^3 \text{ s}^{-1}$) by the quasi absence of floods events, as only one day spate with a flow superior to $30 \text{ m}^3 \text{ s}^{-1}$ was recorded (January 2005). The low water period ($<5 \text{ m}^3 \text{ s}^{-1}$) was quite extended during the summer 2005, lasting from June to October.

2.4. Sampling strategy

2.4.1. Biological compartments studied

2.4.1.1. Sources

All the potential sources of food available to consumers were collected for each sampling period and each site studied. These sources of food can be classified in three main categories:

- **Primary producers:** these include fresh and/or detrital macroalgae, epipellic microphytobenthos, saltmarsh phanerogams and terrestrial macrophytes from the catchment. Saltmarsh plants were only present in BISL. Terrestrial plants sampled corresponded to the leaves of the main species present in the catchment.

- **Reservoirs of particulate organic matter** sampled on the studied sites: these correspond to the suspended Particulate Organic Matter (POM) and Sedimentary organic matter (SOM). POM from the intertidal sites was sampled only from the 2nd survey (November 2005).

- **End-member sources of POM** entering the bay. These end-member sources were the marine POM from the open sea and POM from the catchment. POM inputs from the catchment are delivered essentially by rivers, among which the Liffey constitutes the main source with 75% of the freshwater particulates (Wilson, 2005), and by the sewage effluent discharged into the estuary from the Ringsend Sewage Treatment Plant (STP). The open sea POM was sampled off the bay, away from terrestrial influence, in order to get the typical signature of marine-derived organic matter (east of Kish Bank (53°17,16'N; 05°52,07'W, Fig. 2.1). Freshwater POM from the Liffey river was sampled just before the Islandbridge weir (11kms upstream the river mouth, Fig. 2.1) to avoid tidal influence.

2.4.1.2. Fauna

The dominant species of benthic macro-invertebrates and fishes were sampled in BISL and at the subtidal sites. Dominant primary consumers were sampled in the intertidal sites of Sandymount and Blackrock. The meiofauna compartment was also considered at BISL through the nematodes, which represent 95% of the meiofauna (Bourke, 2001).

2.4.2. Sampling timing

Four sampling surveys were carried out seasonally from 2005 to 2006: in May-June 2005, November-December 2005, March-April 2006 and June-July 2006. The choice of these sampling dates was driven by the *a priori* seasonal variability in the sources production or availability (higher river discharge in winter, spring phytoplanktonic and macroalgal blooms and autumnal seaweed decay) as well as the disponibility of the Celtic Voyager for sampling operations in the subtidal sites.

For each survey, a time-lag was set between the collection of all potential food sources and the consumers to account for the delay in the incorporation of new dietary sources in the consumers tissues. This time-lag was set at one month based on the few data on isotopic turnover rates of species similar to the ones studied here published at the time the study was designed and sampling started (Fry & Arnold, 1982; Tominaga *et al.*, 2003; Jardine *et al.*, 2004; Witting *et al.*, 2004; Suzuki *et al.*, 2005). For each survey, the fauna was thus collected one month after sampling all the potential food sources, except for the subtidal sites where the sampling of all biological compartments had to occur at the same date, due to the vessel booking constraints.

2.5. Variables studied

2.5.1. Environmental factors

The environmental variables considered were the salinity and the water temperature. These measurements were performed seasonally at each of the POM sampling sites,

concomitantly to the water sampling for the other hydrobiological variables. Measurements were made using a WTW LF196 thermosalinometer.

2.5.2. Characterisation of the seston

The variables detailed below were measured to better characterise the seston nature and quality. These were measured for each of the seston *reservoirs* and *end-members* analysed. Samples collection for these analyses was carried out at the same stations where POM samples were collected for stable isotope analysis, *i.e.* at:

- the intertidal sites (Bull Island South Lagoon, Sandymount, Blackrock)
- the subtidal sites (north and south sites)
- the “Kish bank” offshore station, where the open sea POM was collected
- in the Liffey river at Islandbridge

Water sampling for these analyses consisted in the collection of three replicates of ~5L of water, 20cm below the surface at Islandbridge and at the intertidal sites, and at 2m depth for the subtidal sites and at “Kish bank”. In intertidal areas, the sampling was carried around high tide slack. Samples were kept in a dark cool box and within 2hrs after collection, the suspended matter was collected by filtration onto pre-combusted (550°C, 4h) Whatman GF/F filters. For the Chlorophyll *a* analyses, GF/C filters were used. The following variables were measured for each water sample.

2.5.2.1. Total Suspended Matter (TSM)

Concentrations of TSM were measured after drying the filter at 60°C to constant weight and reweighing it. The concentrations of TSM are expressed in mg DW l⁻¹.

2.5.2.2. Particulate Organic Carbon and Nitrogen (POC and PON)

Water samples were prefiltered through a 230µm mesh to remove zooplankton and large detritus and then filtered onto pre-combusted (550°C, 4h) Whatman GF/F filters. The particulate matter thus collected was then acidified (10% HCl) to remove

inorganic carbonates, thoroughly rinsed with Milli-Q water, dried at 60°C and stored at -20°C until analysis. Concentrations of particulate organic carbon and nitrogen were determined using a CHN elemental analyser, and expressed in $\mu\text{g l}^{-1}$.

2.5.2.3. Chlorophyll *a* and phaeopigments

Chlorophyll *a* (Chl *a*) is commonly measured as an indicator of aquatic micro-algae biomass. Sample volumes used for these pigments analysis were between 0.5L and 1L, depending on the seston concentration. Chl *a* and phaeopigments concentrations were determined according to the standard procedure by Aminot & Rey (2000), using a Shimadzu UV 1601 spectrophotometer. Pigments were extracted with 97.8% Acetone, in the dark and on priory frozen filters. Following homogenization using an electrical homogeniser, the homogenate solution was filtered into the spectrophotometer cuvette using a Whatman disposable 0.45 μm nylon membrane “Autovial” to eliminate filters residus. The absorbance was read at 665nm and 750nm wavelengths. Following these readings, 0.1ml of 1% by volume HCl was added into the cuvette. After exactly 2min, the absorbance values at each wavelength were measured to determine total phaeopigments concentration. The Chl *a* and phaeopigments concentrations ($\mu\text{g l}^{-1}$) were calculated according to the equations of Lorenzen (1967).

2.5.3. Carbon and nitrogen stable isotopes

2.5.3.1. Sampling

Suspended POM

Replicates samples of 2L were taken 20cm below the surface, using a weighed plastic bottle, at Islandbridge for the Liffey river and at the intertidal sites. At the “Kish bank” station and at the subtidal sites studied, samples were collected by pumping water at 2m depth. Sewage effluent samples from Ringsend STP were provided by Dublin City Corporation. For each survey, sampling of the POM from the Liffey river and the sewage effluent was carried out weekly during three weeks.

Intertidal sources and consumers

Sediments were collected in triplicates at low tide by scraping the top centimeter of the sediments surface. They were kept in the dark and brought back to the laboratory for SOM extraction.

Microphytobenthos was sampled where dense cover was visible by scraping off the upper 5mm of the sediment and brought back to the laboratory for extraction.

The main fresh and/or detrital macroalgae present at each of the sites were collected by hand at low tide. The species identification of detrital kelps collected proved difficult in most cases as only parts of the fronds were found. Saltmarsh plants were hand-picked in Bull Island South Lagoon from the adjacent lower saltmarsh where *Halimione portulacoides* is the dominant species. *Spartina anglica*, *Salicornia europaea*, *Limonium humile* and *Spergularia* sp. were sampled when present. Detrital leaves from common angiosperm species in the catchment were collected in November 2005 from the south of Dublin Bay and BISL. All these primary producers were kept at -20°C until their preparation for isotopic analysis.

Nematodes, which represent 95% of the meiofauna abundance (Bourke, 2001), were sampled by collecting the upper 2cm of the sediment where most of the meiofauna use to occur (Coull & Bell, 1979) and brought back to the laboratory for extraction.

Macroinvertebrates were collected at low tide by sieving hand-shoveled sediments on a 1mm mesh. Fish and larger mobile invertebrates (i.e. crustaceans) were caught using a seine net (1cm mesh size). Invertebrates were brought back alive to the laboratory and fishes were directly frozen.

Subtidal sources and consumers

Only the south station was sampled in May 2005. The north station was included in the study from November 2005. Samples collection in the subtidal areas was carried out from the R. V. Celtic Voyager.

SOM samples were collected using a Reineck Box core or a multi-core device when weather was permitting. The upper 2 cm of sediments were collected and brought back to the laboratory for SOM extraction.

Macroalgae are almost inexistent in the area sampled, as reported by Brennan *et al.* (1994) and confirmed by divers from TCD SubAqua club during our survey. Detrital

kelps and unidentified algae probably originating from the surrounding rocky areas of Howth and Dun Laoghaire were however collected with the trawl and kept for analysis.

Zooplankton was collected using a plankton net with a 176 μm mesh size.

Macro-invertebrates and fishes were collected using various sampling gears, to get the different components of the fauna. The infauna was collected using a Day Grab and a Reineck Box Core. The epifauna was sampled using the epibenthic sledge, and a beam trawl was used for larger invertebrates and fishes. The grabs and cores were deployed 8 to 15 times while the sledge and the trawl were dragged on a 500m distance. Samples collected from the grabs, cores and sledge were sieved on a 0.5mm sieve. Invertebrates were brought back alive to the laboratory and fishes frozen directly after collection.

2.5.3.2. Samples processing

POM

Within 2hrs after collection, water samples were prefiltered through a 230 μm mesh to remove zooplankton and larger particles. POM was obtained by filtering 1 to 2L (300mL max. for the sewage effluent) onto a pre-combusted (550°C, 4h) Whatman GF/F filter. Filters were briefly acidified with 1N HCl to remove inorganic carbon, thoroughly rinsed with Milli-Q water, dried at 60°C and stored at -20°C until analysis.

SOM

Muddy sediments collected were sieved on 250 μm to remove macroscopic animals and detritus. Sandy sediments were sieved on 63 μm to remove macroscopic organisms and separate sand grains from most of the SOM. The filtrate was dried at 60°C and then ground to powder using a mortar and a pestle. A sub-sample of the SOM sample (<2g) was then acidified (10% HCl), but was not rinsed to prevent any loss of organic matter (Riera *et al.*, 1996). It was dried (45°C) under a fume extractor to evaporate acid, then mixed with Milli-Q water and dried again to eliminate acid residues (twice). The SOM samples were ground to a fine powder and stored (-20°C) prior to analysis.

Microphytobenthos

Mobile benthic diatoms were extracted from the sediment according to the method of Couch (1988) as modified by Riera & Richard (1997). Sediments were thus

spread on flat trays to form a 1cm layer and were covered with a Nytex nylon mesh (63µm), which was itself covered by a 5mm layer of clean and combusted (550°C, 6h) sand (63 to 250µm grain size). The trays were kept moist by spraying filtered seawater (0.45µm) and left under light for 12h, allowing the mobile microalgae to migrate to the sediment surface. These were then collected by scraping the upper 2mm of the sediments, sieved through a 63µm mesh to separate them from the sand grains, and filtered on combusted GF/F filters. Examination of the water filtrate under the microscope revealed that the water contained only microalgae. The filters were quickly acidified (1N HCl), rinsed with Milli-Q water, dried (60°C) and kept frozen (-20°C) until analysis.

Larger primary producers

Algae, terrestrial and saltmarsh plants samples were cleaned of epibionts, briefly washed in 1N HCL, rinsed with Milli-Q water, dried at 60°C, and ground to a powder using a mortar and pestle.

Nematodes

Sediments collected for nematode extraction (~2L) were homogenised and sub-samples were gently sieved through successively 250µm and 63µm sieves to reduce the sample volume and eliminate larger organisms. Nematodes were then hand-picked from the concentrated sediment using a fine hook, which allowed their collection alive and without chemical contaminants. Between 120 and 160 individuals per replicate were collected for muddy sediments (BISL, Sandymount). In clean sediments (Blackrock), 200 individuals at least were collected for each replicate. Nematodes were then starved in filtered seawater (0.45µm, 12h) to clear their gut and collected by filtration on pre-combusted GF/F filters, which were then treated as for POM samples.

Macro-invertebrates and fish

The body size of consumers is an important factor to take into account as their diet, and hence their isotopic composition, can vary due to ontogenetic shifts (Hentschel, 1998; Giraldo *et al.*, 2011). To minimize this source of variation, isotopic analyses were carried out on adult invertebrates of similar size, for all sites and all seasons.

Regarding fish, juveniles were differentiated from adults and labelled as such. Only adults were analysed at the subtidal sites, while both categories were found for some species in Bull Island South Lagoon.

Bivalves were manually cleaned of epibionts. These and other invertebrates were kept alive in filtered seawater (0.45µm) from their sampling site for 12 to 24h to purge their gut contents, taking care to separate them according to their feeding behaviour, and then killed by freezing. Invertebrates and fishes were identified down to species level as much as possible. Shells or external skeletons were removed in the case of molluscs and crustaceans respectively. All animals were briefly acidified (1N HCl) to remove any carbonate debris, thoroughly rinsed with Milli-Q water, dried at 60°C, ground to a fine powder with a mortar and pestle and stored at -20°C until analysis. Invertebrates were analysed whole. Where individual dry weight was insufficient for the mass spectrometer requirements, a few specimens were then pooled. Only dorsal muscle tissue was analysed for fish.

All samples were powdered, weighed according to sample type (from 0.9mg for animal tissues up to 40mg for SOM) and wrapped into tin cups before the analysis.

2.5.3.3. Mass spectrometer measurements

Carbon and nitrogen stable isotopes analyses were carried out in Trinity College Dublin by Dr. Robbie Goodhue and myself.

These analyses were performed by Continuous Flow Isotope Ratio Mass Spectrometry (CF-IRMS), using a CE Instruments 1112 Flash elemental analyser (EA) interfaced to a Thermo Delta^{plus} mass spectrometer via a Conflo III. The amino acid L-Alanine was used as a working standard. The isotopic compositions ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) were expressed as the relative difference between isotopic ratios in the sample and in conventional standards (Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen) according to the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = [(R \text{ sample} / R \text{ standard}) - 1] \times 1000$$

$$\text{where } R = {}^{13}\text{C}/{}^{12}\text{C} \text{ or } {}^{15}\text{N}/{}^{14}\text{N}$$

Analytical precision was ~0.2 ‰ for both carbon and nitrogen.

The C:N ratio was also determined for each sample, from the percentage of organic carbon and organic nitrogen in each sample.

CHAPTER 3

Impact of sewage and green macroalgal blooms on the food web of an intertidal mudflat: the case of Bull Island South Lagoon

3.1. Introduction

By affecting the composition of benthic communities, anthropogenic disturbances resulting in the organic enrichment of natural systems have been shown to alter the structure and functioning of associated food webs (McClelland & Valiela, 1998). The effects of organic matter loadings on benthic communities have been extensively studied and are well documented (Pearson & Rosenberg, 1978; Gray, 1982; Weston, 1990; Gray *et al.*, 2002). In comparison, few quantitative studies have considered the effect of macroalgal blooms on food web structure, and the patterns of incorporation of this algal carbon through the food chains.

Macroalgal blooms driven by the eutrophication of coastal systems are a major ecological and economic problem whose occurrence, frequency and magnitude have dramatically increased worldwide over the past 50 years (Valiela *et al.*, 1997; Ye *et al.*, 2011). Depending on local hydrodynamics, these blooms can exist as vast aggregations of loose algae drifting above the seabed and eventually stranding on the shore or, in sheltered systems, as stationary and cohesive mats formed of attached algae embedded in the sediments. They are predominantly attributed to the proliferation of fine and fragile but fast-growing green algae of the genus *Ulva* (including the genus formerly known as *Enteromorpha*), *Chaetomorpha* and *Cladophora* (Fletcher, 1996; Ye *et al.*, 2011). Ecological effects of these blooms of ephemeral macroalgae have been relatively well documented. When macroalgae start to develop, they may procure food and shelter for some species, but as algal biomass increases, deleterious effects on the seabed and its communities can appear. Macroalgal mats can thus modify the chemistry of the water column and sediments (through high uptake of oxygen at night and of inorganic carbon for photosynthesis; Van Alstyne *et al.*, 2015) and can strongly alter the diversity and community structure of benthic organisms at different levels including primary producers, invertebrates and fish, due to antagonistic effects on species and/or feeding guilds (see reviews by Valiela *et al.*, 1997, Raffaelli *et al.*, 1998, Grall & Chauvaud, 2002; Lyons *et al.*, 2014, Korpinen & Bonsdorff, 2015). These effects have been reported, to different degrees, in a variety of environments (from macro- to atidal and sheltered to exposed systems) and for different types of habitats, such as seagrass beds, subtidal sand and mud habitats, intertidal sandy, muddy and rocky habitats (e.g. Bolam *et*

al., 2000; Cummins *et al.*, 2004; Worm & Lotze, 2006; Lyons *et al.*., 2014; Korpinen & Bonsdorff, 2015). The influence of macroalgal blooms on the trophic functioning of benthic systems has not been as much studied. Macroalgal mats are indeed highly productive and the large amount of organic matter generated can either be exported in particulate and dissolved forms to surrounding areas, or supply the local sediments and its infauna during the course or the bloom, as well as during the burial process (Owens & Stewart, 1983; Pregnall & Rudy, 1985; Rossi, 2006; Ouisse *et al.*, 2011). Hence, Dolbeth *et al.* (2003) found that algal mats enhanced secondary productivity during a macroalgal bloom in the Mondego estuary. During the decaying stage, bioturbation by the infauna can magnify the decomposition of the mats (Raffaelli, 2000; Nordstrom *et al.*, 2006) and detritivorous species may play a notable contribution in the recycling of this organic matter by assimilating a portion of it (Moore *et al.*, 2004; Rossi, 2007). The majority of studies investigating the effects of macroalgal blooms focused on species-specific or primary consumers trophic guilds responses (e.g. Warwick *et al.*, 1982, Raffaelli *et al.*, 1991; Norkko, 1998; Thiel & Watling, 1998; Norkko *et al.*, 2000, Raffaelli, 2000; Österling & Pihl, 2001, Green *et al.*, 2014), revealing contrasted effects from strongly positive to highly negative. In estuarine and sheltered systems, eutrophication has been shown to negatively impact suspension-feeders, while deposit-feeders (e.g. Bachelet *et al.*, 2000; Keats *et al.*, 2004) and/or grazers (Ouisse *et al.*, 2011) were favored. On the other hand, other authors showed suspension-feeders to be fostered by the presence of *Ulva* mats on open sandy beaches (Quillien *et al.*, 2015b). Such differences may be linked to habitat-specific responses (Lyons *et al.*, 2014), and/or correspond to differential and successional responses of benthic organisms to increasing pressure intensity, as evidenced by Tewfik *et al.* (2007) who found distinct functional groups of primary consumers to predominate along an eutrophication gradient. The sound understanding of the effects of a disturbance on the communities functioning and how organisms deal with it however requires getting a broad picture by investigating trophic responses at both species and community level. But few studies have investigated *in situ* the impact and/or role of macroalgal mats on the whole trophic structure and on pathways of energy transfer through the whole food web (Ouisse *et al.*, 2011; Quillien *et al.*, 2016). In an extensive study assessing the food web structure of seagrass communities along an eutrophication gradient in Atlantic Canada, Coll *et al.* (2011) found, at the most impacted sites, grazers and detritivores to be favored whereas groups at higher trophic levels (omnivores, predators) decreased. They also evidenced a reduction of the food web

complexity with fewer trophic groups, fewer trophic pathways and a reduction of the food web length. In an open and dynamic sandy beach enduring serious green tides of opportunistic *Ulva*, Quillien *et al.* (2016) similarly reported a simplification of the food web with an alignment of trophic pathways, supported directly and indirectly by the green algae. In sheltered intertidal flats, such detrimental effects of macroalgal mats on the overall trophic structure are not so well documented (but see Ouisse *et al.*, 2011).

Surrounded by a conurbation of over one million inhabitants, Dublin Bay has a long eutrophication history, receiving substantial amounts of sewage particulate organic matter (POM) and nutrients in the lower estuary. In the estuarine and semi-enclosed mudflat of Bull Island South Lagoon (BISL), this coastal eutrophication has induced for over a century the ephemeral development of macroalgal mats of *Enteromorpha* spp., fueled directly by nutrients or through sediment remineralisation of the sewage POM (Adeney, 1908; Jeffrey *et al.*, 1992; Khan, 1998). Interestingly, the influence of this recurrent macroalgal bloom on the trophic functioning of the mudflat has, to our knowledge, never been investigated. This is even more surprising considering that this site constitutes an important feeding ground for migratory birds and waders in winter and is therefore protected under several national, European and international designations (e.g., National Nature Reserve under the national Wildlife Act, Special Protection Area under the EU Birds directive, UNESCO World Biosphere Reserve). Through a network analysis, Wilson & Parkes (1998) described POM from sewage to play a major role for Dublin bay food web, being mainly processed by detritivores. However, their analysis was carried out at the whole bay scale and did not consider habitat specificities and other anthropogenic constraints (such as the macroalgal bloom) at small spatial scale. The upgrade of the sewage treatment plant (STP) in 2003 resulted in a strong drop of the particulates released in Dublin Bay, while inorganic nitrogen levels were slightly reduced (O'Higgins, 2005). As a result of this upgrade, the role of sewage POM on the bay food web was considered to be very much reduced (Wilson *et al.*, 2007) but this was not assessed *in situ* and trophic effects might reveal more subtle at small spatial scale especially in adjacent habitats such as BISL.

Carbon and nitrogen stable isotopes have largely demonstrated to be a relevant tool for the study of marine benthic food webs, in particular to follow the fate of various organic matter sources, delineate trophic interactions, and to assess the trophic levels of consumers and the food web length (but see details in the general introduction).

Moreover, $\delta^{15}\text{N}$ has been successfully used as an indicator of urbanization and anthropogenic nutrient enrichment since it is typically higher in primary producers tissues, and potentially in higher trophic levels, of such stressed environments (McClelland *et al.*, 1997; Carlier *et al.*, 2008).

Considering the *a priori* influence of sewage in stimulating the development of the macroalgal bloom in BISL (Brennan *et al.*, 1994; Jeffrey *et al.*, 1995; Khan, 1998), and given the changes of the sewage effluent composition since the upgrade of the STP in 2003, this chapter aims to delineate the trophic functioning of Bull Island South Lagoon, to examine the trophic role played by sewage either directly through organic particulates or indirectly through the macroalgal mats it sustains, and to further investigate the influence of the macroalgal mats on the food web structure. This was undertaken through a stable isotope analysis of the benthic food web over time (4 surveys) by assessing two main hypothesis: first, that the bloom-forming green algae (*Enteromorpha* spp.) exhibits a high $\delta^{15}\text{N}$ signature, indicative of the watershed (sewage) influence; second, that the green algal mat influences species-specific diets over time and integrates the benthic food web mostly via deposit-feeders. This study also aims to depict the effects of the green algal mat on the trophic functioning of this sheltered mudflat and more specifically, on the diversity of trophic pathways and on the food web length.

3.2. Supplementary material and methods

3.2.1. Samples collection

Fauna were sampled within the central part of BISL (Fig. 3.1). All potential sources of organic matter to the consumers, local or entering the site with the tide, were considered for collection. The methodologies applied for the collection, processing and analyses of samples are detailed in Chapter 2. The green algae were collected from the mats in May 2005 and June 2006 and from the sediments where they were partly buried in November 2005.

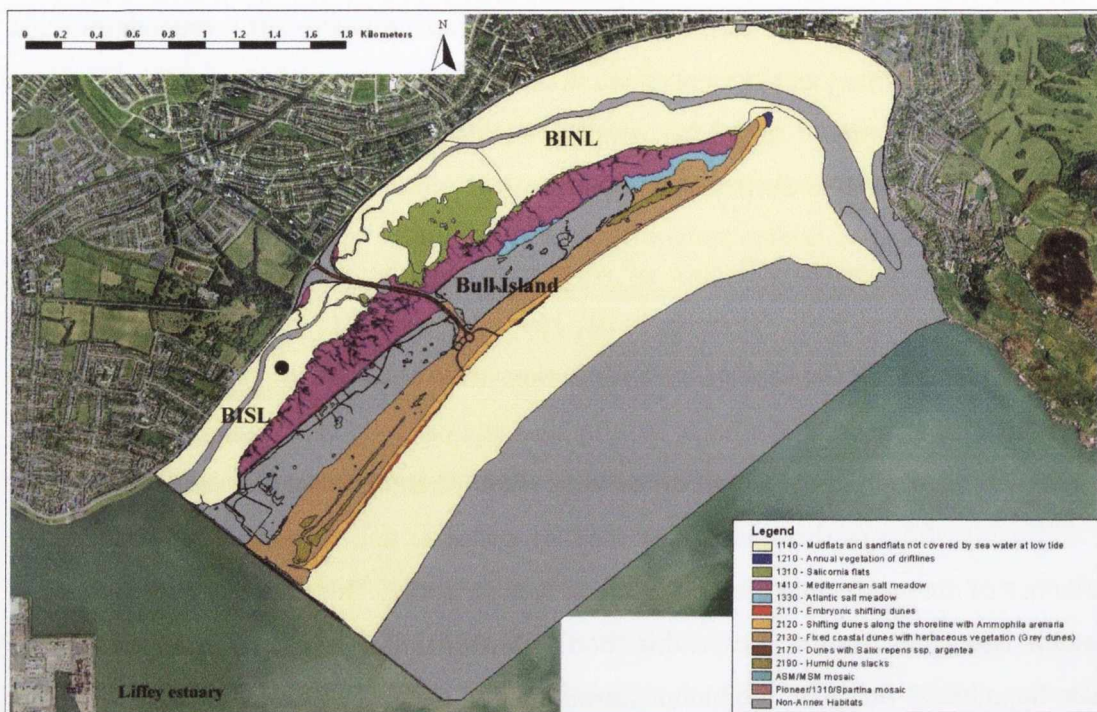


Figure 3.1: Map of Bull Island (NW Dublin Bay), locating the sampling site (black dot) in the intertidal muddy sandflat of so called Bull Island South Lagoon (BISL). BINL stands for Bull Island North Lagoon. Habitats given in the legend relate to the list from Annex 1 of the EU Habitat Directive. Map modified from McCorry & Ryle (2009).

3.2.2. Statistical analysis

Statistical analyses were performed using SPSS. Data normality was verified and the homoscedasticity tested using the Levene test before choosing for parametric or non-parametric analyses. Temporal variation in the isotopic composition of sources and secondary consumers was tested using One-way parametric or non-parametric (Kruskal-Wallis) ANOVA as appropriate, followed respectively by LSD or Dunn's (Zar, 1999) *post hoc* tests. As *S. europaea* was only sampled on two surveys, the T-test was used instead. For the river Particulate Organic Matter (POM) and sewage POM data, which integrated a weekly sampling within each sampling survey, a nested analysis of variance was performed to take into account the weekly variability. The data were inverse-transformed prior to the test to obtain better homogeneity of variances among groups and in some cases, to also correct them for their non-normality. In case the data transformation did not improve the requirements of normality (*i.e.* for the sewage POM $\delta^{15}\text{N}$), a non-parametric Kruskal-Wallis (KW) test was used instead. Temporal and inter-specific variability in the isotopic compositions of the primary consumers were compared

using 2-way ANOVAs. A more stringent significance level of 0.01 was applied to overcome the heteroscedasticity observed between samples, as recommended by Pallant (2007). In the case of an interaction between the two factors, one-way parametric or non-parametric (Kruskal-Wallis) ANOVA was then performed as appropriate.

3.2.3. Dietary analysis

A feeding strategy was assigned to each species based on the literature (Table 3.5). Primary consumers were categorised according to their reported feeding strategy (FS), i.e. as active suspension-feeder (aSF), grazer (G), surface deposit-feeder (SDF), sub-surface deposit-feeder (SSDF) or interface-feeder (IF). Species feeding at higher trophic levels were labelled as predators, whether they may be predators or scavengers. A distinction was further made for fishes according to their foraging behavior (planktivorous, benthivorous, piscivorous) as reported in the literature and FishBase (Froese & Pauly, 2013).

Dietary analyses described thereafter were carried out for the dominant primary consumers present on site. The probability distributions of the food sources contributions to the diets were estimated using the Bayesian isotopic mixing model SIAR (Parnell *et al.*, 2008; Jackson *et al.*, 2009; Parnell *et al.*, 2010) computed in the R statistical program. The Bayesian approach is a robust method for situations where the high number of sources ($> n$ isotopes + 1) exclude the use of linear mixing models and therefore the computation of the exact proportional contribution of end-members to a mixture. Moreover, the SIAR mixing model enabled to account for the quantified sources of uncertainty in the model inputs by incorporating the isotopic signatures of each individual consumer as well as the variance associated to the sources and to the trophic step fractionation factors (as mean and SD values). Sources used for these mixing models were selected based on the analysis of both isotopic data and non-isotopic constraints (see sections 3.3.4 and 3.4 for details).

Considering that the C and N isotopic fractionation between a consumer and its source of food ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$) can vary depending on multiple factors (McCutchan *et al.*, 2003; Vanderklift & Ponsard, 2003; Martinez del Rio *et al.*, 2009), it is preferable when using values from the literature to choose trophic-step fractionation measured for related

species with analogous diets and analysed using similar laboratory methods. However there are few values of fractionation factors available for marine suspension-feeders and deposit-feeders such as the mollusc and polychaete species studied here. Only work by Yokoyama *et al.* (2005a) and Dubois *et al.* (2007) produced isotopic fractionation data for intertidal bivalves using sample preparation methods similar to the present study (analysis of whole body, acidification treatment – see Chapter 2). They measured values of $\Delta\delta^{13}\text{C}$ and especially $\Delta\delta^{15}\text{N}$ close to the ones reported in the review by Post (2002a) for aquatic species. Trophic-step fractionation values based on the results from these three studies were therefore used in the present work, assuming fractionation factors to be the same for all species, seasons and sources. The variance associated with the values obtained from these studies was also considered in the mixing models. The fractionation factors thus used were of $3.4 \pm 0.7 \text{ ‰}$ for $\Delta\delta^{15}\text{N}$ and $0.4 \pm 1.3 \text{ ‰}$ for $\Delta\delta^{13}\text{C}$ (corresponding to the weighed mean and pooled SD of the estimates from Post, 2002; Yokoyama *et al.*, 2005; and Dubois *et al.*, 2007).

The models were also run using a N isotopic fractionation of $2.3 \pm 1.75 \text{ ‰}$ (McCutchan *et al.*, 2003) - corresponding to the mean $\pm$ SD reported by these authors for consumers raised on plants or algal diets - since this $\Delta\delta^{15}\text{N}$ is also commonly used as an average fractionation factor for herbivores. It did not lead to significant changes in our contribution estimates, because of the good isotopic distinction among the sources signatures. Indeed, biais in the estimates of sources contribution to a diet are much reduced when the signatures of these sources are well differentiated (Vander Zanden & Rasmussen, 2001; McCutchan *et al.*, 2003). We are therefore confident in the reliability of our results for the estimations of the sources contributions to the diets of the consumers.

3.2.4. Trophic levels

Trophic positioning relatively to a baseline can be determined using $\delta^{15}\text{N}$ signatures. A trophic level (TL) was thus estimated for each consumer using the equation proposed by Cabana & Rasmussen (1996), in which:

$$\text{TL} = \text{TL}_{\text{base}} + (\delta^{15}\text{N}_{\text{C}} - \delta^{15}\text{N}_{\text{base}}) / \Delta\text{N}$$

where: TL_{base} is the trophic level used as a baseline ($TL_{base} = 2$ in the present case)

$\delta^{15}N_C$ is the $\delta^{15}N$ of the consumer species,

$\delta^{15}N_{base}$ is the average $\delta^{15}N$ of the baseline,

ΔN is the ^{15}N -enrichment per trophic level

A ^{15}N -enrichment value per trophic level of 3.4‰ was used as estimated from the literature for the dietary analysis. Strict primary consumers ($TL=2$) were used as a baseline rather than sources. This permitted to integrate the higher isotopic variability of the food sources (Cabana & Rasmussen, 1996; Post, 2002a) and to avoid using trophic fractionation factors occurring between primary producers and consumers since these factors are reported to be more variable than the ones reported for higher TL (Vander Zanden & Rasmussen, 2001). The primary consumers used for this purpose were the dominant species systematically present along the surveys, i.e. the filter-feeder *C. edule*, the interface-feeders *M. balthica* and *S. plana*, and the deposit-feeders *Hydrobia ulvae* and *Arenicola marina*. Besides being common on site, these five species integrate, through their feeding modes, both the pelagic and benthic production. Trophic levels were calculated per season to account for the temporal variability in the resources availability and their utilisation by primary consumers, as it was observed during this study to induce some temporal variations of the primary consumers $\delta^{15}N$. The uncertainties associated to the estimations of trophic levels were assessed by considering two sources of variations:

- the $\delta^{15}N$ variability recorded for the primary consumers used as the baseline (SD_{base})
- the $\delta^{15}N$ variability associated to each secondary consumer species (SD_C)

An upper and a lower trophic level estimates were therefore calculated as follow:

$$\text{Upper TL} = 2 + [(\text{mean } \delta^{15}N_C + SD_C) - (\text{mean } \delta^{15}N_{base} - SD_{base})] / \Delta N$$

$$\text{Lower TL} = 2 + [(\text{mean } \delta^{15}N_C - SD_C) - (\text{mean } \delta^{15}N_{base} + SD_{base})] / \Delta N$$

3.3. Results

3.3.1. Characterisation of the allochthonous and autochthonous water bodies

This section presents the results of the environmental factors and the hydrobiological variables measured at two end-member stations (the “open sea” station and the Liffey river upstream station of “Islandbridge”) and at the study site (BISL).

3.3.1.1 Environmental factors

Water temperatures at the three stations appear to follow classical seasonal variations for the north-east Atlantic region, with the coldest temperature measured at the end of winter (March 2006, Fig. 3.2).

Salinity measured offshore (“Open sea”) was stable and characteristic of oceanic waters (mean salinity: 33, Fig. 3.3). With a salinity varying from 23 to 30.6, waters entering BISL clearly appear as polyhaline, reflecting the estuarine position of the site. Accordingly with the higher flow recorded for the Liffey river (see Chapter 2), the freshwater influence to BISL was higher in November 2005 (salinity: 23) than in March and June 2006 (salinity: 30).

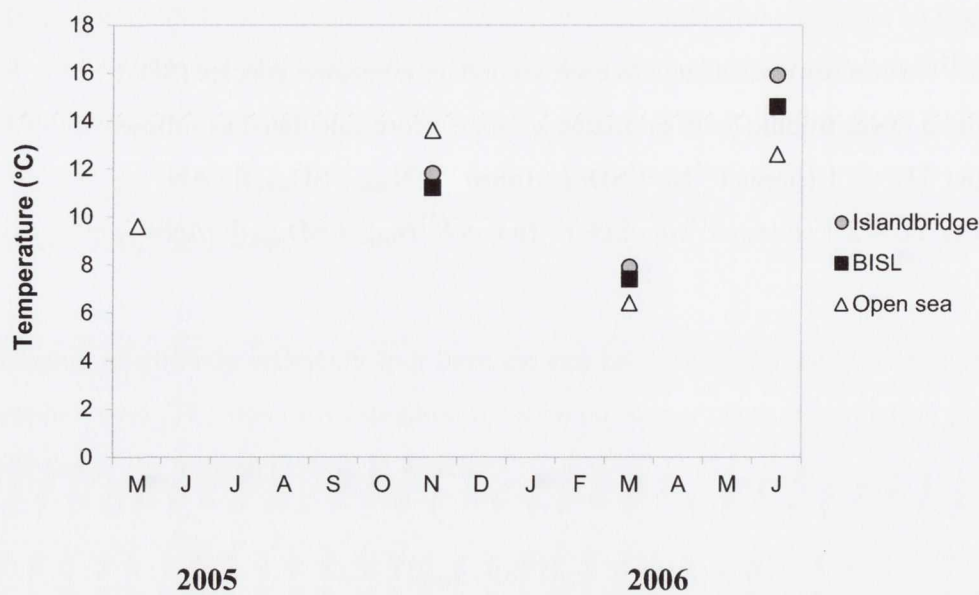


Figure 3.2: Temporal changes of the water temperature from May 2005 to June 2006.

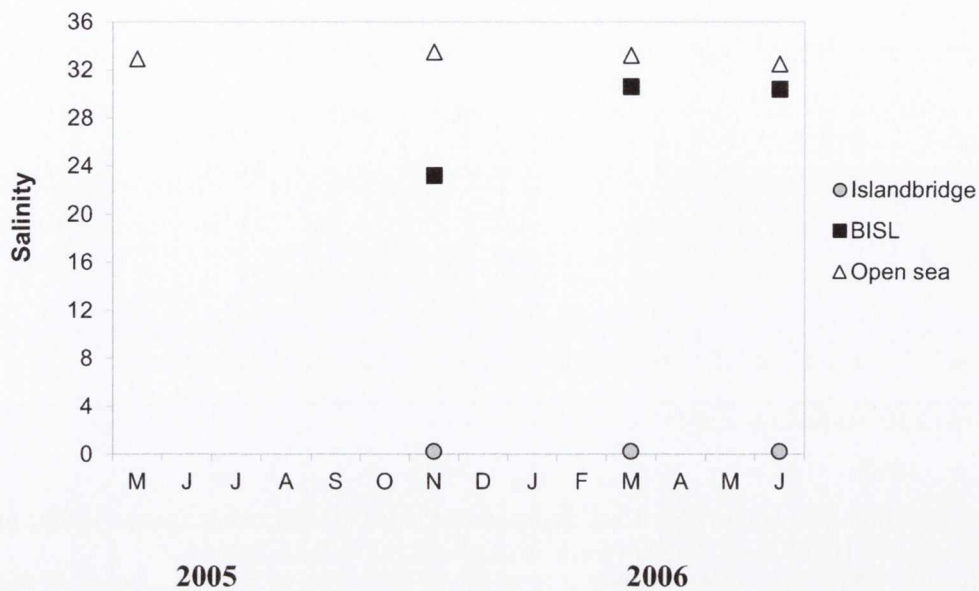


Figure 3.3: Temporal changes of the salinity from May 2005 to June 2006.

3.3.1.2 Hydrobiological variables

Total Suspended Matter (TSM)

Concentrations of Total Suspended Matter (TSM) measured at BISL and Islandbridge are presented in Figure 3.4. Due to laboratory issues, samples from the open sea could unfortunately not be analysed for their TSM concentrations. The TSM levels in the Liffey river were quite low (from 2.5 to 13.2 mg l⁻¹) and consistently lower than the concentrations measured at BISL (from 15.3 to 25.5 mg l⁻¹). TSM concentrations showed similar patterns of variation at both stations with the highest values measured in November 2005.

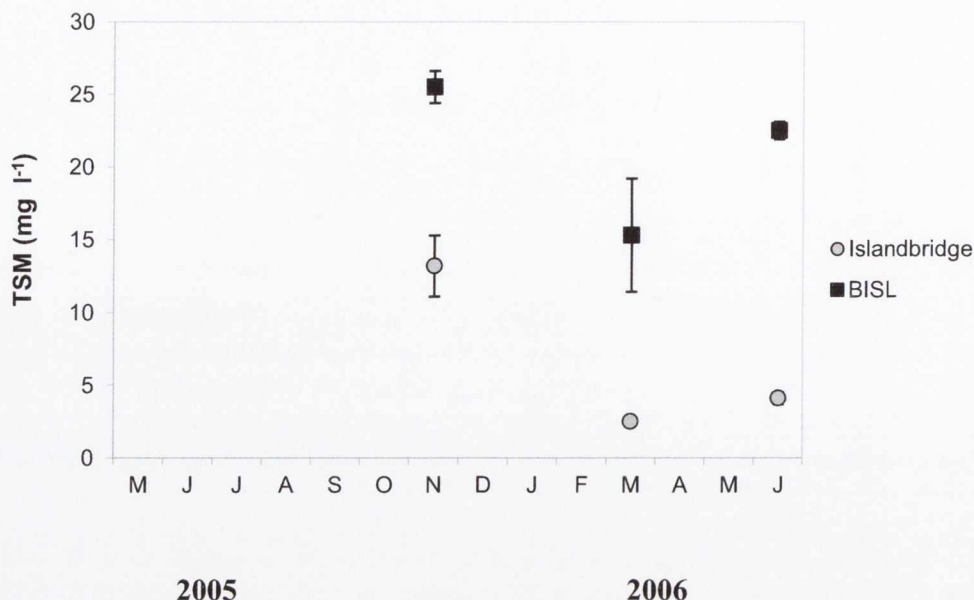


Figure 3.4: Temporal changes of the Total Suspended Matter (TSM) levels (mean $\pm$ SD) at Islandbridge and Bull Island South Lagoon from November 2005 to June 2006.

Particulate Organic Carbon (POC)

Particulate Organic Carbon (POC) measurements give an estimation of the amount of particulate organic material in the water samples. A land to sea gradient of decreasing concentrations was observed independently of the season sampled. The POC levels measured offshore were quite stable and low during the study (mean: $172 \pm 11 \mu\text{g C l}^{-1}$; Fig. 3.5). Concentrations were much higher and variable at Islandbridge (from 461 to $1455 \mu\text{g C l}^{-1}$), where the highest levels were measured in winter. A similar, although less contrasted, seasonal trend was recorded at BISL but unlike the TSM concentrations, POC levels at this site were lower than at Islandbridge.

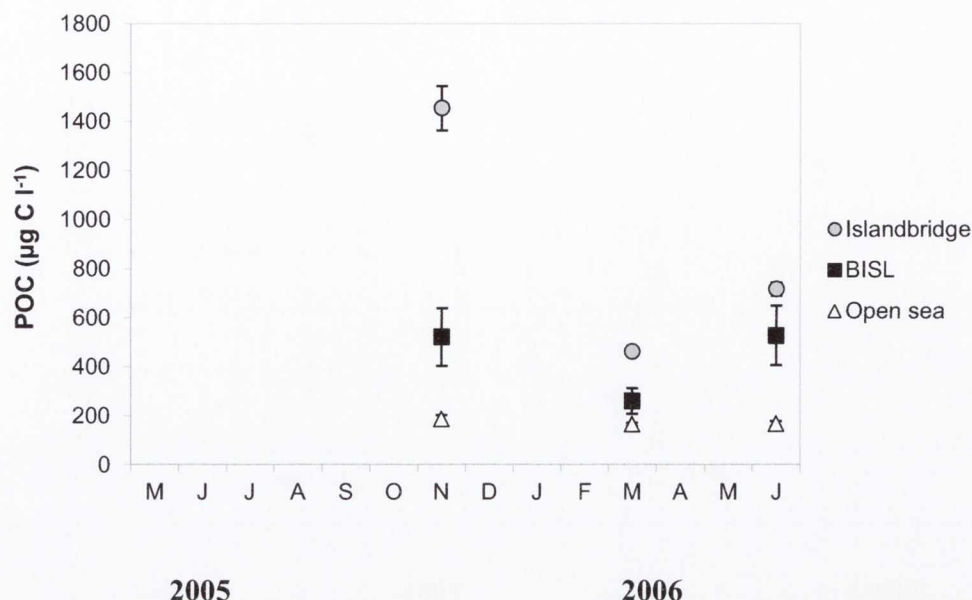


Figure 3.5: Temporal changes of the Particulate Organic Carbon (POC) concentrations (mean $\pm$ SD) from November 2005 to June 2006.

Chlorophyll a (Chl a) and phaeopigments

Temporal changes in the concentrations of Chl *a* and phaeopigments are represented respectively in Figures 3.6 and 3.7.

Marine waters from the “open sea” station exhibited the lowest Chl *a* levels during the study. Concentrations were stable around $0.7 \mu\text{g l}^{-1}$ during the first three sampling periods before increasing to $2 \mu\text{g l}^{-1}$ in June 2006. Surface freshwater (Islandbridge) and estuarine waters from BISL exhibited a stronger seasonal variability of their Chl *a* concentrations, with low levels in winter ($<1.4 \mu\text{g l}^{-1}$) increasing to above $5 \mu\text{g l}^{-1}$ in June 2006, suggesting the occurrence of a spring bloom at the time of the sampling.

Phaeopigments concentrations were higher than Chl *a* concentrations in November 2005 (except in the open sea waters), but were lower than the latter in March and June 2006.

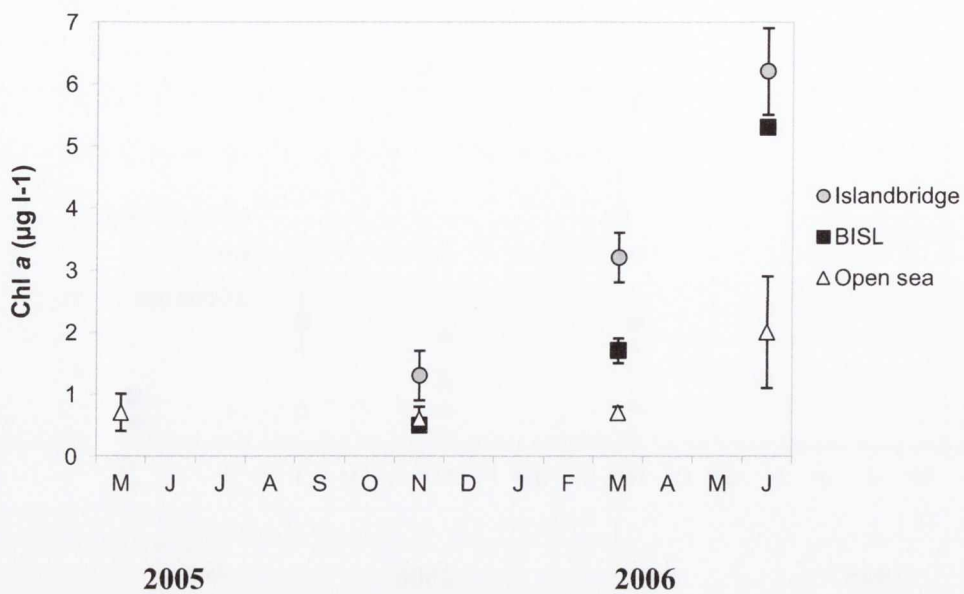


Figure 3.6: Temporal changes of the Chlorophyll *a* concentrations (mean $\pm$ SD) from May 2005 to June 2006.

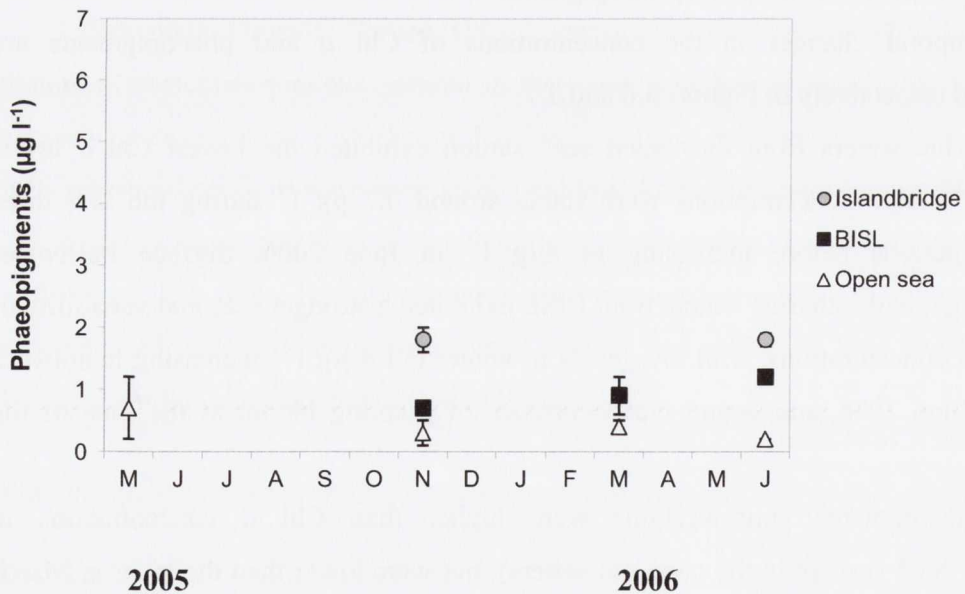


Figure 3.7: Temporal changes of the phaeopigments concentrations (mean $\pm$ SD) from May 2005 to June 2006.

POC / Chl a

In November 2005, the POC/Chl *a* ratio was above 200 at all sites, with average values above 1100 in Islandbridge and BISL (Fig. 3.8). The ratio decreased below 200 in March and June 2006 at both these stations and in June 2006 at the “open sea” station.

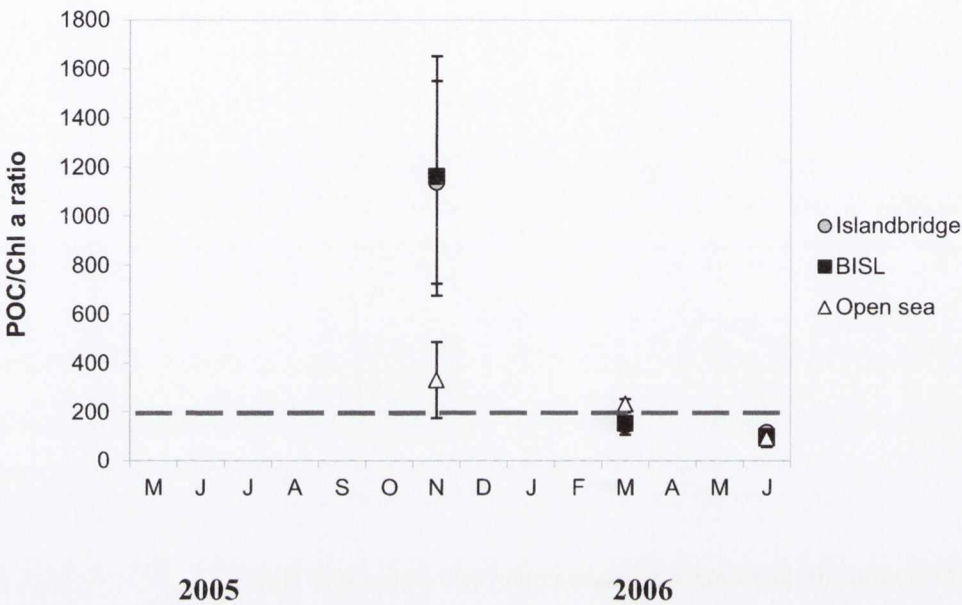


Figure 3.8: POC / Chl *a* ratios (mean ± SD) between November 2005 and June 2006. The dotted line indicates the threshold point of 200 (see Discussion, section 3.4.1).

3.3.2. C:N ratios of the various organic matter sources and reservoirs

The C:N values measured for the end-members POM pools (Liffey POM from “Islandbridge”, the “open sea” POM and the sewage effluent POM) and terrestrial plants are detailed in Table 3.1. Measurements made on the reservoirs and autochthonous sources at BISL are presented in Table 3.2.

Table 3.1 : C:N ratios of the end-members POM pools and terrestrial plants.

	May 2005	November 2005	March 2006	June 2006
Liffey river	6.5 ± 0.3	11.3 ± 0.8	8.6 ± 0.3	6.5 ± 0.5
Open sea	6.8 ± 0.5	12.1 ± 0.4	7.9 ± 0.2	6.9 ± 0.4
Sewage effluent	7.2	6.2 ± 0.5	5.3 ± 0.3	5.8 ± 0.2
Terrestrial plants				
<i>Acer pseudoplatanus</i>	-	34.4 ± 8.7		
<i>Aesculus hippocastanum</i>	-	63.5 ± 1.6		
<i>Fagus sylvatica</i>	-	75.2 ± 12.1		
<i>Populus</i> sp.	-	34.2 ± 0.1		
<i>Prunus</i> sp.	-	72.9 ± 29.9		
<i>Quercus</i> sp.1	-	49.4 ± 6.7		
<i>Quercus</i> sp.2	-	34.9 ± 6.0		
<i>Salix fragilis</i>	-	33.1 ± 15.1		

Table 3.2 : C:N ratios of the primary producers and OM reservoirs at BISL.

	May 2005	November 2005	March 2006	June 2006
POM	-	21.4 ± 2.6	6.0 ± 0.1	6.4 ± 0.5
SOM	7.2 ± 0.1	7.2 ± 0.0	7.0 ± 0.1	8.1 ± 0.4
Microphytobenthos	5.8 ± 0.0	6.1 ± 0.2	5.3 ± 0.0	5.1 ± 0.0
<i>Enteromorpha</i> spp.	9.4 ± 0.8	7.8 ± 0.2	-	9.8 ± 0.7
Saltmarsh plants				
<i>Spartina anglica</i>	11.6 ± 1.6	25.7 ± 3.4	12.4 ± 4.0	18.5 ± 1.8
<i>Halimione portulacoides</i>	13.9 ± 0.3	15.8 ± 1.6	16.8 ± 1.4	20.1 ± 5.3
<i>Salicornia europaea</i>	15.1 ± 0.5	-	-	12.4 ± 2.3
<i>Limonium humile</i>	-	-	9.1 ± 0.2	-
<i>Spergularia</i> sp.	-	-	12.4 ± 2.5	-

The aquatic POM pools globally show an increase of their C:N ratio in winter. C:N ratios of the POM from the Liffey river and from the open sea both ranged from ~6 to 13 with their maximum in November 2005. At BISL, the C:N ratio of the suspended POM averaged 6.2, except in November 2005 when it reached 21. C:N ratios of the SOM kept

stable around 7-8. Saltmarsh plants exhibited C:N ratios varying globally from 9 to 25, but the highest C:N values were measured for detrital leaves of terrestrial plants.

3.3.3. Isotopic signatures

3.3.3.1 Sources $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

3.3.3.1.a) Sources isotopic characterisation

The mean isotopic ratios of all the potential food sources sampled are presented in Table 3.3. Over the four surveys, the main sources of organic matter present in the study area were clearly differentiated by their $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$. The Liffey river POM, terrestrial plants and the C_3 -saltmarsh plants were the most ^{13}C -depleted (overall mean $\delta^{13}\text{C}$ values of -30.4‰, -29.6‰ and -26.7‰, respectively). The sewage effluent also exhibited rather ^{13}C -depleted values, ranging over the year from -26.9‰ to -25.4‰. In contrast, the C_4 saltmarsh grass *Spartina anglica* was the most ^{13}C -enriched (overall mean of -13.7‰), followed by the green macroalgae *Enteromorpha* spp. and the microphytobenthos (overall $\delta^{13}\text{C}$ mean values of -15.4‰ and -16.5‰, respectively). $\delta^{13}\text{C}$ values of the SOM and suspended POM at BISL were intermediate between the above pure sources (overall mean of -21.5‰ and -19.7‰, respectively), except in November 2005 when the site POM was very ^{13}C -enriched ($\delta^{13}\text{C}$ of -15 ‰).

The potential food sources featured a wide spectrum of $\delta^{15}\text{N}$ values, ranging from 0.6‰ for the terrestrial vascular plant *Quercus* sp. to 14.5 ‰ for *Spartina anglica*. The green algae *Enteromorpha* spp. exhibited a high $\delta^{15}\text{N}$ (overall mean: 12.1‰), as well as the saltmarsh plants *Halimione portulacoides* and *Salicornia europaea* (overall mean $\delta^{15}\text{N}$ values of 11.6‰ and 12.7‰, respectively). The various pools of suspended POM were more ^{15}N -depleted than macroalgae and saltmarsh plants. Microphytobenthos (MPB) was amongst the most ^{15}N -depleted sources with an overall $\delta^{15}\text{N}$ mean value of 4.6‰. The $\delta^{15}\text{N}$ signature of the SOM was intermediate between the above pure sources.

Table 3.3: Carbon and nitrogen isotopic ratios (mean $\pm$ SD) of potential food sources.

	May 2005			November 2005			March 2006			June 2006		
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n
POM												
Lifey river	-31.9 $\pm$ 1.3	5.7 $\pm$ 0.2	6	-27.6 $\pm$ 0.2	6.3 $\pm$ 0.4	8	-29.3 $\pm$ 0.3	5.7 $\pm$ 0.3	8	-32.6 $\pm$ 1.3	10.5 $\pm$ 1.2	6
Open sea	-23.0 $\pm$ 1.1	4.5 $\pm$ 0.5	3	-21.0 $\pm$ 0.6	3.8 $\pm$ 1.0	3	-22.4 $\pm$ 0.2	6.2 $\pm$ 0.4	3	-25.0 $\pm$ 0.1	7.0 $\pm$ 0.3	2
Site BISL	-	-		-15.0 $\pm$ 0.2	5.6 $\pm$ 0.3	2	-21.6 $\pm$ 0.1	6.7 $\pm$ 0.2	2	-23.7 $\pm$ 0.4	7.5 $\pm$ 0.5	2
Sewage effluent	-25.8	3.4	1	-25.7 $\pm$ 0.2	8.1 $\pm$ 2.0	9	-26.0 $\pm$ 0.0	3.4 $\pm$ 0.3	9	-26.6 $\pm$ 0.3	8.7 $\pm$ 0.1	6
SOM												
Site BISL	-21.6 $\pm$ 0.9	7.5 $\pm$ 0.1	3	-20.4 $\pm$ 0.1	8.2 $\pm$ 0.1	3	-21.9 $\pm$ 0.1	7.9 $\pm$ 0.3	3	-22.0 $\pm$ 0.1	8.2 $\pm$ 0.2	3
Microphytobenthos	-15.4 $\pm$ 0.4	5.9 $\pm$ 0.1	3	-16.9 $\pm$ 0.3	2.8 $\pm$ 0.2	3	-14.2 $\pm$ 0.1	4.6 $\pm$ 0.1	3	-15.9 $\pm$ 0.2	4.9 $\pm$ 0.3	3
Macroalgae												
<i>Enteromorpha</i> spp.	-15.2 $\pm$ 0.9	11.2 $\pm$ 0.1	3	-15.9 $\pm$ 0.4	12.5 $\pm$ 0.3	3	-	-		-14.9 $\pm$ 0.6	12.6 $\pm$ 0.1	3
Saltmarsh plants												
<i>Spartina anglica</i>	-13.5 $\pm$ 0.3	13.7 $\pm$ 0.6	3	-13.0 $\pm$ 0.2	11.7 $\pm$ 0.3	3	-14.9 $\pm$ 0.7	11.3 $\pm$ 0.9	3	-13.4 $\pm$ 0.6	14.5 $\pm$ 0.3	2
<i>Halimione portulacoides</i>	-27.4 $\pm$ 0.3	12.4 $\pm$ 0.5	3	-28.0 $\pm$ 0.3	12.0 $\pm$ 1.1	3	-26.2 $\pm$ 0.5	10.6 $\pm$ 0.6	2	-26.4 $\pm$ 0.4	12.3 $\pm$ 1.5	2
<i>Salicornia europaea</i>	-27.1 $\pm$ 0.1	11.8 $\pm$ 0.3	3	-	-		-	-		-26.0 $\pm$ 0.1	13.6 $\pm$ 0.2	2
<i>Limonium humile</i>	-	-		-	-		-26.3 $\pm$ 0.3	7.6 $\pm$ 0.7	3	-	-	
<i>Spergularia</i> sp.	-	-		-	-		-26.8 $\pm$ 1.0	9.7 $\pm$ 0.9	3	-	-	
Terrestrial plants (detrital)												
<i>Acer pseudoplatanus</i>	-	-		-29.0 $\pm$ 1.2	5.1 $\pm$ 1.6	3	-	-		-	-	
<i>Aesculus hippocastanum</i>	-	-		-30.4 $\pm$ 0.7	1.5 $\pm$ 1.3	2	-	-		-	-	
<i>Fagus sylvatica</i>	-	-		-29.9 $\pm$ 0.8	1.5 $\pm$ 0.6	3	-	-		-	-	
<i>Populus</i> sp.	-	-		-28.9 $\pm$ 2.7	3.6 $\pm$ 0.0	2	-	-		-	-	
<i>Prunus</i> sp.	-	-		-29.0 $\pm$ 0.8	2.1 $\pm$ 0.0	2	-	-		-	-	
<i>Quercus</i> sp. 1	-	-		-29.1 $\pm$ 1.0	0.6 $\pm$ 0.3	3	-	-		-	-	
<i>Quercus</i> sp. 2	-	-		-30.8 $\pm$ 0.1	5.1 $\pm$ 0.5	2	-	-		-	-	
<i>Salix fragilis</i>	-	-		-29.4 $\pm$ 2.6	7.2 $\pm$ 0.0	3	-	-		-	-	

3.3.3.1.b) Temporal variability of the sources isotopic signatures

Most of the sources collected for at least two sampling seasons displayed some temporal variability of their $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$, except for *Enteromorpha* spp. (Table 3.4). There were significant temporal changes of the site POM $\delta^{13}\text{C}$ which was particularly ^{13}C -enriched in winter 2005 (-15‰) compared to marine POM collected offshore (-21‰), whereas these POM pools had close values at other seasons. $\delta^{13}\text{C}$ of the sedimentary organic matter (SOM) was stable over time but its $\delta^{15}\text{N}$ signature increased significantly in November 2005 and June 2006 (ANOVA, $p < 0.01$). For both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, the Liffey POM showed highly significant differences among seasons ($p < 0.001$). Indeed, the Liffey POM had fluctuating $\delta^{13}\text{C}$ values during the year with the most ^{13}C -enriched values in November 2005 and the most depleted values in June 2006. $\delta^{15}\text{N}$ values were also highly significantly different over time¹ (p -value < 0.001), due to a higher value in June 2006 of about 4 to 5‰ compared with the three other seasons. Sewage POM also showed significant variations of its isotopic signatures over time², with notably lower $\delta^{13}\text{C}$ values in June 2006 compared with November 2005.

¹ It must be noted that the assumptions of normality were not fully met for the nested ANOVA carried out on the river POM $\delta^{15}\text{N}$ values as the Levene's test of homogeneity of variance gave a significant value ($p = 0.002$). However, as the non-parametric analysis of variance (Kruskal-Wallis) carried out on the non-transformed data also gave a highly significant result (KW, $\chi^2 = 16.325$, $df = 3$, $p = 0.001$), the result of the nested ANOVA was considered acceptable.

² Again, the condition for homogeneity of variances was not met (Levene test, $p = 0.014$), but the result was considered acceptable as the Kruskal-Wallis ANOVA carried on the non-transformed $\delta^{13}\text{C}$ data led to a similar level of significance (KW, $\chi^2 = 14.978$, $df = 3$, $p = 0.002$).

Table 3.4: Results of statistical tests on the differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of sources among seasons. Statistic tests are F for ANOVAs and nested ANOVAs, and F', χ^2 and t for Welch ANOVAs, Kruskal-Wallis (KW) ANOVAs and t-tests, respectively. For ANOVAs, degrees of freedom (df) between and within groups (error) or displayed as the 1st and 2nd numbers respectively. Sig.: significance level, ***: $p \leq 0.001$, **: $p \leq 0.01$, *: $p \leq 0.05$, NS: non-significant ($p > 0.05$).

	$\delta^{13}\text{C}$					$\delta^{15}\text{N}$				
	Test	df	Statistic test	p	Sig.	Test	df	Statistic test	p	Sig.
Liffey river POM	Nested ANOVA ^t	3, 6.98	24.383	<0.001	***	Nested ANOVA _t	3, 6.75	34.244	<0.001	***
Open sea POM	KW	3	8.318	0.04	*	ANOVA	3, 7	13.562	0.003	**
Site POM (BISL)	Welch ANOVA	2	753.584	0.005	**	Welch ANOVA	2	10.355	0.100	NS
Sewage effluent	Nested ANOVA ^t	2, 6.08	13.620	0.006	**	KW	3	17.783	<0.001	***
SOM	KW	3	7.476	0.058	NS	ANOVA	3, 8	11.404	0.003	**
MPB	KW	3	10.458	0.015	*	ANOVA	3, 8	144.218	<0.001	***
<i>Enteromorpha</i> sp.	ANOVA	2, 6	2.098	0.204	NS	KW	2	5.956	0.051	NS
<i>Halimione portulacoides</i>	ANOVA	3, 6	6.777	0.024	*	ANOVA	3, 6	1.073	0.428	NS
<i>Salicornia europaea</i>	t-test	3	-11.875	0.001	***	t-test	3	-6.796	0.007	**
<i>Spartina anglica</i>	ANOVA	3, 7	8.520	0.01	**	ANOVA	3, 7	16.727	0.001	***

^t indicates that the variable tested was 'Inverse' transformed.

3.3.3.2 Consumers $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

3.3.3.2.a) Carbon and nitrogen isotopic characterisation of the consumers

Mean C and N isotopic ratios of consumers sampled for each survey are presented in Table 3.5. The indicated feeding strategies for each species were determined on the literature basis. Over the four seasons, the range of $\delta^{13}\text{C}$ values of consumers was narrower than those of the sampled food sources. They varied overall from -21.5‰ for the nematodes to -13.9‰ for juveniles of the stickleback *Gasterosteus aculeatus*, while the sources signatures ranged from -34‰ to -12.7‰ (Fig. 3.9). $\delta^{13}\text{C}$ of primary consumers (i.e. suspension-feeders, surface and sub-surface deposit-feeders, grazers, interface-feeders) varied over the four sampling surveys from -20.5‰ for *Scoloplos armiger* to -14.6‰ for *Hydrobia ulvae*. The range of $\delta^{13}\text{C}$ values of carnivorous species (-18.9‰ to -13.9‰) was overall higher than for omnivorous species (-18.9‰ to -15.3‰). The range of $\delta^{13}\text{C}$ values measured for the meiofauna and primary consumers was clearly narrowed in December 2005, compared to other surveys.

Considering $\delta^{15}\text{N}$, the range of signatures measured varied overall from 8‰ for nematodes to 18‰ for juveniles of *G. aculeatus*. Primary consumers exhibited a large range of $\delta^{15}\text{N}$ values, from 8.9‰ to 14.6‰ over the four surveys, induced by seasonal variations in the $\delta^{15}\text{N}$ of most species. $\delta^{15}\text{N}$ of omnivorous and carnivorous species ranged respectively from 12.2‰ to 15.1‰ and from 12.1‰ to 18‰. Carnivorous fish exhibited larger ranges of values than carnivorous invertebrates, both for C and N isotopes. Among the four most occurring species of omnivorous and carnivorous consumers (*i.e.* collected at least on three sampling occasions), the omnivorous polychaete *Hediste diversicolor* showed stable $\delta^{13}\text{C}$ values but significantly higher $\delta^{15}\text{N}$ values in December 2005 compared with June 2005 and July 2006 (LSD tests, $p < 0.001$). *Carcinus maenas* displayed higher $\delta^{15}\text{N}$ in December 2005 and April 2006. For the benthivorous fish *Pomatoschistus* spp., both C and N isotopic signatures were higher in December 2005 (KW, $p < 0.05$). In July 2006 when most fish species were sampled, zooplanktivorous juveniles of *Clupea harengus* had the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures while juveniles of *G. aculeatus* showed the highest C and N signatures.

Table 3.5: Carbon and nitrogen isotopic ratios (mean $\pm$ SD) of invertebrates and fish from Bull Island South Lagoon (FS: Feeding Strategy; aSF: active suspension feeder, IF: Interface feeder, O: omnivorous, P: Predator (b: benthivorous, pl: planktivorous, ps: piscivorous), SDF: Surface Deposit feeder, SSDF: Sub-Surface Deposit feeder). * pooled organisms, - not sampled.

	FS	June 2005			December 2005			April 2006			July 2006		
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n
Phylum Nematoda*		-20.5 $\pm$ 1.7	10.9 $\pm$ 1.0	3	-18.1 $\pm$ 0.9	10.2 $\pm$ 1.2	3	-21.4 $\pm$ 1.3	8.0 $\pm$ 0.9	2	-21.5 $\pm$ 0.2	9.2 $\pm$ 0.1	3
Phylum Annelida													
Class Oligochaeta*													
Class Polychaeta													
<i>Arenicola marina</i>	SDF	-18.0 $\pm$ 1.4	10.7 $\pm$ 0.4	4	-17.8 $\pm$ 0.2	14.5 $\pm$ 0.2	4	-19.6 $\pm$ 0.5	10.1 $\pm$ 0.7	4	-19.4 $\pm$ 0.5	10.5 $\pm$ 0.1	5
<i>Eteone</i> sp.	P	-	-		-15.6 $\pm$ 0.7	16.6 $\pm$ 0.5	4	-	-		-	-	
<i>Nephtys hombergii</i>	P	-	-					-17.9 $\pm$ 0.9	12.9 $\pm$ 0.2	4	-18.3 $\pm$ 0.4	12.8 $\pm$ 0.5	3
<i>Hediste diversicolor</i>	O	-16.7 $\pm$ 1.7	12.9 $\pm$ 0.7	3	-15.6 $\pm$ 0.3	15.0 $\pm$ 0.2	5	-	-		-16.5 $\pm$ 0.4	13.5 $\pm$ 0.3	3
<i>Notomastus latericeus</i>	SSDF	-	-		-	-		-	-		-18.9	9.3	1
<i>Scotoplanes armiger</i>	SSDF	-19.3 $\pm$ 0.0	10.2 $\pm$ 0.4	2	-	-		-20.0 $\pm$ 0.4	9.8 $\pm$ 0.3	5	-	-	
Dolichopodids larvae					-16.5 $\pm$ 0.4	15.7 $\pm$ 0.8	5	-18.1 $\pm$ 0.3	14.2 $\pm$ 0.3	4	-	-	
Phylum Crustacea													
<i>Carcinus maenas</i>	O	-	-		-18.9	14.7	1	-18.2 $\pm$ 1.1	14.5 $\pm$ 0.4	7	-17.7 $\pm$ 0.7	13.6 $\pm$ 0.7	4
<i>Crangon crangon</i>	P	-	-		-18.0 $\pm$ 0.2	14.8 $\pm$ 1.0	5	-	-		-16.1 $\pm$ 1.0	14.1 $\pm$ 0.5	5
<i>Praunus flexuosus</i>	O	-	-		-17.5 $\pm$ 0.7	13.8 $\pm$ 0.7	2	-	-		-	-	
Phylum Mollusca													
Class Bivalvia													
<i>Cerastoderma edule</i>	aSF	-19.8 $\pm$ 0.7	9.8 $\pm$ 0.2	5	-18.6 $\pm$ 0.2	10.8 $\pm$ 0.4	9	-19.8 $\pm$ 0.3	9.4 $\pm$ 0.2	6	-19.7 $\pm$ 0.2	10.5 $\pm$ 0.4	8
<i>Macoma balthica</i>	IF	-17.0 $\pm$ 2.2	10.6 $\pm$ 0.1	3	-16.7 $\pm$ 0.4	11.8 $\pm$ 0.2	4	-17.5 $\pm$ 1.1	9.7 $\pm$ 0.2	5	-16.8 $\pm$ 0.2	9.3 $\pm$ 0.3	4
<i>Scrobicularia plana</i>	IF	-18.3 $\pm$ 0.6	10.3 $\pm$ 0.4	5	-16.7 $\pm$ 0.3	11.8 $\pm$ 0.2	6	-17.9 $\pm$ 1.5	9.7 $\pm$ 0.5	5	-17.9 $\pm$ 0.7	10.1 $\pm$ 0.9	4
Class Gastropoda													
<i>Hydrobia ulvae</i> *	SDF,G	-15.0 $\pm$ 0.7	10.5 $\pm$ 0.2	2	-15.1 $\pm$ 0.1	12.1 $\pm$ 0.2	2	-15.3 $\pm$ 0.5	11.2 $\pm$ 0.0	2	-15.9 $\pm$ 0.2	11.3 $\pm$ 0.1	2
Phylum Chordata													
Class Osteichthyes													
<i>Atherina presbyter</i>	Pb,pl	-	-		-17.8	12.1	1	-17.7 $\pm$ 0.5	15.4 $\pm$ 0.3	8	-16.2 $\pm$ 1.0	15.8 $\pm$ 0.4	6
<i>A. presbyter</i> Juv.	Pb,pl	-	-		-	-		-	-		-15.4 $\pm$ 0.2	16.2 $\pm$ 0.2	5
<i>Clupea harengus</i> Juv.	Ppl	-	-		-	-		-	-		-18.7 $\pm$ 1.0	14.0 $\pm$ 0.6	8
<i>Gasterosteus aculeatus</i>	Pb,pl,ps	-	-		-	-		-	-		-17.8 $\pm$ 0.5	15.2 $\pm$ 0.7	4
<i>G. aculeatus</i> Juv.	Pb,pl,ps	-	-		-	-		-	-		-14.4 $\pm$ 0.5	17.7 $\pm$ 0.3	3
<i>Pleuronectes platessa</i> Juv.	Pb	-	-		-	-		-	-		-15.5 $\pm$ 0.7	15.5 $\pm$ 0.6	6
<i>Platyichthys flesus</i>	Pb	-	-		-18.9	13.9	1	-	-		-	-	
<i>Pomatoschistus</i> spp.	Pb	-	-		-15.1 $\pm$ 0.1	17.1 $\pm$ 0.6	2	-16.2 $\pm$ 0.4	16.4 $\pm$ 0.5	5	-15.9 $\pm$ 0.3	15.9 $\pm$ 0.3	9

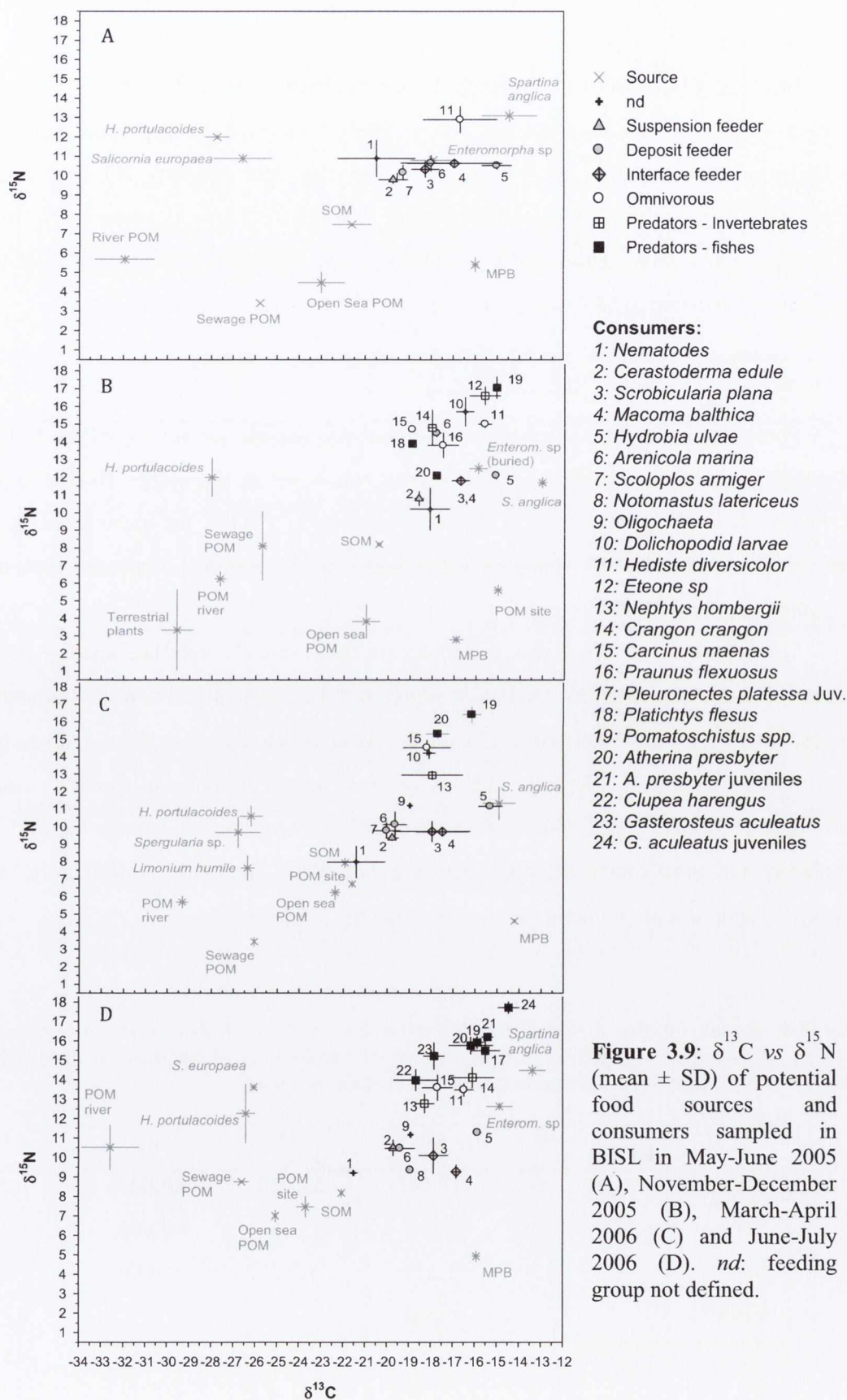


Figure 3.9: $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ (mean $\pm$ SD) of potential food sources and consumers sampled in BISTL in May-June 2005 (A), November-December 2005 (B), March-April 2006 (C) and June-July 2006 (D). nd: feeding group not defined.

3.3.3.2.b) Interspecific and temporal variations in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of primary consumers

Inter-species variations and temporal changes in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of nematodes and primary consumers were assessed using 2-way ANOVA for the most common species, which were systematically collected over the four sampling surveys. These species were the molluscs *Cerastoderma edule* (suspension-feeder), *Macoma balthica* and *Scrobicularia plana* (interface-feeders), *H. ulvae* (grazer/deposit-feeder) and the polychaete *A. marina* (surface deposit-feeder).

$\delta^{13}\text{C}$ of the dominant primary consumers

There was no interaction effect between surveys and species but both these factors had a significant effect (Table 3.6). The survey conducted in December 2005 differed significantly from the 3 other surveys (LSD post hoc tests; $p < 0.001$ for each comparisons cases), as the range of $\delta^{13}\text{C}$ values recorded then was ^{13}C -enriched compared with the three other sampling dates.

The gastropod *H. ulvae* was systematically the most ^{13}C -enriched species (LSD tests, $p < 0,001$). The interface-feeders *S. plana* and *M. balthica* had similar signatures independently of the survey. Their $\delta^{13}\text{C}$ values were significantly lower than *H. ulvae* but higher than nematodes, the deposit-feeder *A. marina*, and the suspension-feeder *C. edule* (LSD tests, $p < 0,001$ in all comparisons cases). These two species, *A. marina* and *C. edule*, were not significantly different regarding their $\delta^{13}\text{C}$. They were significantly ^{13}C -enriched compared with the nematodes, which had the lowest $\delta^{13}\text{C}$.

Table 3.6: Results of the 2-way ANOVA performed on $\delta^{13}\text{C}$ of nematodes and primary consumers collected during the four surveys, using a 0.01 alpha level of significance. df: degrees of freedom, MS: Mean Square, F: statistic test, Sig: significance level

	df	MS	F	Sig
Species	5	37.646	58.710	<0.001
Survey	3	8.497	13.251	<0.001
Species*survey	15	1.226	1.912	0.035
Error	74	0.641		

$\delta^{15}\text{N}$ of the dominant primary consumers

Both the survey and species factors had a significant effect on the data, but there was also a significant interaction between these two factors (Table 3.7), indicating notably differing patterns of $\delta^{15}\text{N}$ temporal variations among species.

Table 3.7: Results of the 2-way ANOVA performed on $\delta^{15}\text{N}$ of nematodes and primary consumers collected during the four surveys, using a 0.01 alpha level of significance. df: degrees of freedom, MS: Mean Square, F: statistic test, Sig: significance level

	df	MS	F	Sig
Species	5	5.987	28.647	<0.001
Survey	3	17.407	83.295	<0.001
Species*survey	15	1.998	9.562	<0.001
Error	74	0.209		

Figure 3.10 displays the evolution of $\delta^{15}\text{N}$ signatures of the dominant primary consumers and nematodes during the study. While $\delta^{15}\text{N}$ mean values of the nematodes tended to decrease over time between June 2005 and April 2006, the interface-feeders *M. balthica* and *S. plana* and the deposit-feeders *A. marina* and *H. ulvae* exhibited a significant ^{15}N -enrichment in December 2005 (Table 3.8), up to nearly 4‰ for *A. marina*. Their $\delta^{15}\text{N}$ then returned to lower levels in April and July 2006. $\delta^{15}\text{N}$ of the suspension-feeder *C. edule* was also significantly higher in December 2005. $\delta^{15}\text{N}$ signatures of the interface-feeders *S. plana* and *M. balthica* were similar, as observed for their $\delta^{13}\text{C}$. *A. marina* and *C. edule* had similar $\delta^{15}\text{N}$ signatures except in December 2005 (Dunn's post hoc, $p < 0.01$), owing to the strong ^{15}N -enrichment of *A. marina*.

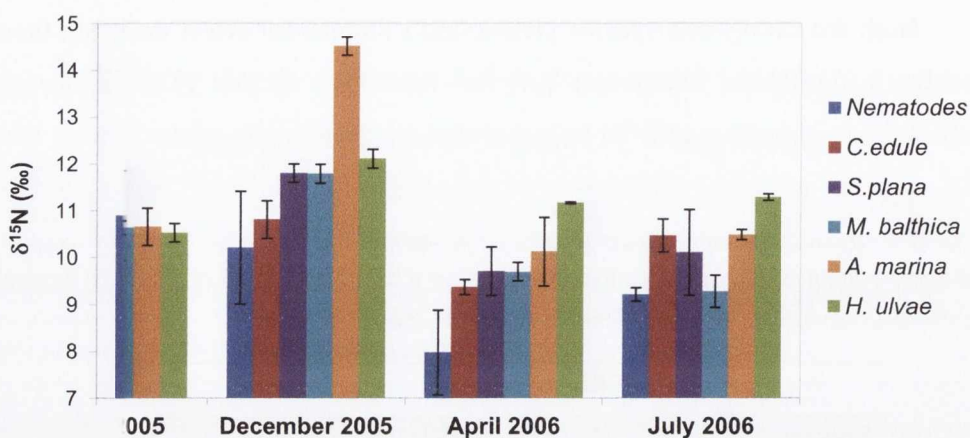


Figure 3.10 Oral variations of the nematodes and the primary consumers $\delta^{15}\text{N}$ signatures (mean $\pm$ SD) June 2005 to July 2006 in Bull Island South Lagoon.

Table 3.8: TI variability of the $\delta^{15}\text{N}$ values of nematodes and primary consumers in BISL. Results of the analysis of variance (ANOVA and Kruskal-Wallis test) among sampling surveys (A: June 2005; B: December 2005; C: April 2006; D: July 2006). Post hoc tests were LSD for ANOVAs and Post hoc for Kruskal-Wallis tests (significance level : 0.05).

Sp	Test	p-values	Post hoc tests
Nes	AOV	0.043	(A > C) = B = D
<i>Cerma edule</i>	AOV	<0.001	B = D > A = C
<i>Hyulvae</i>	AOV	0.001	B > C = D > A
<i>Moalthica</i>	AOV	<0.001	B > A > C = D
<i>Scnria plana</i>	KW	0.004	B > A = C = D
<i>Armarina</i>	KW	0.017	B > C = D = A

In sy, these results show: (1) a gradual ^{13}C -enrichment among primary consumers suspension-feeders and surface deposit-feeders to interface-feeders and then grazerhe interface-feeding bivalves *S. plana* and *M. balthica* to occupy the same isotope during all surveys with similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; (3) similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the suspension-feeder *C. edule* and the deposit-feeder *A. marina* except in Dec 2005; (4) higher $\delta^{15}\text{N}$ of most primary consumers and particularly deposit-feed interface-feeders in December 2005 compared with other dates.

3.3.4. Primary consumers diets: influence of sewage POM anonal shift induced by the macroalgal bloom

Figure 3.11 shows the relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of primary consumers and the potential food sources sampled for each survey. Estimated isotopic compositions of the dietary mixtures are also reported on the plot for each species. Food sources contributions to the diet of the dominant primary consumers species from BISL, and which represent the different feeding groups (i.e. suspension-feeder (SF) *C. edule*, the interface-feeders (IF) *M. balthica* and *S. phaeodactylus* deposit-feeder (DF) *A. marina* and the grazer/DF *H. ulvae*), were estimated using a Bayesian mixing model SIAR. Primary consumers systematically had high $\delta^{13}\text{C}$ values related to ^{13}C -depleted sources from the catchment (river POM, terrestrial plants) and a saltmarsh, which can therefore be excluded as major food sources and were excluded from the SIAR analysis. The analysis considered the following end-members: open sea POM, sewage POM, *Enteromorpha* spp. and the microphytobenthos. Even if *Enteromorpha* spp. was not collected and analysed in March 2006 as intended, this source was included in the model for that survey since it may have been used by consumers at an advanced detrital stage at the end of winter. The isotopic values of detrital (and partly buried) *Enteromorpha* spp. collected in November 2005 were used for this purpose. The site POM and the SOM were not considered in the analysis as they also constitute mixtures of end-member sources, as characterised based on isotopic and non-isotopic variables (see discussion, section 3.4.1), and would therefore have confounding effects on the dietary estimations. In addition, the site POM exhibits strong temporal variations of its $\delta^{13}\text{C}$ which are suspected to occur at high frequencies (see discussion 3.4.1), and thus do not make it an appropriate candidate for the mixing model. The cordgrass *Spartina anglica* was ruled out of the analysis given its relatively low abundance on site and its very poor digestibility that makes it unlikely to be a major food source for the consumers (cf discussion - section 3.4.2).

Contributions of the food sources to the species diet are expressed as the median probability contribution (see Fig. 3.12 for the probability intervals). Sewage POM appears from the model to be a non-negligible source of food in BISL. Estimated

contributions to the species diets varying over time from 5% to 28% (see seasonal values in Appendix 3.1). The suspension-feeder *C. edule* was the main consumer to assimilate this food source (median contribution between 17 and 28%), followed by the deposit-feeder *A. marina* (between 15 and 26%). However, we cannot exclude the contribution of this source to have been slightly overestimated by the model to the detriment of seaPoM, especially for the surveys A (May-June 2005) and C (March-April 2006) given the relative positions of these two sources in the isotopic space and the convex hull implied by the sources. Sewage POM contributed much less to the diets of interface-feeders (i.e. *S. plana* and *M. balthica*) with median contribution values not exceeding 10% except in May-June 2005 (17 and 14% respectively).

The suspension-feeder *C. edule* was always the main species to exploit marine POM (between 21 and 29% over time), followed by the deposit-feeding lugworm *A. marina* (contribution of seaPOM between 20 and 27%, except in December 2005 when this food source contributed only 6% of its diet) which likely used this resource after sedimentation (i.e. through the SOM pool), accordingly with its psammivorous feeding mode. Over the year, interface-feeders and deposit-feeders rather relied on benthic and autochthonous primary producers (Fig. 3.12). Interestingly, the mixing models revealed strong seasonal variations in the resources used by most primary consumers with a trophic shift for all species from spring to the early winter 2005, induced by an increased use of *Enteromorpha* spp. This was particularly obvious for *A. marina*, as the contribution of *Enteromorpha* to its tissues reached over 50% (median value of 66%) by December 2005. The interface-feeders *S. plana* and *M. balthica* mostly relied on the green algae too with median contributions increasing from 33% and 36% respectively in June 2005 to 53% and 52% in December 2005. *Enteromorpha* contributed slightly less to the diet of the suspension-feeder *C. edule* (36% in December 2005), and more surprisingly to the diet of the grazer/deposit-feeder *H. ulvae* (42% in December 2005). These contributions nevertheless remain high. The contribution of *Enteromorpha* to the diet of these primary consumers then decreased during the following months (toward the end of winter and next spring, i.e. between March-April 2006 and June-July 2006). During this period, *Enteromorpha* spp. was progressively (but not totally) replaced by a mixture of other sources, including microphytobenthos which progressively became the dominant food source by July 2006 for all species, with contributions to the diets reaching 73% and 54% for the interface-feeders (*M. balthica* and *S. plana* respectively).

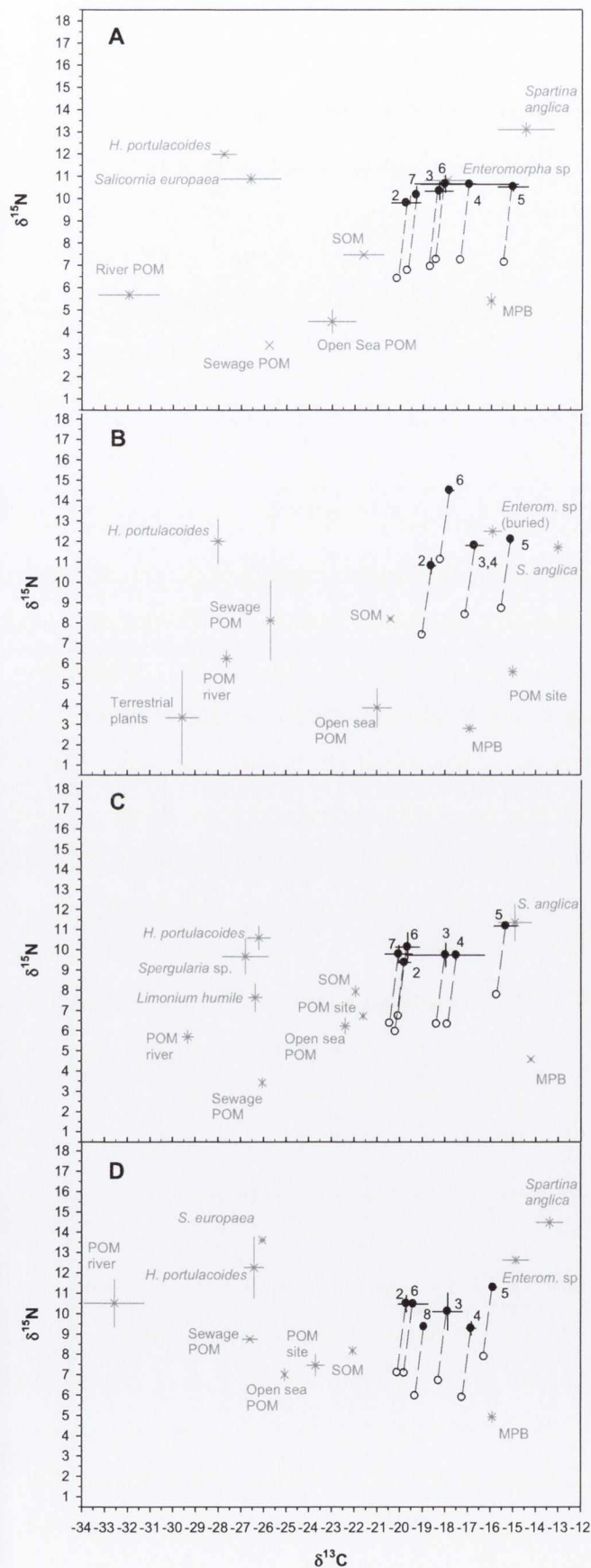
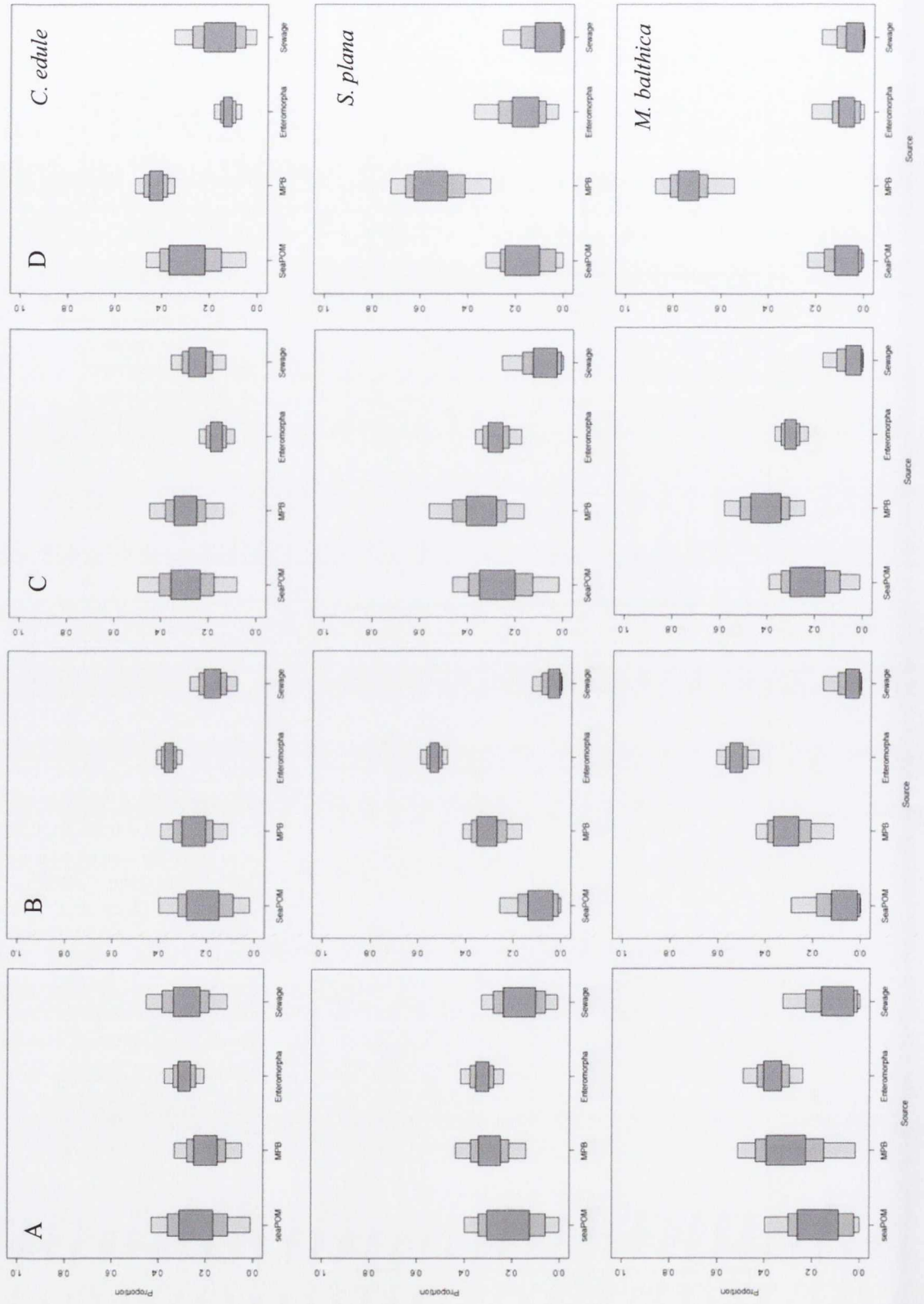


Figure 3.11: $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ (mean $\pm$ SD) of potential food sources and primary consumers sampled in May-June 2005 (A), November-December 2005 (B), March-April 2006 (C) and June-July 2006 (D). Isotopic compositions of the estimated diets were calculated based on mean fractionation factors of 0.4‰ and 3.4‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively.



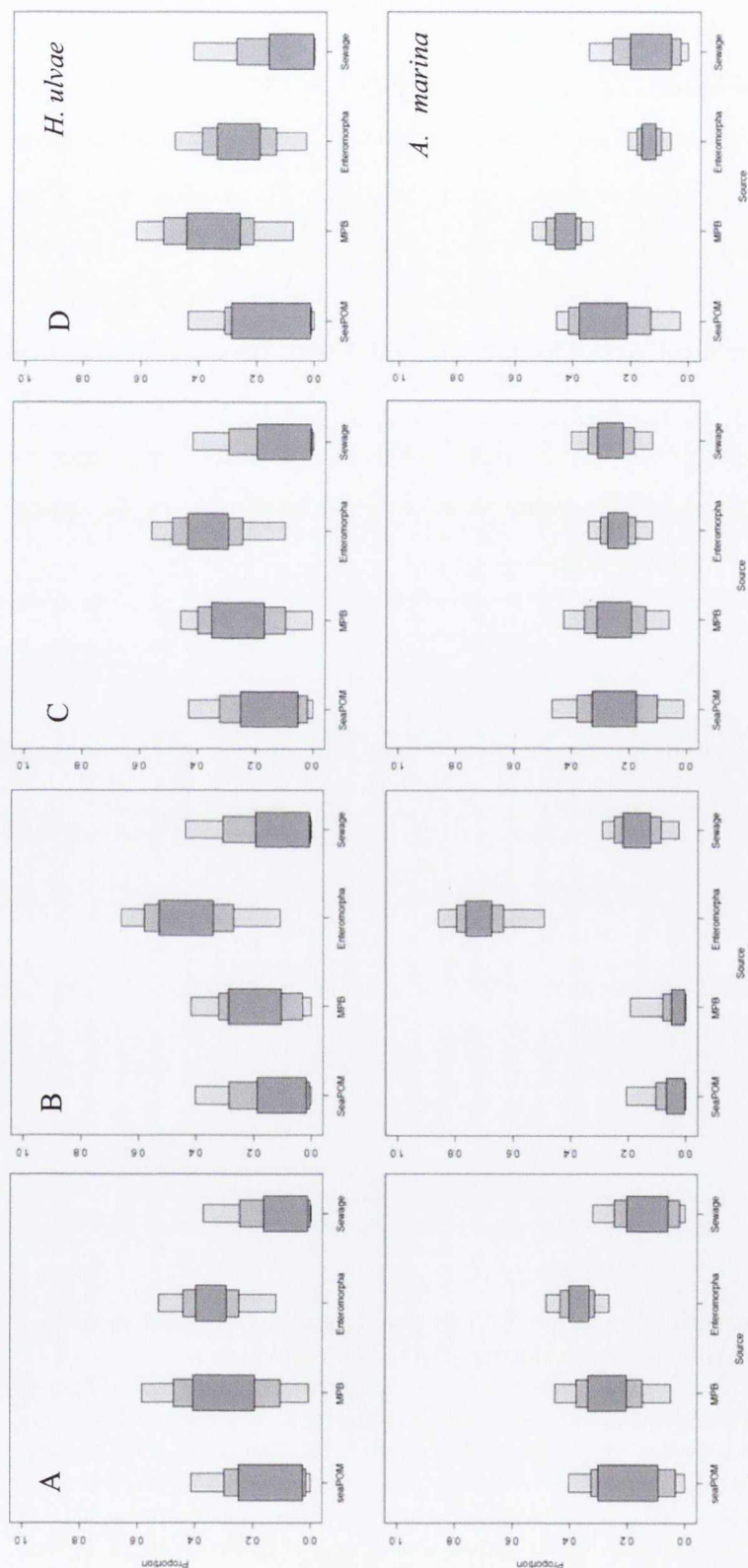


Figure 3.12: Seasonal variability of the relative contributions of the sources to the diets of dominant primary consumers, as estimated by the Bayesian mixing model SIAR, using trophic fractionation values (mean $\pm$ SD) of $3.4 \pm 0.7\text{‰}$ and $0.4 \pm 1.3\text{‰}$ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively. Sources are marine Particulate Organic Matter (seaPOM), microphytobenthos (MPB), *Enteromorpha* spp., and Sewage POM. Surveys are A: May-June 2005, B: November-December 2005, C: March-April 2006 and D: June-July 2006. Contributions are displayed as the 50th, 75th and 95th extent of the probability intervals.

3.3.5. Food chain length and consumers trophic levels

The studied food web was globally organised in 4.2 trophic levels (TL) over the year (Fig. 3.13). Taking into consideration the seasonal variability in the $\delta^{15}\text{N}$ signatures of strict primary consumers and secondary consumers, the food chain length varied between 3.9 and 4.5 TL (Table 3.9). Macro-invertebrates occupied mean TL ranging from 2 for strict primary consumers to 3.4 for secondary consumers (*Eteone* sp. in December 2005 and *C. maenas* in April 2006). Fish were distributed overall from mean TL of 2.1 (*Atherina presbyter* in December 2005) to 4.2 (*G. aculeatus* juveniles). Seasonal variations of TL were observed for some species, such as *Carcinus maenas*, whose TL increased by 0.6 between December 2005 and April 2006. In opposition, other species such as *H. diversicolor* maintained a stable trophic level over the study, despite the strong seasonal raise of their $\delta^{15}\text{N}$ in December 2005.

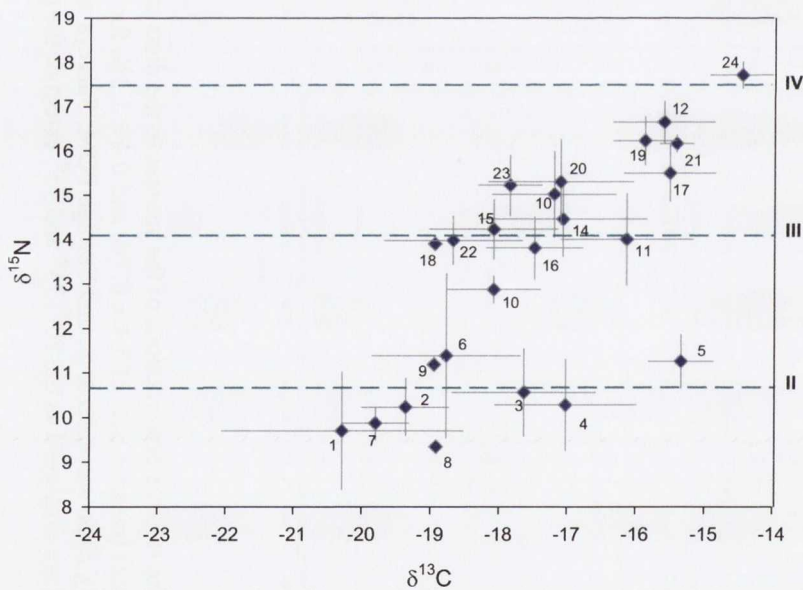


Figure 3.13: $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ values (annual mean $\pm$ SD) of consumers in BSL and theoretical trophic levels (dashed lines), based on a ^{15}N -enrichment of 3.4‰ per trophic level. Consumers are: 1: Nematodes, 2: *Cerastoderma edule*, 3: *Scrobicularia plana*, 4: *Macoma balthica*, 5: *Hydrobia ulvae*, 6: *Arenicola marina*, 7: *Scoloplos armiger*, 8: *Notomastus latericeus*, 9: Oligochaeta, 10: Dolichopodid larvae, 11: *Hediste diversicolor*, 12: *Eteone* sp., 13: *Nephtys hombergii*, 14: *Crangon crangon*, 15: *Carcinus maenas*, 16: *Praunus flexuosus*, 17: *Pleuronectes platessa* Juv., 18: *Platichthys flesus*, 19: *Pomatoschistus* spp., 20: *Atherina presbyter*, 21: *A. presbyter* juveniles, 22: *Chupea harengus*, 23: *Gasterosteus aculeatus*, 24: *G. aculeatus* juveniles.

Table 3.9: Trophic levels estimations for all secondary consumers sampled in BISL from June 2005 to July 2006.

Species	June 2005			December 2005			April 2006			July 2006		
	lower estimates	upper estimates	difference	lower estimates	upper estimates	difference	lower estimates	upper estimates	difference	lower estimates	upper estimates	difference
<i>Scoloplos armiger</i>	1,7	2,2	0,5				1,7	2,3	0,6			
<i>Notomastus latericeus</i>										1,5	1,9	0,4
Dolichopodid larvae				2,5	3,7	1,2	3,0	3,6	0,5			
Nematodes	1,8	2,6	0,8	0,8	2,2	1,5	1,0	1,9	0,9	1,4	1,9	0,5
Oligochaetes							2,2	2,6	0,4	2,0	2,5	0,4
<i>Hediste diversicolor</i>	2,4	3,1	0,7	2,5	3,3	0,9				2,6	3,2	0,6
<i>Eteone sp</i>				2,9	3,9	1,0						
<i>Nephtys hombergii</i>							2,7	3,2	0,5	2,4	3,1	0,7
<i>Praunus flexuosus</i>				2,0	3,1	1,2						
<i>Carcinus maenas</i>				2,4	3,2	0,8	3,1	3,7	0,6	2,6	3,4	0,8
<i>Crangon crangon</i>				2,2	3,5	1,3				2,8	3,5	0,7
<i>Pleuronectes platessa</i> juveniles										3,1	3,9	0,8
<i>Gasterosteus aculeatus</i> juveniles										3,9	4,5	0,6
<i>Gasterosteus aculeatus</i>										3,0	3,9	0,8
<i>Clupea harengus</i> juveniles										2,7	3,4	0,7
<i>Platycthis flesus</i>				2,2	3,0	0,8						
<i>Pomatoschistus</i> spp.				3,0	4,1	1,1	3,6	4,3	0,6	3,4	4,0	0,6
<i>Atherina presbyter</i>				1,7	2,4	0,8	3,4	3,9	0,5	3,3	3,9	0,7
<i>Atherina presbyter</i> juveniles										3,5	4,0	0,5

3.4. Discussion

3.4.1. Characterisation of the sources and the anthropogenic influence on primary producers

Riverine POM from the Liffey exhibited a range of low $\delta^{13}\text{C}$ values typical of freshwater seston derived from terrestrial C_3 angiosperms. $\delta^{13}\text{C}$ of the terrestrial and saltmarsh C_3 plants were also in good agreement with signatures usually reported for these categories of vascular plants (Fry & Sherr, 1984; Cloern *et al.*, 2002). Conversely, $\delta^{15}\text{N}$ signatures of the saltmarsh plants and green macroalgae from Bull Island South lagoon were relatively high compared with values from other sites of western Europe (Créach *et al.*, 1997; Decottignies *et al.*, 2007; Marconi *et al.*, 2011). This trend was particularly marked and consistent over the surveys for *Enteromorpha* spp. with a ^{15}N -enrichment by ~ 3 to 7‰ compared with other measurements in Europe and elsewhere (McClelland *et al.*, 1997; Machás *et al.*, 2003; Schaal *et al.*, 2010; Ouisse *et al.*, 2011). Patterns of nitrogen isotopic composition of macroalgae are poorly related to biogeography but predominantly determined by local factors such as anthropogenic inputs of nitrogen (McClelland *et al.*,

1997; Marconi *et al.*, 2011). Results from this study thus confirm the first hypothesis that Ulvales growing in south Bull Island are greatly influenced by the high nitrogen inputs from the catchment basin and particularly wastewaters, as previously observed in strongly impacted coastal environments (Riera *et al.*, 2000; Cole *et al.*, 2005; Vizzini *et al.*, 2005). The Liffey estuary is indeed considered as highly eutrophied, with nitrogen always in excess, originating from the Liffey river and mostly from treated wastewaters released by the sewage treatment plant of Ringsend into the lower estuary (O'Higgins, 2005). Sewage nitrogen released in the estuary since 2003 is mainly in oxidized forms such as nitrate (O'Higgins, 2005), which are typically ^{15}N -enriched (Michener & Schell, 1994; Domingues *et al.*, 2011).

Pools of POM in estuarine and nearshore environments are typically composed of a mixture of fresh and detrital organic matter, produced *in situ* and/or from allochthonous origin, and present in relative proportions varying depending on environmental and seasonal conditions. Terrestrial POM inputs to Dublin estuary are considered to be essentially delivered by the sewage effluent discharged in Ringsend and the Liffey river, and to mix in the estuary with marine particulates carried in with the tide (Wilson, 2005). Since the only tidal entrance to the studied site is from the estuary, POM from these three end-members (river, sewage and marine POM) could be expected to be present in BISL. In fact, $\delta^{13}\text{C}$ signatures of the suspended particulates collected inshore at BISL were at all time of the year ^{13}C -enriched compared with these three end-member POM pools. Isotopically close to the open sea POM (except in November 2005), POM in BISL always set apart from the river POM, and particularly in winter even though the river flow was higher. Riverine POM therefore appears to have a negligible influence (if any) on BISL, based on these POM data. Compared to the sewage effluent, POM from BISL was always ^{13}C -enriched by at least 3‰ and isotopically closer to the marine POM and/or to ^{13}C -enriched benthic primary producers. While the influence of sewage POM on BISL cannot be ruled out, its importance and contribution to the POM pool may be minor compared to other sources.

Variations in the isotopic signature and organic properties of the site POM pool suggest some high temporal variability of its composition. In November 2005, the POM was particularly ^{13}C -enriched, reaching -15‰. The microphytobenthos was likely to contribute to this POM pool after its resuspension by the tide as commonly observed on

mudflats (Baillie & Welsh, 1980; Guarini *et al.*, 2004; Malet *et al.*, 2008). However, the comparatively higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the POM pool (by 2-3‰) also suggest the incorporation of other sources of organic matter. The POC / Chl *a* ratio is a convenient indicator of the POM quality. It can be used to estimate the relative proportion of fresh algae to detrital organic matter within the POM pool. Low values between 20 and 200 are indeed reported to characterise fresh phytoplankton (Parsons *et al.*, 1977; Cifuentes *et al.*, 1988) whereas higher values indicate the predominance of detrital matter within the POM pool. The high C:N and POC/Chl *a* ratios measured in the POM fraction in November 2005 go to show that this organic matter was largely detrital. Decaying *Enteromorpha* spp. can be expected to be a significant contributor to the POM pool during the fall period. After largely covering the sandflat as thick algal mats during the summer, these green algae were indeed still observed at the sediment surface in November 2005 although mostly buried. Although confined to the eastern margin of the flat and being minor on the site compared with the Ulvales, the C_4 plant *Spartina anglica* might have also entered the POM pool at a detrital state thereby contributing to the high $\delta^{13}\text{C}$ and C:N ratio of the POM, the refractory lignin fraction of this plant becoming dominant as degradation proceeds. The POM collected in November 2005 thus appears as a complex mixture of organic matter mostly detrital in nature, and most likely originating from local ^{13}C -enriched primary producers. $\delta^{13}\text{C}$ signatures of the site POM were much lower and closer to the offshore POM in spring and summer 2006. This site POM was further characterised by low POC/Chl *a* ratios (<200), low C:N ratios (<6.5) and increasing chlorophyll *a* levels (up to $5\mu\text{g l}^{-1}$ in June 2006), suggesting a high proportion of fresh neritic phytoplankton within the POM pool, as to be expected accordingly with the spring bloom timing (O'Higgins, 2005). This tends to confirm that the influence of sewage POM on the POM pool entering BISL is likely minor, at least at these POM sampling dates. The results also outline the high variability of the POM composition in BISL in response to local hydrodynamics and environmental conditions. However these results only constitute a snapshot of a phenomenon that may occur at a much higher frequency, as pointed out in studies involving higher frequency sampling (Rolff, 2000; Nordstrom *et al.*, 2009). To avoid the confounding effects that the use of the site POM may therefore have on diet estimations, sea POM was used for this latter purpose.

Isotopic composition of the SOM was intermediate among the other measured sources, suggesting the SOM pool to result from a mixture of these sources under decomposition,

as it is usually reported for enclosed or semi-enclosed ecosystems (Nichols & Allen, 1981). The contributions of the various sources to the SOM are liable to vary with time depending on the seasonal cycle of growth and senescence proper to each primary producer. The slight but statistically significant $\delta^{15}\text{N}$ increase of the SOM in November 2005 and June 2006 likely resulted from an increased incorporation of ^{15}N -enriched *Enteromorpha* spp., especially as it coincided with high abundances of these algae on site either buried or in a fresh state. Multiple processes however affect SOM stable isotope ratios such that its isotopic signature cannot be regarded as a simple mixture of end-member sources. Indeed, the decomposition of plants often comes along with a modification of their initial $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, either as an increase or a decrease of the isotopic value depending on species and season (Benner *et al.*, 1987; Cloern *et al.*, 2002). SOM also incorporates other sources of organic matter such as secondary producers (e.g. bacteria, dead animal matter). In addition, deposited organic matter rapidly undergoes diagenic processes which further modify its $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Altabet, 1996; Lehman *et al.*, 2002; Chen *et al.*, 2008). These shifts can be caused by the selective removal of compounds inducing the isotopic composition of the residual substrate to be rebalanced (Lehman *et al.*, 2002), or through isotopic discrimination during remineralisation. For instance, it is widely assumed that the preferential utilization of ^{14}N during this process tends to enrich the residual organic substrate in ^{15}N (Owens & Law, 1989; Thornton & McManus, 1994; Bernasconi *et al.*, 1997).

In summary

- Potential sources of food to BISL zoobenthic community were well differentiated by their C and N isotopic ratios.
- High $\delta^{15}\text{N}$ displayed by the green algae *Enteromorpha* spp. confirms the influence of anthropogenic inputs of nitrogen, which are known to predominantly originate from sewage in Dublin Bay, on the green macroalgal bloom occurring in BISL.
- POM inputs from rivers appeared to be negligible in BISL compared to organic matter derived from marine and local sources such as phytoplankton, microphytobenthos and macrophytes.

3.4.2. Food sources sustaining the food web: influence of macroalgal mats on the base of the food web and on species-specific diets

The various sources of food sampled in this study showed a clear differentiation based on their C and N isotopic ratios, allowing their contribution to the benthic food web of BISL to be assessed. Results suggest that ^{13}C -depleted sources from the catchment basin (river POM) and the saltmarsh were not exploited by the primary consumers, which were always much more ^{13}C -enriched. Such a differentiation is indeed too high to be ascribable to an above average isotopic fractionation (Vander Zanden & Rasmussen, 2001). Moreover, riverine POM was not detected at the entrance of the lagoon in the site POM as discussed in the previous section, which supports a negligible role, if any, for consumers. This is also consistent with the very low riverine flow entering Dublin Bay, even in winter, and with the low contribution of river particulates to the bay particulates budget as demonstrated by Wilson *et al.* (2002). Saltmarsh C_3 and C_4 vascular plants contain highly refractory compounds such as high molecular weight polysaccharides, lignin and phenolic compounds, making them much less digestible and palatable for animals than algal material (Findlay & Tenore, 1982; Mann, 1988; Buchsbaum *et al.*, 1991). Vascular plants can be utilized as a food source when they are decomposed by bacteria (Tenore, 1983; Langdon & Newell, 1990). The substantial ^{13}C enrichment measured between primary consumers and C_3 vascular plants however suggests that even through a bacterial mediation, detrital organic matter derived from these plants did not make a significant contribution to the diet of these primary consumers. Tidal amplitude in Dublin Bay estuary is such that the saltmarshes, unlike north American marshes, are irregularly flooded and only inundated during spring tides as most European marshes (Cattrijsse & Hampel, 2006), thus limiting the export and the availability of the saltmarsh plant production to adjacent flat areas. In this context, *Spartina anglica*, whose abundance is very low on the studied site, can also be considered to be a minor source to the fauna from the adjacent flat.

The large range of $\delta^{13}\text{C}$ values displayed by the primary consumers attests the diversity of sources sustaining the food web. The discrimination among the feeding groups along an increasing $\delta^{13}\text{C}$ gradient (from suspension-feeders and deposit-feeders to interface-feeders to grazers) points out different trophic pathways at the base of the food web due

to a differential utilisation of the local sources by these various primary consumers. In this sense, the enlarged group of deposit-feeding species showed some ability to partition the resources among surface and sub-surface deposit-feeders, interface-feeders and the grazer *H. ulvae*. The results further evidenced a seasonal trophic shift of primary consumers and particularly deposit-feeders, induced by the green macroalgal bloom of *Enteromorpha* spp, which confirms the second hypothesis of this work. The sources supporting the food web and the trophic pathways involved are discussed thereafter.

While the influence of sewage particulates on the pool of POM entering Bull Island South Lagoon was not obvious, sewage POM was nevertheless found to contribute to the food web of this mudflat, essentially through the suspension-feeders (mostly *C. edule*) and the deposit-feeder *A. marina*. Even if the contribution of sewage POM to the diet of these species might have been overestimated by the model (see section 3.3.4), this finding challenges the position taken by Wilson *et al.* (2007) in their network analysis of energy flow through Dublin Bay, and emphasises the importance of considering trophic interactions at small-spatial scale, especially in highly dynamic environments such as estuarine systems.

The green macroalgal bloom was shown to greatly influence the primary consumers diet at some time of the year, with an increased proportion of *Enteromorpha* spp. traced in the tissues of the various feeding guilds in December 2005, as shown by the mixing models. This was evidenced owing to the high $\delta^{15}\text{N}$ of the green algae, itself indicative of the anthropogenic influence on the site (McClelland *et al.*, 1997; Cole *et al.*, 2005), which drove the strong $\delta^{15}\text{N}$ increase of the meiofauna and invertebrates primary consumers (up to 4.4‰). These diet changes were reflected in the primary consumers tissues sampled in December 2005 but likely occurred during the previous weeks, i.e. in autumn, due to the delay of isotopic incorporation of new dietary sources into marine invertebrate consumers tissues as estimated from recent studies (as reviewed by Thomas & Crowther, 2015; Vander Zanden *et al.*, 2015). The autumnal period corresponds to the decaying phase of the algal mat, and progressively, to its burial in sediments (Jeffrey *et al.*, 1992; Khan, 1998), but the incorporation of *Enteromorpha* in the food web may have also been progressive from the summer months. In autumn, the high biomass of *Enteromorpha* spp. appeared to be exploited by all primary consumers, including the suspension-feeder bivalve *C. edule*, but was mostly processed by deposit-feeding species (i.e. surface deposit-feeders and interface-feeders). These results are in accordance with Rossi (2007)

who showed deposit-feeders to derive up to 26% of their ^{15}N from decomposing green algae buried in the sediments, and suggested an even higher use of it. In the present study, *Enteromorpha* spp. had a considerable influence of the food web since it constituted more than 50% of the deposit-feeders diets, and up to 66% of it in the case of the lugworm *Arenicola marina*. It was also consumed by the grazer/deposit-feeder snail *Hydrobia ulvae* which made its highest use of it at this period (up to 42% of its diet). A narrowed distribution of the meiofauna and primary consumers $\delta^{13}\text{C}$ was concomitantly recorded in December 2005, suggesting an alignment of trophic pathways at the base of the food web at this time of the year. A linear arrangement of the food web on a sandy beach affected by green tides has been similarly evidenced by Quillien *et al.* (2016). Interestingly, the incorporation of the algal material into the food web was particularly recorded by these authors in autumn during the decomposition of the mats, as in the present study.

Herbivores preference for chlorophytes such as filamentous Ulvales is largely reported (e.g. Goecker & Kåll, 2003; Paul *et al.*, 2006; Van Alstyne *et al.*, 2009). These ephemeral green algae are considered attractive and more edible to grazers as they lack chemical defenses such as deterrent secondary metabolites of perennial species, are nitrogen rich, and have a morphology and structure enhancing their palatability (Littler & Littler, 1980; Hay & Feinical, 1988). Detrital *Enteromorpha* is likely to represent a good alternative to consumers during autumn and winter when the pelagic and benthic microphyte production is low. Nitrogen content of decomposing *Enteromorpha* was indeed increased as shown by its low C:N ratio in November 2005. This may result from a rapid loss and mineralization of the carbon (Robinson *et al.*, 1982; Best *et al.*, 1990) and also from the microbial colonisation of this substrate. This higher nitrogen content confers an increased nutritional value to the algae. Van Alstyne *et al.* (2009) demonstrated an increased attractiveness of *Ulva* to grazing snails when its C:N ratio fell below 8, which was the case in the present study in autumn. Additionally, the fragmentation of the algae thalli as degradation progresses and becomes predominant likely extends the accessibility of this resource to other feeding groups. *Enteromorpha* fragments can be redistributed in the sediments by bioturbating species such as *H. diversicolor* (Raffaelli, 2000; Nordstrom *et al.*, 2006) and might as well be resuspended through tidal- or wind-induced water motion, hence becoming available to a wider range of deposit-feeders (such as *A. marina*) and to suspension-feeders, as observed during this study. The bloom-forming algae have thus been likely used either directly, as well as indirectly through its contribution to the

suspended and sedimentary pools of organic matter. The increase of the SOM $\delta^{15}\text{N}$ observed in November 2005, is indeed likely linked to the degradation of *Enteromorpha* spp., and would support this assumption.

Later during the year, i.e. through winter 2006 and the following spring when taking into account the tissues turnover time, the contribution of the green algae to the diets of primary consumers decreased progressively but never completely disappeared, reflecting the persistency of its effects on the system. This was most obvious for the grazing snail *H. ulvae* which was the first species to exploit this source, along with microphytobenthos, through spring 2006 when the algal mat started to develop again. *H. ulvae* indeed is a well-known grazer of green algae in estuarine environments, and is also known to occupy the algal mats which may further act as a refuge for this gastropod (Raffaelli *et al.*, 1998).

Conversely, the analysis of the interface-feeders tissues in April and then July 2006 showed that *M. balthica* and *S. plana* had switched mostly to microphytobenthos in late spring 2006 as it constituted from 50 to 73% of their food items (median contribution). They relied on benthic sources (microphytobenthos and detrital *Enteromorpha*), and to a lesser extent on plankton/POM in March-April 2006. These two interface-feeding bivalves clearly shared the same trophic niche and overall showed to favor benthic primary producers over particulate and or planktonic matter. These results suggest a preference for deposit-feeding over suspension-feeding in Bull Island South Lagoon. Deposit-feeding can be preferred at low water velocities or when the flux of suspended particulates is low (Lopez & Levinton, 1987). In muddy sediments, deposit-feeding has for example been shown to induce a higher growth rate than suspension-feeding for the interface-feeder polychaete *Pseudopolydora kempji japonica* (Taghon & Greene, 1992).

The suspension-feeder *C. edule* and the deposit-feeder *Arenicola marina* had a higher reliance than interface-feeders on marine POM in late winter and in spring 2006 (as pointed out from the analysis of their tissues in April and July 2006). Owing to their feeding mode, this source may have been processed either directly from the water column during its sedimentation and eventually its resuspension (*C. edule*), or through the incorporation to the SOM pool during/after sedimentation (*A. marina*). In late spring 2006, the contribution of microphytobenthos to the diet of the filter-feeder *C. edule* also increased, underlying the importance of resuspension events in the trophic functioning of this shallow environment, as reported for similar environments (Malet *et al.*, 2008). The

importance of phytoplankton and microphytobenthos in fuelling benthic food webs of coastal mudflats and sheltered sandflats is a common feature of such ecosystems (e.g. Kang *et al.*, 1999; Riera *et al.*, 1999; Machás *et al.*, 2003; Page & Lastra, 2003; Kanaya *et al.*, 2007). Microphytobenthos and phytoplankton can indeed be more nutritive than macroalgae (Valiela *et al.*, 1997) and thereby be preferred when available and sufficiently abundant.

Due to diagenic processes occurring within sediments which may modify the isotopic composition of its components (Lehman *et al.*, 2002; Chen *et al.*, 2008; see discussion section 3.4.1), SOM is considered and used by some authors as an additional source of organic matter in dietary analysis (e.g. Riera, 2010). In the present study, given the uncertainties linked with the SOM composition and because it likely incorporates sedimenting POM and local benthic sources already considered in the mixing models, the choice was made to not consider this organic matter pool in the dietary estimations to avoid any confounding effects and to better depict the origin of trophic sources fueling the food web. I acknowledge however that this pool of organic matter and its associated bacterial community plays a fundamental role in channeling some sources of organic matter (e.g. *Enteromorpha* spp., planktonic matter) to deposit-feeders such as *A. marina* and sub-surface deposit-feeders.

The isotopic signals of nematods, always close to the suspension-feeder *C. edule* or slightly less ^{13}C -enriched, suggest that they essentially exploit POM, probably once sedimented, and potentially through its associated bacterial community (Gerlach, 1978; Pascal *et al.*, 2008). The relatively large range of isotopic values measured for this meiofaunal community nevertheless suggests some partitioning in the exploitation of the POM/SOM components, or may reflect isotopic differences amongst feeding groups (Moens & Vincx, 1997) as these were not differentiated in the present study. The omnivorous polychaete *H. diversicolor* was also characterized by a strong increase of its $\delta^{15}\text{N}$ in December 2005. Common inhabitant of European estuarine mudflats, the feeding behaviour of this species has been extensively studied. *H. diversicolor* can act as a suspension-feeder, switch to deposit-feeding or grazing, and can also behave as a predator or scavenger (see Fauchald & Jumars, 1979 and references therein; Rönn *et al.*, 1988; Riisgård, 1991). This polychaete has been shown to feed on green algae to such an extent that it can profoundly affect the algae biomass and relocate it in the sediments

(Raffaelli, 2000; Nordstrom *et al.*, 2006; Engelsen & Pihl, 2008), as mentioned above. Olivier (1995) acknowledged that this opportunistic species can consume the source of best quality or the most abundant at the moment. In this context, it is highly probable that the increase of the $\delta^{15}\text{N}$ signature of *H. diversicolor* recorded in December 2005 was also related to the consumption of *Enteromorpha* spp..

In summary

- Sewage POM played a moderate trophic role in the estuarine mudflat of BISL, and was mainly processed by the suspension-feeding group and the deposit-feeder *A. marina*.
- Different trophic pathways at the base of the food web linked to a differential use of basal food sources (POM, microphytobenthos, green algae) by primary consumers were evidenced and reflected in the C isotopic discrimination of these consumers.
- The green macroalgal bloom of *Enteromorpha* spp. had a strong influence on all the primary consumers diets, especially during the decomposition of the mat in autumn, becoming their dominant source of food. It was mostly processed by deposit-feeders, reaching 66% of their diet, but it overall tightened the base of the food web at this period of the year, indicating an alignment of trophic pathways.
- The seasonal exploitation of ^{15}N -enriched green macroalgae induced an isotopic shift of the primary consumers $\delta^{15}\text{N}$ from 1.1 to 4.4‰.

3.4.3. Food web structure

Methodological considerations

Given the high N fractionation between a consumer and its prey, trophic levels are classically calculated based on $\delta^{15}\text{N}$ values. As these calculations require a homogenous baseline, primary consumers (TL = 2) are generally used for this purpose to overcome the strong heterogeneity commonly observed in the $\delta^{15}\text{N}$ of primary producers (VanderZanden & Rasmussen, 2001; Post, 2002). Dominant primary consumers in BISL showed significant seasonal variations of their $\delta^{15}\text{N}$ due to the consumption of ^{15}N -

enriched sources at some periods of the year. It appeared necessary to take this source of variation into account by calculating trophic levels per season. Although minor compared with primary producers, the heterogeneity of the primary consumers $\delta^{15}\text{N}$ however complicated the trophic level calculations. For this reason, the $\delta^{15}\text{N}$ variability associated to the primary consumers used as a baseline was considered in the calculations. Another source of uncertainty taken into account was the $\delta^{15}\text{N}$ variability associated with each secondary consumer species (intra-specific variability). It is important to keep in mind that the degree of uncertainty associated to these sources is mirrored when looking at the difference between the upper and lower ranges of the TL estimates. This difference tends for example to be higher in December 2005 when the $\delta^{15}\text{N}$ heterogeneity among the primary consumers was the highest.

Food web length and structure

The food web in BISL was composed of 4 trophic levels (TL) from primary producers to predatory fish, which is consistent with results from previous studies in coastal environments such as enclosed lagoons (Carlier *et al.*, 2008) or shallow open bays (Carlier *et al.*, 2007b; Nordstrom *et al.*, 2009) and is typical of marine environments (Vander Zanden & Fetzer, 2007). While the food web length is considered by some authors to not be linked to resource availability or primary productivity (Pimm & Lawton, 1977; Vander Zanden *et al.*, 1999; Post, 2002b), it has however been showed to be reduced in systems under high anthropogenic stress through eutrophication (Vander Zanden & Fetzer, 2007; Coll *et al.*, 2008; Coll *et al.*, 2011). This effect was not observed in BISL.

Consumers trophic levels determined in the present study from $\delta^{15}\text{N}$ measurements were overall in accordance with their feeding groups identified based on the literature. These TL were stable with time for most species, even if $\delta^{15}\text{N}$ changes were observed at the consumer level (e.g. *H. diversicolor*). Such results point out the importance to calculate TL at a seasonal scale in order to account for shifts of the baseline. The TL of some recognised predatory invertebrates such as *Nephtys hombergii* or *Crangon crangon*, oscillated between 2.2 and 3.5. Such variations are unlikely to result from an omnivorous regime (Fauchald & Jumars, 1979; Pihl & Rosenberg, 1984; Schubert & Reise, 1986), and rather underline the large diversity of prey they exploit,

from meio to macro-invertebrates (Pihl & Rosenberg, 1984). Feeding on nematods with a low $\delta^{15}\text{N}$ as measured in the present study would indeed induce a shift to lower $\delta^{15}\text{N}$ values of their predators and thereby a decrease of their TL value. On the other hand, TL variation observed for the omnivorous green crab *Carcinus maenas* likely results from the consumption of green macroalgae as previously reported by Warwick *et al.* (1982).

The large range of $\delta^{13}\text{C}$ displayed by secondary consumers reveals a diversity of foraging behavior among predators, associated with the different trophic pathways at the base of the food web, as it emerged on the isotopic dual plots (Fig. 3.9) and from the dietary estimations of primary consumers. ^{13}C -depleted predators, such as the planktivorous fish *Clupea harengus* or the benthivorous flounder *Platichthys flesus*, exploited trophic pathways mainly based on planktonic or suspended sources (POM), through pelagic or benthic intermediates. Our isotopic data indeed suggest that *P. flesus* fed on the suspension-feeder *C. edule* (its siphons), which is in accordance with findings of Pasquaud *et al.* (2010) based on stomach contents. Conversely, ^{13}C -enriched species, such as *Pleuronectes platessa*, *Pomatoschistus* spp. and *A. presbyter*, appeared to rely on another trophic pathway based on deposit-feeding species exploiting primarily benthic primary producers (green algae, biofilm). The sand smelt (*Atherina*) is a recognized planktivorous but also an opportunist benthivorous which essentially feeds on benthic preys in shallow waters (Kiener & Spillmann, 1969; Vizzini & Mazzola, 2005). Furthermore, the winter $\delta^{15}\text{N}$ increase of the resident goby *Pomatoschistus* spp. (one of the few predatory fish to have been sampled over three surveys and in December 2005), by following the same trend as for primary consumers, gives evidence of the incorporation of the green algae *Enteromorpha* spp. into the food web up to higher trophic levels.

3.4.4. Effect of the macroalgal bloom on the trophic functioning of the community

This study aimed to assess the effects of the green macroalgal bloom on the trophic structure and functioning of BISL mudflat community. Impacts on the food web length and on the diversity of the trophic pathways connecting top consumers to basal resources were expected. The high production of opportunistic *Enteromorpha* spp. influenced

species-specific diets, particularly at the primary consumers level, but was also channeled to higher trophic levels of the food web, via the deposit-feeders guild mainly (see section 3.4.3 and Fig. 3.9), the high $\delta^{15}\text{N}$ of *Enteromorpha* spp. being reflected at the different trophic levels. The high biomass of *Enteromorpha* spp. likely entered the food web progressively but the effects were significantly recorded at the end of autumn, several weeks after the start of the decay and burial of the mat. At this stage, the algal mat was exploited by all consumers but predominantly processed by deposit-feeders and grazers (section 3.4.2). This result, also recently reported by Park *et al.* (2016), corroborates the assumption that eutrophication, and particularly macroalgal blooms, favors detritivores and grazers in sheltered systems, which could lead to a structural modification of the community. Such a situation was reported by Marques *et al.* (2003) in the Mondego estuary on the most eutrophied sites and persisted with time. The food web of BISL featured different trophic pathways from basal resources up to higher trophic levels (see section 3.4.3), but this trophic functioning was tackled seasonally by the macroalgal bloom. After the bloom (i.e. by late autumn), a reduction of trophic links was indeed observed with the emergence of a dominant trophic pathway based on *Enteromorpha* spp. Severe eutrophication resulting in the overgrowth of some opportunistic primary producers has been recently shown to induce the simplification of the trophic complexity (Coll *et al.*, 2011; Quillien *et al.*, 2016), and also to reduce the food web length (Coll *et al.*, 2011). In the present study, the food web length was typical of marine environments (Vander Zanden & Fetzer, 2007) and the reduction of the number of trophic pathways was seasonal, the consumers managing to switch to other sources at other times of the year, such that the impact of the bloom may be interpreted as intermediate. However, sediments remain enriched with macroalgal organic matter during the wintering period when they are bare, such that *Enteromorpha*-derived organic matter was still traced in the consumers tissues at the next spring period, revealing the persistency of the bloom effects. The recurrence of the bloom every summer moreover maintains the communities in a disturbed state, with likely effects on the whole community structure and on higher trophic levels, including top predators (including shorebirds, waders, fish) of high ecological and economic values, for which the mudflat is an important foraging area. Whereas macroalgal blooms may be profitable to deposit-feeders as a food supply during their decay, they also induce many deleterious effects on the infauna (including deposit-feeders) and epibenthic assemblages. They can indeed limit larval settling, affect foraging behavior of epibenthic species and reduce oxygen penetration to the sediments thereby

affecting burrowed species (Valiela *et al.*, 1997; Raffaelli *et al.*, 1998; Raffaelli, 2000; Wennhage & Pihl, 2007). In this context, it would be worthy to couple such a seasonal food web study with an assessment of the community structure and diversity in order to have a thorough understanding of the biological interactions induced by the algal mats throughout the different stages of the bloom. Measurements of the fauna abundance and biomass would moreover weight the effects of the macroalgal bloom on the food web and the communities structure. Considering the large surface of the flat covered by algal mats every year, most of the lagoon may be affected given that the impact of macroalgal proliferation on communities can be felt at a distance from the mats (Ouisse *et al.*, 2011). Those studies should nevertheless be carried out on a larger spatial scale to account for small-scale spatial variations. Finally, this study clearly showed the necessity of following the fate of algal mats during and after the growing season, thus stressing the importance of considering temporal variability in food web studies of such dynamic and changing environments in order to get a sound idea of the processes underlying the functioning of these ecosystems.

3.5. Conclusion

The green macroalgal bloom impacted the food web functioning in Bull Island South Lagoon. It significantly influenced species-specific diets of all primary consumers and was channeled to higher trophic levels of the food web, mainly via deposit-feeders which derived up to 66% of their food from the green algae. These main effects were recorded several months after the peak production of the green algae, i.e. following its decay and during its burial in sediments. The food web which featured different trophic pathways was simplified at this period and one main energy pathway, based on detrital green algae, emerged. This was reflected in a $\delta^{13}\text{C}$ narrowing at the base of the food web and $\delta^{15}\text{N}$ isotopic shifts at different trophic levels, and depicted through dietary mixing models of primary consumers.

This study highlighted the impact of sewage from Dublin urban centre on the ecosystem of Bull Island South Lagoon, and evidenced its direct and most of all indirect influence on this site of high ecological value. Sewage particulates were thus shown to have a moderate direct effect on the food web, and to be processed mostly by suspension-feeders

and the deposit-feeder *Arenicola marina*. The indirect role played by sewage on the food web was supported by the high $\delta^{15}\text{N}$ of the bloom-forming algae *Enteromorpha* spp., pointing out the role played by these anthropogenic effluents in sustaining the macroalgal bloom.

CHAPTER 4

Differential influence of sewage on intertidal primary consumers across Dublin Bay: a spatio-temporal isotopic analysis of basal sources and primary consumers

4.1. Introduction

Carbon flow within ecosystems is primarily driven by the quality and availability of basal sources to consumers (Hoeinghaus *et al.*, 2007; Pingram *et al.*, 2014). Spatio-temporal variations of consumers' diets and trophic pathways can thus occur in highly dynamic coastal environments such as estuaries, in response to seasonal changes of the sources abundance, and even more when anthropogenic disturbances are affecting the variety and abundance of potential food sources (McClelland & Valiela, 1998).

Dublin estuary receives sewage effluent from the surrounding conurbation, which stimulates annual macroalgal blooms of *Enteromorpha* spp. (Jeffrey *et al.*, 1992; 1995) in the northern part of Dublin bay, particularly in Bull Island South Lagoon. At this site, sewage particulates have been shown in Chapter 3 to have a moderate trophic influence whereas the high biomass of green algae significantly affects the primary consumers diet. The spatial extent of the sewage influence on benthic consumers, particularly in regard of the south part of the bay, has never been investigated *in situ*. Given the local hydrographic conditions which induce the estuarine plume conveying sewage effluents to mainly follow a clockwise motion to exit the bay through the north (ERU, 1992), the southern area can be expected to be less impacted by these anthropogenic inputs, with regard to both nutrients and particulates, particularly since the amount of particulates released in the bay has been reduced following the STP upgrade in 2003.

The general aim of this chapter was to identify the basal sources sustaining the intertidal food web and how their use by consumers vary across Dublin Bay and, more specifically, to assess the spatial influence of sewage across Dublin Bay and its knock-on effects on the diets of primary consumers. Since C and N stable isotopes are powerful tools to delineate trophic interactions in coastal systems (Peterson & Fry, 1987; Michener & Schell, 1994) and because $\delta^{15}\text{N}$ is a good indicator of anthropogenic nutrient enrichment in urbanised areas (McClelland *et al.*, 1997; Cole *et al.*, 2005), this study was undertaken through a spatio-temporal analysis of isotopic signatures of both basal sources and primary consumers at three intertidal sites with presumably different exposure to anthropogenic effluents (Bull Island South Lagoon, Sandymount and Blackrock). The overarching hypothesis was that the influence of sewage is low in the south part of Dublin Bay. This was examined through two sub-hypothesis: 1) autochthonous

macroalgae exhibit different $\delta^{15}\text{N}$ across the bay as a result of their differential exposure to sewage nutrients, i.e. the macroalgae $\delta^{15}\text{N}$ would be much lower in the south of the bay than in the impacted site of BISL; 2) sewage particulates play a minor trophic role for primary consumers in the south part of the bay. Spatial difference in the direct (POM) and indirect (through macroalgae) influence of sewage on primary consumers should therefore induce different contributions of local sources to their diets across the bay and through time.

4.2. Supplementary material and methods

4.2.1. Data analysis

Spatial variations in the C and N isotopic ratios of the sources among the three intertidal sites (BISL, Sandymount and Blackrock) were assessed using the non-parametric Kruskal-Wallis test, followed by Dunn's *post hoc* tests (Zar, 1999). Tests of variations between two types of sources (i.e. macroalgae and microphytobenthos) were performed using non-parametric Mann-Whitney test. Spatio-temporal variations in the C and N isotopic values of the primary consumers from the three intertidal sites could not be assessed using 2-way ANOVA due to strong heteroscedasticity among samples, except for the species *Cerastoderma edule*. Tests of spatial and temporal variations of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of primary consumers were therefore carried out using the non-parametric Kruskal-Wallis test, followed by pairwise multiple comparisons (Dunn's method) when applicable. These tests were realised for feeding groups (deposit-feeders, interface-feeders, and suspension-feeders which were only represented by the cockle *C. edule*), and further for the interface-feeding bivalves and the deposit-feeder *Arenicola marina*. Species sampled at only one occasion and species whose isotopic data corresponded to pooled individuals were not included in the analysis.

4.2.2. Dietary analysis

Contributions of food sources to the diet of the only two species common to the three intertidal sites (*A. marina* and *C. edule*) were estimated for each site with a Bayesian

approach using the mixing model SIAR (Parnell *et al.*, 2008; Jackson *et al.*, 2009; Parnell *et al.*, 2010). Annual mean values of the sources were used for this purpose. Sources considered were the marine Particulate Organic Matter (seaPOM), sewage POM, microphytobenthos (MPB) and macroalgae (all species pooled at each site). As microphytobenthos and macroalgae C and N isotopic signatures were not significantly different in Blackrock (MW, $p > 0.2$ for both isotopes), they have been combined for the model (consistently with recommendations of Phillips *et al.*, 2014), which was otherwise struggling to determine their contributions to the diets. The trophic fractionation factors applied were the same as the ones retained for species analysed in Chapter 3, i.e. 3.4 ± 0.7 ‰ for $\Delta\delta^{15}\text{N}$ and 0.4 ± 1.3 ‰ for $\Delta\delta^{13}\text{C}$ (corresponding to the weighed mean and pooled SD of the estimates from Post, 2002; Yokoyama *et al.*, 2005; and Dubois *et al.*, 2007). Trophic fractionation factors were assumed to be the same for all sources and surveys.

4.3. Results

4.3.1 Potential food sources at intertidal sites

4.3.1.1. Characterisation of the suspended POM

Values of the hydrobiological variables of the suspended POM sampled in Sandymount (SM) and Blackrock (BR) are given in Table 4.1. Values measured for the same variables at BISL, in the Liffey river and at the offshore station of Kish bank (Open sea POM) and further described in Chapter 3 are also reminded in Table 4.1. Levels of Total Suspended Matter (TSM) were highest in November 2005 and June 2006 at both SM and BR. From the three intertidal sites, the highest concentrations were measured in BR for each survey. POM sampled at BR also exhibited the highest content in organic carbon (POC) of all intertidal sites, whatever the surveys. At SM and BR, Chlorophyll *a* (Chl *a*) concentrations were higher in June 2006 than other surveys, as observed for BISL. Chl *a* concentrations were always higher at these three intertidal sites than offshore in June 2006. At all sites, the POC/Chl *a* ratio was low (<130) in June 2006 and high in November 2005. The highest C:N ratios were recorded in November 2005 for each of the

sites. Salinity was relatively stable with time and varied between 31 and 33 at both these sites.

Table 4.1: Characteristics of the seston (mean ± standard deviation) collected in Sandymount (SM) and Blackrock (BR). Results measured in the Bull Island South Lagoon (BISL), in the Liffey river and offshore (Open Sea) and previously described in Chapter 3 are reminded here to facilitate comparisons. POC: Particulate Organic Carbon, Chl *a*: Chlorophyll *a*. In brackets: number of replicates.

Variable	Survey	Intertidal sites			River	Open sea
		SM	BR	BISL		
Totat Suspended Matter (mg L ⁻¹)	May 2005	-	-	-	-	-
	November 2005	32.4 ± 3.3 (3)	34.6 ± 3.3 (3)	25.5 ± 1.1 (3)	13.2 ± 2.1 (3)	-
	March 2006	13.3 ± 5.7 (2)	17.9 ± 0.1 (2)	15.3 ± 3.9 (2)	2.5 ± 0.1 (2)	-
	June 2006	22.8 ± 0.5 (2)	32.7 ± 3.5 (2)	22.5 ± 0.6 (2)	4.1 ± 0.4 (2)	-
POC (µgC L ⁻¹)	May 2005	-	-	-	-	-
	November 2005	389 ± 15 (2)	1013 ± 152 (2)	521 ± 118 (2)	1455 ± 90 (2)	185 ± 17 (3)
	March 2006	161 ± 2 (2)	410 (1)	259 ± 52 (2)	461 ± 9 (2)	164 ± 7 (3)
	June 2006	553 ± 21 (2)	791 ± 27 (2)	527 ± 121 (2)	716 ± 27 (2)	167 ± 12 (2)
Chl <i>a</i> (µg L ⁻¹)	May 2005	-	-	-	-	0.7 ± 0.3 (2)
	November 2005	1.3 ± 0.5 (2)	1.6 ± 0.4 (2)	0.5 ± 0.1 (2)	1.3 ± 0.4 (2)	0.6 ± 0.2 (3)
	March 2006	0.5 ± 0.0 (2)	1.9 ± 0.2 (2)	1.7 ± 0.2 (2)	3.2 ± 0.4 (2)	0.7 ± 0.1 (3)
	June 2006	6.9 ± 0.7 (2)	6.3 ± 0.5 (2)	5.3 ± 0.1 (2)	6.2 ± 0.7 (2)	2.0 ± 0.9 (2)
POC/Chl <i>a</i>	May 2005	-	-	-	-	-
	November 2005	331 ± 136 (2)	666 ± 244 (2)	1163 ± 488 (2)	1137 ± 413 (2)	330 ± 156 (3)
	March 2006	302 ± 4 (2)	236 (1)	152 ± 46 (2)	145 ± 20 (2)	232 ± 20 (3)
	June 2006	81 ± 5 (2)	125 ± 14 (2)	100 ± 21 (2)	116 ± 17 (2)	92 ± 37 (2)
C: N ratio	May 2005	-	-	-	6.5 ± 0.3 (3)	6.8 ± 0.5 (3)
	November 2005	17.7 ± 0.2 (2)	18.1 ± 0.3 (2)	21.4 ± 2.6 (2)	11.3 ± 0.8 (3)	12.1 ± 0.4 (3)
	March 2006	6.4 ± 0.1 (2)	7.4 (1)	6.0 ± 0.1 (2)	8.6 ± 0.3 (3)	7.9 ± 0.2 (3)
	June 2006	6.4 ± 0.5 (2)	7.4 ± 0.1 (2)	6.4 ± 0.5 (2)	6.5 ± 0.5 (3)	6.9 ± 0.4 (3)

4.3.1.2. Isotopic signatures of the sources

Table 4.2 presents the mean isotopic ratios (averaged over surveys) of the sources collected at the three intertidal sites. Seasonal isotopic values of each source from Sandymount and Blackrock are detailed in Appendix 4.1 and 4.2 respectively. Mean δ¹³C values of the sources collected at SM varied from -19.8‰ (POM) to -13.2‰ (MPB). At BR, mean δ¹³C of the potential food sources varied from -23.1‰ (stranded *Desmarestia*) to -15.9‰ (detrital *Ceramium* sp.). Macroalgae collected at this site were all detrital. They belonged to different species but most of them had δ¹³C between -18 and -16‰, very close (and not significantly different; MW, p=0.49) to the δ¹³C signature of MPB at

this site. Most sources displayed strong spatial and/or temporal isotopic variations. The POM collected in the south intertidal area of the bay was characterised by a high $\delta^{13}\text{C}$ temporal variability linked with a strong ^{13}C -enrichment of the POM in November 2005 at both SM and BR, as for BISL. $\delta^{13}\text{C}$ of the POM thus varied from -22.4‰ to -16‰ at SM and from -21.6‰ to -14.4‰ at BR (Appendix 4.1 and 4.2). The POM also showed a strong decrease of its $\delta^{15}\text{N}$ at SM in November 2005. SOM displayed relatively stable $\delta^{13}\text{C}$ values at each intertidal site and these values differed significantly among the three sites (KW, $p<0.001$), showing a gradient of decreasing $\delta^{13}\text{C}$ from SM to BR to BISL. Likewise, MPB displayed significant spatial variations of its $\delta^{13}\text{C}$ (KW, $p=0,001$) with higher values at SM than BISL or BR (Table 4.2).

The range of $\delta^{15}\text{N}$ values of sources collected at BISL was much larger than for the south of the bay (Table 4.2) due to the high $\delta^{15}\text{N}$ signal ($> 11\text{‰}$) of *Enteromorpha* spp. and most of the saltmarsh plants at BISL (see Table 3.3 for details). $\delta^{15}\text{N}$ of *Enteromorpha* spp. was significantly higher (KW, $p = 0.001$) at BISL than at SM and BR (difference of about 4‰) and the $\delta^{15}\text{N}$ values were much more stable over time at BISL. MPB also displayed significant spatial variations of its $\delta^{15}\text{N}$ signature with higher values at BR than at SM and BISL (KW, $p=0.001$). At SM, $\delta^{15}\text{N}$ of the potential sources ranged in average from 4.7‰ to 8.6‰ (Table 4.2). At BR, $\delta^{15}\text{N}$ of the sources were relatively close, varying in average between 6.2‰ and 8.7‰ but the SOM displayed a high temporal variability with $\delta^{15}\text{N}$ varying between 6 and 9.8‰ (Appendix 4.2).

Table 4.2: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (overall mean ‰ $\pm$ sd) of the sources collected at the three intertidal sites of Bull Island South Lagoon (BISL), Sandymount (SM) and Blackrock (BR). n: number of samples, sd: standard deviation.

Sources	BISL						SM						BR					
	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
	n	mean	sd	mean	sd		n	mean	sd	mean	sd		n	mean	sd	mean	sd	
POMsite	6	-20,1	4,1	6,6	0,9		6	-19,8	3,0	6,7	1,8		5	-18,7	3,8	7,5	1,2	
SOM	12	-21,5	0,8	7,9	0,3		9	-18,4	0,5	6,7	1,1		10	-20,1	0,6	7,7	1,7	
Microphytobenthos	12	-15,6	1,0	4,6	1,2		12	-13,2	1,7	4,7	2,2		11	-17,7	1,7	7,0	1,0	
Chlorophyta																		
<i>Enteromorpha</i> spp. / <i>Ulva</i> sp.	9	-15,4	0,7	12,1	1,1		8	-15,3	2,0	8,3	1,9		4	-18,4	1,0	8,2	0,8	
Ochrophyta																		
Fucales													18	-16,7	1,0	7,3	0,8	
Kelps													5	-17,0	2,5	6,2	0,4	
<i>Ectocarpus</i> sp.							1	-16,2		8,6			2	-19,0	0,1	8,2	0,1	
<i>Desmarestia</i> sp.													2	-23,1	0,3	7,5	0,4	
Rhodophyta																		
<i>Ceramium</i> sp.													2	-15,9	1,3	8,7	0,1	

4.3.2. Isotopic signatures of primary consumers

$\delta^{13}\text{C}$ values displayed by primary consumers varied from -23.1‰ to -14.4‰ at Sandymount (Table 4.3) and from -25.1‰ to -16.6‰ at Blackrock (Table 4.4). The range of $\delta^{15}\text{N}$ values of these macro-invertebrates was relatively narrow at SM (1‰, 1.6‰, 1.8‰ and 1.4‰ in June 2005, December 2005, April 2006 and July 2006, respectively) and varied overall from 8.2‰ to 10.5‰ between June 2005 and July 2006. At BR, this range of $\delta^{15}\text{N}$ values was large (4.2‰, 3.1‰, 4‰ and 3.8‰ in June 2005, December 2005, April 2006 and July 2006, respectively) and related to a ^{15}N -enrichment of polychaetes compared to bivalves. $\delta^{15}\text{N}$ of macro-invertebrates ranged at BR between 8.7‰ and 13.6‰.

Figure 4.1 displays seasonal mean values of $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ of feeding groups and species sampled at the three intertidal sites for the four surveys. Strict suspension-feeders were only represented by the cockle *C. edule*. As for sources, most consumers displayed significant spatio-temporal isotopic variations. Deposit-feeders were particularly well differentiated among the three sites by their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, as were interface-feeders to a lesser extent, and the variation of their isotopic values among sites was greater than temporal variations at any site except at BISL (Fig. 4.1). *A. marina* displayed spatio-temporal isotopic patterns very similar to those of deposit-feeders, and could therefore be considered as representative of this feeding group. Suspension-feeders, deposit-feeders and interface-feeders all showed significant spatial variations of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (KW, $p < 0.001$ in all cases) while temporal variations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were only significant for *C. edule* and interface-feeders (Table 4.5). All feeding groups and species displayed higher $\delta^{13}\text{C}$ values at SM than at other sites (Dunn's *post-hocs*, Table 4.5). Regarding $\delta^{15}\text{N}$, interface-feeders and *C. edule* had overall significantly higher $\delta^{15}\text{N}$ at BISL than at other sites (KW, $p < 0.001$). A 2 way-ANOVA performed for *C. edule* however indicated a strong interaction ($p < 0.001$) between the spatial and temporal factors (which both had a significant effect with $p < 0.001$ in both cases). This species was ^{13}C -enriched at all sites in December 2005, but also at SM in April 2006. Deposit-feeders displayed significantly higher $\delta^{15}\text{N}$ at BR than SM and BISL ($p < 0.001$). The highest $\delta^{15}\text{N}$ of all sites was however recorded at BISL in December 2005 for *A. marina* (Fig. 4.1).

Table 4.3: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the primary consumers sampled from June 2005 to July 2006 in Sandymount. Elements of taxonomic classification are indicated in brackets: Amph.: Amphipoda, Biv.: Bivalvia, Gastr.: Gastropoda, Pol.: Polychaeta.
 FS: Feeding Strategy; SF: suspension-feeder, G: Grazer, IF: Interface-feeder, SDF: Surface Deposit-feeder, SSDF: Sub-Surface Deposit-feeder. sd: standard deviation; n: sample size; * indicates that several individuals were pooled.

Species	FS	JUNE 2005						DECEMBER 2005						APRIL 2006						JULY 2006						
		$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			
		n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	
<i>Cerastoderma edule</i> (Biv.)	SF	5	-19,4	1,0	9,1	0,2	11	-17,6	0,2	9,4	0,1	5	-18,2	0,4	9,5	0,3	5	-19,2	0,4	9,5	0,3					
<i>Angulus tenuis</i> (Biv.)	IF	5	-16,7	0,7	8,2	0,3	11	-15,7	0,7	9,0	0,2	6	-17,2	0,2	9,3	0,2	7	-16,8	0,2	8,3	0,4					
<i>Macoma balthica</i> (Biv.)	IF	5	-14,9	0,7	8,4	0,4	10	-15,4	0,6	9,1	0,4	5	-16,7	0,3	9,4	0,4	6	-15,6	0,4	9,1	0,5					
<i>Scrobicularia plana</i> (Biv.)	IF						1	-15,6		8,9		4	-16,6	0,2	9,9	0,2										
<i>Gammarus locusta</i> (Amph.)	SDF, G											1*	-17,0		7,0											
<i>Hydrobia ulvae</i> (Gastr.)	SDF, G											1*	-14,6		8,7		2*	-14,8	0,1	8,3	0,0					
<i>Arenicola marina</i> (Pol.)	SDF	2	-14,4	0,3	8,6	0,2	2	-15,1	0,6	10,4	1,7	4	-16,0	0,6	10,3	0,5	4	-15,6	0,1	9,7	0,7					
<i>Euclymene lombricoides</i> (Pol.)	SSDF											2*	-16,6	0,1	9,5	0,0										
<i>Heteroclymene robusta</i> (Pol.)	SSDF	2	-16,5	0,4	8,9	0,2	2*	-15,9	0,0	8,7	0,1	1*	-15,9		9,7		2*	-16,5	0,1	8,4	0,1					
<i>Notomastus latericeus</i> (Pol.)	SSDF											3	-14,6	0,4	9,8	0,6	4	-15,5	0,3							
<i>Orbinia latreillii</i> (Pol.)	SSDF	3	-16,1	0,5	9,2	0,1																				
<i>Scoloplos armiger</i> (Pol.)	SSDF											6	-14,7	0,5	9,7	0,5	5	-16,6	0,6	10,6	0,0	3	-15,5	0,2	9,7	0,5

Table 4.4: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the primary consumers sampled from June 2005 to July 2006 in Blackrock. Elements of taxonomic classification are indicated in brackets: Biv.: Bivalvia, Pol.: Polychaeta.
 FS: Feeding Strategy; SF: suspension-feeder, G: Grazer, IF: Interface-feeder, SDF: Surface Deposit-feeder, SSDF: Sub-Surface Deposit-feeder. sd: standard deviation; n: sample size; * indicates that several individuals were pooled.

Species	FS	JUNE 2005				DECEMBER 2005				APRIL 2006				JULY 2006							
		n	$\delta^{13}\text{C}$	sd	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	sd	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	sd	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	sd	$\delta^{15}\text{N}$				
<i>Cerastoderma edule</i> (Biv.)	SF	5	-19,4	0,7	9,3	0,4	5	-18,4	0,3	9,5	0,2	5	-19,3	0,2	9,7	0,3	5	-21,1	0,7	9,8	0,2
<i>Angulus tenuis</i> (Biv.)	IF	16	-19,0	0,9	8,7	0,3	15	-17,9	0,4	10,2	0,1	7	-18,6	0,1	9,6	0,3	8	-18,5	0,4	9,6	0,4
<i>Lanice conchylega</i> (Pol.)	IF						1	-19,2		11,5		6	-18,2	0,3	11,5	0,5	5	-18,8	0,6	11,3	0,5
<i>Owenia fusiformis</i> (Pol.)	IF						1	-17,8		12,1		3	-17,8	0,1	11,0	0,4	4	-18,8	0,2	11,9	0,1
<i>Arenicola marina</i> (Pol.)	SDF	4	-17,9	0,3	10,9	0,3	3	-17,9	0,8	12,2	0,8	5	-18,1	0,7	12,2	0,9	5	-17,2	0,5	13,0	0,1
<i>Notomastus latericeus</i> (Pol.)	SSDF	3	-17,1	0,3	12,9	0,1	3	-17,1	0,4	12,6	0,9	4	-16,6	0,5	13,2	0,7	4	-16,6	0,1	13,4	0,2
<i>Orbinia latreillii</i> (Pol.)	SSDF											1	-17,6		13,6						

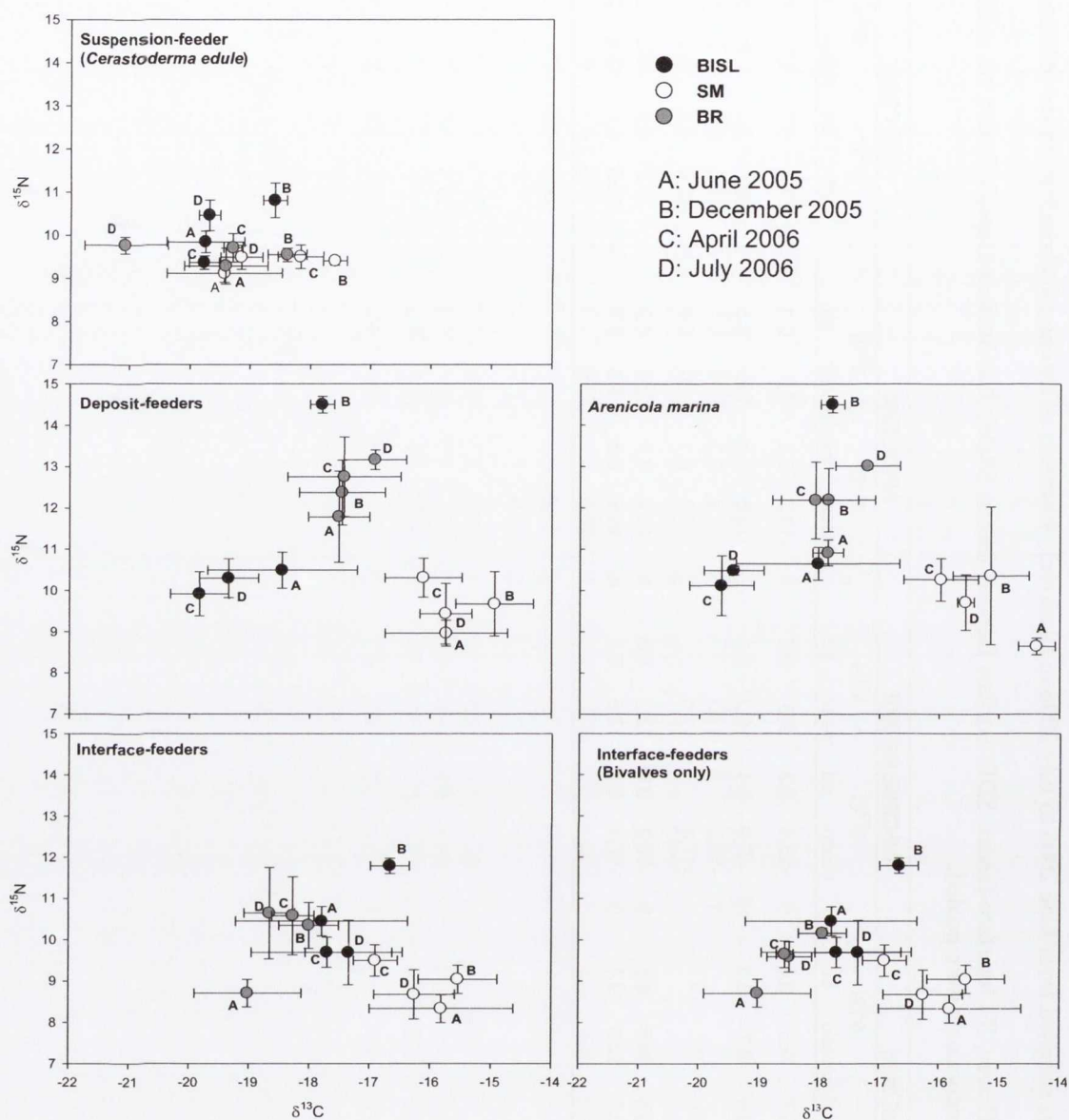


Figure 4.1: Spatio-temporal variations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean $\pm$ standard deviation) of primary consumers collected at the intertidal sites of Bull Island South Lagoon (BISL), Sandymount (SM) and Blackrock (BR).

Table 4.5: Results of the non-parametric ANOVA (Kruskal-Wallis tests) performed on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of primary consumers collected during the four surveys at the three intertidal sites. Sites are: 1: Bull Island South Lagoon, 2: Sandymount and 3: Blackrock. Surveys are 1: June 2005, 2: December 2005, 3: April 2006, 4: July 2006.

$\delta^{15}\text{N}$	site		survey	
	p-value	Dunn's <i>post hoc</i> test	p-value	Dunn's <i>post hoc</i> test
<i>Arenicola marina</i>	p<0.001	1 = 3 > 2	p=0.034	2 > 1, 2 = 3, 4 = 1
<i>Cerastoderma edule</i>	p<0.001	1 > 3 = 2	p=0.010	4 > 1, 1 = 2, 3 = 4
Deposit-feeders	p<0.001	3 > 1 = 2	p=0.655	
Interface-feeders	p<0.001	1 > 3 > 2	p<0.001	3 > 1, 2 > 1, 4 > 1, 3 = 4
Interface-feeders (Bivalves only)	p<0.001	1 > 3 > 2	p<0.001	2>1, 2>4, 3>1, 2=3, 3=4, 4=1
$\delta^{13}\text{C}$	site		survey	
	p-value	Dunn's <i>post hoc</i> test	p-value	Dunn's <i>post hoc</i> test
<i>Arenicola marina</i>	p<0.001	2 > 3 = 1	p=0.740	
<i>Cerastoderma edule</i>	p<0.001	2 > 1 = 3	p<0.001	2>1, 2>3, 2>4, 3>4, 3=1, 1=4
Deposit-feeders	p<0.001	2 > 3 > 1	p=0.055	
Interface-feeders	p<0.001	2 > 1 > 3	p<0.001	2>1, 2>3, 2>4, 1=4
Interface-feeders (Bivalves only)	p<0.001	2 > 1 > 3	p<0.001	2>1, 2>3, 2=4, 4=1

4.3.3. Contribution of food sources to the diet of *Arenicola marina* and *Cerastoderma edule*

The sources contributions (seaPOM, sewage POM, MPB and macroalgae) to the diet of *A. marina* and *C. edule* were estimated for each site. The relative contribution of MPB and macroalgae to the diet of consumers could not be determined at BR as the isotopic compositions of these two food sources were too close (MW, $p > 0.2$).

On an annual basis, sewage POM made a much lower contribution to the diet of the deposit-feeder *A. marina* in the south of the bay (median value <7% at both SM and BR) than in BISL (20%, Fig. 4.2). The contribution of marine POM to its diet was also lower

in SM (12.5%) and BR (6.5%) than in BISL (21.7%). Benthic primary producers (micro and macroalgae) were overall the dominant food sources for *A. marina* but their relative contributions varied among sites. Over the year, the diet of *A. marina* was mostly sustained by *Enteromorpha* spp. in BISL (37%) while MPB was the first food resource for the polychaete in SM (41%). The contribution of both these sources reached 85% in BR. When looking at seasonal variations, a strong diet shift clearly appears in BISL with the substantial increase of the contribution of *Enteromorpha* spp. to *A. marina* tissues recorded in December 2005, during the decomposition of the macroalgal mats (Fig. 4.3), while the diet was more stable over the year in SM.

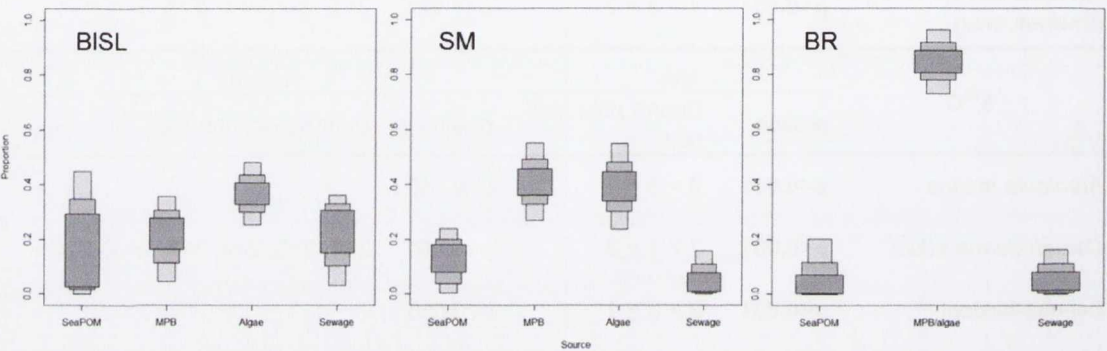


Figure 4.2: Contribution of food sources to the diet of *Arenicola marina* from the three intertidal sites, over the four surveys. Sites are BISL: Bull Island South Lagoon, SM: Sandymount, BR: Blackrock. POM: Particulate Organic Matter, MPB: Microphytobenthos. Contributions are displayed as the 50th, 75th and 95th extent of the probability intervals.

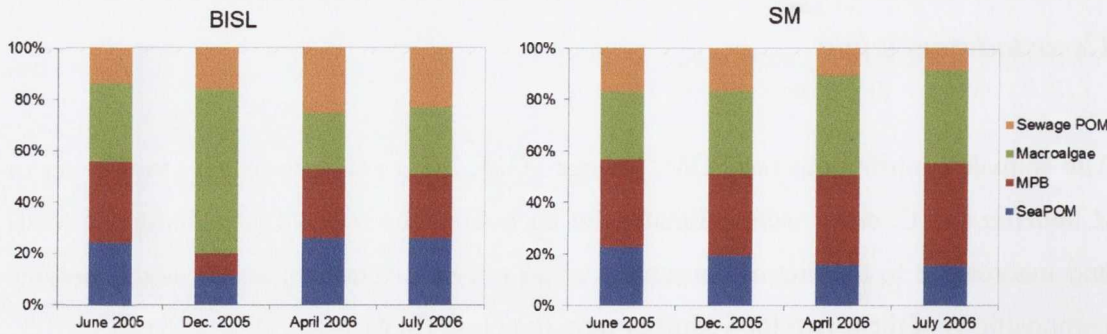


Figure 4.3: Seasonal changes of the relative contribution (median value) of the food sources to the diet of *Arenicola marina* in Bull Island South Lagoon (BISL) and Sandymount (SM). POM: Particulate Organic Matter, MPB: Microphytobenthos.

Sewage POM also made a lower contribution to the diet of the suspension-feeder *C. edule* in the south of the bay, particularly in BR (8%), compared to BISL (25%, Fig. 4.4). In SM, its contribution to the diet of *C. edule* was estimated by the model to 15%. *C. edule* exhibited quite similar diets between SM and BISL on an annual basis, relying mainly on marine POM (median contributions of 28 and 33% respectively in BISL and SM), closely followed by MPB (27 and 26.5% respectively). The contribution of marine POM to its diet was higher in BR (39%). Seasonal variations were not as clear cut as for *A. marina* but *C. edule* however derived a higher proportion of its food from *Enteromorpha* spp. in December 2005 in BISL (Fig. 4.5). At SM, benthic sources made a higher contribution to the diet of the cockle in December 2005 and April 2006, consistently with the ^{13}C enrichment of the cockle. The large contribution of macroalgae may however be contested at this site (see discussion).

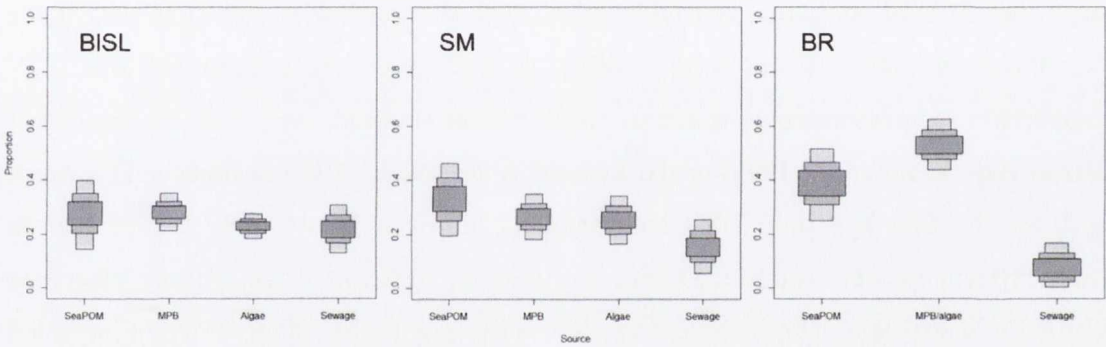


Figure 4.4: Contribution of food sources to the diet of *Cerastoderma edule* from the three intertidal sites, over the four surveys. Sites are BISL: Bull Island South Lagoon, SM: Sandymount, BR: Blackrock. POM: Particulate Organic Matter, MPB: Microphytobenthos. Contributions are displayed as the 50th, 75th and 95th extent of the probability intervals.

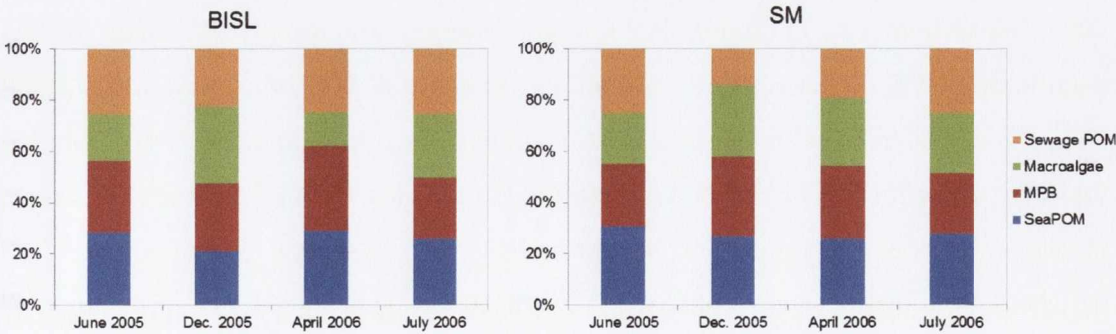


Figure 4.5: Seasonal changes of the relative contribution (median value) of the food sources to the diet of *Cerastoderma edule* in Bull Island South Lagoon (BISL) and Sandymount (SM). POM: Particulate Organic Matter, MPB: Microphytobenthos.

4.4. Discussion

4.4.1. Spatio-temporal variations of the basal sources isotopic signatures

Autochthonous primary producers showed strong spatial variations of their isotopic signatures (on C, N or both) among the intertidal sites, as well as some temporal variations at some or all the sites. $\delta^{15}\text{N}$ of macroalgae is acknowledged to be influenced by the nitrogen source and to constitute an indicator of anthropogenic nitrogen loadings which tend to induce a ^{15}N -enrichment of macrophytes (McClelland *et al.*, 1997; Cole *et al.*, 2005; Marconi *et al.*, 2011). In Sandymount, $\delta^{15}\text{N}$ of *Enteromorpha* spp. was much lower than at BISL and other coastal sites reported as impacted by anthropogenic inputs (Vizzini *et al.*, 2005; Baeta *et al.*, 2009). In fact, green algae from SM had $\delta^{15}\text{N}$ comparable to measurements made in relatively unimpacted sites such as Marennes-Oleron Bay (Kang *et al.*, 1999) or the Roscoff Aber (Riera, 2010) in France. This result confirms the first hypothesis that anthropogenic nitrogen inputs from sewage have a lower influence on the south of Dublin Bay than on BISL, as it would have otherwise resulted in a stronger ^{15}N -enrichment of the macroalgae in SM, as measured in impacted sites such as BISL (see discussion on this site in Chapter 3) and elsewhere. *Enteromorpha* spp. however displayed a large seasonal variability of its $\delta^{15}\text{N}$ at Sandymount, while it was very stable at BISL. Seasonal variation in the isotopic composition of macrophytes has been previously reported elsewhere (Vizzini & Mazzola, 2003; Nordstrom *et al.*, 2009). As nitrogen sources are usually the main factors determining $\delta^{15}\text{N}$ of macrophytes as said above, seasonal variability may likely result from the use of different nitrogen sources (nitrate (NO_3^-) or ammonium (NH_4^+)) during the year. According to Vizzini & Mazzola (2003), a higher $\delta^{15}\text{N}$ of macrophytes tissues in late winter-early summer may be induced by the preferential use of nitrate (^{15}N -enriched, Domingues *et al.*, 2011) made available during winter. Later in summer, as nitrate levels become progressively depleted, primary producers would turn to the use of recycled ammonium which is usually more ^{15}N -depleted (Michener & Schell, 1994). Our results could support this assumption since *Enteromorpha* spp. from SM displayed their

highest $\delta^{15}\text{N}$ values in March and their lowest $\delta^{15}\text{N}$ values during summer. In contrast, the stable $\delta^{15}\text{N}$ signal observed over time at BISL for *Enteromorpha* spp. (see details in Chapter 3) suggests that these green algae can there rely on the same ^{15}N -enriched nitrogen source throughout the year without facing any depletion of it, as also evidenced by the excessive proliferation of this opportunistic green algae at BISL every year. And indeed, BISL is directly exposed to the lower estuary waters which are hyper-nutriented with nitrogen in oversupply (O'Higgins, 2005). This excess of nitrogen is being constantly fuelled by inputs of oxidized forms of nitrogen loaded in the lower estuary, essentially with treated sewage effluent (O'Higgins, 2005). Unlike $\delta^{15}\text{N}$, the $\delta^{13}\text{C}$ of *Enteromorpha* spp. was similar at BISL and SM and characteristic of this type of green algae (Raven *et al.*, 2002). They were on the other hand ^{13}C -enriched compared with detrital green macroalgae sampled at BR. This differentiation may be linked with isotopic fractionation taking place during decomposition processes, as reported by Hill & McQuaid (2009) or Cloern *et al.* (2002).

Microphytobenthos displayed a relatively large range of $\delta^{13}\text{C}$ values across Dublin Bay which overlapped the $\delta^{13}\text{C}$ reported in the literature for this category of primary producer, essentially for cohesive sediments (i.e. ranging from -17.6‰ to -12‰ for the following studies : Currin *et al.*, 1995; France, 1995; Riera & Richard, 1996; Herman *et al.*, 2000; Page & Lastra, 2003; Kang *et al.*, 2006; Malet *et al.*, 2008). Consistent spatial variations were observed in the $\delta^{13}\text{C}$ of intertidal MPB across Dublin Bay with their lowest $\delta^{13}\text{C}$ always measured in the sandy sediments of BR and the highest signatures at SM. The $\delta^{13}\text{C}$ of marine primary producers is known to depend on the source of dissolved inorganic carbon (DIC) used (HCO_3^- and/or dissolved CO_2), and we may expect this source to be a factor of variation given that $\delta^{13}\text{C}$ of DIC can vary spatially and seasonally within an estuary (Coffin *et al.*, 1994). Several other factors have been reported to affect the $\delta^{13}\text{C}$ of planktonic microphytes such as the species and the nutrient conditions (Thompson & Clavert, 1994; Brutemark *et al.*, 2009), the size and morphology of the cells (Popp *et al.*, 1998), or the pH (Thompson & Clavert, 1994). Factors affecting the isotopic composition of MPB have not been as much studied as for the phytoplankton, probably due to the sampling difficulties associated with its position within sediments and the complexity of processes occurring within sediments. It can reasonably be expected though that several of the variables mentioned above (such as

species or nutrient conditions) can also influence the $\delta^{13}\text{C}$ of MPB, likely along other specific processes taking place at the sediment-water interface or within the sediments.

With $\delta^{13}\text{C}$ varying between -22 and -14‰, POM sampled in the south part of Dublin Bay was at all time of the year much more ^{13}C -enriched than the Liffey waters (mean $\delta^{13}\text{C}$: -30.1‰), suggesting that the Liffey POM has a minor influence on this part of the bay. In March and June 2006, the POM $\delta^{13}\text{C}$ of SM and BR was representative of marine POM (Fry & Sherr, 1984), as sampled offshore in the Irish sea (mean $\delta^{13}\text{C}$: -22.6‰, details in Chapter 3). Moreover, the POM was essentially composed of phytoplankton in June 2006 as indicated from the high Chl *a* concentrations and the low POC/Chl *a* ratios (Parsons *et al.*, 1977). In contrast, the strong ^{13}C -enrichment of the POM at both sites (SM and BR) in November 2005 (by 5 to 6.6‰ compared with offshore POM) points out a high contribution of ^{13}C -enriched sources, such as MPB or macroalgae, to the POM pool. Microphytobenthos (MPB) resuspension with the superficial sediments is a well-known and common phenomenon in intertidal muddy environments, essentially due to physical factors such as wind-driven waves and tidal action (De Jonge & Beusekom, 1995; Ubertini *et al.*, 2012). Benthic microalgae, whose assemblages are typically dominated by diatoms in intertidal sediments of western Europe (e.g. Underwood, 1994; Ribeiro *et al.*, 2003; Haubois *et al.*, 2005; Jesus *et al.*, 2009), can hence become a major component of the water column plankton in intertidal flats, especially in winter (Malet *et al.*, 2008). It has for instance been shown to represent up to 89% of the plankton (Guarini *et al.*, 2004) and contribute to up to 30% of the Chl *a* levels in the overlying water column (De Jonge & Van Beusekom, 1992). Macroalgae have also been shown to be noticeable contributors to POM pools, especially near rocky substrates, by inducing a ^{13}C -enrichment of the POM (Bustamante & Branch, 1996; Kaehler *et al.*, 2006). In the present study, both MPB and macroalgae presented the highest $\delta^{13}\text{C}$ of all sources which make them likely to have induced the ^{13}C -enrichment of the POM. The concomitant and strong ^{15}N -depletion of the POM in SM in November 2005 nevertheless suggests that MPB (^{15}N -depleted at this site compared to macroalgae) was the main source inducing the POM ^{13}C -enrichment at this site. At BR, micro and macroalgae had very similar C and N isotopic signatures which did not permit to delineate their relative contribution to the POM pool.

4.4.2. Spatio-temporal isotopic variations of primary consumers across Dublin Bay indicate differential sewage influence

In coastal food web studies, carbon stable isotopes are useful indicators of the origin and type of organic matter sources encountered (Peterson *et al.*, 1985; Simenstad & Wissmar, 1985; Cifuentes *et al.*, 1996). They allow for the distinction between ^{13}C -depleted terrestrial sources and ^{13}C -enriched marine sources (Fry & Sherr, 1984; Gearing *et al.*, 1984). They furthermore permit the differentiation between pelagic sources such as phytoplankton ($\delta^{13}\text{C}$ of $\sim -22\text{‰}$) and benthic primary producers such as microphytobenthos which are more ^{13}C -enriched than marine or estuarine phytoplankton (Maberly *et al.*, 1992; France, 1995). In the south part of Dublin Bay (SM and BR), macro-invertebrates were always very ^{13}C -enriched (by 10 to 15.7‰ depending on species and surveys) compared to the river POM (mean $\delta^{13}\text{C}$ of -30.1‰). This suggests that riverine POM is not a significant component of the primary consumers diet of the south of the bay, and matches with the fact that river POM was not distinctly detected in the POM collected at the south sites, as discussed based on both isotopic and other hydrobiological variables. This is also consistent with the dominant clockwise water motion regime in Dublin Bay, such that the Liffey plume is essentially brought out of the walled estuary to the north of the bay with tidal waters and exit the bay off Howth (ERU, 1992). For these reasons, the river POM was not included in the dietary analysis performed on the two dominant primary consumers species.

Between-sites comparisons of the isotopic signatures of primary consumers evidenced large spatial variations, either coupled to significant temporal variations (e.g. for the suspension-feeder *C. edule*), or largely overriding the temporal variations (for deposit-feeders, their main representative *A. marina*, and to a lesser extent for interface-feeders). The isotopic signature of a consumer characterises the isotopic composition of its food items, meaning a trophic fractionation taking place during metabolic reactions (De Niro & Epstein, 1978, 1981; Martinez del Rio *et al.*, 2009). Spatial variations in the isotopic signature of a given consumer species may thus: a) result from a modification of its diet with the use of different food sources with different isotopic compositions (trophic shift); b) reflect spatial variations in the isotopic signature of the food sources at the base, but without major diet changes for the primary consumer (isotopic shift); c) or result from

both sources of variations. In the present study, both possibilities could be considered given that 1) the studied sites were characterised by different environmental conditions with different exposure to anthropogenic effluents as evidenced in the previous section, and with contrasting abundances of autochthonous macroalgae; 2) autochthonous primary producers displayed strong spatial variations of their isotopic ratios across the bay.

The mixing model analysis carried out for the two species representatives of the suspension-feeder (i.e. *C. edule*) and the deposit-feeder (i.e. *A. marina*) guilds in Dublin Bay, and which occurred at all sites, enabled to delineate the trophic interactions between sources and consumers isotopic variations across sites and through time. For both feeding guilds, sewage POM was found to have a minor and lower influence in Sandymount and Blackrock than in the estuarine site of Bull Island South Lagoon, thus supporting the second hypothesis of this work that the direct influence of sewage on primary consumers (through its POM) is minor in the south of Dublin Bay.

The deposit-feeder *A. marina* was found to exploit different sources across the bay, and in proportions which appear linked with their abundance and availability on sites. If primary producers (macroalgae and microphytobenthos) were overall the first sources of food for this deposit-feeder, their contributions to its diet varied both spatially and seasonally. *A. marina* exhibited a strong seasonal response to high organic matter inputs from macroalgae in BISL (see details in Chapter 3), where opportunistic blooms of *Enteromorpha* spp. occur every year. Such seasonal variations of its diet were not recorded in SM where the autochthonous macroalgal production is very much lower. Unlike the south of the Bay, the macroalgal production at BISL has been related, through the high $\delta^{15}\text{N}$ of *Enteromorpha* spp. (see section 4.3.1), to anthropogenic inputs of nitrogen, which come from sewage essentially (O'Higgins, 2005), thus highlighting the indirect impact of sewage. In SM, the mixing model output suggested a relatively constant contribution of algae to the diet of the lugworm all along the year, in even higher proportions than what this polychaete assimilates in BISL when algal mats are covering the mudflat (Fig. 4.3). This is however unlikely that macroalgae would make such a permanently high dietary contribution at SM. Indeed, given the relatively low macroalgal production on this site, only a high and continual tidal input of particles of macroalgae could explain such a high contribution of macroalgae to the diet of *A. marina* (and so of deposit-feeders) relative to BISL. Such high advection of macroalgae up to SM appears

however unlikely for two reasons: 1) stranded macroalgae were never observed continuously or in high abundance on the site during the study; and 2) the isotopic compositions of the site POM measured at SM did not reveal any major contribution of macroalgae, but rather of resuspended MPB, and this only in November 2005. In fact, the contribution of macroalgae to the diet of *A. marina* may have been overestimated by the SIAR model to the detriment of MPB, as suspected from the high and negative correlation of MPB and macroalgae given by the model.

The suspension-feeder *C. edule* displayed more homogenous and more ^{13}C -depleted isotopic signatures across the bay than deposit-feeders. This different pattern reflected their dominant reliance on marine planktonic matter. *C. edule* however showed notable isotopic variations over time. Unlike BISL where this variability occurred for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ due to the opportunist consumption of ^{15}N -enriched green macroalgae detritus in winter, cockles from the south of the bay showed temporal variations of their $\delta^{13}\text{C}$ only, which were related to a higher consumption of benthic sources during the autumn and winter periods. Macroalgae may have been consumed in Sandymount but, for the same reasons as discussed above for *A. marina*, it is unlikely that it would have reached such high contributions as expressed by the model. In this part of the bay, the ^{13}C -enrichment of cockles was most likely driven by a higher consumption of resuspended MPB in winter. Locally resuspended MPB has indeed been reported by various authors to play a major trophic role for intertidal bivalves, and particularly for the cockle *C. edule*, during periods of lower phytoplankton production (Kang *et al.*, 1999; Kang *et al.*, 2003; Page & Lastra, 2003).

Interface-feeders have the ability to take their food from either the surface of the sediment or from the water column (Levinton, 1991; Taghon & Greene, 1992). In Dublin Bay, interface-feeders, mostly represented by Tellinid bivalves, displayed a clear isotopic differentiation among sites and isotopic signatures close to the deposit-feeders ones (or more ^{13}C -enriched). This suggests that they mostly rely on benthic sources. It was demonstrated in the previous chapter that at BISL, these interface-feeders exploited mostly detrital *Enteromorpha* during the autumn 2005 (as measured from their tissues in December 2005) -thus inducing their ^{15}N -enrichment-, while they relied mostly on MPB later in the year. Macroalgae were also ^{15}N -enriched relatively to other sources at SM but in contrast, interface-feeders from that site always displayed ^{15}N -depleted signatures, especially compared with deposit-feeders, indicating a minor or at least lower reliance on

macroalgae than strict deposit-feeders. The $\delta^{13}\text{C}$ of the interface-feeders in SM further suggests that they essentially feed on MPB during the year. In opposition, interface-feeders (and particularly the bivalve *Angulus tenuis*) from the sandflat of BR displayed C and N isotopic signatures intermediate between the cockle *C. edule* and deposit-feeders, suggesting that suspended POM likely plays a higher trophic role for them on this sandflat than on the muddier sites. Interface-feeders can switch feeding mode according to the availability of food (Hummel, 1985; Taghon & Greene, 1992; Kamermans, 1994). At BR, sediments are dominated by clean sands with a low organic matter content compared with the muddier sites. Moreover, the sediment Chl *a* concentration, which constitutes a proxy of microphytes biomass, has been measured to be about 5 times lower at BR than at BISL (Hayes, 2005). This could explain why interface-feeders may have a stronger need to switch to feeding on planktonic matter in that site than in muddier ones hosting a higher microphyte-benthic biomass such as at BISL or SM. Physical forces such as hydrodynamic conditions could have also influenced the feeding behaviour of the interface-feeders (Levinton 1991) given that BR is less sheltered than the other two sites. The latter author indeed demonstrated that interface-feeding Tellinids progressively switch to suspension-feeding at higher water velocities combined with bottom sediment transport.

4.5. Conclusion

The present study indicated that the influence of sewage effluents on intertidal benthic habitats of Dublin Bay is localized to the north area. In the estuarine mudflat of Bull Island South Lagoon, sewage indirectly affects the primary consumers through the green macroalgal bloom it sustains, as confirmed by the high $\delta^{15}\text{N}$ of *Enteromorpha* spp. at this site. Sewage particulates also have a direct but moderate influence on primary consumers as a food source. In contrast, the south of the Bay was relatively unimpacted by these effluents, with regard to both their role as nutrients for macroalgal production, and their trophic role for primary consumers.

In the absence of major anthropogenic disturbance at the southern site of Sandymount, primary consumers displayed a higher temporal stability of their diets than consumers

from BISL enduring macroalgal blooms. Excluding the temporary diet shift caused by the macroalgal bloom at BISL, the suspension-feeder *Cerastoderma edule* had relatively similar diets between BISL and Sandymount, relying primarily on planktonic matter according to its feeding mode. It however demonstrated its trophic plasticity, adapting to changing environmental conditions (e.g. lower phytoplanktonic biomass, higher frequency of resuspension events in winter) by making a higher exploitation of benthic sources during the winter months in the south of the bay. In the absence of disturbance effects, interface-feeders displayed isotopic signatures suggesting a relatively consistent diet but which tend to differ according to the habitat occupied and the associated environmental conditions. Interface-feeding bivalves thus appeared to make a higher reliance on POM in sandy habitats than muddy habitats.

CHAPTER 5

Seasonal survey of the subtidal benthic food web of Dublin Bay

5.1. Introduction

Food webs are a core aspect of ecological studies, providing a holistic view of ecosystem functioning through the analysis of energy flow and resource-consumer interactions within a community. The identification of trophic pathways can further provide a basis for modelling communities and ecosystem dynamics once coupled to a quantification of species and trophic components (Baird & Ulanowicz, 1989; Christensen & Pauly, 1992; Baird & Ulanowicz, 1993).

Large efforts have been put in the description of marine food webs in a variety of ecosystems and habitats in the last twenty years, owing particularly to the advent and the development of stable isotopes techniques and associated analytical tools (Michener & Schell, 1994; Layman *et al.*, 2012). Major trophic pathways and the sources sustaining them in coastal systems have been described (see general introduction for a detailed description) yet these studies underline different trophic pathways, even in similar ecosystems, depending on local biotic and abiotic factors. The importance of temporal studies for a sound delineation of trophic interactions in response to sources availability and variability has also been demonstrated (Goering *et al.*, 1990; Riera & Richard, 1997; Vizzini & Mazzola, 2003; Akin & Winemiller, 2006; Carlier *et al.*, 2007b; Nordstrom *et al.*, 2009). Changes in food web interactions and structure may further occur when a natural or man-induced disturbance affects some of its components. Such a disturbance can induce shifts of the community structure by impacting the base of the food web and eventually trophic interactions among consumers (Layman *et al.*, 2007; Sabo *et al.*, 2010). A sound understanding of trophic interactions in ecosystems is thus crucial, particularly when such systems are undergoing human disturbances, in order to improve the prediction of impacts and apply appropriate managements measures on the source of pressure. Such assessments notably imply to identify the resources supporting food webs, the trophic position of organisms and the structure and length of the food web.

The present study undertook such a food web analysis of the subtidal benthic communities in Dublin Bay. This shallow sandy bay, surrounded by a large urban centre, has indeed long been suffering of organic enrichment from sewage effluents. The trophic functioning of the subtidal communities and how they cope with these anthropogenic

inputs is however largely unknown (Wilson *et al.*, 2007). Through a C and N stable isotopes analysis carried out at two subtidal sites through 4 surveys, this study aims to fill this lack of knowledge and depict the pathways of carbon flow through Dublin Bay subtidal food web. In particular, it aimed to 1) identify the sources of organic matter supporting the food web and, 2) characterize the trophic structure through an assessment of the consumers' trophic levels and the food web length. Since this study occurred just after the upgrade of the Sewage Treatment Plant which was followed by a substantial drop (83%) of the amount of POM discharged, it was hypothesized that sewage POM would have a minor influence as a food source for subtidal benthic consumers.

5.2. Supplementary material and methods

5.2.1. Samples analysis

The methodology applied for the collection, processing and analysis of samples are detailed in Chapter 2. Due to technical issues, megafauna species and fish have not been analysed for the survey of April 2006.

5.2.2. Data analysis

Given the large sample sizes and the homogeneity of variance positively assessed (Levene test), the distribution and relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ individual values of invertebrates were compared between sites for each survey using a covariance analysis (ANCOVA). As there was no significant difference, samples from these two sites were then pooled for each survey. Because of small samples sizes, seasonal differences in the C and N isotopic composition of sources, species and trophic groups were assessed with non-parametric Kruskal-Wallis or Mann-Whitney tests. A hierarchical cluster analysis (Bray-Curtis similarity index, average grouping method) was carried out on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ seasonal mean values of consumers from all surveys to identify dominant groupings. Groups were validated by a SIMPROF test (5%). To avoid confusion in sub-groupings of primary consumers which could derive from seasonal variations of their isotopic

signatures, hierarchical cluster analyses were further performed for each survey on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean values of primary consumers.

A trophic level (TL) was estimated for each species and each season using the equation proposed by Cabana & Rasmussen (1996) and a mean ^{15}N -enrichment factor of 3.4‰ (Minagawa & Wada, 1984; Post *et al.*, 2000), such that:

$$\text{TL} = \text{TL}_{\text{base}} + (\delta^{15}\text{N}_{\text{C}} - \delta^{15}\text{N}_{\text{base}}) / 3.4$$

where: TL_{base} is the trophic level used as a baseline ($\text{TL}_{\text{base}} = 2$ in the present case)

$\delta^{15}\text{N}_{\text{C}}$ is the $\delta^{15}\text{N}$ of the consumer species

$\delta^{15}\text{N}_{\text{base}}$ is the mean $\delta^{15}\text{N}$ of the baseline

Suspension-feeders were used as a baseline (seasonal mean values) as they displayed relatively close $\delta^{15}\text{N}$ values. This formula using primary consumers rather than POM as a baseline has the advantages to integrate the higher isotopic variability of the food sources and to not involve trophic enrichment factors occurring between primary producers and consumers, as these are reported to be more variable than the ones reported for higher TL (Vander Zanden & Rasmussen, 2001).

5.3. Results

5.3.1 Isotopic composition of potential food sources

$\delta^{13}\text{C}$ signatures of the potential food sources ranged from -32.6‰ (river POM) to -16.8‰ (detrital kelps). The river POM and the sewage effluent systematically had the most negative $\delta^{13}\text{C}$ values (mean of -30.4‰ and -26‰ respectively, see details in chapter 3) and detrital kelps the most positive values along the surveys (see Fig. 5.6 and 5.7). Kelps samples featured a wide range of $\delta^{13}\text{C}$ (from -22.9‰ to -16.8‰), larger than for other unidentified algae whose signatures varied around -20.8‰ (± 0.8). Sedimentary and particulate organic matter (SOM and POM respectively) had intermediate $\delta^{13}\text{C}$ signatures among other sources. The $\delta^{13}\text{C}$ of the POM measured in the bay (POM site) was ^{13}C -depleted in June 2006 (-24.3‰), thus differing significantly (KW, $p < 0.05$) from other surveys (-22.7‰ in average). This POM sampled within the bay was always close to the

POM sampled offshore (POM open sea) regarding their $\delta^{13}\text{C}$. The $\delta^{13}\text{C}$ of the open sea POM varied from -25‰ in June 2006 to -21‰ in November 2005 (see Table 3.3, Chapter 3).

The range of $\delta^{15}\text{N}$ measured for the potential food sources varied from 3.4‰ (sewage effluent) to 11.1‰ (detrital kelps). $\delta^{15}\text{N}$ of the open sea POM varied from 3.8‰ in November 2005 to 7‰ in June 2006. $\delta^{15}\text{N}$ of the site POM did not vary significantly among seasons (KW, $p=0.156$) and had an average $\delta^{15}\text{N}$ of 5.7‰.

The C and N isotopic composition of SOM was also very stable in time (KW, $p=0.418$ and $p=0.328$ for C and N respectively) and in space (MW tests between north and south sites: $p>0.05$ for each survey). $\delta^{13}\text{C}$ of the SOM (mean: -21.7‰) was relatively close to the POM signatures, apart from June 2006 when the $\delta^{13}\text{C}$ of the POM decreased. The SOM (mean $\delta^{15}\text{N}$: 7.4‰) was ^{15}N enriched by 1‰ (May 2005, June 2006) to 2.9‰ (November 2005) compared to the site POM.

Chlorophyll *a* (Chl *a*) concentration measured on the sites varied between 0.5 and $1\mu\text{g l}^{-1}$ in November 2005, March and June 2006 (Table 5.1). The POC/Chl *a* ratio decreased to 150 during that last survey. This trend was similar to what was measured offshore at the open sea station but with lower Chl *a* levels in Dublin Bay than offshore. Low POC/Chl *a* ratios measured in June 2006 indicate that the POM was essentially composed of living phytoplanktonic cells while higher ratios above 200 in November 2005 and March 2006 indicate the dominant detrital nature of the POM (Parsons *et al.*, 1977; Cifuentes *et al.*, 1988). C:N ratios of the POM varied between 6 and 9 except in November 2005 when it was measured at 12 for the open sea POM and at 20 for the site POM. C:N ratios of detrital kelps were higher than for other sources (apart from the site POM in November 2005) including the SOM.

Table 5.1: Biochemical characteristics (C:N ratio, Chlorophyll *a* (Chl *a*) concentration and Particulate Organic Carbon (POC) to Chl *a* ratio) of the potential food sources for the subtidal food web. n: sample size, POM: Particulate Organic Matter, SOM: Sedimentary Organic Matter. Results relative to the general POM end-members (river, sewage and open sea) have been previously described in Chapter 3 and are reminded here to facilitate comparisons.

Source	Date	n	C:N (mean±SD)	Chl <i>a</i> (µg l ⁻¹)	POC/Chl <i>a</i>
POM river	May 2005	6	6.5 ± 0.3	-	-
	November 2005	8	11.3 ± 0.8	1.3	1137
	March 2006	8	8.6 ± 0.3	3.2	145
	June 2006	6	6.5 ± 0.5	6.2	116
POM Sewage	May 2005	1	7.2	-	-
	November 2005	9	6.2 ± 0.5	-	-
	March 2006	9	5.3 ± 0.3	-	-
	June 2006	6	5.8 ± 0.2	-	-
POM Open Sea	May 2005	3	6.8 ± 0.5	0.7	-
	November 2005	3	12.1 ± 0.4	0.6	330
	March 2006	3	7.9 ± 0.2	0.7	232
	June 2006	3	6.9 ± 0.4	2	92
POM site	May 2005	3	6.5 ± 0.2	-	-
	November 2005	5	20.0 ± 3.2	0.5	292
	March 2006	3	8.5 ± 1.0	0.9	285
	June 2006	6	7.8 ± 1.1	1.0	150
SOM	May 2005	3	6.8 ± 0.2	-	-
	November 2005	6	9.0 ± 1.9	-	-
	March 2006	5	7.7 ± 0.4	-	-
	June 2006	5	7.1 ± 0.2	-	-
Detrital kelps	May 2005	2	18.1 ± 1.1	-	-
	November 2005		-	-	-
	March 2006 (sp.1)	1	12.3	-	-
	March 2006 (sp.2)	1	11.5	-	-
	June 2006 (sp.3)	1	17.2	-	-
	June 2006 (sp.4)	3	14.8 ± 0.6	-	-
Other detrital algae	May 2005		-		
	November 2005	5	5.7 ± 0.9	-	-
	March 2006	4	3.8 ± 0.4	-	-
	June 2006	2	4.3 ± 0.5	-	-

5.3.2 Isotopic composition of consumers

A total of 83 species were analysed (Table 5.2). Annelids were the most represented taxa (28 species) followed by Molluscs (18 species including 13 bivalves), Crustaceans (12 species) and fish (10 Actinopterygii and 2 Elasmobranchii), Echinoderms (10 species) and Cnidarian Anthozoa (2 species). Considering functional groups, predators constituted the most represented feeding group with 26 invertebrates and 12 fish species. It was followed by suspension-feeders (16 species) and deposit-feeders (15 species).

5.3.2.1 Spatial variations

The number of invertebrate species collected in the south site was consistently higher relative to the north site (a total of 65 species versus 30 species respectively), while the number of fish species collected was similar in both sites. Only the south site was sampled in May 2005. The distribution of invertebrates isotopic values within the bivariate delta space was analogous between the two sites for each survey (Fig. 5.1) and there was no significant difference between sites in the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of invertebrates (ANCOVA, $p>0.4$ for each season).

Table 5.2: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean values) of the consumers sampled from May 2005 to June 2006 in the subtidal area of Dublin Bay (north and south sites). FS: Feeding Strategy; aSF: active suspension-feeder, pSF: passive suspension-feeder, DF: deposit-feeder, SSDF: sub-surface deposit-feeder, IF: interface-feeder, O: omnivorous, P: Predator; FS of fish: Pi: benthivorous (invertebrates), Pp: piscivorous; n: sample size.

CODE	Species	FS	MAY 2005					NOVEMBER 2005					MARCH 2006					JUNE 2006				
			n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
				mean	sd	mean	sd		mean	sd	mean	sd		mean	sd	mean	sd		mean	sd	mean	sd
1	Zooplankton		2	-19.6	0.4	8.2	0.2						2	-21.1	0.0	9.9	0.7	4	-24.0	0.3	9.4	0.4
Annelida																						
2	Aphaelochaeta filiformis	DF																4	-21.3	1.2	11.5	0.6
3	Aphrodita aculeata	P						2	-17.1	0.6	14.4	0.6						3	-18.5	1.3	14.1	0.4
4	Chone fauveli	aSF						3	-20.1	0.1	11.1	0.1						2	-20.2	0.0	11.2	0.1
5	Euclymene lombricoides	SSDF						2*	-19.9	0.0	10.4	0.3										
6	Glycera sp	P						1*	-18.7		13.6		1	-20.9		13.7						
7	Glycera tridactyla	P	1	-18.6		12.9							6	-19.7	0.6	14.0	0.5	6	-19.4	0.5	13.8	0.6
8	Goniada maculata	IF																1*	-19.8		14.6	
9	Heteroclymene robusta	SSDF	2	-21.4	0.0	10.0	0.0	2*	-20.2	0.1	10.5	0.0	2*	-20.7	0.1	10.6	0.0	2*	-20.6	0.2	10.6	0.0
10	Hydroides norvegicus	aSF						1	-19.9		11.3											
11	Jasmineira elegans	aSF	1	-20.4		10.1																
12	Lagis koreni	DF						2*	-20.2	0.1	9.7	0.0										
13	Lanice conchylega	IF																2	-22.7	1.0	10.1	0.0
14	Lepidonotus squamatus	P																1	-19.3		13.6	
15	Lumbrineris laireilli	P																2	-20.3	1.2	12.6	0.1
16	Mugilona mirabilis	DF																2*	-20.8	0.5	9.6	0.3
17	Meltingeriaella castanea	P	2	-17.9	0.4	12.9	0.2	5	-18.5	0.6	13.1	0.5						1	-18.5		13.4	
18	Melinia palmata	DF						4	-19.7	0.2	11.3	0.4						4	-21.8	0.6	10.4	0.6
19	Nephtys hombergii	P	1	-17.0		12.5		5	-18.8	0.4	12.8	0.3	3	-19.0	0.5	13.5	0.4	6	-19.3	1.4	12.5	0.6
20	Nephtys sp	P	3	-19.1	0.4	12.1	0.1															
21	Notomastus latericeus	SSDF	2	-19.0	0.2	10.0	0.3						1	-17.8		13.2						
22	Ophelia acuminata	DF						1	-18.8		10.1							1	-20.4		10.8	
23	Owenia fusiformis	IF																3	-20.0	0.2	11.2	0.5
24	Phyllodoce greenlandica	P	2	-19.2	0.2	11.7	0.3	7	-19.3	1.3	12.1	1.0	4	-20.4	1.2	11.7	0.5					
25	Pista cristata	DF											4	-21.8	0.6	12.0	0.3					
26	Sigalion mathildae	P																3	-18.7	0.3	11.7	0.4
27	Sthenelais limicola	P						3	-18.5	0.5	13.1	0.1										
28	Terebellides stroemlii	DF											1	-21.5		11.2						
29	Tharyx mariani	DF																1	-20.3		12.1	
Crustacea																						
Amphipoda																						
30	Ampelisca brevicornis	aSF/DF						1*	-17.0		10.3											
Cirripedia																						
31	Balanus sp	aSF						2	-22.4	0.7	10.4	0.9										
Decapoda																						
32	Corystes casiveiaunus	P	2	-17.9	0.8	13.2	0.6	1	-18.6		15.1							6	-19.6	0.6	13.0	1.3
33	Crangon almanii	P						6	-17.6	0.3	14.4	0.7										
34	Crangon crangon	P						15	-16.9	1.1	14.4	0.6						1	-17.1		14.4	
35	Gastrosaccus spinifer	O						1*	-17.0		12.1											
36	Hyas coarctatus	O						1	-18.9		13.3											
37	Inachus dorsetiensis	O						4	-19.1	0.6	13.7	0.8						2	-18.5	0.2	13.6	0.3
38	Locarcinus depurator	O	4	-17.9	1.1	12.8	0.4	9	-18.3	0.4	13.1	0.4						7	-18.4	0.3	13.2	0.3
39	Macropodia rostrata	O						1*	-18.7		13.4											
40	Pagurus bernhardus	O	2	-19.5	0.7	12.4	0.0	5	-19.3	0.7	13.0	0.8						6	-19.9	0.6	12.8	0.3
41	Pandalus moriaugui	P						9	-18.1	0.4	13.9	0.4										
Cnidaria																						
42	Alcyonium digitatum	aSF/C	2	-17.4	0.1	11.7	0.4	6	-18.9	0.7	12.4	0.8						3	-18.9	0.3	11.9	0.0
43	Metridium senile							1	-17.8		15.1		1	-17.8		14.8		1	-18.5		14.0	
Echinodermata																						
44	Acrocidia brachiata	IF						6	-22.5	0.6	11.8	0.3	5	-22.5	0.3	10.9	0.3	10	-21.5	0.5	10.9	0.2
45	Amphura filiformis	IF						3*	-20.2	0.1	12.1	0.2						2	-18.7	0.4	14.1	1.6
46	Asterias rubens	P	4	-17.3	1.0	12.6	0.7	19	-17.9	1.0	13.3	0.4						7	-19.0	0.4	13.5	0.4
47	Astropecten irregularis	P						1	-16.7		15.9							2	-18.7	0.4	14.1	1.6
48	Crossaster pauposus	P	1	-16.3		14.2												3	-17.6	1.1	15.5	0.4
49	Echinocardium cordatum	DF						1	-22.7		10.4		3	-23.6	0.8	9.9	0.5	3	-24.0	0.3	9.4	0.1
50	Leptosynapta naerens	DF	2	-19.7	0.1	10.7	0.1	3	-18.9	0.6	11.2	0.3	1	-19.3		11.2		1	-18.5		11.7	
51	Ophiotrix fragilis	pSF						13	-21.8	0.8	12.1	0.4										
52	Ophiura albida	O	2	-17.5	1.5	10.5	0.8						2	-20.3	1.0	11.0	0.1	2	-19.1	1.0	11.9	0.2
53	Ophiura ophiom	O	4	-18.0	1.0	12.1	0.6	21	-17.8	0.6	13.9	0.5	9	-19.8	0.5	12.3	0.4	8	-18.7	0.7	12.1	0.2
Mollusca																						
Bivalvia																						
54	Acanthocardia sp	aSF						2	-21.2	0.1	10.3	0.2	1	-20.7		9.3		1	-21.7		9.2	
55	Aequipecten oerularis	aSF						4	-18.6	0.5	11.4	0.3										
56	Chamelea gallina	aSF	4	-20.8	0.8	9.7	0.4	3	-21.3	0.6	11.0	0.2	4	-21.6	0.6	9.9	0.4	2	-21.9	0.2	9.9	0.1
57	Dosinia exoleta	aSF						4	-20.8	0.6	10.8	0.1	5	-21.3	0.9	10.1	0.4	4	-21.3	1.1	10.1	0.2
58	Ensis siliqua	aSF											1	-20.2		10.0						
59	Gari ferverensis	aSF	2	-20.5	0.0	8.4	0.5	1	-21.6		9.9							3	-20.6	0.3	9.4	0.1
60	Mactra stultorum	aSF											2	-21.4	1.1	10.2	0.3					
61	Modiolus modiolus	aSF	2	-20.4	1.5	8.8	0.9															
62	Nucula nitidosa	SSDF	9	-18.7	0.7	8.8	0.4	9	-18.6	0.2	10.3	0.5	17	-19.2	0.2	9.4	0.3	12	-19.5	0.6	9.3	0.4
63	Nucula nuclei	SSDF											7	-19.0	0.3	9.0	0.2					
64	Pharus legumini	aSF	3	-20.1	0.7	9.3	0.1						4	-20.4	0.1	10.2	0.2	5	-21.0	0.5	9.9	0.4
65	Polittapes virgineus	aSF											2	-20.7	0.2	9.4	0.1					
66	Thracia phaseolina	aSF	2	-22.5	0.1	8.8	0.2	4	-20.4	0.3	10.2	0.1	3	-21.1	0.3	9.6	0.0	5	-22.9	0.7	9.1	0.2
Cephalopoda																						
67	Loligo forbesii	P						2	-18.6	0.3	14.4	0.6										
68	Sepioteuthis atlantica	P						5	-18.5	1.1	13.1	1.2										
Gastropoda																						
69	Buccinum undatum	P	2	-17.2	0.7	12.2	0.1	9	-18.0	1.1	12.3	0.5						5	-18.0	0.5	12.6	0.3
70	Lunatia catenata	P																1	-19.0		11.8	
71	Philine aperta	P	3	-18.0	0.7	10.4	0.6	3	-18.1	0.1	12.0	0.6						4	-18.2	0.4	10.9	0.1
Nemertea																						
72	Cerebratulus sp	P	1	-18.7		12.7																
Chordata																						
73	Amoglossus itema	PI						1	-17.6		14.6											
74	Callionymus lyra	PI	1	-15.8		13.3		9	-17.7	0.2	13.8	0.1						4	-17.9	0.1	13.9	0.5
75	Eutrigla gurnarus	PI,p						13	-16.7	0.6	15.7	0.5										
76	Gadus morhua	PI,p						5	-17.3	0.4	16.8	0.7										
77	Limanda limanda	PI,p	2	-19.4	0.8	13.3	0.1	21	-18.0	0.6	15.0	0.6						12	-18.1	0.6	14.0	0.5
78	Merlangius merlangus	PI,p						8	-16.9	0.4	16.2	0.7										
79	Platichthys flesus	PI,p	2	-16.6	3.3	14.3	2.4															
80	Pleuronectes platessa	PI	3	-16.6	0.7	13.1	0.3	15	-17.4	0.7	14.3	0.7						25	-18.0	0.7	13.6	0.6
81	Raja clavata	PI,p	4	-16.6	0.9	14.6	0.3	1	-16.3		15.6							4	-16.9	0.4	14.5	0.4
82	Scyliorhinus canicula	PI,p	1	-17.1		14.5		3	-16.4	0.3	15.5	0.2						1	-16.6		14.7	
83	Solea solea	PI						1	-18.5		14											

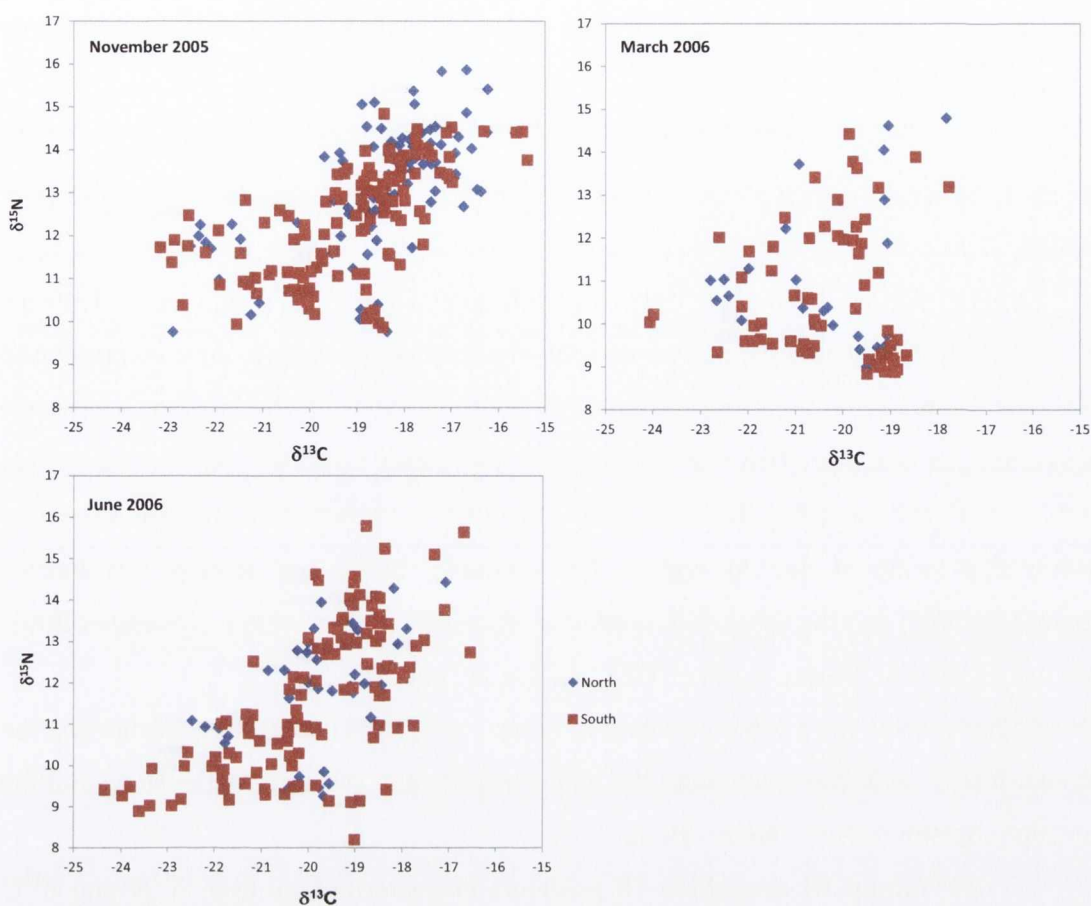


Figure 5.1: $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ distribution of invertebrates (individual values) from the north and south sites of Dublin Bay in November 2005, March 2006 and June 2006.

5.3.2.2 Food web structure and temporal variations

$\delta^{13}\text{C}$ values displayed by the consumers ranged over the year from -24‰ to -16.8‰. Without considering the survey of March 2006 (for which megafauna and fish could not be analysed), the ranges of $\delta^{13}\text{C}$ varied slightly among surveys (6.4 to 7.4‰). $\delta^{15}\text{N}$ values scattered over the year from 8 to 17.5‰. The range of $\delta^{15}\text{N}$ values did not vary much (6.3, 7.1 and 6.4 ‰ in May 2005, November 2005 and June 2006 respectively).

Cluster analysis performed on the average seasonal values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of each consumer species (83 species + zooplankton) resulted in the identification of three main trophic groups, based on 90% similarity (Fig 5.2). These groupings differed mostly

on $\delta^{15}\text{N}$ (Fig. 5.3), and were relatively concordant with assumed trophic levels of feeding groups identified from the literature (see Table 5.2):

- Group I gathered together the primary consumers with the lowest $\delta^{15}\text{N}$ values ($<12\text{‰}$), including suspension-feeders (SF), deposit-feeders (DF) and interface-feeders (IF).

- Group II clustered species with intermediate $\delta^{15}\text{N}$ signatures between 11.4‰ and 14.6‰. These species were mostly omnivorous and carnivorous invertebrates, plus the common sole *Solea solea*. The suspension-feeder anthozoan *Alcyonium digitatum* was also classified within that group indicating a stronger reliance on animal prey. Some species generally considered as primary consumers were also classified within this group II due to high $\delta^{15}\text{N}$ signatures: the queen scallop *Aequipecten opercularis* (SF) and the ophiuroid *Amphiura filiformis* (IF) sampled in November 2005, and the polychaete *Tharyx mariani* (DF) sampled in June 2006.

Three other species were either clustered in group I or group II depending on surveys: the deposit-feeder *Ophelina acuminata*, the omnivorous brittle star *Ophiura ophiura* and the predatory opisthobranch *Philine aperta*.

- Group III assembled 18 predators characterized by high $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures. Among them, 8 fish species considered to feed on both fish and invertebrates, the echinoderm *Crossaster papposus* and shrimps (genus *Crangon*) were included for each of the surveys they were sampled. Seven other species clustered in this group for some but not all of the surveys sampled: 3 predatory demersal fish species considered in the literature to feed on invertebrates only (the European plaice *Pleuronectes platessa*, the common dab *Limanda limanda* and the dragonet *Callionymus lyra*), predatory invertebrates such as the seastar *Astropecten irregularis*, the sea mouse *Aphrodita aculeata* and the sand crab *Corystes cassivelaunus* in November 2005, and the plumose anemone *Metridium senile* in November 2005 and March 2006. At other times of the year, these species appeared in group II.

The cluster analysis did not reveal any clear pattern of seasonal variation at the scale of the whole food web or at a feeding group level.

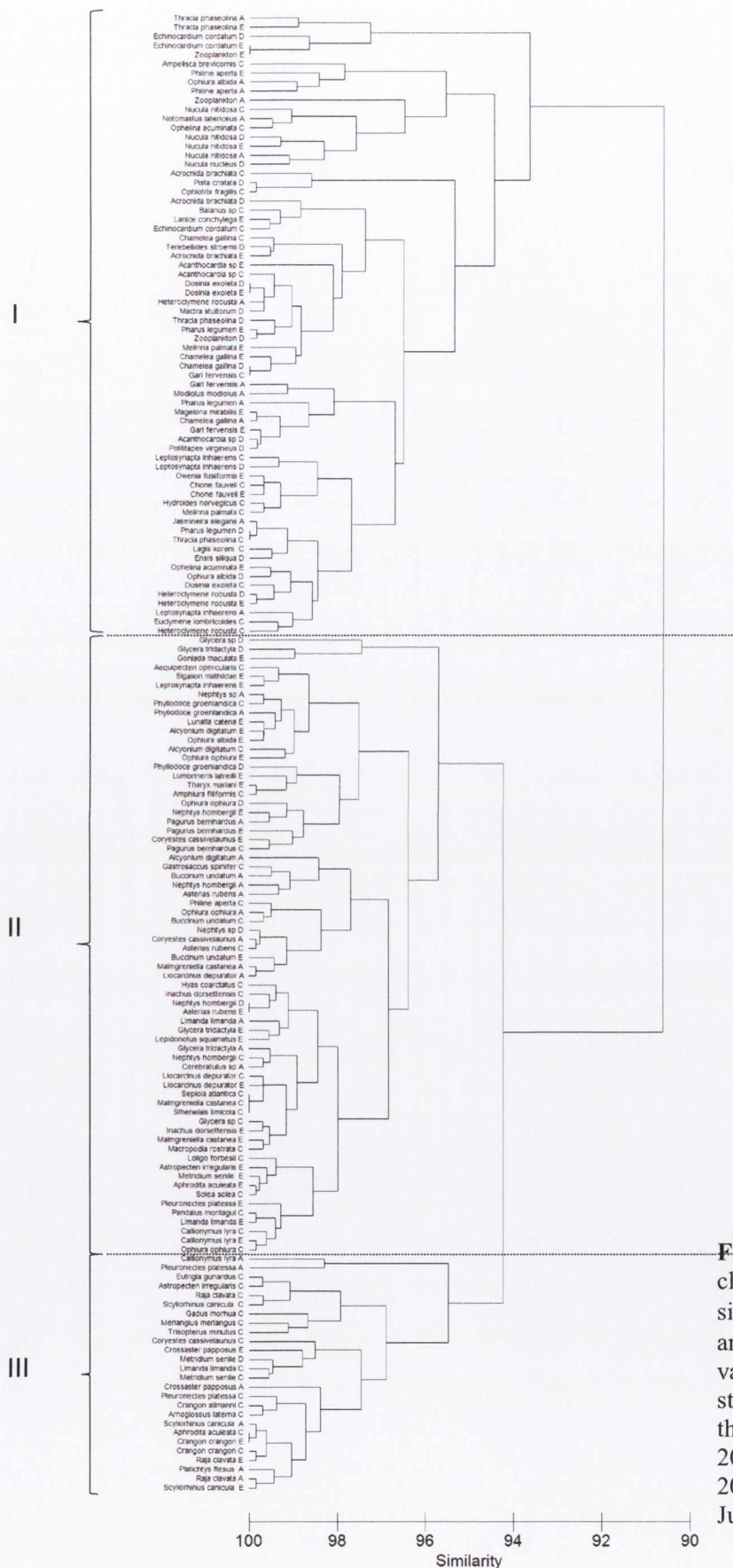


Figure 5.2: Hierarchical clustering (Bray-Curtis similarity index) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean seasonal values of 84 species studied sampled during the four surveys (A: May 2005; C: November 2005; D: March 2006; E: June 2006).

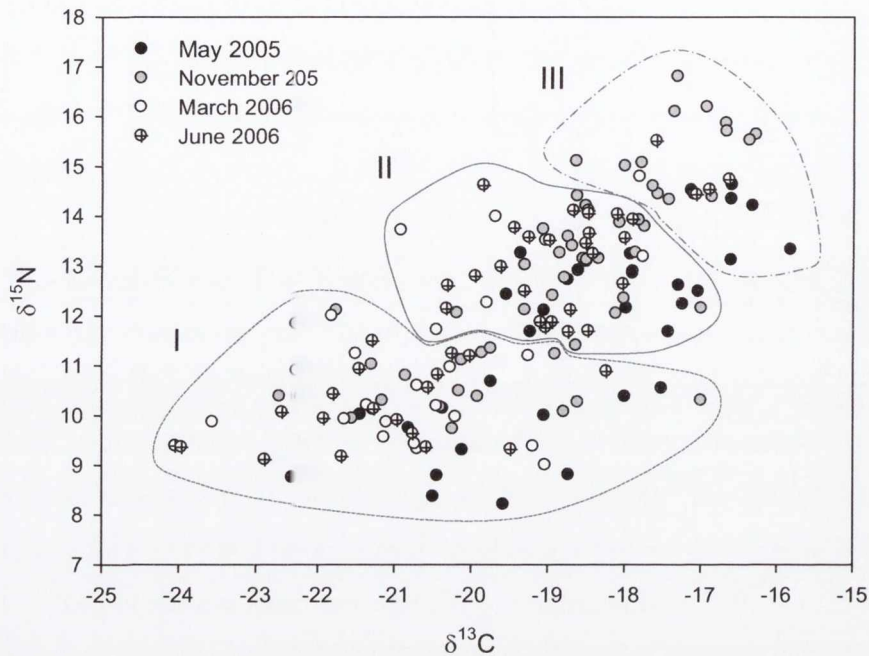


Figure 5.3: Distribution of carbon and nitrogen stable isotope ratios (mean $\pm$ SD) among consumers sampled from May 2005 to June 2006. Groups (encircled) were determined from the hierarchical cluster analysis.

The two following sections explore potential temporal variations among primary and secondary consumers.

Primary consumers

Primary consumers displayed a large variability of carbon isotopic ratios with ranges of $\delta^{13}\text{C}$ values of 3.8‰ in May 2005 up to 5.6 and 5.7‰ in June 2006 and November 2005 respectively (Fig. 5.4). Such large range of $\delta^{13}\text{C}$ signatures were particularly observed for deposit-feeders (either the surface or sub-surface feeders), whose values encompassed the range of $\delta^{13}\text{C}$ measured for suspension-feeding species. The range of $\delta^{15}\text{N}$ signatures displayed by primary consumers was less extended (2.3‰ and 2.4‰ in May and November 2005 respectively to 3‰ in March and June 2006; Fig. 5.5) than for $\delta^{13}\text{C}$. $\delta^{15}\text{N}$ values of deposit-feeders also extended over the range displayed by suspension-feeders.

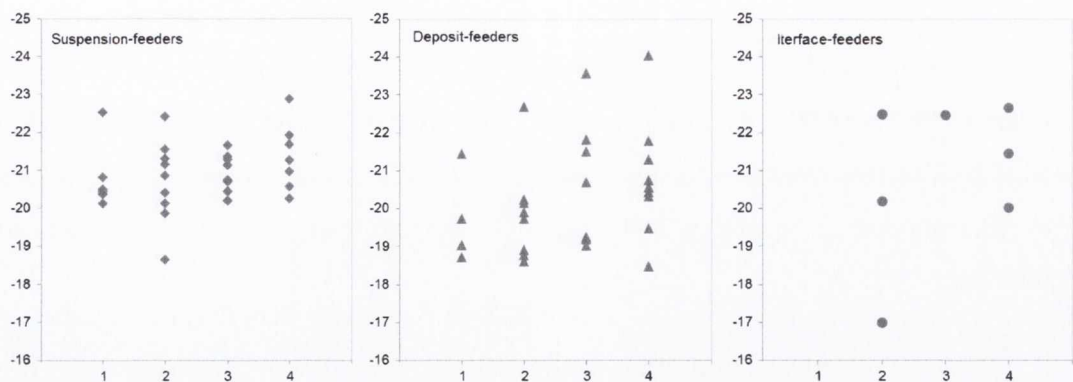


Figure 5.4: Temporal variability in the $\delta^{13}\text{C}$ of primary consumers (mean value of each species) feeding groups as identified from the literature (active suspension-feeders, deposit-feeders (feeding at both the surface or sub-surface) and interface-feeders. Cores on the X axis refer to sampling surveys. 1: May 2005; 2: November 2005; 3: March 2006; 4: June 2006.

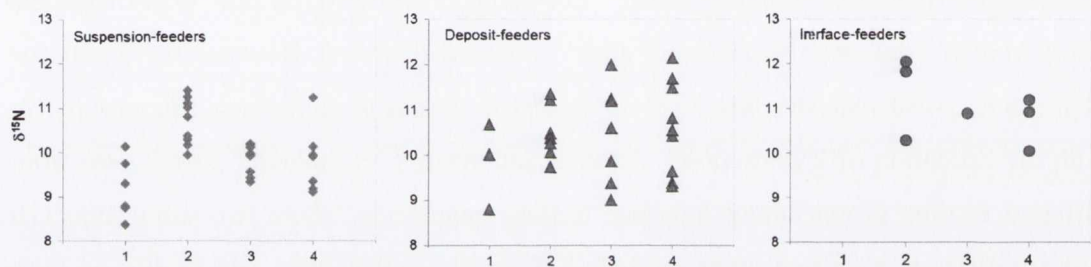


Figure 5.5: Temporal variability in the $\delta^{15}\text{N}$ of primary consumers (mean value of each species) feeding groups as identified from the literature (active suspension-feeders, deposit-feeders (feeding at both the surface or sub-surface) and interface-feeders. Cores on the X axis refer to sampling surveys. 1: May 2005; 2: November 2005; 3: March 2006; 4: June 2006.

Variance of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of deposit-feeders did not vary significantly among surveys (KW, $p>0.05$ in both cases). Significant temporal variations were however observed at species level for both carbon and nitrogen isotopic ratios of *Melinna palmata* (MW, $p=0.021$ for $\delta^{13}\text{C}$ and $p=0.042$ for $\delta^{15}\text{N}$) and *Nucula nitidosa* (KW, $p<0.001$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) with low $\delta^{13}\text{C}$ in June 2006 and high $\delta^{15}\text{N}$ in November 2005 compared with other surveys. The interface-feeding brittle star *Arocnida brachiata* also exhibited significant variations of its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (KW, $p=0.002$ in both cases).

Range of $\delta^{13}\text{C}$ signatures of suspension-feeders did not vary significantly among surveys (KW, $p>0.1$). Only the bivalve *Thracia phaseolina* showed, unlike other species, a significant temporal variation of its $\delta^{13}\text{C}$ (KW, $p=0.008$) due to a ^{13}C -depletion in May 2005 and June 2006. $\delta^{15}\text{N}$ of suspension-feeders fluctuated more with time, with ranges

significantly higher in November 2005 and lower in May 2005 than other surveys (KW, $p=0.001$, Dunn's *post* tests). These trends were observed at species level for *Dosinia exoleta* (KW, $p=0.001$), *Pharus legumen* (KW, $p=0.028$) and *T. phaseolina* (KW, $p=0.014$). The bivalve *Gari fervensis*, *Chamelea gallina* and *Acanthocardia* sp. were also ^{15}N -enriched in November 2005 although these differences were not statistically significant.

Hierarchical clustering performed for each survey on the primary consumers $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ resulted in identification of four to five groups and subgroups (Sg) for each survey (Appendix 5.ig. 5.6 and 5.7). Primary consumers tended to cluster according to both their feeding and taxonomic group. The sub-surface deposit-feeder heart urchin *Echinocardium coran* displayed the most ^{13}C -depleted values, while the amphipod *Ampelisca brevicornis* was the most ^{13}C -enriched, followed by the deposit-feeders *Leptosynapta inhaerens* (holothuroid) and *N. nitidosa* (bivalve). Between these extreme $\delta^{13}\text{C}$ values, suspension-feeding bivalves tended to cluster at all seasons (Subgroup 1b), with the exception of *phaseolina*. Another sub-group (1c) gathered polychaetes from different feeding grounds and/or interface-feeding ophiuroids. These two sub-groups (1b and 1c) differed from each other on their $\delta^{13}\text{C}$ in November 2005 and mostly on their $\delta^{15}\text{N}$ values in March 2005 and June 2006.

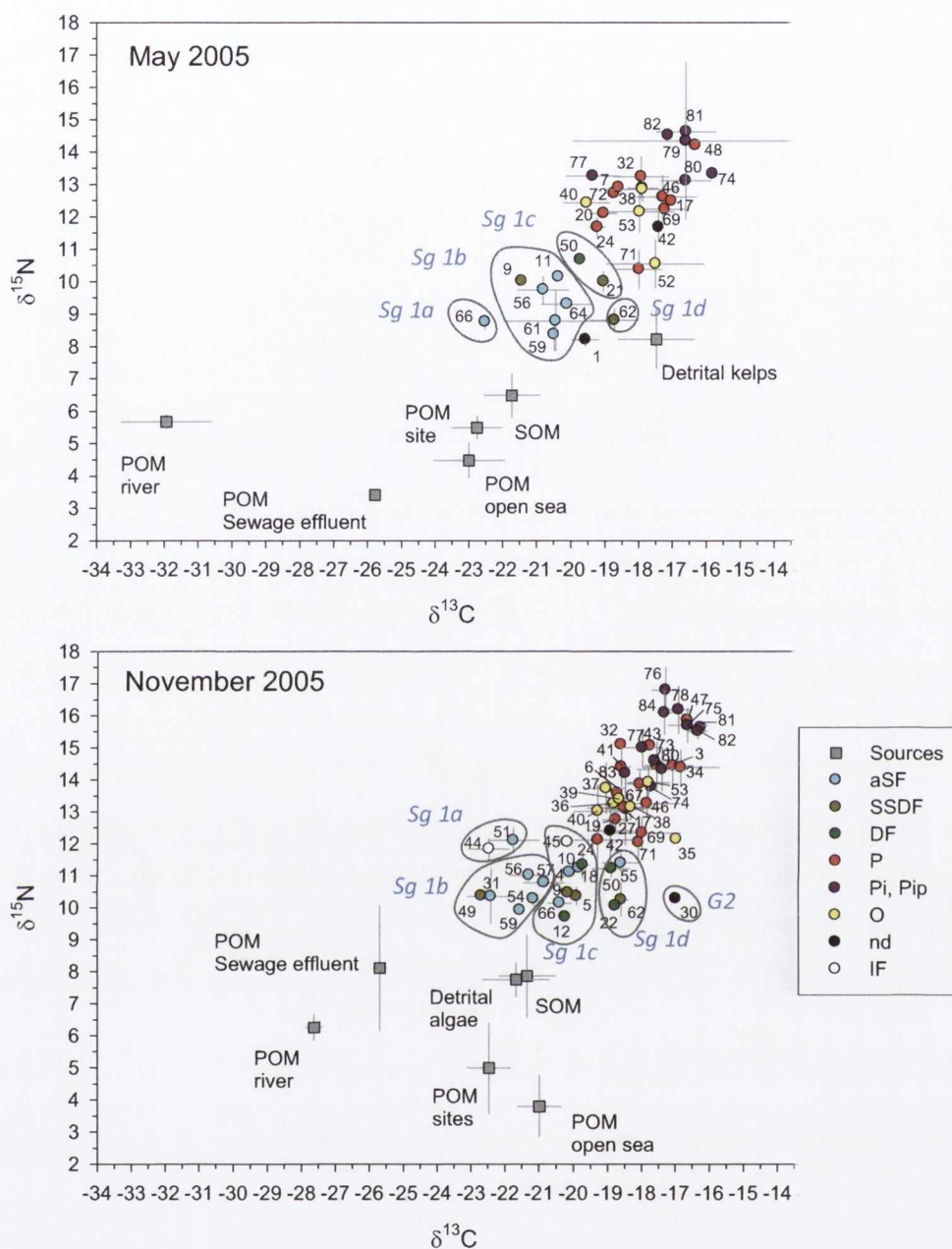


Figure 5.6: $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ (mean $\pm$ SD) of sources and consumers in Lin Bay in May 2005 and November 2005. Color codes correspond to feed strategies (from literature): SF: Suspension-Feeders, SSDF: Sub-Surface Det-Feeders, DF: Deposit-Feeders, IF: Interface-Feeders, O: Omnivorous, Predators (invertebrates); Pi: Benthivorous fish (on invertebrates), Pp: Piscivore fish. nd: feeding type not clearly determined. Species codes are indicated in e 5.2. Sg: sub-group, G: Group.

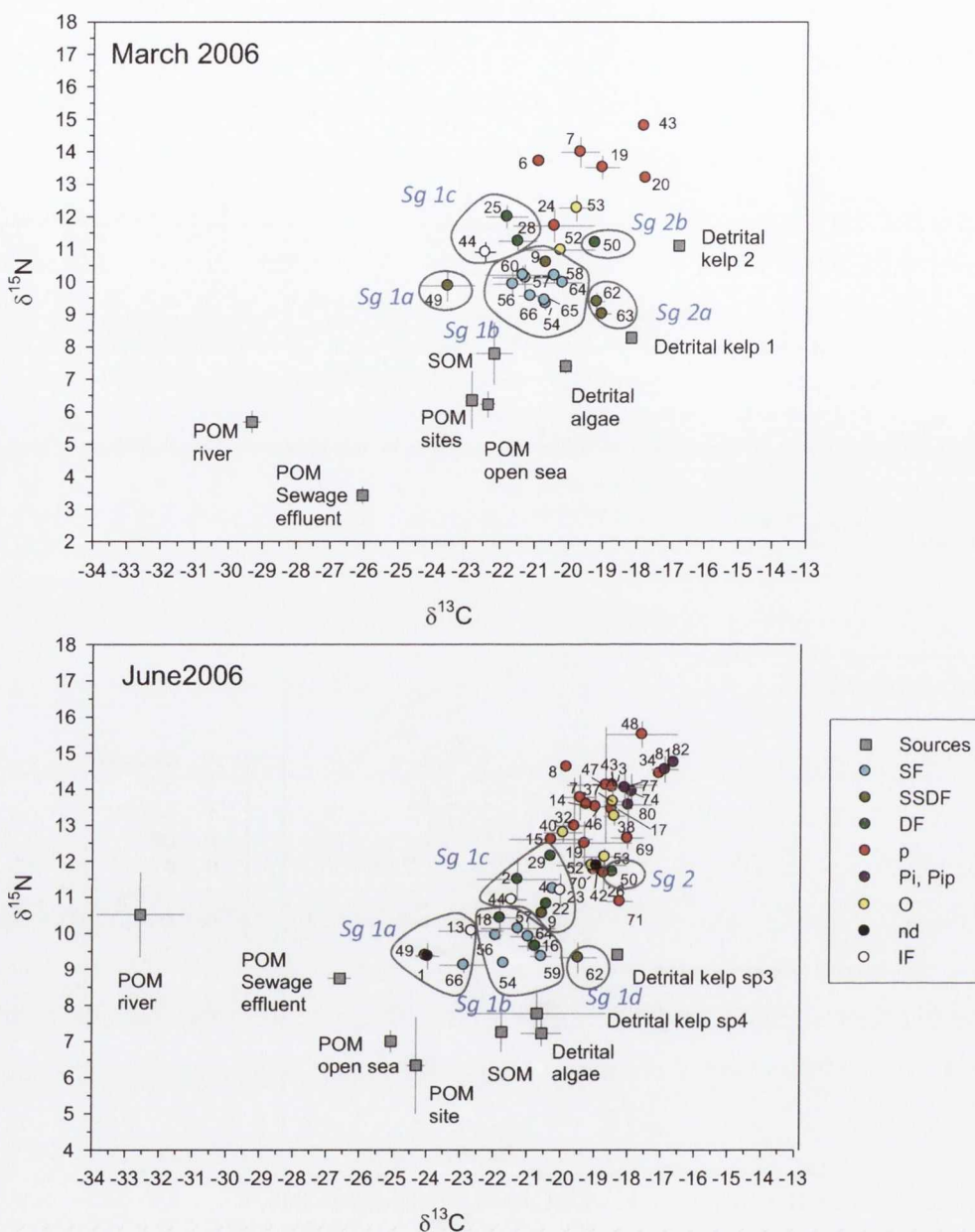


Figure 5.7: $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ (mean $\pm$ SD) of sources and consumers in Dublin bay in March and June 2006. Color codes correspond to feeding strategies (from literature): SF: Suspension-Feeders, SSDF: Sub-Surface Deposit-Feeders, DF: Deposit-Feeders, IF: Interface-Feeders, O: Omnivorous, P: Predators (invertebrates); Pi: Benthivorous fish (on invertebrates), Pp: Piscivorous fish. nd: feeding type not clearly determined. Species codes are indicated in Table 5.2. Sg: sub-group, G: Group.

Secondary consumers

Omnivorous species and benthivorous fish (Pi) did not show seasonal variations in the variance of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (KW, $p > 0.05$ in all cases). $\delta^{13}\text{C}$ of predatory invertebrates (P) and fish feeding on both invertebrates and fish (Pi,p) did not vary among surveys (KW, $p = 0.180$ and $p = 0.907$ respectively) but their $\delta^{15}\text{N}$ did (KW, $p = 0.032$ and 0.001 respectively). Unlike suspension-feeders, there was no consistent pattern in the $\delta^{15}\text{N}$ temporal variations of the different species of predatory invertebrates. Fish categorised as Pi,p displayed a wider range of $\delta^{15}\text{N}$ values in November 2005 (1.8‰) than in May 2005 (1.4‰) and June 2006 (0.7‰). This was related to: i) higher $\delta^{15}\text{N}$ in November 2005 than at other surveys of fish sampled at several occasions (e.g. *L. limanda*, *Raja clavata* and *Scyliorhinus canicula*); ii) the collection of ^{15}N -enriched species such as *Gadus morhua*, *Merlangius merlangus*, *Eutrigla gunardus* and *Trisoptera minutus* in November 2005 only.

Food web structure

The benthic food web of Dublin Bay was organised on 3.8 trophic levels (Table 5.3a, b). All species sampled at several occasions showed stable trophic levels among surveys. Species classified as omnivorous displayed a mean trophic level of 2.8, suggesting a strong reliance on animal prey as a food source. Predatory invertebrates were spread between trophic levels 2.4 and 3.6 (mean TL = 3). Fish species all had a trophic level above 3, with benthivorous fish displaying trophic levels of 3-3.1 and fish categorised as both benthivorous and piscivorous having trophic levels between 3.2 and 3.8.

Table 5.3a: Trophics (TL) calculated for species sampled in May 2005, November 2005, March 2006 and Jun.

SPECIES	Feeding guild	May 2005	Nov. 2005	March 2006	June 2006	mean TL	stdev TL
Zooplankton		1,7			1,9	1,8	0,1
<i>Acanthocardia</i> sp	aSF		1,9	1,9	1,8	1,9	0,0
<i>Aequipecten opercularis</i>	aSF		2,2			2,2	
<i>Balanus</i> sp	aSF		1,9			1,9	
<i>Chamelea gallina</i>	aSF	2,2	2,1	2,0	2,0	2,1	0,1
<i>Chone fauveli</i>	aSF		2,1		2,4	2,3	0,2
<i>Dosinia exoleta</i>	aSF		2,0	2,1	2,1	2,1	0,0
<i>Ensis siliqua</i>	aSF			2,0		2,0	
<i>Gari fervensis</i>	aSF	1,8	1,8		1,9	1,8	0,1
<i>Hydroides norvegica</i>	aSF		2,2			2,2	
<i>Jasmineira elegans</i>	aSF	2,3				2,3	
<i>Mactra stultorum</i>	aSF			2,1		2,1	
<i>Modiolus modiolus</i>	aSF	1,9				1,9	
<i>Pharus legumen</i>	aSF	2,0		2,1	2,0	2,1	0,0
<i>Polititapes virgineus</i>	aSF			1,9		1,9	
<i>Thracia phaseolina</i>	aSF	1,9	1,8	1,9	1,8	1,9	0,1
<i>Alcyonium digitatum</i>	aSF/C	2,7	2,5		2,6	2,6	0,1
<i>Ampelisca brevicornis</i>	aSF/DF		1,9			1,9	
<i>Acrocnida brachiata</i>	IF		2,3	2,3	2,3	2,3	0,0
<i>Amphiura filiformis</i>	IF		2,4			2,4	
<i>Lanice conchylega</i>	IF				2,1	2,1	
<i>Owenia fusiformis</i>	IF				2,4	2,4	
<i>Ophiotrix fragilis</i>	pSF		2,4			2,4	
<i>Echinocardium cordatum</i>	SSDF		1,9	2,0	1,9	1,9	0,1
<i>Euclymene lumbricoides</i>	SSDF		1,9			1,9	
<i>Heteroclymene robusta</i>	SSDF	2,2	1,9	2,2	2,2	2,2	0,1
<i>Notomastus latericeus</i>	SSDF	2,2				2,2	
<i>Nucula nitidosa</i>	SSDF	1,9	1,9	1,9	1,9	1,9	0,0
<i>Nucula nucleus</i>	SSDF			1,8		1,8	
<i>Aphaelochaeta filiformis</i>	DF				2,5	2,5	
<i>Lagis koreni</i>	DF		1,7			1,7	
<i>Leptosynapta inhaerens</i>	DF	2,4	2,2	2,4	2,6	2,4	0,2
<i>Magelona mirabilis</i>	DF				2,0	2,0	
<i>Melinna palmata</i>	DF		2,2		2,2	2,2	0,0
<i>Ophelina acuminata</i>	DF		1,8		2,3	2,1	0,3
<i>Pista cristata</i>	DF			2,6		2,6	
<i>Terebellides stroemii</i>	DF			2,4		2,4	
<i>Tharyx mariani</i>	DF				2,7	2,7	
<i>Gastrosaccus spinifer</i>	O		2,4			2,4	
<i>Hyas coarctatus</i>	O		2,8			2,8	
<i>Inachus dorsettensis</i>	O		2,9		3,1	3,0	0,2
<i>Macropodia rostrata</i>	O		2,8			2,8	
<i>Ophiura albida</i>	O	2,4		2,3	2,6	2,4	0,1
<i>Ophiura ophiura</i>	O	2,9	2,9	2,7	2,7	2,8	0,1
<i>Pagurus bernhardus</i>	O	2,9	2,7		2,9	2,8	0,1
<i>Liocarcinus depurator</i>	O	3,1	2,7		3,0	2,9	0,2

Table 5.3b: Trophic levels (TL) calculated for species sampled in MD5, November 2005, March 2006 and June 2006.

SPECIES	Feeding guild	May 2005	Nov. 2005	March 2006	Jun	mean TL	stdev TL
<i>Aphrodita aculeata</i>	P		3,1			3,2	0,1
<i>Asteria rubens</i>	P	3,0	2,8			2,9	0,2
<i>Astropecten irregularis</i>	P		3,5			3,4	0,2
<i>Buccinum undatum</i>	P	2,9	2,5			2,7	0,2
<i>Cerebratulus</i> sp	P	3,0				3,0	
<i>Coryistes cassivelaunus</i>	P	3,2	3,3			3,1	0,2
<i>Crangon allmanni</i>	P		3,1			3,1	
<i>Crangon crangon</i>	P		3,1			3,2	0,2
<i>Crossaster papposus</i>	P	3,5				3,6	
<i>Glycera</i> sp	P		2,8	3,2		3,0	0,2
<i>Glycera tridactyla</i>	P	3,1		3,2		3,2	0,1
<i>Goniada maculata</i>	P					3,4	
<i>Lepidonotus squamatus</i>	P					3,1	
<i>Loligo forbesii</i>	P		3,1			3,1	
<i>Lumbrineris latreilli</i>	P					2,8	
<i>Lunatia catena</i>	P					2,6	
<i>Malmgreniella castanea</i>	P	3,1	2,7			3,0	0,2
<i>Metridium senile</i>	P		3,3	3,5		3,3	0,1
<i>Nephtys hombergii</i>	P	3,0	2,6	3,1		2,9	0,2
<i>Nephtys</i> sp	P	2,9		3,0		2,9	
<i>Pandalus montagui</i>	P		2,9			2,9	
<i>Philine aperta</i>	P	2,3	2,4			2,4	0,0
<i>Phyllodoce groenlandica</i>	P	2,7	2,4	2,6		2,6	0,2
<i>Sepiolo atlantica</i>	P		2,7			2,7	
<i>Sigalion mathildae</i>	P					2,6	
<i>Sthenelais limicola</i>	P		2,7			2,7	
<i>Amoglossus latema</i>	Pi		3,1			3,1	
<i>Callionymus lyra</i>	Pi	3,2	2,9			3,1	0,2
<i>Pleuronectes platessa</i>	Pi	3,2	3,1			3,1	0,0
<i>Solea solea</i>	Pi		3,0			3,0	
<i>Eutrigla gunardus</i>	Pi,p		3,5			3,5	
<i>Gadus morhua</i>	Pi,p		3,8			3,8	
<i>Limanda limanda</i>	Pi,p	3,2	3,3			3,2	0,0
<i>Merlangius merlangus</i>	Pi,p		3,6			3,6	
<i>Platichthys flesus</i>	Pi,p	3,5				3,5	
<i>Raja clavata</i>	Pi,p	3,6	3,5			3,5	0,1
<i>Scyliorhinus canicula</i>	Pi,p	3,6	3,4			3,5	0,1
<i>Trisopterus minutus</i>	Pi,p		3,6			3,6	

5.4. Discussion

5.4.1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the POM and SOM

Carbon and nitrogen isotopic signatures of the POM from the sampling sites were relatively close to the signatures of the POM collected offshore, away from terrestrial influence. Excluding November 2005, $\delta^{13}\text{C}$ of the site POM differed from terrestrial sources from the catchment basin, such as riverine and wastewater POM, suggesting a negligible influence of the catchment on waters from the outer Dublin Bay during most of the year. The ^{13}C -depletion of the site POM compared to marine POM in November 2005 however suggests a slightly higher influence of the catchment, and so potentially of sewage, on organic matter pools at this time of the year.

With $\delta^{13}\text{C}$ ranging from -25 to -21‰, the carbon signatures of suspended POM (from the bay and from offshore) were within the range of values reported for marine waters of temperate ecosystems (8 to -25‰; e.g. Gearing *et al.*, 1984; Wainwright & Fry, 1994). The POM was ^{13}C -depleted in June 2006 when phytoplankton dominated (as indicated by the low POC/Chl *a* ratio) and significantly departing from measurements made at other seasons. Carbon isotopic composition of marine POM can typically fluctuate strongly with time (e.g. Wainwright & Fry, 1994; Rolff, 2000; Lorrain *et al.*, 2002; Savoye *et al.*, 2003). In phytoplankton-dominated POM, $\delta^{13}\text{C}$ depends on a combination of biotic and abiotic factors and processes, which can make it difficult, based on bulk POM, to identify the main causes behind the variations measured. For example, $\delta^{13}\text{C}$ of phytoplankton can differ among phytoplankton species (Brutemark *et al.*, 2009), between growth rate and phases for a same species (Burkhardt *et al.*, 1999; Brutemark *et al.*, 2009), or among size fractions of plankton (au *et al.*, 1990). It is also influenced, among other factors, by dissolved inorganic carbon concentrations (Fry, 1996), as well as irradiance and nutrients (Thompson & Clavert, 1994). Rolff (2000) nevertheless reported a ^{13}C -enrichment of the POM synchronously with the spring phytoplankton bloom, followed by a $\delta^{13}\text{C}$ decrease in June-July at the end of the bloom. Although caution must be taken in the interpretation of results from the present study due to low temporal resolution of the POM collection, a similar relationship may have applied and could explain the low $\delta^{13}\text{C}$ values of POM in

June compared to March and May. Indeed, the sampling likely took place toward the end of the spring bloom whose peak typically occurs in late April-early May in Dublin Bay (O'Higgins, 2005). The presence of macroalgal detritus in the water column has been reported by Kaehler (2000) to induce a ^{13}C -enrichment of the suspended POM. Such a shift was observed offshore in November 2005 but not within Dublin Bay where it could be rather expected. Macroalgal detritus may therefore be a minor component within the suspended particulates pool in Dublin Bay.

Sedimentary organic matter had a stable isotopic composition, overall close to the POM one, indicating that SOM was mainly derived from marine planktonic matter. The SOM enrichment in ^{15}N compared with the suspended POM may result from the selective removal of the lighter isotope (^{14}N) during remineralisation processes (Altabet, 1996). Moreover, deposited phytoplankton may undergo a ^{13}C enrichment across the microbial loop (Goering *et al.*, 1990; Hobson *et al.*, 1995), resulting in higher $\delta^{13}\text{C}$ of the detrital SOM relatively to the suspended POM, as measured in June when phytoplankton dominated the POM pool. In contrast, terrestrial OM which was characterised by low $\delta^{13}\text{C}$ does not appear to be a significant contributor to the SOM pool as this would have otherwise induced a shift to more negative $\delta^{13}\text{C}$ values of the SOM, as seen elsewhere (Simenstad & Wissmar, 1985; Thornton & McManus, 1994; Yu *et al.*, 2010).

5.4.2 Trophic sources sustaining the food web

The subtidal food web of Dublin Bay showed a relatively linear distribution of $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$, suggesting a single major pathway of organic matter transfer. The food web was stable in time and consumers were ^{13}C -enriched compared to terrestrial sources of organic matter such as sewage and riverine POM, suggesting that these sources have a minor or negligible influence as trophic resources for subtidal communities, as it would have otherwise induced a shift of the food web or part of it to C-depleted values. A minor trophic role of sewage POM in the outer bay was expected due to the upgrade of the sewage treatment plant in 2003 which induced a substantial drop of the amount of particulates released (O'Higgins, 2005), and this result tends to confirm this hypothesis.

River inflow to Dublin Bay (main river is the Liffey with an annual mean discharge of $12 \text{ m}^3 \text{ s}^{-1}$) is relatively low compared with other European rivers whose POM inputs have been previously traced in marine food webs (Riera & Richard, 1997; Riera *et al.*, 2000; Darnaude *et al.*, 2004b) and its low influence on the food web is therefore not surprising.

Apart for *A. opercularis* which was quite ^{13}C -enriched relative to other suspension-feeding species, suspension-feeders had homogenous ranges of $\delta^{13}\text{C}$ across surveys, between -23 and -20‰. When estimating their theoretical food sources by subtracting average trophic fractionation factors (between 0.5 and 2‰ for C and ~ 3.4 ‰ for N; Minagawa & Wada, 1984; Post, 2002a; Yokoyama *et al.*, 2005a; Dubois *et al.*, 2007) from suspension-feeders mean isotopic signatures, POM (by considering the whole range of values measured over the four surveys) appears as the main source of food to these consumers, accordingly with their recognised feeding mode. In November 2005 however, the significant $\delta^{15}\text{N}$ increase of suspension-feeders suggest a higher assimilation than for other surveys of ^{15}N -enriched particulates, such as SOM whose isotopic ratios better correspond to the computed theoretical food source. Although suspension-feeders are known to filter material sedimenting from the water column, two observations can support a higher trophic exploitation of the SOM in early winter: 1) the increasing occurrence of windy meteorological events in autumn and winter affects hydrodynamical conditions in shallow waters, and classically enhances water mixing and resuspension of sediments during this period of the year. Particulate matter in suspension above the seabed then becomes a mixture of sedimenting OM from the water column and deposited matter resuspended, whose access to suspension feeders is therefore extended; 2) SOM had a higher nutritional value in November 2005 (mean C:N ratio = 9) than the POM (C:N between 18 and 25), possibly due to microbial colonization of the detrital OM composing the SOM (Mann, 1988), such that suspension feeders may have selectively fed on that source within the particulates pool. Heterotrophs are indeed considered to favor the consumption of food items with the lowest C:N ratios in order to fulfill their nutritive requirements (Elser *et al.*, 2000; Dorenbosch & Bakker, 2011). Suspension-feeders have different capacities to particles selection such that they may use fractions of different size, quality or origin within the heterogeneous pool of particulates made available to them (Kiorboe & Mohlenberg, 1981; Hawkins *et al.*, 1998). That may explain the higher variability of isotopic ratios among suspension-feeders in November 2005, leading them to be classified in different sub-groups by the cluster analysis. While

bivalves clustered together (Sg 1b), the grouping of the bivalve *T. phaseolina* and the Sabellida polychaetes *Chone fauveli* and *Hydroides norvegica* (all suspension-feeders) with deposit-feeding species in November 2005 (Sg 1c) suggests that these three suspension-feeders may have even more strongly relied on resuspended particles from the SOM than other suspension-feeding bivalves. It thus appears that through natural resuspension events, SOM may play an increased trophic role for benthic suspension-feeders in autumn and winter, when phytoplankton production is low. Such a scenario has already been highlighted by Carlier *et al.* (2007a) in the bay of Banyuls sur mer (NW Mediterranean sea) and likely is relatively common in shallow and semi-open coastal ecosystems of soft substrate.

The values of $\delta^{13}\text{C}$ displayed by deposit-feeders were consistent with the fact that they mainly feed on (or in relation with) the pool of SOM. The strong overlap between the $\delta^{13}\text{C}$ of suspension-feeders and deposit-feeders additionally supports the tight coupling between marine POM sedimenting from the water column and SOM and the importance of sediment resuspension in Dublin Bay. Deposit-feeders exhibited a large range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ indicating different dietary compositions within this feeding guild. This differentiation may be linked with different feeding behaviors and various levels of selectivity among species, based on the quality and/or size of the “particles” (Fauchald & Jumars, 1979). A species’ ability to select food is related to its biological traits like the presence and the structure of tentacles and palps (Fauchald & Jumars, 1979; Compton *et al.*, 2008), but it may also occur internally during the digestion process for some species such that, based on Fauchald & Jumars (1979), most species analysed here may present a certain extent of selectivity in their diet. Given that the SOM constitutes a heterogeneous pool including notably detrital organic matter of different quality and origins, bacteria, microheterotrophs and meiofauna (protists, larvae, etc), a differential use and assimilation of the various organic components of the SOM pool could have led to the $\delta^{13}\text{C}$ differentiation among the broad deposit-feeders group. The large range of $\delta^{15}\text{N}$ displayed by deposit-feeders, spanning over two trophic levels (1.7 to 2.7) moreover support this hypothesis, by suggesting that ^{15}N -enriched species preyed on microheterotrophs or small invertebrates from the sediments.

Macroalgal-derived detritus can be considered as another source of food which may have been used by some of the primary consumers displaying higher $\delta^{13}\text{C}$. In particular, the

amphipod *A. brevicornis* which clearly differentiated from other species due to its ^{13}C -enrichment may have significantly exploited this resource. The suspension-feeding clam *A. opercularis* may have also fed on macroalgal detritus, but to a lesser extent than the amphipod. These detritus may originate from fucoids and kelps growing on rocky substrates bordering the bay, from the sandy upper infralittoral fringe east of Bull Island where blooms of *Ectocarpus* regularly occur, or eventually from Bull Island lagoons. They may have been exported to the adjacent soft substrates by tidal advection and supply food to benthic organisms via the detrital chain as reported by several authors (Duggins *et al.*, 1989; Bustamante & Branch, 1996; Soares *et al.*, 1997). Nevertheless, the trophic role of macroalgae to subtidal species may be minor in Dublin Bay compared with pelagic sources and just limited to a few consumers. It would have otherwise resulted in a more general shift of the base of the food web to ^{13}C -enriched signatures. This low contribution might be related to a relatively low macroalgal standing stock at the scale of the bay. Macroalgal biomass was indeed estimated by Wilson & Parkes (1998) to 225t DW over the whole bay, which is, for example, more than a hundred fold lower than estimations of kelp biomass given by Soares *et al.* (1997) for sites on the west coast of South Africa where kelp were reported to significantly contribute to the diet of nearshore clams.

Microphytobenthos is another ^{13}C -enriched source shown to have an important trophic role in some coastal ecosystems (Riera & Richard, 1996; Kang *et al.*, 2003; Grall *et al.*, 2006). It can however be considered negligible in the outer Dublin Bay where waters are highly turbid and the euphotic layer very shallow (O'Higgins, 2005) such that light conditions are likely insufficient to enable microphytobenthic production in this area.

5.4.3 Food web structure

The food web was organised into 3.8 trophic levels. This is comparable to findings for other subtidal benthic food webs in Europe (Grall *et al.*, 2006; Carlier *et al.*, 2007a; Le Loc'h *et al.*, 2008) and in agreement with an average food chain length of 4 trophic levels in marine ecosystems (Vander Zanden & Fetzer, 2007). Species TL estimated from this study were overall in good agreement with ecological and biological information from

the literature, but also revealed a high degree of omnivory, reflected in the $\delta^{15}\text{N}$ continuum from the primary consumers at the base up to top-predators (Fig. 5.6 and 5.7). Predatory invertebrates displayed a relatively large range of $\delta^{15}\text{N}$, corresponding to TL of 2.4 to 3.6, indicating a diversity of prey linked with various feeding behaviours. Indeed, the sunstar *C. papposus* (TL= 3.6) actively feeds on large invertebrates including predators such as asteroides. The grouping of this species with top-predators by the cluster analysis is thus in agreement with its feeding mode. Carnivorous polychaetes such as scale worms (e.g. *Malmgreniella castanea*, *Lepidonotus squamatus*), Nephtyidae or Glyceridae are typical active predators and/or scavengers and their TL of ~ 3 was in agreement with this. A few species of predators had on the other hand lower TL than could be expected (e.g. *P. aperta*) suggesting a mixed diet with preys from lower TL. As suggested in the previous section, omnivory appeared to occur among deposit-feeders, which exhibited TL from 1.7 to 2.7. In the case of terebellids polychaetes for example (TL of 2.4 and 2.6), a mixed diet would be in agreement with Fauchald & Jumars (1979) who reported them to feed on detritus, unicellular algae but also on small invertebrates and larvae. Species initially considered as omnivorous accordingly displayed intermediate trophic levels (from 2.4 to 3) but their relatively high $\delta^{15}\text{N}$ suggested an increased reliance on predation, especially for decapods. Omnivory in the subtidal food web thus appeared to be essentially directed upward, i.e. with a stronger reliance on animal prey (from higher TL) in the mixed diets rather than the opposite (i.e. feeding on basal sources). This tendency can indeed be expected in a system where primary producers are not well represented as in the study area and where detritivory is important. Omnivory is common in marine food webs (Thompson *et al.*, 2007). It increases feeding options to omnivorous organisms and thus optimise their chance to meet their nutritional requirements (Buck *et al.*, 2003). According to Namba *et al.* (2008), omnivory has a stabilizing effect on the system especially when it is directed toward predation as observed in the present study. It may also play a role in the resilience of the ecosystem by lessening the severity and/or duration of changes induced by disturbances (Fagan, 1997).

The subtidal food web was stable both in space, indicating no major changes in food supply among the studied sites, and through time. The food web length and structure did not appear to differ among seasons. Variations in the isotopic composition of primary consumers, particularly suspension-feeders, revealed an adaptative strategy in response to the seasonal change in food supply (both in quality and quantity), highlighting the trophic

plasticity of these primary consumers in response to changing environmental conditions in soft substrates.

Some seasonal isotopic variations also occurred at species level in other feeding guilds. These species isotopic variations may be the result of temporal modification in the composition of their diet but these would have been minor as it did not impact the structure of the food web: it never induced a major variation of TL, neither it indicated major changes of feeding pathways from the basal sources. These temporal variations at species level may otherwise be a reflection of seasonal isotopic variations at lower trophic levels. Intraspecific isotopic variations can also result from other factors such as ontogenetic diet changes (Hentschel, 1998; Rossi *et al.*, 2004) or physiological processes linked with the reproductive cycle or the lipid content (Martinez del Rio *et al.*, 2009). However, maximum care was taken to analyse species of similar size, and in the case of larger species, to analyse their muscle whose lipid content is typically lower (Pinnegar & Polunin, 1999) such that we are confident these sources of variation have been minimized.

5.5. Conclusion

The benthic food web of Dublin Bay outer subtidal exhibited a relatively linear structure and was shown to tightly rely on marine organic matter, originating primarily from marine phytoplankton and detritus, and to a lower extent from macroalgal detritus. Terrestrial inputs of organic matter delivered by rivers and wastewaters were found to have a minor influence on the food web. The structure of the subtidal food web appeared stable across the bay and through time. The detrital pathway seemed to play an important role for the food web over the year, through a strong benthic-pelagic coupling. Isotopic data suggest some important resource partitioning by primary consumers, and the trophic adjustment displayed by suspension-feeders in response to food supply of various qualities likely plays a role in the stability of the food web. A strong degree of omnivory was also detected within this food web which was composed of 3.8 trophic levels from the base up to top predatory fishes.

GENERAL CONCLUSION & PERSPECTIVES

General conclusion

The limited knowledge of the trophic functioning of Dublin Bay, a valuable ecosystem yet characterised by some ecological dysfunctions linked to on-going issues of nutrients and organic matter enrichment from a large urban centre, led to the implementation of this thesis work. This study aimed to characterise the trophic structure and functioning of Dublin bay benthic communities, to identify the basal sources supporting the food web and the major trophic pathways, and to assess the impacts of anthropogenic disturbances from sewage on the trophic functioning of the benthic system. In particular, I investigated, in the context of a changing regime of sewage discharge, the direct and indirect effects of sewage on food webs through organic particulates and through the macroalgal blooms it sustains. Carbon and nitrogen stable isotopes analysis is a powerful tool to get an overall time-integrated picture of food webs in poorly investigated coastal ecosystems. This approach was thus particularly relevant to depict the pathways of organic matter transfer and the structure of the food web within Dublin Bay system. An extensive field study covering five sites featuring contrasted oceanic influence and exposition to the watershed (from inshore and estuarine conditions to offshore and northern vs southern parts of the bay) has thus been implemented to characterize the whole food web functioning over time. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were applied on a seasonal basis (four surveys) in combination with hydrobiological analysis of the POM to characterise the trophic role of various food sources and their use by consumers (from meiofauna to large predatory fish) over more than a year which is a relevant timescale in temperate areas.

Anthropogenic particulates delivered with sewage were shown to have a low and very localised effect on the food web. They moderately contributed to the diet of primary consumers in the estuarine mudflat of Bull Island South Lagoon and were essentially processed by suspension-feeders (the cockle *Cerastoderma edule*) and deposit-feeders (mostly the lugworm *Arenicola marina*). The influence of these sewage particulates was minor or negligible in the others environments of the bay studied, i.e the south intertidal and the subtidal areas. This result is likely linked with the upgrade of the sewage treatment plant in 2003 which induced a drop of the amount of particulates released in the bay. On the other hand, my analysis confirmed, through the $\delta^{15}\text{N}$ displayed by

macroalgae, the role of sewage effluents in sustaining opportunistic blooms of green macroalgae (*Enteromorpha* spp.) in Bull Island South Lagoon. This influence of anthropogenic nitrogen was not detected in the south part of the bay.

Dublin Bay benthic food web as a whole was shown to rely predominantly on particulate organic matter of marine origin and on benthic primary producers. However, the food sources exploited by the fauna, the trophic pathways involved and their temporal patterns varied largely between the intertidal and the subtidal systems.

The food web of the subtidal area was stable both in space and time and was mostly supported by planktonic organic matter which was transferred to predators by one main trophic pathway, whereas macroalgae detritus were a minor food source for consumers. The detrital pathway played an important role over the whole year, through a strong benthic-pelagic coupling enhanced by resuspension events in winter. By switching between suspended Particulate Organic Matter (POM) and Sedimentary organic matter (SOM), suspension-feeders showed a high trophic plasticity in response to seasonal changes of food supply (POM vs resuspended SOM), thus revealing their adaptation to naturally changing environmental conditions, which likely plays a role in the stability of the food web. Large range of isotopic values indicated some food partitioning among deposit-feeders which seemed to exploit different fractions of the SOM pool. As observed in many temperate coastal benthic food webs, the subtidal food web of Dublin Bay was organised in ~4 trophic levels. It was also characterised by a high degree of omnivory directed toward predation, which is considered as an indicator of stability (Fagan, 1997; Namba *et al.*, 2008).

Benthic primary producers played a higher trophic role for primary consumers in the intertidal area than in the subtidal one. Hence the food web in these environments (intertidal mudflats and sandflats) was mostly supported by microphytobenthos, marine POM (mainly composed of phytoplankton or marine detritus depending on seasons) and detrital macroalgae. In sites unaffected by macroalgal blooms, primary consumers displayed a relative trophic consistency over time and the results showed that consumers tended to utilise food sources produced nearby them. Deposit-feeding species mostly exploited MPB and macroalgal organic matter, and to a lesser extent some POM through the SOM pool. The suspension-feeding cockle *Cerastoderma edule* relied on the same sources but in different proportions, accordingly with its feeding mode. It exploited

essentially POM but this species adapted to varying food sources availability in the water column (lower phytoplanktonic production, resuspension events) by a shift to a higher exploitation of benthic resources during the winter period. Species able to switch between both feeding modes (i.e. interface-feeders) showed stronger spatial variations. In the absence of anthropogenic disturbance, the isotopic signatures of interface-feeders indicated a relatively consistent diet but which tended to differ according to the habitat occupied and the associated environmental conditions. Interface-feeding bivalves thus appeared to make a higher reliance on POM in semi-exposed sandy habitats and on MPB in muddier habitats.

Primary consumers from the site impacted by green macroalgal blooms of *Enteromorpha* spp. (BISL) had relatively similar diets to the ones from unimpacted sites at some periods of the year, but they also strongly reacted to pulses of organic matter induced by the macroalgal bloom. The macroalgal biomass appeared to be incorporated in the local food web mainly through the detrital pathway during the decomposition and burial of the mats, as revealed by the $\delta^{15}\text{N}$ shift of the consumers' tissues at the end of autumn. It was particularly assimilated by deposit-feeders, which derived up to 66% of their food from it. The high production of opportunistic *Enteromorpha* spp. was nevertheless shown by this study to influence species-specific diets of most consumers, from primary consumers (all feeding groups: suspension-feeders, deposit-feeders, grazers), including the meiofauna compartment, to omnivorous species. It was channeled to upper trophic levels of the food web, via the deposit-feeder guild mainly. While no effect on the food web length (which reached four trophic levels) was recorded, my results nevertheless show that macroalgal blooms of *Enteromorpha* spp. affected the whole food web structure periodically. Several trophic pathways, based on a differential use of basal sources (plankton, MPB, macroalgae, POM detrital matter) by primary consumers were indeed depicted from the base to the top of the food web at the end of spring, in the absence of algal mats. But in winter following the collapse of the macroalgal mat and its burial, the food web was simplified and one main trophic pathway, based on detrital *Enteromorpha* spp., emerged. This was reflected in a $\delta^{13}\text{C}$ narrowing at the base of the food web and $\delta^{15}\text{N}$ isotopic shifts at different trophic levels, and depicted through dietary mixing models of primary consumers. Such a simplification of the food web complexity as a consequence of macroalgal blooms has been recently reported in other environments such as macrotidal sandy beaches (Quillien *et al.*, 2016) but, to my knowledge, is a new finding for sheltered

tidal systems. The food web structure thus appeared to oscillate between two states of trophic complexities during the year, and questions therefore arise on the long-term effects of the bloom on the system structure and functioning. The recurrence of the bloom every year moreover maintains the communities in a disturbed state, with likely effects on the benthic community structure and diversity, and on higher trophic levels, including top predators of high ecological and economic values (fish, shorebirds, waders), for which the mudflat is an important foraging area.

The spatio-temporal approach implemented for this study proved relevant to characterise the food web of Dublin Bay and to partly depict the effects of anthropogenic urban inputs on the ecosystem functioning. My results clearly highlight that the impact of sewage effluents on the food web is now mostly indirect, via the eutrophication symptoms it induces such as the green macroalgal bloom in BISL. This study provide further insights on macroalgal blooms effects on the food web structure of sheltered systems, which are of paramount importance given that the occurrence of macroalgal blooms is a spreading phenomenon, increasing in frequency and intensity worldwide.

Perspectives

This first investigation of the benthic food web of the Dublin Bay draws the overall structure of trophic connections between food sources, meiofauna and macrofauna and depicts the main pathways of organic matter transfer. From these results and considerations, two main aspects would be worth to consider for future research:

1/ The first one would be to reconsider the Ecological Network analysis (ENA) of energy flow through the bay which have been previously realised (Wilson & Parkes, 1998; Wilson *et al.*, 2007) in light of these new insights on the pathways of organic matter transfer in Dublin Bay. Further, I suggest that these models should better consider the effects of sewage influence at small spatial scale (e.g. moderate influence of sewage POM in BISL).

2/ Impact of macroalgal blooms on benthic communities:

The characterisation of the terms of incorporation of macroalgae in the food webs by this thesis work offers interesting perspectives for future work in order to better depict the

impact of macroalgal blooms on the functioning of coastal ecosystems. This is particularly interesting regarding the international context of increasing eutrophication issues with macroalgal blooms (Ye *et al.*, 2011). The large ^{15}N -enrichment of the green algae compared to the other sources, allowed a nice isotopic distinction among sources to estimate the contribution of this algae in the diet of the consumers. For this essential reason, the site of Bull Island South Lagoon fills some necessary conditions to pursue the research.

Aspects to consider further would be to better characterize the temporal terms of integration of macroalgae into the food web, by both nematodes and macro-invertebrates. For this, a pre-requisite will be to determine precisely the turn-over time of consumers tissues (for a selection of species based on the result from this study, such as *Arenicola marina* or deposit-feeding bivalves) and precise fractionation factors applicable to these consumers, for the tissue considered. Analysing tissues known to have a short turn-over such as the digestive gland (~1 week) could also be particularly useful in this regard.

Stable isotope data obtained in the present study at BISL were very insightful to understand (at least partly) the fate of *Enteromorpha* spp. in the food web. However it gives a rather qualitative picture of interactions occurring (each analysed species contributes in a similar way to the general structure of the food web). Given the side-effects macroalgal blooms are known to have on benthic communities (see General Introduction), it appears essential to bring a more realistic picture of the interactions taking place by taking into account the relative occurrence of each species in the investigated ecosystem (in terms of abundance, biomass, production). Some questions indeed rose about the results from this study, as discussed in Chapter 3. Hence, are populations of deposit-feeders enhanced by macroalgae they heavily feed on? Or on the other way, are they also impacted even if they heavily rely on the algae as a food source? Which effect does this have on upper trophic levels? Thus, the trophic links identified in this work should be superimposed with a quantitative analysis of the community structure (biodiversity and biomass data). This would give weight to the different consumers (or feeding guilds) of community and then would help to quantify carbon fluxes from sources to consumers and among consumers. By that way, trophic indices characterizing the food web complexity which have been recently developed could be applied to biomass-weighted or productivity-weighted isotopic signals of consumers and would allow a

better identification of major trophic interactions on a dynamic perspective. Such a coupled study should be made at a larger spatial scale than the one considered here to account for small-scale variation and eventual patchiness of the algal mats distribution. It should also be performed on a relatively high temporal frequency.

Further studies on the food web in Dublin Bay should also take into consideration the following points to improve the characterisation of the food sources:

One requisite to take into consideration is to better characterise the assimilated food sources by measuring isotopes more frequently in rapid-changing sources and/or by coupling isotopes with additional trophic markers.

- Carbon and nitrogen isotopic signal of microalgae (phytoplankton, and to a lesser extent microphytobenthos) did show important temporal variations due to their rapid turn-over and variable growth and depending on nutrient conditions. Patterns of variation of these microphytes are not well known. For instance, it is not clear if the isotopic variations observed during this study reflect seasonal patterns or if, very likely, they vary at much higher frequency (day scale). As a result, microphytobenthos could be sampled on a monthly basis to better characterise its pattern of variability in order to take into account robust mean and deviation signals in the isotope mixing models.
- Some sources appeared to not discriminate by their isotopic signal during this study (e.g. green macroalgae and microphytobenthos in BR), thereby limiting their use in mixing models. Lipids biomarkers such as fatty acids may be applied for future studies.
- Coupled measurements of the hydrobiological variables (Total Suspended Matter, Chlorophyll pigments concentrations, C/N ratio and POC/Chl *a* ratio) with the isotopic characterisation of the particulate organic matter (POM) proved to be insightful to assess the quality and the origin of the organic matter composing the POM and therefore made available to suspension-feeders. This double approach also gave insights on the dynamic processes occurring at the local scale in intertidal areas (e.g. microphytobenthos resuspension) and suggested a high temporal variability of the POM composition and quality in these shallow intertidal environments. It would be useful to better characterise the POM composition in order to precise the environmental and trophic conditions

suspension-feeders are exposed to. This would involve an intensification of the sampling frequency of both the POM and the potential source of organic matter composing it in the intertidal areas. Considering the time this requests to acquire and analyse the samples, a bi-monthly sampling should be appropriate.

Finally, this work calls for the use of complementary techniques (such as additional trophic markers or specific-compounds analysis) to better discriminate the potential food sources and better understand their trophic roles. Indeed, conclusions derived from stable isotope approach sometimes have interpretation limits. As observed in most of the isotopic food web studies, the origin of organic matter contained in the sediment was very difficult to characterise since the SOM is a very complex pool of fresh and detritic sources mixed with bacteria, protozoans and small meiofauna. In this perspective, fatty acids should be very useful to specifically delineate the importance of some components of the SOM pool (Meziane *et al.*, 1997). For instance, the contribution of bacteria in the diet of deposit-feeding invertebrates could be efficiently determined since bacteria have very specific odd-branched fatty acids which may be easily traced among the primary consumers. Sulfur stable isotopes are also helpful to distinguish potential food sources that occur in different redox conditions, e.g. for sources that are close to the redox transition zone of the sediment. Green macroalgae that stay buried in the sediment for a long time may thus exhibit a different sulphur signal than fresh macroalgae.

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Appendices

Appendices list

- Appendix 3.1:** Median probability contribution (%) of the sources to the diet of the primary producers from the Bull Island South Lagoon, as estimated from the SIAR mixing model using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ trophic-step fractionation of $0.4 \pm 1.3\text{‰}$ and $3.4 \pm 0.7\text{‰}$, respectively.
- Appendix 4.1:** $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the sources sampled from May 2005 to June 2006 in Sandymount
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Appendix 3.1 : Median probability contribution (%) of the sources to the diet of the primary producers from the Bull Island South Lagoon, as estimated from the SIAR mixing model using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ trophic-step fractionation of $0.4 \pm 1.3\text{‰}$ and $3.4 \pm 0.7\text{‰}$, respectively. In brackets: 5-95th percentiles of the credible intervals.

	May-June 2005	Nov-Dec 2005	March-April 2006	June-July 2006
<i>Cerastoderma edule</i>				
SeaPOM	23 (1-43)	21 (2-40)	29 (8-49)	28 (5-47)
Microphytobenthos	19 (5-33)	25 (11-39)	29 (14-45)	43 (35-51)
<i>Enteromorpha</i> spp.	29 (20-37)	36 (30-41)	16 (9-24)	12 (7-18)
Sewage POM	28 (11-45)	17 (7-27)	24 (13-36)	17 (1-35)
<i>Scrobicularia plana</i>				
SeaPOM	21 (0-40)	11 (0-26)	26 (1-46)	16 (0-33)
Microphytobenthos	29 (13-44)	30 (16-41)	35 (16-56)	54 (31-72)
<i>Enteromorpha</i> spp.	33 (24-41)	53 (48-59)	28 (17-37)	18 (2-38)
Sewage POM	17 (1-33)	5 (0-12)	10 (0-26)	11 (0-26)
<i>Macoma balthica</i>				
SeaPOM	19 (0-40)	11 (0-29)	22 (1-39)	10 (0-23)
Microphytobenthos	30 (2-51)	30 (11-44)	41 (24-58)	73 (54-87)
<i>Enteromorpha</i> spp.	36 (24-49)	52 (43-60)	30 (23-37)	8 (0-22)
Sewage POM	14 (0-32)	6 (0-16)	6 (0-17)	7 (0-18)
<i>Hydrobia ulvae</i>				
SeaPOM	18 (0-41)	17 (0-40)	20 (0-43)	18 (0-44)
Microphytobenthos	30 (1-58)	20 (0-42)	25 (1-46)	35 (8-61)
<i>Enteromorpha</i> spp.	34 (12-52)	42 (11-66)	36 (10-56)	27 (3-48)
Sewage POM	15 (0-37)	18 (0-44)	18 (0-42)	15 (0-42)
<i>Arenicola marina</i>				
SeaPOM	20 (0-40)	6 (0-21)	25 (1-47)	27 (3-46)
Microphytobenthos	26 (5-45)	2 (0-19)	25 (6-43)	43 (33-54)
<i>Enteromorpha</i> spp.	37 (26-48)	66 (49-86)	24 (12-34)	14 (6-21)
Sewage POM	15 (0-32)	17 (2-29)	26 (12-40)	16 (0-35)

Appendix 4.1: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the sources sampled from May 2005 to June 2006 at Sandymount. sd: standard deviation; n: sample size.

Sources	MAY 2005						NOVEMBER 2005						MARCH 2006						JUNE 2006					
	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd
POM site		-			-		2	-16,0	0,4	4,5	0,3	2	-21,1	0,1	7,0	0,1	2	-22,4	0,1	8,4	0,3			
SOM	3	-18,9	0,3		6,6	0,2		-		-		3	-18,5	0,1	6,9	2,0	3	-17,9	0,1	6,8	0,5			
Microphytobenthos	3	-14,2	0,2		4,0	0,2	3	-11,5	0,3	5,9	0,2	3	-15,3	0,1	7,1	0,0	3	-12,0	0,2	1,6	0,5			
Macroalgae																								
<i>Ectocarpus</i> sp. detrital		-			-		1*	-16,2		8,6			-		-			-		-				

Appendix 4.2: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the sources sampled from May 2005 to June 2006 at Blackrock. sd: standard deviation; n: sample size.

Sources	MAY 2005						NOVEMBER 2005						MARCH 2006						JUNE 2006					
	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd
POM site		-			-		2	-14,4	0,2	6,3	0,3	1	-21,3			2	-21,6	0,3	8,7			2	-21,6	0,3
SOM	3	-20,3	0,8	7,2	0,2		2	-20,7	0,1	9,8	0,2	2	-19,5	0,1		3	-19,7	0,2	6,0			3	-19,7	0,2
Microphytobenthos	3	-17,7	0,6	8,0	0,9		2	-15,5	0,0	5,9	0,1	3	-19,4	2,1		3	-17,7	0,2	6,9			3	-17,7	0,2
Chlorophyta																								
<i>Enteromorpha</i> sp. detrital	2	-17,6	0,2	8,3	0,1		-			-			-			-			-			-		
<i>Ulva</i> sp. detrital	2	-19,2	0,8	8,1	1,3		-			-			-			-			-			-		
Ochrophyta																								
<i>Ascophyllum nodosum</i> detrital		-			-		2	-17,5	1,3	5,8	0,3		-						1	-15,8				
<i>Fucus serratus</i> detrital	3	-16,3	1,1	6,4	0,5		2	-16,7	2,3	7,3	0,5		-			2	-16,8	0,1	8,3			2	-16,8	0,1
<i>Fucus spiralis</i> detrital	3	-16,8	0,2	7,6	0,2		2	-17,2	1,7	7,9	0,2		-						-					
<i>Fucus vesiculosus</i> detrital	3	-16,3	0,8	7,7	0,1					-			-						-					
Kelps detrital	3	-18,0	1,4	6,4	0,3		2	-15,4	3,5	5,8	0,1		-						-					
<i>Ectocarpus</i> sp. detrital		-			-					-			-						-					
<i>Desmarestia aculeata</i> detrital	2	-23,1	0,3	7,5	0,4					-			-						-			2	-19,0	0,1
Rhodophyta																								
<i>Rhodospira rubra</i> detrital	2	-10,0	1,0	5,1	0,1					-			-						-					

Appendix 5.1 : Hierarchical clustering (Bray Curtis similarity Index, Group Average Method) performed on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean values of primary consumers from Dublin Bay subtidal area for each of the four surveys.

