

Ecosystem services and environmental impacts associated with commercial kelp aquaculture.

A thesis submitted to the National University of Ireland, Galway
for the degree of Doctor of Philosophy

by

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July 2017



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Declaration

I, *Aimée Walls*, certify that this thesis is all my own work and that the results presented here are to the best of my knowledge correct. I have not obtained a degree in this University or elsewhere on the basis of any of this work. Contributions by other authors were made to the articles presented here, as outlined on the cover page of each chapter.

Signed:

Date:

Funding

The Dr. Tony Ryan Research Trust are gratefully acknowledged for their support and for enabling me to carry out the research for this thesis.

Funding from the Thomas Crawford Hayes Award Trust, NUI Galway, Ryan Institute Travel Support Scheme, Marine Institute Networking and Travel Grant Scheme, The British Phycological Society, and The Marine Biological Association are also greatly acknowledged.

Thesis Abstract

Wild kelp forests represent one of the most productive habitats on Earth, supplying ecosystem services, including, but not limited to, biodiversity, habitat provision, food web subsidy, nutrient cycling, carbon sequestration and coastal defence. Ecosystem services associated with wild kelps have been examined by researchers for more than six decades. Currently, with the development and growth of seaweed aquaculture the ecosystem services and potential impacts associated with cultivated kelps need to be assessed.

World aquaculture production continues to grow year on year with approximately 131.4 million tonnes of fish, aquatic animals and aquatic plants produced in 2014. The cultivation of aquatic plants, predominantly marine species of seaweeds, accounts for over 20 % of this total production (by weight) which too has seen rapid growth of almost 8 % per year over the past decade. Seaweed cultivation is traditionally dominated by Asian countries, however, over the last 15 years the industry has also expanded in Europe. This interest has been supported by feasibility studies and pilot-scale farms being set up to begin to develop the industry and advance cultivation techniques of kelps native to European waters. Observations of seaweed farms suggest that both the growing kelp and its associated infrastructure could provide ecosystem services, in particular, habitat for associated species altering the ecosystem structure and functioning of the surrounding area. Additionally, carbon from the highly productive kelps released as detritus could be assimilated by other species and incorporated into the food web. This primary productivity may also negatively impact the benthic environment beneath the farm causing increased organic enrichment of the seabed.

Little research has been conducted into the communities associated with cultivated kelps. The habitat created by farmed kelps may act as a novel habitat and not simply an expansion of the existing habitat created by wild kelps, due to differences in kelp morphology, age and position within the water column. A comparative study between wild (benthic) and cultivated (suspended) holdfasts communities of *Laminaria digitata* at an experimental research site on the west coast of Ireland, demonstrated that cultivated kelps provide a distinct habitat for species compared to their wild counterparts. Subsequently, an extensive investigation of the communities associated with cultivated kelp (*Alaria esculenta*) was conducted at a commercial-scale seaweed farm in Ventry Harbour, Co. Kerry

(southwest coast of Ireland). These studies assessed the properties of the whole farms and the individual parts (frond and holdfast) of the kelp sporophyte as a habitat for communities. Kelps are a major source of primary productivity and using stable isotope analysis it was found that cultivated kelps (*A. esculenta* and *Saccharina latissima*) are incorporated into the food web via the suspension-feeding mollusc *Mytilus edulis*. Additionally, a multivariate analysis of the Ecological Status of benthic communities suggested no negative impacts from the deposition of kelp detritus on the seabed. The commercial kelp farm in Ventry Harbour was located above a *Zostera marina* bed which is a key habitat under the EU Habitats Directive and OSPAR Commission, yet, this macrophyte community was not adversely impacted by the presence of the farm.

Results from this study show that kelp aquaculture sites provide additional ecosystem services beyond the supply of biomass. These services increase the ecological importance of farms, with multifunctional uses of farms and their infrastructure suggested. Negative impacts caused by detrital deposition on the benthos were not evident at the study site which is provisionally a very positive result for the sector as future expansion and development of the industry may not be limited by detrimental effects on the surrounding environment. However, this is one of the first studies of its kind to identify and assess the ecosystem services and impacts of commercial seaweed (kelp) farms in Europe. Many more factors such as farm scale, seaweed species, site location and hydrodynamics need to be researched in order to fully understand the ecological interactions and impacts (positive or negative) of commercial seaweed cultivation in the future.

Chapter 1

Introduction

1.1 General introduction

World aquaculture production

World aquaculture production continues to grow year on year with approximately 131.4 million tonnes of fish, aquatic animals and aquatic plants produced in 2014 (FAO 2016). The cultivation of aquatic plants, predominantly marine species of seaweeds, accounts for over 20 % of this total production which too has seen rapid growth of almost 8 % per year over the past decade (FAO 2016). Over 33 % of the 27.3 million tonnes of annual global aquatic plants produced in 2014 was from the two kelp species *Laminaria japonica* and *Undaria pinnatifida* (FAO 2016). ‘Kelp’ is a generic term that refers to large brown seaweeds including those of the order Laminariales, which dominate rocky reefs throughout the world’s temperate seas (Mann 2000, Steneck et al. 2002). Asian countries are consistently the leading producers of macroalgae in the world (FAO 2014, 2016) and kelp farming in particular is currently dominated by China and Korea (FAO 2014, 2016). However, over the last 15 to 20 years, an interest in kelp cultivation has developed in European countries such as, Ireland, Spain, Scotland, Norway and Denmark, (Kraan et al. 2000, Kerrison et al. 2015, Peteiro et al. 2016) to support the already well-established industry associated with wild kelp harvesting. This interest has been supported by desk-based feasibility studies (Bruton et al. 2009) and pilot-scale farms (Edwards & Watson 2011, Sanderson et al. 2012, Marinho et al. 2015) being set up to advance the cultivation techniques of kelps native to European waters to begin to develop the seaweed aquaculture industry. Scientific interest in cultivating kelp has been expanding over recent years; over 70 % of all studies found using an International Scientific Index (ISI) search of ‘Laminaria and Aquaculture’ were published in 2007-2016 with 65 studies compared to just 27 studies published over the previous 6 decades from 1945-2006.

Kelp biomass (cultivated and wild harvested) is utilised for a variety of traditional low-tech uses, such as, the provision of food for human and animal consumption as either unprocessed biomass, e.g. ‘sea vegetables’ or as dietary supplements (Jaspars & Folmer 2013). Fresh biomass has traditionally been collected and used on the land as a fertilizer for nutrient poor soils or is added as a concentrated liquid supplement (Guiry & Blunden 1991). Alginates and other products are extracted from kelp and used as food additives, and in the textile and pharmaceutical industry for bulking, gelling and stabilizing processes (Guiry & Blunden 1991, Smit 2004, Bixler & Porse 2011). In addition, kelp biomass has

been targeted as the development of high-tech uses expands, such as, bioactive compounds (Holdt & Kraan 2011), biopolymers (Bella et al. 2015) and biofuel production (Wargacki et al. 2012, Fasahati et al. 2015).

Macroalgal cultivation

In Europe, kelp aquaculture farms are generally situated in nearshore coastal environments with average water depths ranging from 6–20 m (Edwards & Watson 2011, Kerrison et al. 2015, Marinho et al. 2015, Peteiro et al. 2016). Semi-exposed sites with good current flow yet sheltered from the open ocean are ideal to provide the nutrients required for biomass growth without damage of the crop and infrastructure during storms (Edwards & Watson 2011). A typical farm set-up (Fig. 1.1 and Fig. 2.3 Chapter 2) consists of a header rope suspended approximately 1 m below the surface by buoys and kept in position by anchor ropes, chain and anchors (variable, but commonly 1-1.5 tonne concrete blocks). Most often the header rope is seeded with juvenile kelp along its length. However, seeding can occur on vertical ropes called dropper ropes (approximately 3 m in length) which are occasionally suspended from the header rope to increase the surface area of the farm (Edwards & Watson 2011, Peteiro et al. 2016, Walls et al. 2016, 2017). Cultivated kelps differ from their wild counterparts as they are suspended from ropes in the water column whereas, wild kelps are attached to the seafloor (Walls et al. 2016).

The reproductive cycle, or life history of kelp alters between a large and structurally complex macroscopic sporophyte phase ($2n$) and a microscopic filamentous gametophyte phase (n) (Kain 1979). The cycle starts when the unilocular sporangia containing zoospores mature in dark raised patches called sori. The sori develop on the mid to distal central portions of blades in *Laminaria* species and in specialised structures called sporophylls that develop on the stipes of the *Alaria esculenta*. This occurs between April and November on the west coast of Ireland depending on environmental conditions and kelp species. For cultivation, these blades containing sori and sporophylls are collected in the field and are induced to release their spores by leaving them in cool dark conditions for 18-24 hrs (Edwards & Watson 2011). Then by immersing the sori in cool water the spores are released. Spores develop in mass cultures of male and female gametophytes and gametophyte culture biomass is increased slowly in the laboratory under a controlled light and temperature regime for approximately 3-5

months (Edwards & Watson 2011). In preparation for deployment, a further change in laboratory conditions induce gametophyte cultures to develop juvenile sporophytes of up to 5 mm in length. The sporophytes are then sprayed onto culture string or directly onto polypropylene dropper ropes and left to attach to the substrate for 5-8 weeks in laboratory tanks. Once the sporophytes have reached sufficient size (approximately 10 mm) and weather conditions at sea are suitable they can be deployed on the longline structure. Deployment usually occurs between winter months of October and February. Cultured string is wound around the heavier polypropylene header rope and if dropper ropes are being used they are simply suspended vertically from horizontal header rope at 1.5 m intervals. The sporophytes are left to grow with a maximum yield of 6-8 wet kg m⁻¹ usually achieved after 6-7 months (Irish statistics) with harvest usually occurring in late spring early summer (Edwards & Watson 2011).

1.2 Aquaculture ecosystem interactions

It has long been established that aquaculture practices can impact on the local ecosystem and in particular the benthic environment; these impacts include organic loading of the sediments and associated biogeochemical changes caused by the bio-deposition of faeces and pseudofaeces at culture sites (Crawford et al. 2003, Kalantzi & Karakassis 2006, Forde et al. 2015, O'Carroll et al. 2016). However, many of these studies have focused on finfish (Silvert & Sowles 1996, Kalantzi & Karakassis 2006) and shellfish (Stenton-Dozey et al. 1999, Crawford et al. 2003, Dubois et al. 2007, O'Carroll et al. 2016) aquaculture. Seaweed cultivation is an extractive cultivation method meaning it assimilates macronutrients (phosphorous and nitrogen which is often in the form of ammonium) required for growth from the environment with no need for the addition of supplementary feed or nutrients (Chopin & Sawhney 2009). As a consequence, seaweed farms are assumed to have a more benign impact on the benthos when compared to finfish or shellfish aquaculture (Soto 2009, Roberts & Upham 2012).

Despite the widely-held view that kelp cultivation interactions with the local ecosystem are mild, some such interactions have the potential to create negative impacts on the environment. Species of kelp are among the largest sources of primary productivity in marine habitats (Mann 1973, Reed et al. 2008) and this primary productivity enters the food web through two routes; direct grazing on kelp tissue or detrital pathways. Much of the standing stock in temperate kelp beds is

released either as particulate organic matter (POM) also called detritus or as dissolved organic matter (DOM) (Krumhansl & Scheibling 2012). Krumhansl and Scheibling (2012) estimate that > 80 % of kelp production enters the carbon cycle as POM or DOM. Kelp detritus can range in size from small particles to whole thalli depending on how the biomass is removed. There are three main ways tissue can be lost; 1) Whole thalli are removed from breakage at the stipe or when the holdfast becomes detached from its substratum, either rocks or boulders in wild kelp forests or suspended rope substratum at cultivated sites. 2) Parts of the frond can break off removing large pieces from the frond. 3) Erosion of the distal ends of fronds can occur as tissue is continually lost through decay and natural senescence (Zhang et al. 2012, Krumhansl & Scheibling 2012).

The impacts of detrital deposition from macroalgal cultivation on the benthos could be analogous to the impacts caused by the bio-deposition of faeces and pseudofaeces from finfish and shellfish aquaculture on benthic communities. That is, smothering of the benthos can occur from organic enrichment of the sediment causing an anoxic environment to develop. The response of benthic communities to this type of organic enrichment generally follows the succession model outlined by Pearson and Rosenberg (1978). This model outlines how benthic infaunal community structure changes along a gradient of increasing organic enrichment to oxygen depletion. According to the Pearson and Rosenberg (1978) model, with increasing proximity to an enriched or disturbed site a reduction in biodiversity, abundance and biomass of macrofauna occurs. In addition to potential organic enrichment, benthic communities can also be affected by shading of the seabed from the kelp biomass and farm infrastructure sited above. Eklöf et al. (2006) suggested that negative effects on seagrass could be due to shading by farmed algae. The degree of shading probably increases gradually over the culture period (October to June) as the kelp biomass grows thus the impact would be at its strongest towards the end of the cultivation period (Eklöf et al. 2006). Impacts from kelp farms may not be isolated to the footprint of the farm; there could be wider reaching implications for the area where they are situated. As seaweeds take up nutrients from the environment to grow, depending on the scale of the farm, competition for nutrients may occur with other photosynthetic organisms in the area (Aldridge et al. 2012). Local patterns of water movement are also suggested to be altered by artificial structures associated with the farm (Firth et al. 2016, Smale et al. 2013). There is also increasing concern that artificial structures (e.g., energy infrastructure, aquaculture and coastal defences) are acting as ‘stepping stones’

between regions that facilitate the spread of invasive species (Airoldi et al. 2015, Firth et al. 2016). The mechanisms behind artificial structures and invasive species are poorly understood within the field of coastal ecology (Firth et al. 2016). Additionally, seaweed farmers have a responsibility to cultivate kelp species which are native to the region and from the same genetic pool as the wild populations growing within the area of the farm. This will help prevent the spread of non-native species and dilution of genetic diversity if farmed individuals settle and reproduce with wild populations (Cottier-Cook et al. 2016). However, this is not a legal obligation and is not always undertaken, for example the cultivation of the non-native kelp *Undaria pinatifida* in France and Spain.

Ecosystem services provided by cultivated kelp

Supplementary to the algal biomass produced by seaweed farms, aquaculture practices may benefit the areas where these farms are situated through the provision of additional ecosystem services. Ecosystem services are defined as the goods and services provided by an ecosystem that benefit humans (Millennium Ecosystem Assessment 2005). Ecosystem services associated with kelp farms have yet to be fully quantified however, they are expected to be similar to those provided by wild kelp. As habitat forming species, kelps are hugely important primary producers which play important roles in food web subsidy, carbon sequestration, uptake of excess nutrients, coastal defence and environmental restoration and provide nursery grounds and protection from predators for juvenile invertebrates and fish (Smale et al. 2013). Further, the infrastructure (ropes and buoys) of the farm itself can boost the services provided by the cultivated biomass such as habitat provision and coastal defence. See Fig. 1.1 for some of the potential ecosystem services and impacts caused by kelp cultivation.

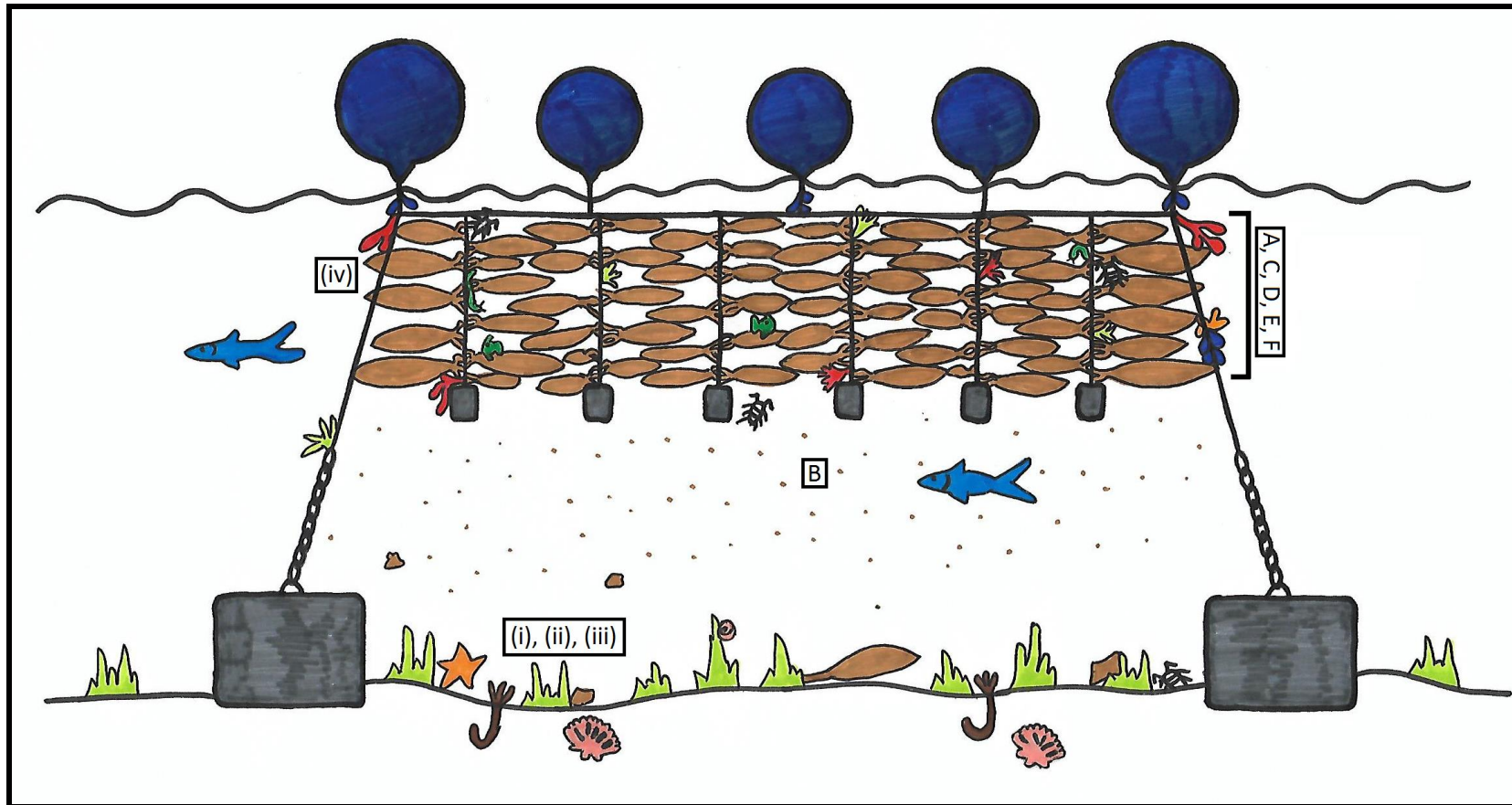


Fig. 1.1. Potential ecosystem services provided by the infrastructure and biomass of a commercial kelp farm: A) Habitat provision (kelp biomass and farm infrastructure); B) Food web subsidy; C) Coastal defence D) Carbon sequestration; E) Mitigation of eutrophication and F) Environmental restoration. The ecosystem services of kelp cultivation are accompanied by potential impacts: i) Organic enrichment of sediments; ii) Shandling of the benthos; iii) Competition for nutrients and iv) facilitation of nuisance or invasive species.

Habitat provision

Kelps are habitat forming species or ecosystem engineers (Jones et al. 1994), exerting control over entire communities by modifying the environment and resources available to other organisms (Bertness & Callaway 1994, Jones et al. 1997). Extensive research has been conducted over the last 60 plus years on kelp-associated faunal assemblages in the northeast Atlantic and has shown that kelp support significant biodiversity (Ebling et al. 1948, Sloane et al. 1957, Jones 1971, Moore 1971, 1973, Norton et al. 1977, Norderhaug et al. 2002, Christie et al. 2003, Blight & Thompson 2008, Walls et al. 2016, 2017, Teagle et al. 2017). For example, Christie et al. (2003) found on average approximately 130 species and 8,000 individual species on single *Laminaria hyperborea* sporophyte in Norway.

As a habitat former, a fully developed kelp sporophyte directly provides three distinct primary habitats; the holdfast, the stipe, and the frond, while established epiphytes (often on the stipe) provide a secondary habitat for colonisation (Teagle et al. 2017). These biogenic habitats differ distinctly in structure and as a result community diversity and composition of organisms utilising the habitats also differ (Teagle et al. 2017). The holdfast is generally found to host more species than other parts of the kelp (Jones 1972, Moore 1972, Thiel & Vásquez 2000, Teagle et al. 2017) with species richness per holdfast typically in the region of 30-70 macrofaunal species (Christie et al. 2003, Blight & Thompson 2008). The relatively high biodiversity of the holdfast is thought to reflect the complex physical structure provided by the holdfast. The intertwining layers of branched haptera of the holdfast provides a number of holes and crevices (Christie et al. 2003). These interstitial spaces may represent favourable habitat for colonising fauna, potentially providing protection from predators and during periods of adverse environmental conditions (Norderhaug et al. 2002). The holdfast functions as a sediment trap accumulating detritus which acts as a food source for many of the organisms inhabiting the structure (Moore 1972). The holdfast also provides a stable environment which is persistent over seasons and years (Schaal et al. 2009); with the lifespan of the holdfast being the same as that of the kelp individual (Christie et al. 2003). The age of kelp individuals varies hugely between species and populations, with *Laminaria hyperborea* living for an average of 10 years (5-15 years Kain 1963, Christie et al. 2003). Species composition and richness of associated fauna are strongly influenced by volume and complexity of the holdfast habitat (Jones 1971, Norderhaug et al. 2007, Blight & Thompson 2008,

Tuya et al. 2011). This stability contrasts with the seasonally fluctuating habitat experienced by stipe-associated epiphytes (Norderhaug 2004) and to the annually renewing frond habitat (Christie et al. 2003, 2007).

The frond and uncolonized stipes of kelps provide little protection to organisms from predators and grazers as they are two-dimensional, and thus have lower faunal densities than the holdfast (Christie et al. 2003). In addition, fronds and stipes are exposed to faster water currents and greater effects of wave action. Kelp stipes are often colonised by a highly abundant and diverse flora and fauna, which varies considerably in space and time (Christie et al. 2003). The rough stipes of *L. hyperborea* in particular are frequently colonised by many epiphytic algal species especially foliose red algae (Christie et al. 1998, Norderhaug 2004). The kelp frond is continually worn away, abraded and replaced by new tissues which keep it relatively clear of epibionts. However, during the summer months when kelp growth slows to a minimum and water temperatures and irradiance increase, opportunistic hydroids and bryozoans settle on the fronds (Edwards & Watson 2011, Peteiro & Freire 2013, Førde et al. 2016, Walls et al. 2017) and can become abundant.

While numerous studies conducted over the last 6 decades have assessed the role of kelp as biogenic habitat formers with an extensive review by Teagle et al. (2017), the role of cultivated kelp has been virtually neglected in the literature up until approximately 5 years ago. Observations of the fauna growing on existing seaweed farms suggest that both the longline infrastructure and the growing kelp biomass may function as a habitat or refuge for a number of species (M. D. Edwards pers. obs.). There are, however, at least three reasons why the cultivated habitat may not act simply as an extension of existing kelp habitats for the associated species. (1) The holdfast morphologies of wild and cultivated kelps differ. Wild kelps tend to grow a characteristic flattened or slightly conical holdfast when attached to rock (Teagle et al. 2017). While, cultivated kelps are seeded onto ropes for growth, resulting in a different morphology, formed by intertwined haptera (Fig. 2.1b Chapter 2; Fig. 1b Walls et al. (2016)). (2) Depending on culture practices, kelp may be seeded fresh to rope for each growing season, so cultivated kelps are younger, on average, than those from wild stands. (3) Finally, cultivated kelp are suspended from ropes in the water column; this will alter both the hydrodynamic environment (discussed as an influence on epifauna by Moore 1972) and the accumulation of sediments thought to provide the bulk of carbon supply to

the associated species (Schaal et al. 2012). Changing from a benthic to a suspended growth form may also alter the environmental conditions experienced by kelp epifauna: changes in depth (Coleman et al. 2007), salinity (Jones 1973), oxygen availability (Scarratt 1961), and temperature (Scarratt 1961).

Food web subsidy

Wild kelp forests represent some of the most productive habitats on Earth (Mann 1973, 2000, Reed et al. 2008) and are a major source of primary productivity in temperate zones (Steneck et al. 2002). In the Atlantic, kelp primary production can be in excess of $1,000 \text{ g C m}^{-2} \text{ year}^{-1}$ (Mann 1973, Smale et al. 2013), while primary production from phytoplankton in coastal temperate regions is typically between 100 and $300 \text{ g C m}^{-2} \text{ year}^{-1}$ (Mann 2000). Kelp primary productivity enters the food web through two routes; direct grazing on kelp tissue or via detrital pathways. Only a few species are known to directly graze on fresh kelp tissue including the gastropods *Patella pellucida* and *Lacuna vincta* and the sea urchins *Strongylocentrotus droebachiensis* and *Paracentrotus lividus* (Steneck et al. 2002, Molis et al. 2010, Leclerc et al. 2013a). Due to the high C:N ratios of fresh kelp tissue, degradation by bacteria needs to occur before the primary production is available to other consumers (Duggins et al. 1989, Norderhaug et al. 2003). Much of the standing stock in temperate kelp beds is released as particulate organic matter (POM) (detritus) and dissolved organic matter (DOM) (Krumhansl & Scheibling 2012).

While the importance of wild kelp detritus to the diet of some organisms has been identified (Duggins et al. 1990, Fredriksen 2003, Norderhaug et al. 2003, Schaal et al. 2012, Leclerc et al. 2013b), there is still much that is to be learnt about trophic ecology even in some of the most well-studied organisms (see Notman et al. 2016, who allude to the fact that the common limpet *Patella vulgata* feeds on drift kelp in the northeast Atlantic). Furthermore, the significance of cultivated kelp detritus to these organisms and the incorporation of this primary productivity source into the food web remains unknown. In contrast to wild kelp, the amount of production from cultivated kelp is likely to be lower as harvest of cultivated kelp biomass usually occurs in early summer and hence there is no further production of kelp occurring once it has been harvested (Yoshikawa et al. 2001).

Coastal defence

Kelp forests prevent and alleviate damage caused by flooding and storm events (Smale et al. 2013). They provide a buffer against storms by wave dampening and attenuation reducing the velocity of breaking waves and altering water motion (Lovas & Torum 2001). This causes a reduction in coastal erosion and movement of sand and pebbles from adjacent beaches (Mork 1996, Lovas & Torum 2001). Very little information is available on the degree of storm protection provided by kelp forests compared to other coastal habitat formers such as mangroves (Smale et al. 2013, Firth et al. 2016). The different morphologies of species found in different biogeographic regions will strongly influence the magnitude of wave dampening (Smale et al. 2013). It is hypothesised that seaweed farms could play a role in coastal protection from severe storm activity in a similar fashion to natural kelp beds. Seaweed farms are located in inshore waters close to land and could be employed to help dissipate the intensity of wave action.

Carbon sequestration

Seaweeds play an important role in carbon sequestration and kelps have been identified to function in the mitigation of climate change impacts (Duarte et al. 2017). Kelps extract nutrients from the surrounding waters and carbon dioxide (CO₂) from the atmosphere and through photosynthesis convert these to carbon, in the form of kelp biomass. This primary productivity can be released through loss from sporophytes (fall-off and break-off) and erosion of detritus from the distal ends of the fronds (Zhang et al. 2012, Krumhansl & Scheibling 2012). This carbon can be buried in sediments (Zhang et al. 2012), sequestered in continental shelf sediments or in the deep sea (Krause-Jensen & Duarte 2016), thus acting as a CO₂ sink. However, cultivated seaweed biomass is removed at harvest in early summer, thus the net primary production available for sequestration is likely to be lower than that of wild kelps (Duarte et al. 2017). Yet, this biomass can be used as biofuel directly replacing fossil fuels (Kraan 2013, Chen et al. 2015). This process may mitigate the impacts of climate change by reducing CO₂ emissions derived from fossil fuel use. However, the specific role of kelps in carbon sequestration is currently unknown, and could be negligible if primary productivity is remineralised quickly and not sequestered to storage habitats.

Mitigation of eutrophication

Finfish and to a lesser extent shellfish aquaculture can cause nutrient enrichment of the surrounding waters through the bio-deposition of faeces and pseudofaeces and the release of excess feed at the culture site (Crawford et al. 2003, Kalantzi & Karakassis 2006, Forde et al. 2015). These excess nutrients can cause eutrophication of the waters which impacts water quality and both benthic and pelagic faunal communities (Chopin et al. 2008). The cultivation of seaweeds in the proximity of finfish aquaculture in Integrated Multi-Trophic Aquaculture (IMTA) is seen as a step forward in the reduction of impacts from fish farming through bioremediation (Neori et al. 2004, Troell et al. 2009). This practice uses seaweeds as biofilters to remove nutrients from the water, thus lessening the environmental impact of finfish aquaculture. In contrast to other potential ecosystem services and impacts of kelp farms their role in nutrient uptake has been well researched due to the commercial interest in IMTA (Troell et al. 2003, 2009, Neori et al. 2004, Chopin 2006, 2013, Neori 2007, Barrington et al. 2009). However, the contribution of organisms, e.g., suspension feeders, attached to seaweed farms to the overall biofiltration capacity of the farm has been overlooked. Attached organisms such as bivalves could have a significant role filtering particulate organic matter in addition to kelp assimilating dissolved organic matter.

Environmental restoration

Environmental restoration is defined as the process of ‘re-establishing, following degradation by human activities, a sustainable habitat or ecosystem with a natural structure and functioning’ (Elliott et al. 2007 and references therein). The restoration of kelp forests (Carney et al. 2005, Yu et al. 2012, Campbell et al. 2014) and the transplantation of habitat forming species (Perkol-Finkel et al. 2012, Ferrario et al. 2016) onto artificial structures has gained increased interest recently in attempts to mitigate the potential negative anthropogenic impacts of ocean sprawl (Airoldi & Beck 2007, Firth et al. 2016). Deployment of seaweed lines could assist in habitat restoration by supplying spores and gametophytes to wild kelp beds that have been damaged by anthropogenic impacts. Colonisation of the re-established wild beds could occur from settlement of larvae or migration of mobile fauna from the developed community on the cultivated kelp. The extent of such ‘seeding’ influences are likely to increase with the time that longlines are kept in the water.

1.3 Themes of thesis

Suitability of Ventry Harbour site

With an increase in demand for biomass to supply the established and new uses of kelp, the European seaweed industry is beginning to diversify into aquaculture, complimenting the more traditional approach of wild harvesting of kelp and other species. One of Europe's largest commercial seaweed farms was established on the southwest coast of Ireland in Ventry Harbour, County Kerry by Dingle Bay Seaweeds in 2009. Development of the seaweed cultivation industry in Ireland has steadily advanced over the last 20 years, mainly through national and international research projects and industry support in particular from Bord Iascaigh Mhara (Edwards and Watson, 2011). A number of small licensed sites (fewer than 10 sites at less than 5 ha in size) were often used, but at 18 hectares, Dingle Bay Seaweeds site at Ventry Harbour, Co. Kerry is Ireland's largest commercial operating seaweed farm (M.D. Edwards pers. comm.).

Ventry Harbour is a moderately sheltered and shallow embayment orientated towards the southeast, approximately 2.5×1.5 km (3.75 km²) with a wide mouth opening into Dingle Bay. *Zostera marina* (seagrass) is extensively distributed throughout the sandy seabed, leading to a rocky boulder reef towards the mouth of the bay. The licensed seaweed farm is orientated northwest to southeast, and located to the westerly side of Ventry Harbour (Fig. 3.1 Chapter 3; Fig. 1 Walls et al. (2017)). The depth underneath the farm is approximately 6 m at the northwestern end before gently sloping to 20 m at the eastern edge of the farm at mean low water spring tide (MLWS). The tidal range in Ventry Harbour is between 0.6 and 4.0 m. The longline structures are similar to the set-up in Chapter 2 (Fig. 2.3 Chapter 2; Fig. 3 Walls et al (2016)), however the farm in Ventry consists of 3 parallel units of 280 m linear longlines suspended approximately 1.5 m below the sea surface (also see Taelman et al. (2015) for schematic of cultivation equipment). For commercial operations seaweed is cultivated directly on the header rope, however for experimental work for this thesis at the Ventry site 1 m droppers were used. The longlines were kept in position by buoys attached to the header rope and by 1500 kg anchor blocks at either end of the lines. The farm cultivates the kelps *Alaria esculenta* and *Saccharina latissima* for human consumption, animal feed and use in cosmetic products.

The availability, location and scale of this commercial kelp farm has lent itself to the investigation of certain ecosystem services and impacts and not others. For example, to examine the role of kelp farms in coastal defence the use of an experimental site is not necessarily a requirement. Knowledge on the hydrodynamic environment around such structures could be gained through computer modelling and theory, thus analysis of this ecosystem service was not a priority for this thesis. Also, as mentioned above numerous studies have already been conducted on the role of kelp in bioremediation. An established IMTA site with cultivation of other trophic groups such as fish and bivalves would be required to develop this research area further from what is currently known in the literature.

While the site in Ventry is not ideally suited to a thesis on hydrodynamics or IMTA, it is ideal for a very practical experimental approach to investigate the ecological impacts and ecosystem services associated with kelp farming. The role of wild kelp in habitat provision is very important as demonstrated by the vast amount of studies conducted on the subject. The role of cultivated kelp in habitat provision requires research and Ventry Harbour provides a model system for this work. However, to firstly understand if there are differences in habitat between wild and cultivated kelp a preliminary study was conducted at an experimental research site in Ard Bay near Carna Research Station, Co. Galway (Chapter 2; Walls et al. (2016)). Here, wild and cultivated individuals of the same kelp species (*Laminaria digitata*) were growing nearby to each other and thus the site provided a suitable location for comparison of their holdfast structures. Holdfasts were believed to be the most appropriate part of the kelp to begin with a primary investigation as they support the highest species richness and diversity of the kelp individual (Jones 1972, Moore 1972, Thiel & Vásquez 2000, Christie et al. 2003, Teagle et al. 2017). Also, the holdfast is subject to a high degree of variation with the change from benthic (wild) to suspended (cultivated) habitats. Faunal communities inhabiting these holdfasts were assumed to have differing compositions for several reasons. (1) The most obvious difference between wild and cultivated kelp is the alteration in habitat from benthic to suspended growth substrates which may affect the composition of associated kelp fauna. In addition to alterations in abiotic conditions such as hydrodynamic environment (Moore 1972), sedimentation rates (Schaal et al. 2012) and depth (Coleman et al. 2007) etc, differences in ecological processes could also occur. (2) Dispersal abilities of fauna inhabiting benthic kelps could inhibit their ability to settle on cultivated kelps which are suspended over the seabed. (3) Morphologies of cultivated kelp holdfasts

differ to those of their wild counterparts: morphology has been found to be the best physical predictor to explain variation in epifaunal assemblage structure between holdfasts of differing morphologies (Tuya et al. 2011). In addition to assessing faunal community differences between holdfasts, some ambiguity in the measurement of the habitat provided by holdfasts was discovered in the literature. We attempt to highlight this issue and present the best method to conduct these measurements, in the hope that future studies use the corrected method which will allow comparisons between studies using the same method more straightforward.

Once an assessment of the variations between wild and cultivated holdfasts were established, a full-scale study could be designed and conducted at the Ventry Harbour site. Figure 1.2 shows the distinct parts of the cultivated kelp *Alaria esculenta* (frond with midrib, stipe, sporophylls and holdfast) and their potential role in habitat provision. We began with an assessment of the holdfast habitat provided by the kelp *Alaria esculenta* which was our model species for all our work carried out at Ventry Harbour. There is a paucity of literature on the ecology of fauna associated with wild *A. esculenta*, possibly due to difficulties in sampling extremely wave-exposed rocky shores where this species is typically found (Kraan et al. 2000). Secondly, the other parts of the kelp individual were assessed; the stipe of *A. esculenta* is very short and sporophylls were small at time of sampling so our focus was on the long narrow frond. Wild kelp fronds have been shown to support the lowest diversity and richness of epifaunal species (reviewed in Teagle et al. 2017) of all kelp habitats, however they do provide key habitats for sessile species e.g. the bryozoan *Membranipora membranacea* in particular. From a commercial point of view the frond is the most important part of the kelp as it provides the bulk of the biomass for which kelps are cultivated. A major challenge to the industry is to minimise the undesirable attachment of these fouling organisms as their presence decreases the productivity of farms (Fletcher 1995, Peteiro & Freire 2013) and impacts on the commercial value of the crop (Park & Hwang 2012). The successional development of these communities on wild or cultivated kelp fronds is not well understood and this knowledge would not only allow quantification of the habitat provided by cultivated kelp fronds but also inform farmers when the best time to harvest their crop is to achieve a harvest of optimal quality and yield. Observations of seaweed farms (infrastructure and algal biomass) suggest that the whole farm functions as a unit, providing habitat for associated mobile species such as amphipods, molluscs and fish, arriving as larvae or colonizing as adults. An assessment of these mobile assemblages enables the

quantification of ecosystem services provided by the farm as a whole (not just the growing kelp biomass), and whether or not the fauna are attracted to the presence of the biomass or the structure of the farm. Understanding the role of the farm infrastructure in habitat provision could have implications for cultivation practices. For example, harvesting practices could be altered to leave holdfasts, stipes and small fronds and associated communities in place for longer duration while harvesting the larger fronds which provide the bulk of usable biomass.

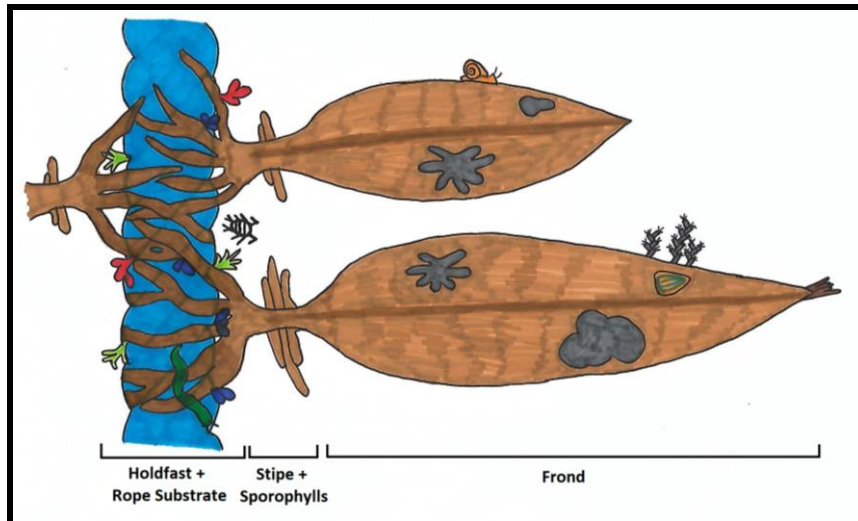


Fig. 1.2. Habitat provided by an individual cultivated *Alaria esculenta* sporophyte

The scale of production (approximately 30-50 tonnes fresh weight per year, M. Murphy, pers. comm.) at the farm site in Ventry Harbour also allows the evaluation of the fate of the primary production which is lost as detritus from the farm. This detritus provided by kelp farms could provide both ecosystem services through its incorporation into the food web and negative impacts on the benthic environment below the farm. The location of the farm aids in both these studies. It is assumed that cultivated kelp detritus can be consumed by suspension feeders and detritivores much the same way as wild kelp detritus has been shown to be incorporated. The diet of suspension-feeding organisms attached to the farm structures can be compared with nearby organisms along the adjacent shoreline to understand if detrital cultivated kelp is consumed by organisms. This comparison can be made using stable isotope analysis to compare the isotopic signatures of suspension-feeder's diets. If the quantity of detritus produced is great and settlement to the seafloor occurs, impacts on the benthic communities may follow. Comparisons of the communities beneath the farm can be made to communities in similar habitats away from the direct impact of the farm. Ventry Harbour is located above a *Zostera marina* seagrass bed, which is recognised as an important habitat

under the EU habitats Directive and as a threatened or declining habitat under OSPAR (OSPAR Commission 2008). This habitat is not uncommon in bays suitable for seaweed cultivation and future location of farms, thus there is a need to assess the impact of the farm on such an important habitat.

1.4 Aims of the thesis

The commercial kelp farm at the Ventry Harbour site in Co. Kerry provides an excellent opportunity to detail the ecological interactions between a commercial-scale kelp aquaculture farm and the surrounding ecosystem. This thesis investigates the role of kelp farms in habitat provision and food web dynamics in addition to examining the potential negative impacts caused by loss of primary productivity from the farm. These topics are addressed in the following aims of the thesis.

- To compare the habitat provided by wild and cultivated *Laminaria digitata* at the aquaculture research site in Ard Bay, Co Galway.

The hypothesis tested in this study was that cultivated (suspended) kelp and wild (benthic) kelp holdfast faunal communities were different due to variations in habitat and growth forms between kelps. We included habitat volume as a covariate in statistical analysis as volume of space within the holdfasts is emphasised in the literature as being a key factor in species richness and abundance of associated fauna. [Chapter 2]

- To assess the role of the distinct parts of the cultivated kelp *Alaria esculenta* and the whole seaweed farm unit at Ventry Harbour, Co. Kerry in habitat provision for associated fauna and flora.

Cultivated *A. esculenta* holdfasts were used to test the hypothesis that seeded ropes are different to ropes otherwise deployed at sea (an ‘ecological priming’ effect) and the development of faunal assemblages is different across primed and unprimed treatments. Seaweed lines are ‘primed’ with juvenile *A. esculenta* sporophytes and were compared with ‘unprimed’ clean rope to test this hypothesis. [Chapter 3]

The development of fouling communities on the fronds of cultivated *A. esculenta* over a typical growing season was assessed. Although this is descriptive work and does not formally test a hypothesis of ecosystem service, an important facet of habitat provision and aquaculture was evaluated: the predictability of communities from one growing season to the next. Also, artificial kelp mimics were used to test whether local frond density could affect fouling communities. [Chapter 4]

The habitat specificities of mobile assemblages associated with different parts of the farm (biomass and infrastructure) were tested by comparing mobile assemblages sampled with *A. esculenta* droppers to mobile assemblages sampled with clean polypropylene droppers, which represented the farm infrastructure. The communities were sampled at various times during the cultivation season. [Chapter 5]

- To determine the potential ecosystem services and negative impacts created by loss of primary productivity (detritus) from cultivated kelp.

It is expected that cultivated kelp farms supply detrital material to the surrounding waters as wild kelps have been shown to do. Using stable isotope analysis, we tested the hypothesis that cultivated kelp is incorporated into the food web via suspension-feeding organisms. We compared the stable isotopic ratios of the suspension-feeding mollusc *Mytilus edulis* and its putative food sources at a control and a kelp aquaculture site. [Chapter 6]

To assess the impact of kelp detritus on the benthic environment we tested the hypothesis that benthic communities are different when comparing beneath the farm to control sites. Additionally, changes in particle grain size, total organic matter and *Zostera marina* biomass were studied over a two-year period. The benthos beneath the farm and three representative control sites near the farm were sampled to assess the impacts in an asymmetrical BACI design. [Chapter 7]

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Chapter 2

Potential novel habitat created by holdfasts from cultivated *Laminaria digitata*: Assessing the macroinvertebrate assemblages

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Published: Aquaculture Environment Interactions 8 (2016) 157-169

Role: Lead author, responsible for overall sampling (preparation and collection), data processing, data analysis and writing of manuscript. Maeve Edwards assisted with the conception of the paper, sampling design and sampling. Robert Kennedy and Richard Fitzgerald assisted with analysis and interpretation of the data for earlier drafts of the manuscript. Andrew Blight provided data used in the analysis and comments on final draft of the manuscript. Mark Johnson contributed to interpretation and analysis of the data and provided guidance and comments on drafts of the manuscript. Data used in this chapter was collected by Aimée Walls and Maeve Edwards as part of Aimee's final year undergraduate project.

Abstract

Interest in the cultivation of native kelp species is increasing within Europe. Observations of seaweed farms suggest that they may act as a habitat for associated species, potentially altering the richness of the local area. Previous studies have generally focused on species associated with wild kelps, showing the holdfast to be relatively species-rich. Little research has, however, been conducted on the species associated with cultivated kelps. The habitat created by cultivated kelp holdfasts may act as a novel habitat and not simply an expansion of existing kelp habitat, due to differences in holdfast age, holdfast morphology and holdfast position in the water column. *Laminaria digitata* from the west of Ireland were examined to test if these differences result in the fauna of cultivated (suspended) holdfasts being distinct from wild (benthic) stands. To place the results in a broader context, patterns were compared to holdfast richness relationships observed in comparable studies from the NE Atlantic. Total abundance of holdfast epifauna was similar across benthic and suspended holdfasts from the west of Ireland, although species richness was higher in suspended samples. Richness and abundance in suspended kelp holdfasts were consistent with the range of values recorded in other wild kelp studies. There were significant differences in assemblage composition between holdfast types. The distributions of faunal feeding types did not, however, vary between suspended and benthic kelps. Suspended holdfasts in the west of Ireland represented a novel habitat with higher species richness and a different species assemblage when compared to adjacent benthic kelps.

Keywords: *Laminaria*; Macroalgae; Aquaculture; Holdfasts; Macroinvertebrate; Multivariate analysis; Habitat volume

2.1 Introduction

Kelps (large seaweeds of the order Laminariales) are an existing and potentially expanding resource for the provision of food, fertilizers, food additives (e.g. alginates), pharmaceutical products (Guiry & Blunden 1991, Smit 2004), bioactive compounds (Holdt & Kraan 2011), biopolymers (Bella et al. 2015) and biofuel production (Fasahati et al. 2015). Scientific interest in cultivating the Laminariales has been increasing with a doubling of the number of studies found using an ISI search of ‘Laminaria and aquaculture’ in 2005–2014 compared to the previous decade. Kelp farming is currently dominated by Japanese kelp *Laminaria japonica* and Wakame *Undaria pinnatifida*, together accounting for an annual production of over 7 million tonnes wet weight in 2012 (FAO 2014). Although there is currently little seaweed biomass cultivated in Europe, feasibility studies (e.g. Bruton et al. 2009) and experimental farms are being established to advance the cultivation of kelps native to the region (including several research projects, e.g. AtSea 2015 [www.atsea-project.eu], EnAlgae 2015 [www.enalgae.eu], MAB3 2015 [www.mab3.dk]).

As habitat-forming species and primary producers, kelps are often considered to be important parts of the ecosystem and providers of ecosystem services. The values of the kelp-associated ecosystem services (products, climate regulation, education, tourism, aesthetic) are currently not well quantified (Costanza et al. 2014). In contrast, there is an extensive literature on the species found living associated with kelps (e.g. Sloane et al. 1957, Schultze et al. 1990, Christie et al. 2003). The holdfast is generally found to host more species than other parts of the kelp (Jones 1972, Moore 1972, Thiel & Vásquez 2000, Arroyo et al. 2004). The relatively high biodiversity of the holdfast is thought to reflect the benefits of a more complex physical structure (the spaces within the holdfast as compared to relatively smooth stipes and fronds), a more sheltered and sediment-rich local environment and the longer time available for colonization when compared to annually renewing parts of the kelp, like fronds (Christie et al. 2003, 2007). Species composition of associated fauna have been found to be affected by differences in habitat architecture and size of other macrophytes (Knowles & Bell 1998, Christie et al. 2009).

Any increase in kelp farming will create new habitat for the associated species, potentially altering the richness and function of the areas where farms are

sited. The majority of work conducted into the impact of seaweed cultivation on surrounding environments has been conducted either in tropical waters e.g. Zanzibar, East Africa (Eklöf et al. 2005) or with other seaweed species e.g. *Gracilaria* in Chile (Buschmann et al. 1996). The impacts of kelp cultivation in temperate seas are not greatly represented in the literature. However, the UK Crown Estate commissioned a report to understand the effects of large-scale seaweed farming off the west coast of Scotland (Aldridge et al. 2012). Using ecosystem-based modelling techniques Aldridge et al. (2012) concluded that the effect of seaweed farming on nutrient concentrations is expected to be ‘marginally significant’; this conclusion reflects a focus on the effects of nutrient removal on plankton productivity, and the effects of habitat creation were not investigated. Studies on the impacts of aquaculture have often focused on a relatively narrow range of pressures, although see Huntington et al. (2006). Evaluating the impact of seaweed aquaculture may be difficult as operations can be in areas away from the natural habitat and ecological reference points are not well defined. Although several of the pressures identified by Huntington et al. (2006) potentially associated with seaweed aquaculture have yet to be characterised, the creation of novel seaweed habitat represents a further process that requires consideration.

Observations of the fauna growing on existing seaweed farms suggest that both the longline infrastructure and the growing kelps may function as a habitat or refuge for a number of species (M. D. Edwards pers. obs.). There are, however, at least 3 reasons why the cultivated habitat may not act simply as an extension of existing kelp habitat for the associated species. 1) The holdfast morphologies of wild and cultivated kelp differ. Wild kelps tend to grow a characteristic flat or slightly conical holdfast when attached to rock (Fig. 2.1a). Cultivated kelps are seeded onto ropes for growth, resulting in a different morphology, formed by intertwined haptera (Fig. 2.1b). Such differences in the arrangement of space within holdfasts are thought to affect the associated community (Vasquez 1993, Tuya et al. 2011). 2) Depending on culture practices, kelp may be seeded fresh to rope for each growing season, so cultivated holdfasts are younger, on average, than those from wild stands. 3) Finally, cultivated kelps are suspended from ropes in the water column; this will alter both the hydrodynamic environment (discussed as an influence on epifauna by Moore 1972) and the accumulation of sediments thought to provide the bulk of carbon supply to the associated species (Schaal et al. 2012). Changing from a benthic to a suspended growth form may also alter the environmental conditions experienced by kelp epifauna: changes in depth

(Coleman et al. 2007), salinity (Jones 1973), oxygen availability (Scarratt 1961), and temperature (Scarratt 1961). Ecological processes may also be altered between benthic and suspended growth forms. Potential changes include variations in the presence of structural epibiota e.g. sponges (Smith 1996, Anderson et al. 2005a), changes in predation pressures on the communities (Vasquez 1993, Dumont et al. 2011) and variation in recruitment associated with dispersal abilities of adults and larvae (Edgar 1987, Thiel & Vásquez 2000, Norderhaug et al. 2002). To emphasize the differences in habitat between the wild and cultivated holdfasts, we refer to wild holdfasts as benthic holdfasts and cultivated holdfasts as suspended holdfasts. But it is important to note that this is not the only difference between the 2 holdfast types.



Fig. 2.1. Morphology of (a) benthic and (b) suspended *Laminaria digitata* holdfast

This study set out to test whether the differences in habitat and growth form of suspended kelp result in a holdfast fauna that is different from benthic stands. Studies of holdfast fauna emphasize that the patterns of species richness and abundance depend on the volume of space in the holdfast (e.g. Anderson et al. 2005a). We therefore included habitat volume as a covariate in statistical analyses of community structure. The goals of this study were, firstly, to test the difference in species richness and abundance between benthic and suspended *L. digitata* holdfast communities. Secondly, to put samples from the west of Ireland in a broader context, the patterns were compared to holdfast richness relationships generated in comparable studies from the NE Atlantic. Multivariate tests were conducted on benthic and suspended holdfasts to assess differences in species

composition. Furthermore, to assess the functional roles of the 2 holdfast types, differences in feeding strategies of their associated fauna were compared.

2.2 Materials and Methods

2.2.1 Study sites

Laminaria digitata holdfasts were collected at Mweenish Island, Galway, on the west coast of Ireland; from an established suspended longline structure and from the closest accessible stand of natural *L. digitata* (Fig. 2.2). Both locations were moderately exposed with southwest winds, situated approximately 10 m offshore, with a tidal range of 2.5–5 m. The suspended site ($53^{\circ} 18' \text{ N}$, $9^{\circ} 53' \text{ W}$), at Ard Bay, held a single longline structure (Fig. 2.3) over sandy substratum, in water depth of approximately 8 m at mean low water spring tide (MLWS). The 60 m longline structure consisted of a header rope suspended approximately 1 m below the sea surface by buoys and kept in position by anchor ropes and 500 kg weights at either end. Polypropylene dropper ropes (10 mm diameter, 3 m in length) with 1 kg weights were seeded with juvenile *L. digitata* sporophytes and suspended from the header rope 2 m apart. Dropper deployments were made in November 2010 and January 2011. The benthic site of natural stands of *L. digitata* ($53^{\circ} 17' \text{ N}$, $9^{\circ} 51' \text{ W}$) was approximately 200 m long, on rocky substratum and in a water depth of 2 m at MLWS.

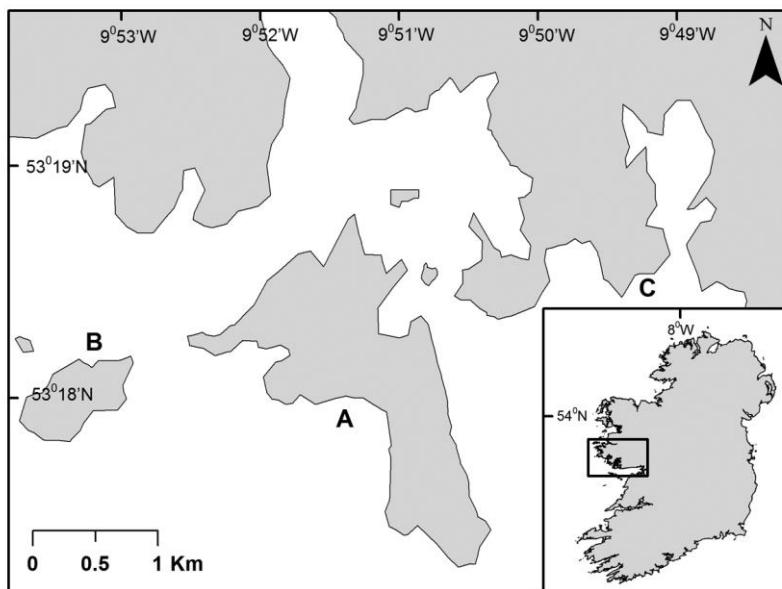


Fig. 2.2. Location of sampling sites at Mweenish Island, Galway, Ireland. A: benthic sampling site; B: suspended longline sampling site in Ard Bay; C: location of Ryan Institute's Carna Research Laboratory

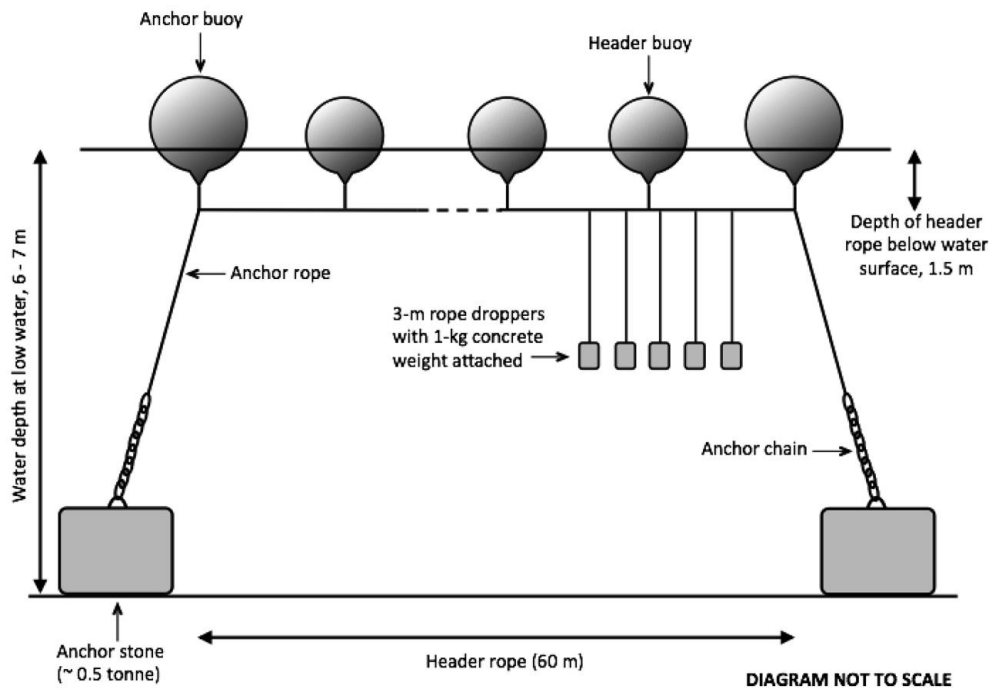


Fig. 2.3. Schematic of suspended longline structure in Ard Bay

2.2.2 Sampling protocol for the benthic and suspended *L. digitata* populations

Samples were taken using SCUBA on 20th October 2011 for the benthic samples and on the 4th November 2011 for the suspended samples. Five random *L. digitata* individuals in 5 replicate areas ($n = 25$) within the benthic site were collected using a mesh bag (1 mm mesh size). The entire plant was enclosed in the bag, the holdfast of the individual was pried away from the substratum using a dive knife and enclosed in the bag which was secured using string. At the suspended site, 5 dropper ropes from both deployment dates (November and January, $n = 10$) were collected using open-ended mesh bags (1 mm mesh size). The bag was slipped over the middle 1 m section of each dropper enclosing entire kelp sporophyte and secured using string, taking care not to disturb the fauna. Unfortunately, it was not possible to remove the stipe and frond material from the suspended holdfasts during sampling as this would involve cutting fronds from suspended holdfasts while diving, potentially losing dislodged animals. All stipe and frond material was therefore collected in the field for both benthic and suspended holdfasts so that both treatments were comparable. Both the benthic and suspended samples were transferred back to the nearby laboratory (Fig. 2.2) and processed within 8 h. The benthic samples were removed from the mesh bags and the holdfast was cut at the base of the stipe using a scalpel and measurements were taken to calculate habitat volume (described below). The suspended samples were

removed from the mesh bags and a 10 cm rope section of continuous holdfast material was excised (Fig. 2.1b) from the 1 m dropper section. The fronds and stipes were removed leaving only the 10 cm section of holdfast biomass; individual holdfasts could not be removed as the morphology of suspended holdfasts mean the haptera grow intertwined with each other. The rope substratum was removed and measurements were taken to calculate habitat volume (described below). After all measurements were taken both benthic and suspended holdfasts were stored in sealable plastic bags containing 70 % ethanol until further processing.

2.2.3 Habitat volume measurements

To compare holdfast communities a standard unit of habitat volume was used, i.e. the volume of space within the holdfast haptera available for colonisation by epibionts. There are 2 main methods used to measure the habitat volume; these methods were compared to investigate whether results from different studies are comparable. The first approach is an algebraic method described by Jones (1971) and the second uses displacement to calculate habitat volume, described by Sheppard et al. (1980) and used more recently by Blight & Thompson (2008). A methodological miscalculation was discovered in both methods (Jones 1971, Blight & Thompson 2008) when calculating tissue volume; the tissue weight should have been *divided* by specific gravity and not multiplied as stated in the original texts. We compared the algebraic and displacement methods (adjusting for the miscalculation) using additional data obtained from Blight & Thompson (2008). Analysis of these data suggests that holdfast volume can be compared across studies that use the algebraic or displacement methods (regression of volume [displacement] against volume [algebraic] is significant, $R^2 = 0.4556$). For our samples, the habitable volume of benthic and suspended holdfasts was calculated using the corrected Jones (1971) algebraic method. From the 10 cm suspended section, the holdfast volume was calculated by treating the holdfast structure as rectangular cuboid in shape.

2.2.4 Sample processing

Holdfast samples were removed from bags and washed over large trays with freshwater. For both benthic and suspended samples, holdfast haptera were carefully removed with frequent pauses for collections of exposed fauna (Jones 1971). Haptera were rinsed with freshwater and the remaining contents in the trays and bags were washed over a 0.5 mm sieve and stored in 70 % ethanol for later

identification. All collected fauna were sorted from the residue and identified down to species level where possible using (Hayward 1988, Hayward & Ryland 2002), with taxonomy cross-checked (using WoRMS Editorial Board 2016), enumerated and stored in 70 % ethanol.

2.2.5 Statistical analyses

Species richness and total abundance were compared across holdfast type (benthic or suspended) using holdfast volume as a covariate in ANCOVA. The optimal regression model, with homogenous slopes or heterogeneity of slopes (a holdfast type \times holdfast volume interaction), was found to minimise the value of the Akaike information criterion (AIC). To determine if the suspended samples from Ard Bay sown at different dates (November and January) could be pooled, the data were initially analysed separately from benthic samples. The relationship of seeded date and species abundance and richness was tested using ANCOVA with habitat volume as the covariate.

Following the determination of whether sowing date should be kept as a separate independent variable, data from this study (benthic and suspended *L. digitata*) were compared to natural stands of *L. digitata* and *L. ochroleuca* sampled off the southwest coast of England near Plymouth (Blight & Thompson 2008). Algal counts were removed from the Plymouth data set as we did not enumerate algae. Other potential sources of northeast Atlantic holdfast richness and abundance data could not be compared directly as the studies, did not present the data in a comparable form or the definition of holdfast volume was different. If suspended holdfasts represent similar habitat to epifauna as benthic holdfasts, then there were expected to be few differences in slope of richness against holdfast volume and no differences in intercept value. Unfortunately, the Blight & Thompson (2008) study scored species presence–absence, so direct comparisons of abundance cannot be made. However, comparison of benthic and suspended holdfast abundance was possible.

Alongside differences in species richness and abundance, the differences between the assemblage structures of fauna from benthic and suspended holdfasts from Ireland were compared with a multivariate test. A Simpson's dissimilarity matrix was generated from the presence–absence data for the 2 treatments (1 benthic [5 levels] and 1 suspended [2 levels; November and January]) using the

PopTools (Hood 2014) add-on in Excel. Simpson's dissimilarity has the advantage that it only measures the turnover of species and is not affected by changes in species richness between samples (Baselga 2010). This makes dissimilarities measured by Simpson's index easier to interpret than in the case for indices that mix the turnover and species richness components of dissimilarity (e.g. Sørensen's index). Multidimensional scaling (MDS) (Shepard 1962, Kruskal 1964a, b) ordination was carried out using PRIMER V6® (Clarke & Gorley 2006), giving the position of each holdfast in two-dimensional space based on its species composition. Nested ANOSIM was used to test for differences between the faunal assemblages of the holdfast types and between spatially (benthic) or temporally (suspended) separated samples within holdfast type. Post-hoc results were pooled for a summary representation of differences between groups. Expected number of species for rarefaction analysis was also conducted using PRIMER V6®.

To test for an effect of holdfast type on the functional roles of associated species, fauna were grouped into categories based on their feeding strategies: suspension feeder, deposit feeder, carnivore, herbivore, or omnivore. Where species spanned these categories, their predominant mode of feeding was recorded (Sheppard et al. 1980). Chi-squared analysis was used to test the hypothesis that feeding types were randomly associated with holdfast type (benthic or suspended). This chi-squared test involved a contingency table, although 4 of the expected values were <5. In a clarification of the rule of thumb that chi-squared is unreliable with expected values <5, Zar (1996) suggests that a better threshold is for the average expected frequency to be at least 6 when testing at 0.05 level of significance. For the chi-squared test presented in the current study, the average expected frequency was 9.4. The test was therefore considered reliable.

2.3 Results

A total of 2434 individuals representing 61 taxa over 42 families were identified: 1801 individuals from the benthic and 633 individuals from the suspended holdfasts. Nematodes, polychaetes and molluscs were the 3 most frequently found groups in the benthic holdfasts, and accounted for 48, 26 and 13 % of abundance (number of individuals), respectively, whereas amphipods, polychaetes and decapods were the 3 most frequently found groups in the suspended holdfasts and accounted for 30, 27 and 16 % of abundance, respectively. Thirty-one percent of all taxa were unique to benthic holdfasts including the

sponges *Halichondria panicea*, *Hymeniacion perleve*, the polychaete *Prionospio multibranchiata* and the gastropod molluscs *Lacuna parva* and *Onoba semicostata*. Thirteen percent of all taxa were unique to suspended holdfasts including the decapod crustacean *Hippolyte varians*, the ascidian *Ascidia mentula*, and the polychaetes *Dasybranchus* spp. and *Nereimyra punctata*. A total of 58 % of all taxa were common to both holdfast types. Where species are rare, presence in a particular type of holdfast may not be distinguishable from random placement. As species occur more frequently, random placement of individuals becomes an unlikely hypothesis for cases where all individuals are found in a single holdfast type. Random placement is an unlikely hypothesis for the exclusivity of *Hippolyte varians*, *Dasybranchus* spp. and *Nereimyra punctata* in suspended holdfasts (binomial test, $p < 0.05$). Similarly, the observations of *Prionospio multibranchiata* and *Hymeniacion perleve* in benthic holdfasts were too frequent for random placement to be a suitable hypothesis (binomial test, $p < 0.05$). A full list of species recorded, their abundance and the taxonomic level to which they were identified is provided in Appendix A Table A1.

Species abundance in suspended holdfasts was not affected by habitat volume or holdfast deployment date (habitat volume: $F_{1,7} = 1.69$, $p > 0.05$; holdfast deployment date: $F_{1,7} = 0.14$, $p > 0.05$; interaction was not fitted in the optimum model). Abundance data from suspended holdfasts from 2 different sowing dates were therefore pooled. In the ANCOVA comparing abundance-volume relationships between holdfast types, the number of associated individuals was related to habitat volume, but not the source of the holdfast (Table 2.1, Fig. 2.4). The pooled regression slope relating abundances to habitat volume had $R^2 = 0.4861$ and slope $1.19 (\pm 15.796 \text{ SE})$.

Table 2.1. ANCOVA data from *Laminaria digitata* number of individuals vs. holdfast types (benthic and suspended) with habitat volume as a covariate. *Significant difference at $p < 0.001$

Source	df	SS	MS	F	p
Habitat volume	1	48503	48503	28.93	<0.001*
Holdfast type	1	1467	1467	0.86	0.361
Error	32	54669	1708		
Total	34	104693			

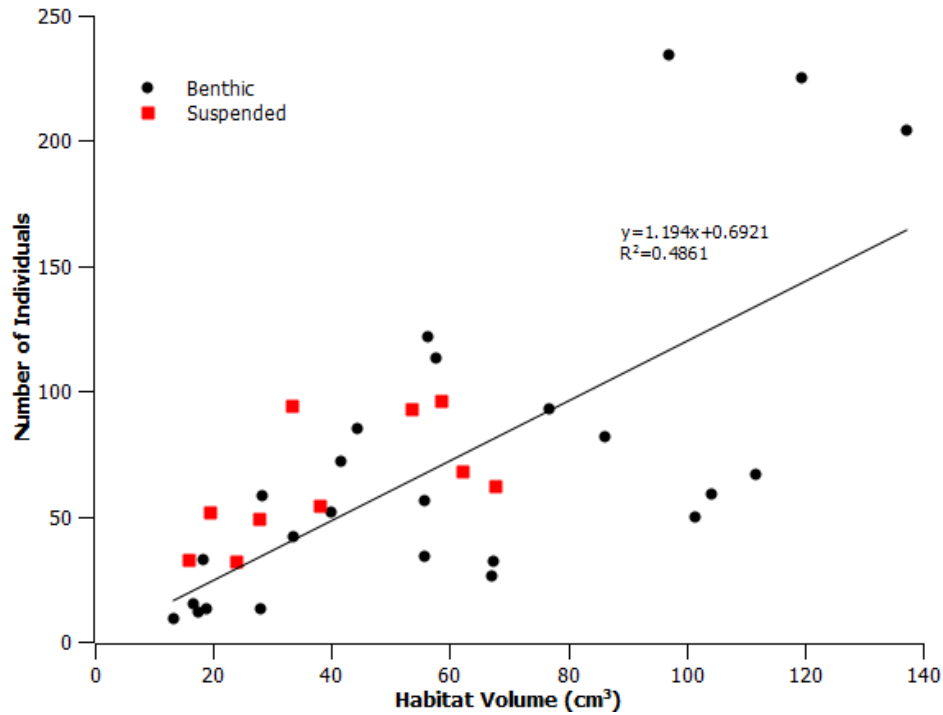


Fig. 2.4. The relationship between holdfast habitat volume and number of individuals per holdfast for benthic and suspended *Laminaria digitata*

Species richness in suspended holdfasts was affected by both habitat volume and holdfast deployment date (habitat volume: $F_{1,7} = 6.54$, $p < 0.05$; holdfast deployment date: $F_{1,7} = 5.64$, $p < 0.05$; interaction was not fitted in the optimum model). Species richness values from *Laminaria digitata* sown in November and January were therefore kept separate for further analysis. The combined data for species richness showed evidence for an interaction between habitat volume and type (Table 2.2, Fig. 2.5). Species richness always increased with habitat volume, but the rate of increase and mean richness differed among benthic and suspended *L. digitata* and *L. ochroleuca*. Benthic *L. digitata* holdfasts from Plymouth were the most species-rich, followed by *L. ochroleuca*. For the material collected in the west of Ireland, suspended holdfasts were generally more species-rich than benthic holdfasts of an equivalent volume. Holdfasts sown in January were more species-rich than holdfasts sown 2 months earlier in November considering their smaller volume.

Table 2.2. ANCOVA data from number of species vs. holdfast types with habitat volume as a covariate. There are 5 holdfast types: the *Laminaria digitata* and *L. ochroleuca* from the Blight & Thompson (2008) study benthic material collected in Ireland and 2 sowing dates of suspended *L. digitata* holdfasts from aquaculture longlines (present study). The 2 sowing dates were not pooled for species richness as a preliminary ANCOVA suggested inhomogeneity of slopes. *Significant difference at $p < 0.05$

Source	df	SS	MS	F	p
Habitat volume (V)	1	11.3	160.02	10.64	0.002*
Holdfast type (T)	4	1425.82	8.60	0.57	0.684
V × T	4	245.47	61.37	4.08	0.006*
Error	55	827.35	15.04		
Total	64	2509.94			

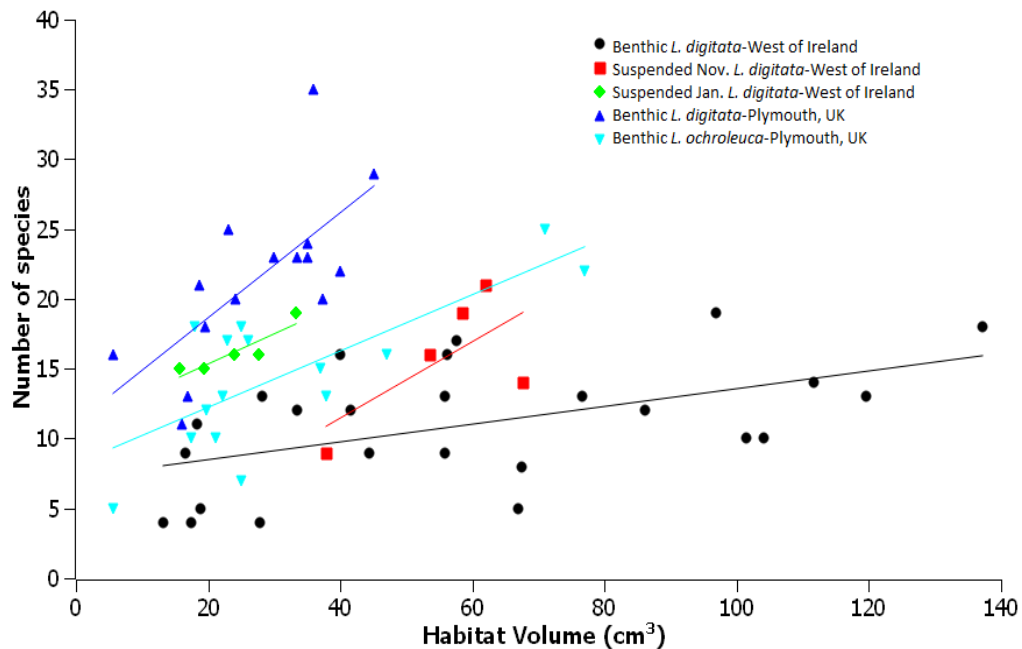


Fig. 2.5. The relationship between holdfast habitat volume and number of species per holdfast for Blight & Thompson (2008, Plymouth, UK) benthic *Laminaria digitata* ($r^2 = 48\%$, slope = 0.376, SE = 0.545) and benthic *L. ochroleuca* ($r^2 = 56\%$, slope = 0.202, SE = 0.262), and, from the current study (west Ireland), benthic *L. digitata* ($r^2 = 27\%$, slope = 0.063, SE = 0.164), suspended November *L. digitata* ($r^2 = 81\%$, slope = 0.276, SE = 5.019) and suspended January *L. digitata* ($r^2 = 45\%$, slope = 0.214, SE = 6.951)

Suspended *L. digitata* holdfasts held the same number of individuals as benthic holdfasts, but had more species in samples of equivalent volume. These

patterns are reflected in rarefaction plots (Fig. A1 Appendix A), with suspended holdfasts having more species per individual counted.

Some separation and clustering of epifaunal assemblages of *L. digitata* holdfasts can be seen for the 2 treatments (1 benthic [5 levels B1–B5] and 2 suspended (2 levels, November and January) in the MDS plot (Fig. 2.6). A stress value of 0.21 in the MDS indicates that the data are only partially represented by a two-dimensional plot and little reliance should be placed on the finer detail of the plot (Clarke & Warwick 1994). However, the broad-scale pattern shows a separation of the benthic holdfast samples and the suspended November and January holdfast samples.

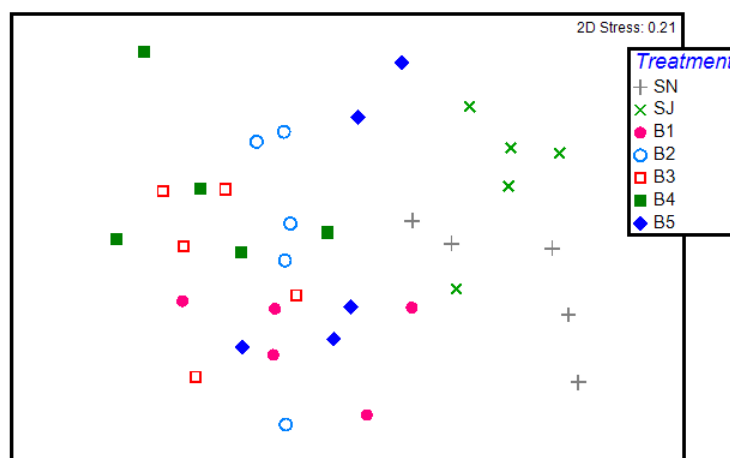


Fig. 2.6. Two-dimensional MDS plot of 35 holdfasts: 25 benthic samples (B1–B5, spatially separate locations in kelp bed) and 5 suspended samples (different droppers from longline) sown in November (SN) and 5 sown in January (SJ), based on presence-absence Simpson's dissimilarity matrix of faunal species collected from each holdfast

The nested ANOSIM indicated significant differences in assemblage composition among holdfast types: between sowing dates and between samples collected in separate areas of the natural kelp bed sampled ($R = 0.383$, $p < 0.01$). There was also significant difference in structure between holdfast types ($R = 1$, $p < 0.05$). The R value of 1 indicates that all samples from suspended holdfasts were more similar to another suspended sample than they were to any benthic sample (and vice versa). The R values from post-hoc tests were pooled to summarise the pattern of pair-wise differences between the 'within' groups of 5 samples (7 groups in total, 5 benthic and 2 suspended). There was a high dissimilarity between the suspended samples from the November and January sowing ($R = 0.82$, no SE as only one comparison can be made), whereas the Benthic-Benthic pairs from separate areas of the same bed were relatively similar (mean $R = 0.29$, $SE = 0.030$).

Benthic-Suspended pairs were generally quite different, as would be expected from the overall result (mean $R = 0.80$, $SE = 0.255$).

The differences in faunal assemblage across holdfast type did not seem to reflect any pattern of variation in categorization of feeding strategy (Table 2.3). The distribution of feeding types was not significantly different from a random allocation to holdfast types (chi-squared test, $\chi^2 = 3.161$, $df = 4$, $p > 0.1$).

Table 2.3. Number of epibiotic species in each feeding category for benthic and suspended *Laminaria digitata* holdfasts

No. of species	Benthic	Suspended
Suspension feeders	16	12
Deposit feeders	19	12
Carnivores	13	14
Omnivores	1	3
Herbivores	3	1

2.4 Discussion

2.4.1 Benthic and suspended holdfast fauna in comparison with other studies

Kelps collected in Ireland show a pattern where suspended holdfasts hold similar numbers of individuals for an equivalent habitat volume as benthic holdfasts, but the species richness was higher in suspended holdfasts. Although the distribution of feeding types did not change across holdfast types, there were differences in the structure of the associated assemblage (identified using multivariate tests). The relatively high richness in suspended holdfasts from Ireland was within the range of values observed from samples taken off Plymouth by Blight & Thompson (2008).

The results from Ireland can be compared to other studies to assess if the relatively low species richness recorded for benthic holdfasts was due to the low numbers of individuals sampled or other factors. Unfortunately, the Blight & Thompson (2008) study did not record individuals so the comparisons for abundance need to be drawn from elsewhere. Comparisons with previous studies are slightly complicated by the different methodologies employed for the measurement of volume: whether estimated as holdfast volume or habitat volume. To compare studies, we calculated the mean abundance per 10 ml of holdfast

volume and the mean abundance per 10 ml of habitat volume (Table 2.4). This provides data that can be compared across studies using different denominators. Comparisons are also complicated by mesh size when sampling and the taxonomic resolution to which organisms were identified. For mesh size the studies we selected for comparison varied from 250 μm to 1 mm or were unreported, and the taxonomic resolution of these studies ranged from identification to species level or only specialist groups were identified, i.e. peracarid crustaceans studied by Thiel & Vásquez (2000). However, these differences show no patterns in the results calculated but should be further studied. Mean abundance per 10 ml holdfast volume for our benthic and suspended *Laminaria digitata* samples fell within the range of previous studies in Europe of kelps *L. hyperborea* (Christie et al. 2003), *Saccorhiza polyschides* and *L. ochroleuca* (Tuya et al. 2011), and from southern hemisphere studies, including *Ecklonia radiata* (Smith et al. 1996, Anderson et al. 2005a), and *Lessonia trabeculata*, *L. nigrescens* and *Macrocystis integrifolia* (Thiel & Vásquez 2000). Mean abundance per 10 ml habitat volume for our benthic and suspended holdfasts was compared with *L. hyperborea* (years 2–7) from the UK (Jones 1971) correcting for miscalculation (Table 2.4). We conducted the same comparison between studies but for mean species per 10 ml holdfast volume and per 10 ml of habitat volume (Table 2.5). Our *L. digitata* holdfast values again fell within the range of other studies, with Blight & Thompson (2008) holdfasts being more species rich per 10 ml of holdfast volume, and our habitat volume values were similar when compared with other available studies (Jones 1971).

Table 2.4. Comparison of mean abundance per 10 ml of holdfast volume or habitat volume across a number of studies from varying locations and varying kelp species (*L.*: *Laminaria*, *S.*: *Saccorhiza*, *E.*: *Ecklonia*, *M.*: *Macrocystis*). Range values given when differential samples from species, location and other factors were provided

Location	Species	Abundance per 10 ml	Abundance per 10 ml	Study
		holdfast volume	habitat volume	
West of Ireland	<i>L. digitata</i>	82.5	11.89	Present study, benthic
West of Ireland	<i>L. digitata</i>	51.26	17.54	Present study, suspended
Norway	<i>L. hyperborea</i>	17.75–63.1 ^a		Christie et al. (2003)
Northern Portugal	<i>S. polyschides</i>	79.93		Tuya et al. (2011)
Northern Portugal	<i>L. ochroleuca</i>	114.03		
New Zealand	<i>E. radiata</i>	47.67		Anderson et al. (2005)
Australia	<i>E. radiata</i>	9.9–62.9		Smith et al. (1996)
United Kingdom	<i>L. hyperborea</i>		1.78–9.59	Jones (1971)
Chile	<i>L. trabeculata</i>	41.68		Thiel & Vásquez (2000)
Chile	<i>L. nigrescens</i>	44.97		
Chile	<i>M. integrifolia</i>	12.21		

^aExcluding stn 1 outlier = 352.73

Table 2.5. Comparison of mean species per 10 ml of holdfast volume or habitat volume across a number of studies from varying locations and varying kelp species (*L.*: *Laminaria*, *E.*: *Ecklonia*, *M.*: *Macrocystis*). Range values given when differential samples from species, location and other factors were provided

Location	Species	Species per 10 ml holdfast volume	Species per 10 ml habitat volume	Study
West of Ireland	<i>L. digitata</i>	16.21	2.41	Present study, benthic
West of Ireland	<i>L. digitata</i>	14.04	4.96	Present study, suspended
SW England	<i>L. hyperborea</i>	32.85		Blight & Thompson (2008)
SW England	<i>L. ochroleuca</i>	20.04		
Norway	<i>L. hyperborea</i>	0.61–1.58 ^a		Christie et al. (2003)
New Zealand	<i>E. radiata</i>	7.17		Anderson et al. (2005)
United Kingdom	<i>L. hyperborea</i>		0.42–5.99	Jones (1971)
Chile	<i>L. trabeculata</i>	8.55		Thiel & Vásquez (2000)
Chile	<i>L. nigrescens</i>	10.73		
Chile	<i>M. integrifolia</i>	5.25		
^a Excluding Stn 1 outlier = 37.27				

Overall, in the context of data from other studies, we can say that the relatively low species richness of our benthic holdfasts (as seen in Fig. 2.5) is not likely to be a sampling artefact from material with a particularly low abundance of individuals, as our values for mean species and abundance per 10 ml of holdfast and habitat volume are within the bounds of values from previous authors.

2.4.2 Composition of fauna

The 3 most dominant groups, in terms of abundance, in benthic holdfasts were nematodes, polychaetes and molluscs and, for suspended holdfasts, the dominant epifaunal groups were amphipods, polychaetes and decapods. These results agree with previous studies of the Laminariales from European waters including Blight & Thompson (2008) from the UK and Christie et al. (2003) from Norway, Tuya et al. (2011) from Portugal; also from the Southern Hemisphere, Smith et al. (1996) found that amphipod crustaceans and polychaete worms accounted for 78 % of the total number of organisms counted in *E. radiata* holdfasts sampled in Australia, and Włodarska-Kowalczyk et al. (2009) found dominant taxa in the dominant kelp species in the Arctic were polychaetes, molluscs and amphipods. Although nematodes were present in these studies, they were not a dominant group as found in our benthic samples. The presence of nematodes can suggest a source of organic pollution (Platt et al. 1984); however, this is unlikely as Ard Bay is situated in a sparsely populated area in west Galway. There may be nematode population increases in response to transient resource availability in the present study as increased detrital food source after kelp die back in autumn (Krumhansl & Scheibling 2012). Moore (1971) studied the nematode fauna of kelp holdfasts in the UK and found, among other species, omnivorous species that exploited the sediment feeding niche provided by holdfasts. Nematodes were found in less abundance in suspended holdfasts, which could be due to the different hydrodynamic environment around these suspended holdfasts, inhibiting the accumulation of detrital material within. Juvenile decapods were a dominant group in our suspended holdfasts and, although present in other studies (Christie et al. 2003, Tuya et al. 2005), they were generally not found to be dominant in previous work (but see Ojeda & Santelices 1984). Decapods are omnivorous and may prefer increased water flow on the suspended holdfasts, which are suspended in the water column compared to benthic holdfast on the seabed.

Although the most dominant groups vary slightly between studies, their contribution as a proportion of overall epifaunal abundance varies considerably; probably due to species, location and time of sampling. Smith (2000) found that, regardless of kelp species, the same functional groups tend to dominate. Polychaete worms and peracarid crustaceans are the most abundant, with molluscs, decapod crustaceans, echinoderms and ascidians providing the majority of the additional taxa (Smith 2000). The fauna found in our suspended samples was not functionally dissimilar to that in natural kelp beds.

2.4.3 Do species richness and abundance increase as habitat volume increase?

Our results show that the epifaunal abundances and species richness increase when habitat volume within the holdfast increases, agreeing with other studies (Ojeda & Santelices 1984, Smith et al. 1996, Thiel & Vásquez 2000, Blight & Thompson 2008, Tuya et al. 2011). However, our data did not reach a plateau as found in Anderson et al. (2005a) who suggested that encrusting species increase in size over time as holdfasts age and may decrease the overall space within the holdfast. No difference in age or size between organisms inhabiting the farmed and benthic holdfasts was observed. Although standardization of the age of *L. digitata* was not possible, as *L. digitata* cannot be aged accurately, no exceptionally large holdfasts were observed or sampled in our benthic kelp populations. We estimate the benthic holdfasts to be between 3 and 5 yr old in comparison to suspended holdfasts that were 11-13 month old. Thus, age of the holdfasts did not seem to affect the size of the individuals inhabiting them, e.g. smaller individuals were not found on the younger suspended kelps. For future studies with kelp species that can be accurately aged, e.g. *L. hyperborea*, standardization should be employed when comparing holdfasts.

2.4.4 Holdfast as sampling unit

Kelp holdfasts represent a discrete sampling unit which is easily collected and which have been used by many studies for the purpose of monitoring pollution (Jones 1971, Moore 1971, Sheppard et al. 1980, Smith 1993), stress (Smith 2000) and evaluating patterns of biodiversity at different taxonomic resolutions in marine ecosystems (Anderson et al. 2005a, b). Fauna in kelp holdfasts often have higher species richness and abundance compared to other adjacent habitats and kelp forests offer uniform habitats across large spatial scales; these are just a few of the advantages of using kelp holdfasts as a sampling unit (Smith 2000).

However, as is clear from attempts to synthesize the literature, problems occur in the definition of volume when comparing different studies, i.e. holdfast volume vs. habitat volume. Standardization is needed across all future studies with habitable volume (space available for colonisation by fauna within holdfast) being our preferred method. Holdfast volume and habitat volume are correlated (data from correlation for benthic holdfasts, $R^2 = 0.81$; for suspended holdfasts: $R^2 = 0.87$). However, habitable volume is more logically consistent as its use avoids possible issues in deciding where the stipe should be cut, which is an important measurement in holdfast volume. Habitable volume is also a more reasonable measure to use, as the indirect nature of holdfast volume as an estimate of habitat can cause problems when correlation does not hold, e.g. comparison across different holdfast morphologies. Further, care must be taken not to continue to use Jones' (1971) miscalculation for tissue volume. In addition to defining holdfast/habitat volume, other issues arise when using holdfasts as a sampling unit, including variations in the age of the holdfasts sampled (which should be standardised where possible) and the process of separating the stipe and frond material from the holdfast. Most studies remove the stipe and frond before collection of the holdfast (but see Thiel & Vásquez 2000, Włodarska-Kowalczyk et al. 2009). Separation of the stipe and frond from the holdfast at the time of sampling seems the least likely to introduce artefacts; however, for this study, we were unable to do so due to the major disturbance it would have caused to the suspended holdfasts, and benthic samples were therefore treated the same for comparison. Tuya et al. (2011) suggests that loss of animals during sampling is likely low since animals tend to attach to the holdfast in response to the detected disturbance. Observations of frond fauna suggest fronds are mainly dominated by sessile organisms such as hydroids and bryozoans and less mobile species, e.g. *Patella pellucida* and other gastropod species. Also, the stipes of *L. digitata* are smooth, unlike the rough *L. hyperborea* stipes; thus, epiphytes and their associated fauna are rare. For these reasons, we suggest that collecting stipe and frond material along with the holdfasts may not have caused significant intra-sample confusion of attached fauna. Finally, both species identities and abundances should be recorded for deeper understanding and more robust comparisons between studies so that richness per individual counted can be compared and rarefaction curves calculated.

2.4.5 Do cultivated kelps provide a novel habitat?

The evidence from this study suggests that suspended holdfasts represents a novel habitat for fauna which is different to the habitat provided by benthic kelp species. The species found on suspended kelps are not unexpected for benthic kelp populations. The multivariate differences, however, demonstrate that suspended kelps can complement diversity in an area to produce higher richness than that just provided by benthic kelp. This was a preliminary study of suspended kelp faunal communities with comparisons to the existing literature. It should be noted that comparisons are somewhat limited as only 1 benthic site was sampled and that further sampling of other benthic sites is required to determine the full characteristics of benthic populations. However, it seems likely that site-specific characteristics will affect the degree of difference between suspended and benthic epifauna. Similarly, holdfast age and seasonal effects are likely to affect comparisons. In this respect, it is remarkable that suspended kelp were only in the sea for 11-13 months, but had higher species richness than adjacent holdfasts from an established kelp bed. Even though holdfasts have been shown to be the tissues hosting the highest richness and abundance of fauna on kelp (Jones 1972, Moore 1972, Christie et al. 2003), the stipe and frond faunal communities can host different assemblages in terms of composition and structure (Christie et al. 2003) and, thus, also need to be analysed. If the communities associated with suspended kelps were found to be beneficial to the ecosystem, different harvesting techniques could be employed to maintain the holdfast, stipe and some frond material to allow the community to continue to grow.

2.4.6 Causes of differences in faunal assemblages

As this is a preliminary study, the specific mechanisms that cause the difference in assemblage structure between the 2 holdfast types cannot be determined, however, the 3 main factors identified; morphology, age and habitat may help explain the variation in species richness. As seen in Fig. 2.1, the morphologies of the holdfasts are distinctly different. Tuya et al. (2011) found holdfast morphology to be the best physical predictor to explain variation in epifaunal assemblage structure between 2 holdfasts with different morphologies. Vásquez (1993) noted that in drifting *Macrocystis pyrifera*, without substrate limiting its vertical growth, the holdfasts acquire a cylindrical morphology. This, he suggests, could be a reason for differences in faunal diversity and density

between drifting and attached holdfasts. As discussed above, the age of our benthic and suspended holdfasts varied considerably. Interestingly, Sheppard et al. (1980) found species richness increased for 3 different age classes of *L. hyperborea* holdfasts. Thus, age should be studied as a possible factor to explain the differences in faunal communities. Change in habitat from benthic to suspended has many associated changes which could all cause variations in faunal communities. Changes in abiotic conditions, such as hydrodynamic environment (Moore 1972), sedimentation rates (Schaal et al. 2012), depth (Coleman et al. 2007), salinity (Jones 1973), oxygen availability (Scarratt 1961) and temperature (Scarratt 1961) are all associated with changes in kelp fauna. These factors could be measured relatively easily in the field to determine their degree of influence on suspended communities. The influences of habitat on ecological processes are a little more difficult to estimate. Dispersal abilities of fauna inhabiting benthic holdfasts can affect their ability to inhabit suspended holdfasts. Highly mobile organisms and those with pelagic larval dispersal rates will have a greater opportunity to inhabit and establish on suspended kelps. Other animals with direct development of offspring or low mobility rates may not be able to establish on the suspended holdfasts or may need more time. Thiel & Vásquez (2000) showed that the distribution of peracarid aggregations may be a consequence of their reproductive biology as they are brooders and juveniles recruit to the immediate vicinity of their mother and may stay and reproduce in the natural holdfast. *Fabricia stellaris* was found in our benthic holdfast but was not present in our suspended samples and so could be an example of a species which was limited by its dispersal abilities, and could not reach the suspended holdfasts, as its offspring brood in the mother's tube and move very short distances (Hayward & Ryland 2002). As shown by Dumont et al. (2011) predation pressures on suspended structures may be considerably less than those of benthic habitats. Some species may thrive in suspended habitats but may not establish in similar benthic communities due to predation by other organisms.

2.4.7 Implications

This study identifies a habitat value associated with suspended kelp holdfasts as, although they hold similar numbers of individuals for equivalent volume as benthic holdfasts, the species richness of macroinvertebrates was greater in suspended populations. The habitat may provide a structure for fauna to inhabit or act as a food source. The presence of an epifaunal assemblage suggests possible

ecosystem services provided by seaweed farms that should be quantified. Ecosystem services are the goods and services provided by an ecosystem that benefit humans (Millennium Ecosystem Assessment 2005). In addition to biodiversity, seaweed farms may provide nursery grounds and protection from predators for juvenile invertebrates and fish similar to that of benthic kelp forests (Smale et al. 2013). Kelp also assists in the uptake of excess nutrients that cause eutrophication, reflected in the use of kelps for Integrated Multi-Trophic Aquaculture (IMTA) (Neori et al. 2004, Troell et al. 2009). The filter feeding organisms attached to kelp may influence particulate and plankton concentrations by biofiltration. It is also possible that the deployment of seaweed lines could assist in habitat restoration by supplying spores and gametophytes where benthic kelp beds have been destroyed by anthropogenic impacts. The extent of such ‘seeding’ influences will increase with the time that longlines are left in the water. Harvesting practices are not yet harmonized and holdfasts and small fronds may potentially remain in place for some time after the fronds are removed. The potential additional benefits need to be assessed and quantified, allowing a valuation of seaweed aquaculture beyond the basic price for the crop.

Acknowledgements

The authors acknowledge the staff at the Ryan Institute’s Carna Research Station for their support over the duration of this project. In addition, they thank Dr. Adrian Patterson from NUI Galway’s Benthic Ecology Laboratory for taxonomic support and Mr. Antaine Conneely and Mr. David Moran for sampling assistance. The authors also acknowledge Martin Thiel and 2 anonymous reviewers for their comments that improved the manuscript. A.M.W. is currently funded by the Dr. Tony Ryan Research Trust, NUI Galway.

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Chapter 3

Ecological priming of artificial aquaculture structures: kelp farms as an example

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Role: Lead author, responsible for overall sampling (preparation and collection), data processing, data analysis and writing of manuscript. Mark Johnson, Maeve Edwards and Louise Firth contributed to the conception and design of the sampling regime, analysis and interpretation of the data and reviews of manuscript drafts.

Abstract

The continued development of the aquaculture industry is contributing to the proliferation of artificial structures in the marine environment. Observations of seaweed farms (infrastructure and biomass) suggest they act as a habitat for associated species. Seaweed farms differ from other forms of artificial infrastructure as the material deployed already has marine organisms (i.e. culture species) growing on it. This ‘priming’ of ropes with juvenile sporophytes may affect future development of communities by facilitating colonizing species or suppressing competitors. We call this process ‘ecological priming’: the provision of a biological platform that influences the successional development of specific communities. The communities that developed on ropes primed with *Alaria esculenta* individuals were compared to unprimed ropes to assess the ecological priming effect, at a commercial kelp farm on the southwest coast of Ireland. Species richness increased over two cultivation seasons and species composition was consistent between years, with distinct communities developing on primed and unprimed treatments. Species occurrence on primed ropes was predictable with no predictable pattern occurring on unprimed ropes. Multivariate tests indicated distinct communities between treatments, with suppression of other algal species and potential facilitation of some species by the presence of *A. esculenta* on primed ropes. Cultivated kelp holdfasts represent a habitat for distinct assemblages that reflect ecological priming of the substratum.

Keywords: Ecological Priming; Artificial structures; Aquaculture; *Alaria esculenta*; Macroalgae; Holdfasts; Epibionts; Predictability

3.1 Introduction

Over the last few decades there has been rapid proliferation of artificial structures in the marine environment (Firth et al. 2016b) to enable the exploitation of the ocean's energy and food resources. Novel structures include oil and gas platforms, marine renewable energy installations and aquaculture facilities (Chapman & Underwood 2011, Firth & Hawkins 2011). The term 'ocean sprawl' has been used to describe this expansion of coastal and marine infrastructure (Firth et al. 2016a,b) and is gaining recognition as one of the biggest threats to marine ecosystems (Airoldi & Beck 2007, Firth et al. 2013, Dafforn et al. 2015).

Specifically, the aquaculture industry has grown dramatically over the last 50 years to an all-time high of 101 million tonnes live weight in 2014 (FAO 2016). Within the aquaculture sector, the cultivation of aquatic plants (dominated by marine macroalgae), is also expanding rapidly: by almost 8 % per year over the past decade (FAO 2016). Over 33 % of the 27.3 million tonnes of global annual aquatic plant production came from just two kelp species (*Laminaria japonica* and *Undaria pinnatifida*) (FAO 2016). Kelp species are cultivated to produce biomass to supply the many traditional (e.g. food) and expanding uses (e.g. biofuels) of kelp (Guiry 1989, Walls et al. 2016). Observations of the artificial infrastructure associated with seaweed farms and the kelp biomass itself suggest that farms provide important ecosystem services such as habitat provision (Park et al. 1990, Peteiro & Freire 2013, Førde et al. 2016, Walls et al. 2016, 2017), protection from predators, and farms may act as nursery grounds for juvenile fish species, similar to that of wild kelp forests (Smale et al. 2013, Walls et al. 2016). However, cultivated kelps are grown suspended from ropes in the water column whereas wild kelps grow attached to the benthos, and this alteration of environments could modify the provision of these services (Walls et al. 2016). Seaweed farms differ from other forms of artificial infrastructure in that the material placed in the sea already has marine organisms growing on it. This 'priming' of ropes with juvenile sporophytes may affect subsequent development of the fouling community by facilitating colonizing species or suppressing competitors. The intended consequence of seeding ropes with sporophytes is that a thick growth of harvestable kelp biomass develops. We term this process 'ecological priming' and define it as the practice of providing a biological platform that influences the successional development of specific communities. In this study, artificial structures (ropes), seeded with organisms, juvenile kelp (*Alaria esculenta*) sporophytes, will be referred to as

‘primed’ structures and conversely artificial structures with no seeding will be referred to as ‘unprimed’ structures.

To date the majority of research on the role of kelp as a habitat has focused on the holdfast structure (Jones 1971, Moore 1973, Schultze et al. 1990, Smith et al. 1996, Thiel & Vásquez 2000, Christie et al. 2003, Blight & Thompson 2008, Walls et al. 2016, Teagle et al. 2017). This focus on holdfasts is due in part to the relative ease in collecting these discrete sampling units (Walls et al. 2016) and because the holdfast is generally found to host the highest diversity of all kelp structures (i.e holdfast, stipe and frond) (Jones 1971, Thiel & Vásquez 2000, Norderhaug et al. 2002, Christie et al. 2003, Arroyo et al. 2004). Within an individual holdfast, species richness typically reaches 30 – 70 macrofaunal species (Jones 1971, Thiel & Vásquez 2000), but in some cases, may reach up to 90 species (Christie et al. 2003). This relatively high biodiversity is thought to reflect the complex physical structure provided by the holdfast. The branched root-like shape of the holdfast provides a number of holes and crevices (Christie et al. 2003). This interstitial space may represent favourable habitat for colonising fauna, potentially providing protection from predators and during periods of adverse environmental conditions (Norderhaug et al. 2002). The holdfast functions as a sediment trap accumulating detritus, which acts as a food source for many of the organisms inhabiting the structure (Moore 1972). The holdfast also provides a stable environment which is persistent over seasons and years (Schaal et al. 2009); with the lifespan of the holdfast being the same as that of the kelp individual (Christie et al. 2003). This stability contrasts with the seasonally fluctuating habitat experienced by stipe-associated epiphytes (Norderhaug 2004) and to the temporally renewing frond habitat (Christie et al. 2003, 2007). It must be noted that, depending on cultivation practices, entire kelp individuals including the holdfast can be harvested from the farm thus the lifespan of cultivated holdfasts is only as long as the cultivation period.

A number of biotic and abiotic factors may influence the assemblages found on both droppers seeded with kelp and on submerged infrastructure like ropes. Where kelps form holdfasts, biotic factors include holdfast age, morphology and habitat volume. Wild holdfasts are perennial; Sheppard et al. (1980) found that species richness increased for three different age classes of *Laminaria hyperborea* holdfasts. Age is linked to habitat volume, as holdfasts continually grow through the addition of more haptera and more space is enclosed within. Here, habitat

volume is defined as the space available for colonisation by organisms within the holdfast (see Walls et al. (2016)). The volume and structure of these interstitial spaces have been shown to impact the diversity and abundance of associated assemblages (Jones 1971, Thiel & Vásquez 2000, Blight & Thompson 2008, Tuya et al. 2011, Walls et al. 2016). The distinct holdfast morphologies of wild and cultivated *L. digitata* were suggested as the cause of variations in species richness and community composition by Walls et al. (2016). Wild kelps tend to grow a characteristic flat or slightly conical holdfast when attached to rock (Fig. 2.1a Chapter 2; Fig. 1a Walls et al. (2016)), whereas, cultivated kelps are seeded onto ropes, resulting in a different morphology, formed by intertwined haptera around the rope substratum (Fig. 3.2a). Alterations in abiotic conditions experienced by the holdfast can also cause variations in the assemblages inhabiting the holdfast (Smith 2000, Walls et al. 2016). Smith (1996) found differences in community structure between holdfasts sampled at 2 depths (2 m and 6 m), however depth also influences sediment load, structural complexity and water turbulence (Smith 2000). Changing from a benthic to a suspended substratum can alter both the hydrodynamic environment and sedimentation rates experienced by fauna (Walls et al. 2016). Shifts in other abiotic conditions that influence holdfast assemblages (and by extension, rope-attached assemblages) include hydrodynamic environment (Moore 1972), sedimentation rates (Schaal et al. 2012), oxygen availability (Scarratt 1961), and temperature (Scarratt 1961).

In this study, we focus on the assemblage that develops on suspended ropes primed with the cultivated kelp *Alaria esculenta*. Kelp-associated holdfast epibionts were surveyed at different times during the two cultivation periods to track changes in community composition and development. We compared these primed rope communities with communities that developed on unprimed ropes. The comparison of primed and unprimed treatments allows for an estimation of the effect of ‘ecological priming’. We tested the hypothesis that a priming effect affects the development of assemblages differently across primed and unprimed treatments. Univariate tests examined whether richness of primed and unprimed assemblages varied as experimental treatments were colonised. Multivariate comparisons were made to examine the changes in species identity across colonizing assemblages. If a priming effect exists, it should make colonization sequences more predictable. This hypothesis was tested by comparing the relative timings (as mean occurrence-day) for species to be found on treatments. An assessment of an ecological priming effect associated with cultivated kelps is novel

and, if present, may have important implications for habitat restoration and enhancement of artificial structures. If the development of primed communities is predictable, this would increase the capacity for planning and management in the seaweed cultivation industry. Also, if cultivated holdfasts are found to have distinct assemblages when compared to unprimed treatments, this suggests they supplement the habitat service provided by artificial structures, such as mooring and anchor ropes, with the farm providing an alternative habitat for associated communities.

3.2 Materials and Methods

3.2.1 Study site

This study was conducted in the southwest coast of Ireland in Ventry Harbour, County Kerry ($52^{\circ} 06' 49.45''\text{N}$, $-10^{\circ} 21'20.17''\text{W}$; Fig. 3.1) at the largest operating commercial seaweed farm in Ireland (18 ha site). Ventry Harbour is a moderately sheltered and shallow embayment orientated towards the southeast, approximately 2.5×1.5 km (3.75 km^2) with a wide mouth opening into Dingle Bay. *Zostera marina* (seagrass) is extensively distributed throughout the sandy seabed, leading to a rocky boulder reef towards the mouth of the bay. The licensed seaweed farm is orientated northwest to southeast, and located to the westerly side of Ventry Harbour (Fig. 3.1; Fig. 1 Walls et al. (2017)). The depth underneath the farm is approximately 6 m at the northwestern end before gently sloping to 20 m at the eastern edge of the farm at mean low water spring tide (MLWS). The tidal range in Ventry Harbour is between 0.6 and 4.0 m. Irradiance values, obtained from nearby Valentia weather observatory ($51^{\circ} 56' 23'' \text{N}$, $-10^{\circ} 14' 40'' \text{W}$), ranged from $5,447 - 63,823 \text{ J cm}^{-2}$ for 2014. Sea surface temperature data were obtained from the M3 offshore weather buoy located approximately 56 km southwest of Mizen head ($51^{\circ} 13' 0'' \text{N}$, $-10^{\circ} 33' 0'' \text{W}$), and ranged from $10.1 - 17.6 \text{ }^{\circ}\text{C}$ for 2014. Although offshore values are less extreme than inshore values, Ventry Harbour is a well flushed bay so values are broadly representative. The longline structure and design is similar to the set-up in Fig. 2.3 Chapter 2 (Fig. 3 Walls et al. (2016)) however, the farm in Ventry consists of 3 parallel units of 280 m linear longlines suspended approximately 1.5 m below the sea surface, and the dropper ropes used in this study were 1 m in length. The longlines were kept in position by buoys attached to the header rope and by 1500 kg anchor blocks at either end of the

lines. The farm cultivates the kelps *Alaria esculenta* and *Saccharina latissima* for human consumption, animal feed and use in cosmetic products.

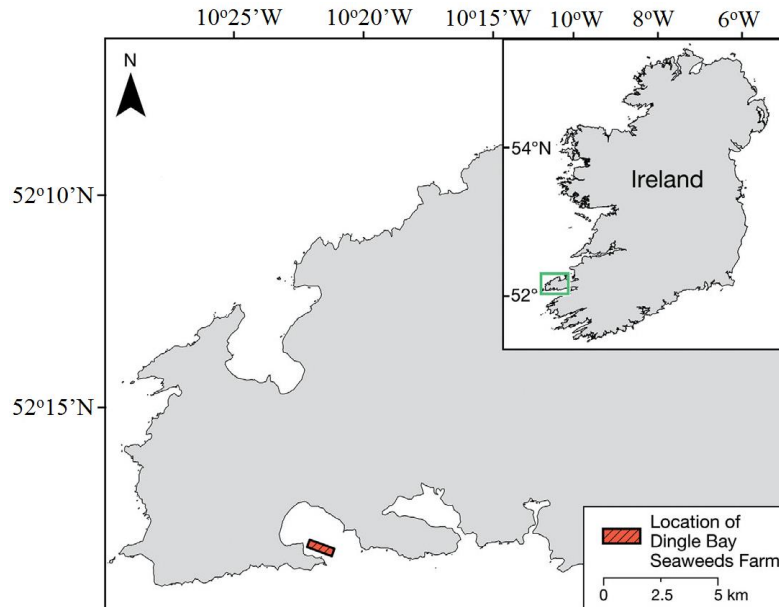


Fig. 3.1. Dingle Bay Seaweeds farm and sampling site at Ventry Harbour, County Kerry, Ireland

3.2.2 Experimental set-up

Experimental treatments were based on 1 m polypropylene dropper ropes (10 mm diameter) with two initial set-ups. Treatment 1 “primed droppers” consisted of ropes sprayed directly with juvenile *A. esculenta* sporophytes that had been developed from gametophyte cultures held in the seaweed hatchery at the NUIG Carna Research Station (County Galway), following standard industry protocols (Edwards & Watson 2011). The primed ropes were left to develop under controlled growth conditions (Edwards & Watson 2011) for between 5 to 12 weeks. Sporophytes were ca. 10 mm at time of deployment. Treatment 2 dropper ropes consisted of clean polypropylene rope and will be referred to as “unprimed droppers” hereon in. Unprimed droppers were submerged in tanks of seawater under the same laboratory conditions as the primed droppers for the same length of time prior to deployment. At deployment, all dropper replicates had a 1 kg concrete weight attached to the end of the rope and were deployed vertically on the longline header rope and spaced 1.5 m apart to mitigate against rubbing and tangling (Walls et al. 2016). Dropper ropes were suspended at a depth of between 1.5 to 2.5 m below the surface of the water which is a depth range experienced at commercial seaweed farms. Each dropper was randomly assigned to a location on the longline

header rope prior to deployment. Primed ($n = 35$) and unprimed ($n=35$) droppers were deployed on the 18th February 2014 for the 2013/2014 growing season (Year 1); deployment was delayed in Year 1 due to winter storms in early 2014. The experiment was repeated for the 2014/2015 growing season (Year 2), when the primed ($n = 35$) and unprimed ($n = 35$) treatments were deployed on the 15th December 2014.

3.2.3 Sampling protocol for primed and unprimed droppers

All samples were collected by SCUBA divers. In April, May and June 2014 and 2015 five droppers were randomly chosen and collected from the primed and unprimed treatments using open-ended mesh bags (150 x 55 cm, 0.5 mm mesh size). If a dropper was not uniformly covered in developing sporophytes (i.e. showed evidence of rubbing or entanglement), another dropper replicate was selected. The mesh bag was carefully slipped up over the dropper and tightly secured at top and bottom (just above the weight) using cable ties, enclosing the entire 1 m dropper and kelp biomass. The focus for the current study was to compare the assemblages associated most closely with the dropper rope. There are potential issues of habitat extent when comparing the assemblages of entire kelp sporophytes with organisms attached to unprimed rope (where large kelp blades did not develop in the experimental time period). We therefore compared the near-rope assemblages of the holdfast with those on unprimed droppers.

All samples were transferred back to nearby facilities at Dingle Oceanworld Aquarium for initial processing within 6 hrs. The sampling technique of bagging fronds and holdfast before separating the material on land potentially risks mixing species attached to the frond with those of the holdfast. However, fronds mainly host attached organisms such as hydroids and bryozoans and less mobile species (Walls et al. 2017) so this form of contamination is likely to be minimal. The alternative, of cutting fronds in situ, risks dislodging loosely attached species on one treatment (primed ropes), but not the other (unprimed ropes). The mesh bags were untied before randomly pre-selected 10 cm sections ($n = 3$) of each dropper were excised from the 1 m dropper (Fig. 3.2a and b). In the rare event that any randomly selected section of primed dropper was not entirely covered in holdfast structures, an alternative section was chosen. The frond and stipe material of primed samples were cut just above the holdfast and stored in sealed plastic bags containing 100 % ethanol for a separate study. The 10 cm primed and unprimed

rope sections were stored in separate sealed plastic bags containing 100 % ethanol. All samples were transported back to the laboratory for further processing.



Fig. 3.2. Morphology of 10 cm section (a) primed *Alaria esculenta* holdfast, (b) unprimed section

3.2.4 Sample processing

The 10 cm replicate primed *A. esculenta* and unprimed sections were removed from the plastic bag and all material including, kelp holdfasts, epiphytes and fauna were cleaned from the substratum. Due to the morphology of cultivated kelp holdfasts individual holdfasts could not be removed as the haptera grow intertwined with each other (see Fig. 2.1b Chapter 2; Fig. 2b Walls et al. (2016) for comparison of morphology of wild and cultivated kelp holdfasts). All collected epibionts from primed and unprimed samples were washed over a 0.5 mm sieve and stored in 100 % ethanol for later identification. All collected flora and fauna were identified to species level where possible using (Hayward 1988, Hayward & Ryland 2002, Bunker et al. 2012). Taxonomy was cross-checked using web resource (WoRMS Editorial Board 2016) and samples were stored in 100 % ethanol.

3.2.5 Statistical analysis

The impact of priming ropes with kelp sporophytes was examined using univariate tests of diversity, multivariate tests of assemblage structure and regressions of mean occurrence-days for different treatment and year combinations.

To compare functional diversity between primed and unprimed treatments, species were grouped into categories based on morphology (algae): thin filamentous algae, foliose algae and leathery macrophyte (Steneck & Dethier 1994, Eriksson et al. 2002); and feeding strategies (fauna): suspension feeder, detritivore,

carnivore, omnivore or herbivore. Where species spanned these categories, their predominant model of feeding was recorded (Sheppard et al. 1980). Occurrences of functional groups were pooled by treatment/month combinations and tested using a two-way sampling month crossed with treatment model with functional group richness as a response variable.

Univariate analysis of variance (ANOVA) was used to examine differences between species richness among primed and unprimed treatments for droppers (random factor) nested within the interaction of treatment (fixed factor) crossed with month (fixed factor) crossed with year (fixed factor).

The differences between assemblage composition from different months and years for the primed and unprimed treatments were compared using multivariate tests. A Simpson's dissimilarity matrix was generated from the presence/absence data for the 6 primed and 6 unprimed treatments and different sampling dates (April, May and June, 2014 and 2015). Simpson's dissimilarity values are 0 when assemblages from separate samples are identical and scaled to 100 when there are no species in common between separate samples. Simpson's dissimilarity has the advantage that it only measures the compositional turnover and is not affected by the relative difference in species richness between samples (Baselga 2010). This makes dissimilarities measured by Simpson's index easier to interpret than is the case for indices that mix turnover and species richness components of dissimilarity (e.g. Sørensen's index). The PRIMER software used for multivariate analysis does not calculate Simpson's dissimilarities, so these were calculated from species presence/absence data in EXCEL using the PopTools add-on (Hood 2014) and dissimilarity matrices were subsequently imported into PRIMER. All samples were ordinated using a multidimensional scaling plot (MDS) (Shepard 1962, Kruskal 1964a, b) in PRIMER V6® (Clarke & Gorley 2006), giving the position of each 10 cm section sample in two-dimensional space based on its species composition.

Permutational multivariate analysis of variance (PERMANOVA, Anderson et al. 2008) was used to test for differences in multivariate species assemblages among primed and unprimed treatments for droppers (random factor) nested within the interaction of treatment (fixed factor) crossed with month (fixed factor) crossed with year (fixed factor), based on 9999 unrestricted permutations of raw data. This design however returned a Negative Pseudo-F value for year factor crossed with

month and treatment. This is sometimes caused when levels of one factor are completely contained within the other factor level, e.g. mean of one level are closer to the mean of the other level than they are to their own mean. We then analysed several different PERMANOVA designs which avoid the term with no result or a negative associated with it. A suitable design removed the year factor and had sampling date as a fixed factor with 6 levels (April-14, May-14, June-14, April-15, May-15 and June-15) and treatment as a fixed factor with 2 levels. PERMDISP routine revealed that the variation in multivariate dispersion (around the centroid) was not significant ($p > 0.5$) for all factors (year, month and treatment).

Where significant differences between sampling dates and treatments were detected, a variation on SIMPER analysis was conducted to highlight the species that contributed most to the observed differences. The SIMPER programme in PRIMER could not be used as Simpson's dissimilarity is not compatible with the algorithm used in the programme. Instead, we conducted a SIMPER-like analysis, hereafter referred to as a test of species influence (Walls et al. 2017), by comparing the observed dissimilarity within and between samples when all species were included to the dissimilarities generated by excluding each species individually. The average between-group and within-group dissimilarities were compared to see whether omitting a species made the groups appear more or less similar. In summary, if a species makes groups more similar when excluded from the matrix, it suggests that the species makes a contribution to the observed differences between groups. Ratios of between to within-group dissimilarities were normalized to facilitate comparisons. Any species greater than one standard deviation of the mean normalized score was identified as having an above average contribution to the overall dissimilarity between the groups.

To test the predictability of the presence of species on primed and unprimed droppers from Year 1 (2014) to Year 2 (2015), we used day of year to calculate the central tendency of species occurrence. The central tendency is the average day when a species was observed for all three sampling dates (April, May & June), weighted by occurrence when observed during each sampling date. Day of year is the number assigned to a whole solar day that starts at 1 on the 1st of January and finishes at 365 on 31st of December (non-leap year), e.g., sampling took place on 7th April 2015 which is day of year 97. The central tendency method as described by Colebrook (1979) can identify changes in the timing of seasonal cycles (Edwards & Richardson 2004, Moore et al. 2011). To test if species arrival

times were similar between the different treatment droppers within the same year the mean species arrival times were compared between treatments within years 2014 and 2015 separately. Regression analysis was conducted using Minitab v16 to test if arrival times of commonly occurring species were significantly related. A slope close to 1 is expected if the timing of species presences is the same from year to year. If the relationship between occurrence days is significant, but the slope is not close to 1, this implies that the order of species occurrence is similar between sets of samples, but the rate of species arrival varies between years.

3.3 Results

In total, we recorded 81 species inhabiting the primed and unprimed 10 cm dropper sections. 54 species were recorded on 2014 primed sections and 63 species were recorded on the 2015 primed sections. A full list of species recorded and their occurrence is provided in Appendix B Table B1. 28 % of all taxa were unique to primed samples including the lumpsucker *Cyclopterus lumpus*, the ascidian *Ciona intestinalis*, and the polychaete *Nereimyra punctata*. Only, 6 % of taxa (5 species) were sampled from the unprimed ropes, 4 of these were algal species including the kelps *Saccorhiza polyschides* and *Saccharina latissima*, the brown algae *Desmarestia viridis* and the green algae *Ulva* sp. The only faunal species unique to unprimed samples was the gastropod *Patella pellucida* which is usually associated with the kelp fronds on which it exclusively feeds. As only one individual was recorded, the occurrence of this species is not particularly informative. Differences in the species richness and community composition of primed and unprimed 10 cm sections did not appear to reflect any replicate-specific variation in sample depth along the 1 m dropper or location of the dropper lines within the farm.

3.3.1 Community composition and development of epibionts on primed and unprimed treatments.

Thin filamentous algae were the most dominant algal functional group for both treatments, with algal diversity higher (4/6 dates) in unprimed treatments when compared to primed treatments in the same month. Suspension-feeding organisms were the most common faunal groups for both treatments, followed by omnivores and detritivores (Table 3.1). Functional group richness increased with time since deployment ($F_{2,6} = 5.7$, $p < 0.05$), but there were no effects of treatment.

Table 3.1. Number of species in each functional group for primed (P) and unprimed (UP) dropper treatments sampled in April, May and June 2014 and 2015.

Functional Groups	April	April	May	May	June	June	April	April	May	May	June	June
	2014	2014	2014	2014	2014	2014	2015	2015	2015	2015	2015	2015
	P	UP	P	UP	P	UP	P	UP	P	UP	P	UP
Thin Filamentous Algae	1	2	2	3	3	4	4	4	3	4	4	5
Foliose Algae	0	0	0	0	0	0	0	0	0	1	0	2
Leathery Macrophyte	1	0	1	1	1	2	1	1	1	3	1	3
Suspension Feeder	5	3	9	9	15	12	8	7	16	13	22	17
Detritivore	2	2	6	3	7	4	3	4	8	5	6	7
Carnivore	1	1	5	6	6	1	4	1	6	3	7	4
Omnivore	7	4	8	8	10	8	6	5	8	7	10	10
Herbivore	0	0	1	1	1	2	0	0	1	1	1	1
Other	0	0	0	0	0	0	0	0	0	1	1	1

Species richness at the seaweed farm site in Ventry increased from time of deployment until the end of the study, for both primed and unprimed treatments. Apart from the accumulation of species with time, the patterns of species richness were not consistent across factors. Significant differences in species richness were recorded for almost all of the factors tested including the dropper nested within the interaction of treatment (primed and unprimed) crossed with month crossed with year from the ANOVA (Fig. 3.3, Table 3.1). Species richness within Year 2 is higher in the primed treatment than in the unprimed treatment for each sampling month. For Year 1 primed was higher than unprimed species richness for April samples, however, richness was lower for primed samples from May and June than unprimed samples.

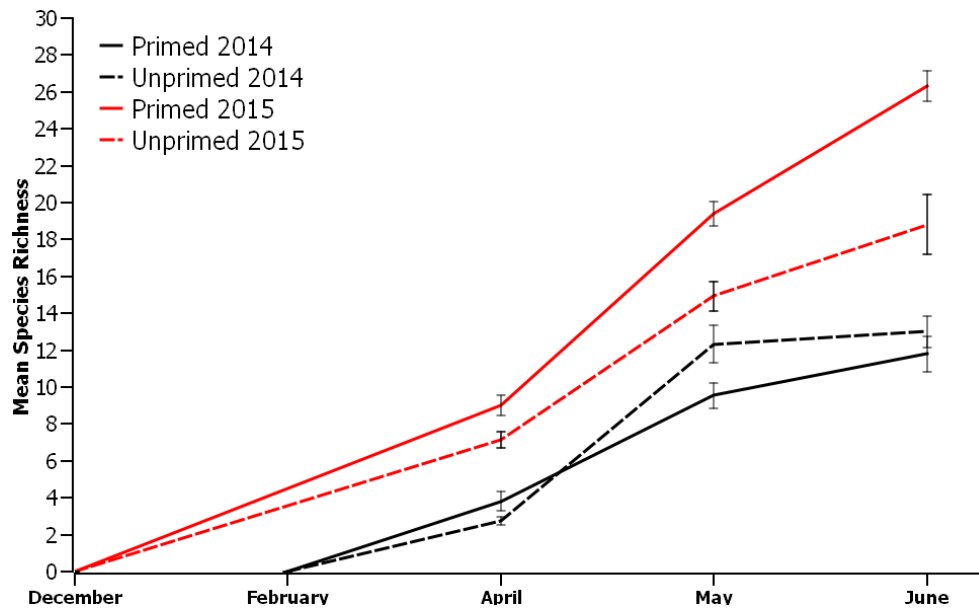


Fig. 3.3. Species richness (mean \pm SE) on primed *Alaria esculenta* holdfast sections and unprimed sections sampled in April, May and June 2014 and 2015. Species richness represents the number of taxa identified on 10 cm section ($n = 3$) from each dropper ($n = 5$).

Table 3.2. ANOVA data of species richness with dropper (1 to 5) nested in month (April, May and June) nested in year (2014 and 2015) crossed with treatment (primed and unprimed). Significant difference at * $p < 0.05$ and ** $p < 0.001$

Source	<i>df</i>	SS	MS	F	p
Year	1	2247.2	2247.2	138.86	0.000*
Month	2	4434.03	2217.02	136.99	0.000*
Treatment	1	149.42	149.42	9.23	0.004*
Year x Month	2	231.63	115.82	7.16	0.002*
Year x Treatment	1	352.8	352.8	21.8	0.000*
Month x Treatment	2	43.68	21.84	1.35	0.269
Year x Month x Treatment	2	133.63	66.82	4.13	0.022*
Dropper (Year x Month x Treatment)	48	776.8	16.18	1.97	0.002*
Error	120	988.0	8.23		

Due to the high stress of the MDS plot (0.25 Fig. 3.4a) patterns of differences among assemblages from separate years, months and treatments were difficult to examine. A stress value of 0.25 indicates the data are only partially represented by the two-dimensional plot and little reliance should be placed on the finer detail of the plot (Clarke & Warwick 1994). However, the broad-scale pattern shows a separation of early April communities to later May and June communities which show little separation, with month also being significant from the PERMANOVA analysis (Table 3.3, $P < 0.001$). There is also a separation of community assemblages between primed and unprimed treatments. This pattern is evident from the PERMANOVA analysis with species assemblage composition differing between treatment (primed and unprimed) from the analysis using the full design; interestingly year, and the interaction of dropper nested within year crossed with month crossed with treatment were also significantly different (Table 3.3, $p < 0.05$). However, this design returned a ‘Negative’ pseudo-F value for year factor and year crossed with treatment. This may be caused by one of the levels of our year factor being contained within the other factor level (Fig. 3.4b).

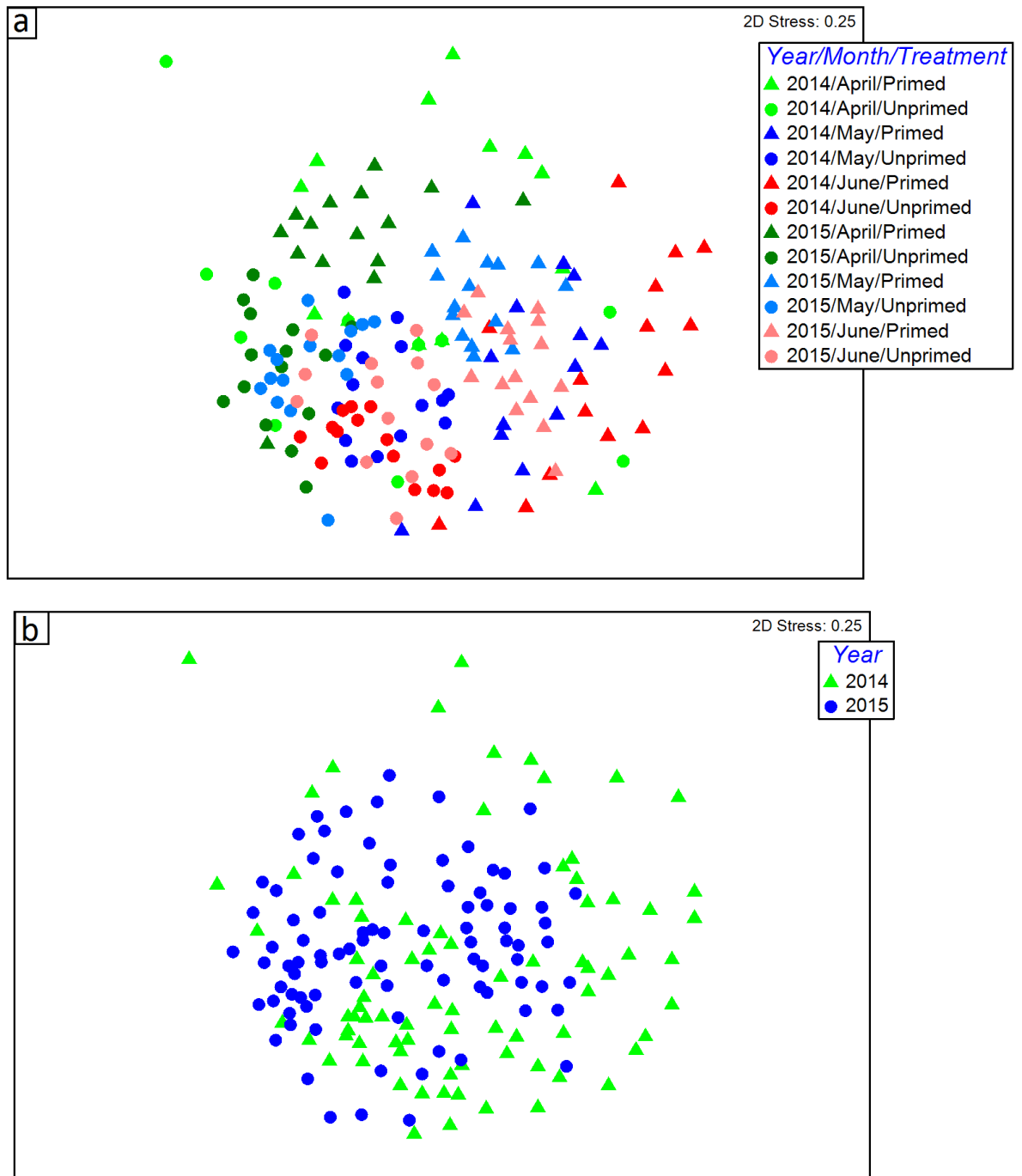


Fig. 3.4a. Two-dimensional MDS plot of 180 primed and unprimed 10 cm samples: 90 from primed treatment and 90 from unprimed treatment, based on presence-absence Simpson's dissimilarity matrix of species collected from each primed and unprimed section (stress = 0.25). Fig. 3.4b. Two-dimensional MDS plot showing how treatment may be the cause of the 'Negative' pseudo-F value in the full PERMANOVA design.

Table 3.3. Permutational multivariate analyses of variance based on Simpsons dissimilarity matrix of presence-absence data of holdfast assemblages for sampling date (April-14, May-14, June-14, April-15, May-15 and June-15) and treatment (primed and unprimed). All tests were conducted using unrestricted permutation of raw data with 9999 permutations.

Significant difference at ** $p < 0.001$

Source	<i>df</i>	SS	MS	<i>F</i>	P (perm)
Year	1	0.694	0.694	7.939	0.001**
Month	2	2.032	1.016	11.628	0.0001**
Treatment	1	3.067	3.067	35.098	0.0001**
Year x Month	2	-0.820	-0.410	NEGATIVE	
Year x Treatment	1	-5.697 x10 ⁻²	-5.697 x10 ⁻²	NEGATIVE	
Month x Treatment	2	0.352	0.176	2.016	0.1016
Year x Month x Treatment	2	0.524	0.262	2.997	0.0231*
Dropper (Year x Month x Treatment)	48	4.2	8.75 x10 ⁻²	2.194	0.0001**
Residuals	119	4.746	3.988 x10 ⁻²		
Total	178	14.764			

To overcome the problem of negative pseudo-F values, we analysed a number of alternative PERMANOVA designs and found the results to be stable across these designs. The most interpretable design removed the year factor and had sampling date as a fixed factor with 6 levels (April-14, May-14, June-14, April-15, May-15 and June-15) and treatment as fixed factor with 2 levels. This design enabled analysis of the year factor interactions without returning a negative result in the PERMANOVA table. The results from this updated design showed, sampling date, treatment, dropper nested within sampling date crossed with treatment were significant ($p < 0.01$) (Table 3.4). A comparison of the dropper nested within year crossed with month and treatment interaction and dropper nested within sampling date crossed with treatment interaction values for sums of squares and mean squares between the two outputs from alternative designs (Table 3.3 and 3.4) are the same (sums of squares: 4.746 and mean squares: 8.750x10⁻²), confirming that the negative output was not causing any unusual results.

Table 3.4. Permutational multivariate analyses of variance based on Simpsons dissimilarity matrix based on presence/ absence data of holdfast assemblages for sampling date (April-14, May-14, June-14, April-15, May-15 and June-15) and treatment (primed and unprimed). All tests were conducted using unrestricted permutation of raw data with 9999 permutations. Significant difference at ** $p < 0.001$

Source	df	SS	MS	F	P (perm)
Sampling Date	5	1.938	0.388	4.435	0.0001**
Treatment	1	3.067	3.067	35.098	0.0001**
Sampling Date x Treatment	5	0.825	0.165	1.887	0.064
Dropper (Sampling Date x Treatment)	48	4.2	8.75×10^{-2}	2.194	0.0001**
Residuals	119	4.746	3.988×10^{-2}		
Total	178				

A test of species influence was conducted to determine which taxa were the major contributors to the observed dissimilarity in assemblage structure between primed and unprimed treatments within the same sampling months (Table 3.5). Most of the species responsible for dissimilarity between treatments were algae and sessile faunal species. The species with higher occurrence on the primed samples were from a variety of different phyla and dissimilarities were not characterised by any specific group. These included the polychaete *Harmothoe* sp. present in May 2014 samples, the bryozoan *Electra pilosa* which contributed to differences between treatments in both June 2014 and May 2015, the amphipod *Gammarellus homari* present in April 2015 and the bivalve molluscs *Anomia eippium* and *Hiatella arctica* which were responsible for some of the differences observed in June 2015. The unprimed treatment was generally characterised by a higher occurrence of algal species during each sampling month. The filamentous brown algae sp. and the red alga *Ceramium* sp. were major contributors to the observed differences between treatments and were present in May 2014 and 2015 and June 2015. *Laminariales* juveniles were present in higher occurrence in all sampling months except May 2014 when they did not contribute to observed dissimilarities. The red algae *Polysiphonia* sp. and *Lomentaria clavellosa* were present in June 2015. The only non-algal species which contributed to the dissimilarity between treatments with higher occurrence in the unprimed treatment was *Harpacticoida* indent. present in June 2014. From the dissimilarity scores May 2014 and April 2015 treatments are less dissimilar than June 2015, however primed and unprimed treatments sampled in June 2014 and May 2015 are the most dissimilar (> 2.00 dissimilarity score, Table 3.5).

Table 3.5. Test of species influence to determine the species contributing to observed differences in the structure of assemblages between primed and unprimed treatments. Dissimilarity scores are the ratio of average between-group dissimilarities to within-group dissimilarities for each pairwise comparison. Normalized score is the reduction in dissimilarity score when excluding the species of interest, normalized to mean = 0, SD = 1 using the mean and standard deviation of all individual species' scores. A higher loss in dissimilarity indicates that a species is important in distinguishing the dates compared.

Months	Dissimilarity score all species included	Species responsible for dissimilarity	Normalized score	Species occurrence (max. 15 mo ⁻¹)	
				Primed	Unprimed
May 2014 Primed & Unprimed	1.56	Filamentous brown algae sp.	4.25	1	< 14
		<i>Ceramium</i> sp.	1.72	2	< 12
		<i>Harmothoe</i> sp.	1.34	6	> 2
June 2014 Primed & Unprimed	2.00	<i>Electra pilosa</i>	4.13	12	> 0
		<i>Laminariales</i> juv.	3.28	0	< 15
		<i>Harpacticoida</i> indent.	1.94	3	< 15
April 2015 Primed & Unprimed	1.62	<i>Laminariales</i> juv.	4.70	0	> 12
		<i>Gammarellus homari</i>	1.37	10	< 0
May 2015 Primed & Unprimed	2.06	Filamentous brown algae sp	4.42	0	< 14
		<i>Laminariales</i> juv.	4.37	0	< 14
		<i>Electra pilosa</i>	1.19	15	> 1
		<i>Ceramium</i> sp.	1.15	7	< 14

Table 3.5. Continued

Months	Dissimilarity score all species included	Species responsible for dissimilarity	Normalized score	Species occurrence (max. 15 mo ⁻¹)		
				Primed	Unprimed	
June 2015 Primed & Unprimed	1.84	<i>Ceramium</i> sp.	4.09	4	<	15
		Filamentous brown algae sp.	3.07	1	<	11
		<i>Polysiphonia</i> sp.	2.64	5	<	14
		<i>Laminariales</i> juv.	1.89	5	<	12
		<i>Anomia epiphium</i>	1.59	15	>	1
		<i>Hiatella arctica</i>	1.10	15	>	2
		<i>Lomentaria clavellosa</i>	1.06	1	<	8

3.3.2 Predictability of holdfast assemblages on primed and unprimed treatments

Shared species between and within treatments included algal species and both sessile and mobile faunal species. The temporal pattern of shared holdfast species was consistent between years for the primed treatment; however, this pattern was not the same in the unprimed treatment. The regression of mean species occurrence in 2014 and 2015 was significant in primed treatments ($P < 0.001$) (Table 3.6, Fig. 3.5a). In contrast, patterns of mean species arrival on unprimed treatments were not consistent between 2014 and 2015 ($P > 0.05$; Table 3.6, Fig. 3.5b).

Recruitment by shared species to both treatments was similar within a year. The timing of species occurrence was consistent across treatments within the same year for both 2014 and 2015. The regression slope relating mean day of year of shared species occurrence samples was significant within both years ($p < 0.05$) (Table 3.6, Fig. B1a and B1b Appendix B).

Table 3.6. Comparisons of mean species occurrence between treatments and years. Significance at ** $p < 0.001$

Comparisons	R ²	Slope & Std Error	Intercept & Std Error	Regression P-value
Primed-2014 vs. 2015	0.3844	0.659 (± 0.1352)	52.363 (± 20.2934)	$< 0.001^{**}$
Unprimed-2014 vs. 2015	0.0234	0.206 (± 0.2286)	124.269 (± 35.1214)	0.373
2014- Primed vs. Unprimed	0.4982	0.534 (± 0.0979)	69.951 (± 14.7025)	$< 0.001^{**}$
2015- Primed vs. Unprimed	0.3712	0.658 (± 0.1322)	55.103 (± 20.2058)	$< 0.001^{**}$

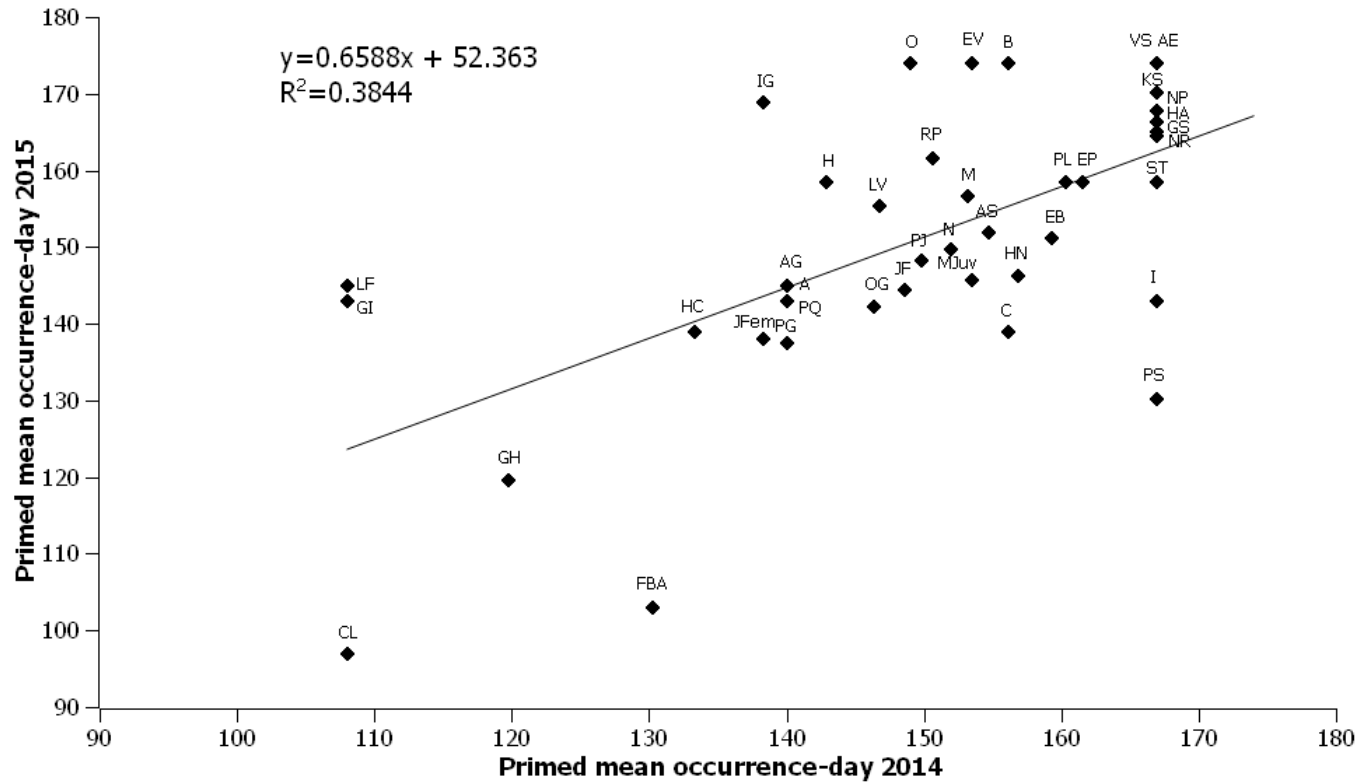


Fig. 3.5a. The relationship between mean day of year occurrences for all shared species in 2014 and 2015 on primed holdfast samples.

Legend: CL: *Cyclopterus lumpus*; LF: *Laomedea flexuosa*; GI: *Gammarus insensibilis*; GH: *Gammarellus homari*; FBA: filamentous brown algae sp; HC: Harpacticoid Copepods; JFem: *Jassa* sp. female; IG: *Idotea granulosa*; PG: Pycnogonida indent.; A: *Aora* sp.; AG: *Aora gracilis*; PQ: *Polycera quadrilineata*; H: *Harmothoe* sp.; OG: *Obelia geniculata*; LV: *Lacuna vineta*; JF: *Jassa falcata*; O: Ostracoda indent.; PJ: *Parajassa pelagica*; RP: *Rissoa parva*; N: Nematoda indent.; M: *Myrianida* sp.; EV: *Eulalia viridis*; MJuv: *Mytilus* sp. juvenile; AS: *Asterias* sp.; B: *Balanus* sp.; C: *Ceramiales* sp; HN: *Hardametopa nasuta*; EB: *Eusyllis blomstrandii*; PL: *Pisidia longicornis*; EP: *Electra pilosa*; ST: *Spirobranchus triqueter*; NR: *Nereiphylla rubiginosa*; NP: *Nereimyra punctate*; GS: *Gitana sarsi*; KS: *Kellia suborbicularis*; HA: *Hiatella arctica*; I: *Idotea* sp.; PS: *Polysiphonia* sp; AE: *Anomia epiphium*; VS: *Vesicularia spinosa*

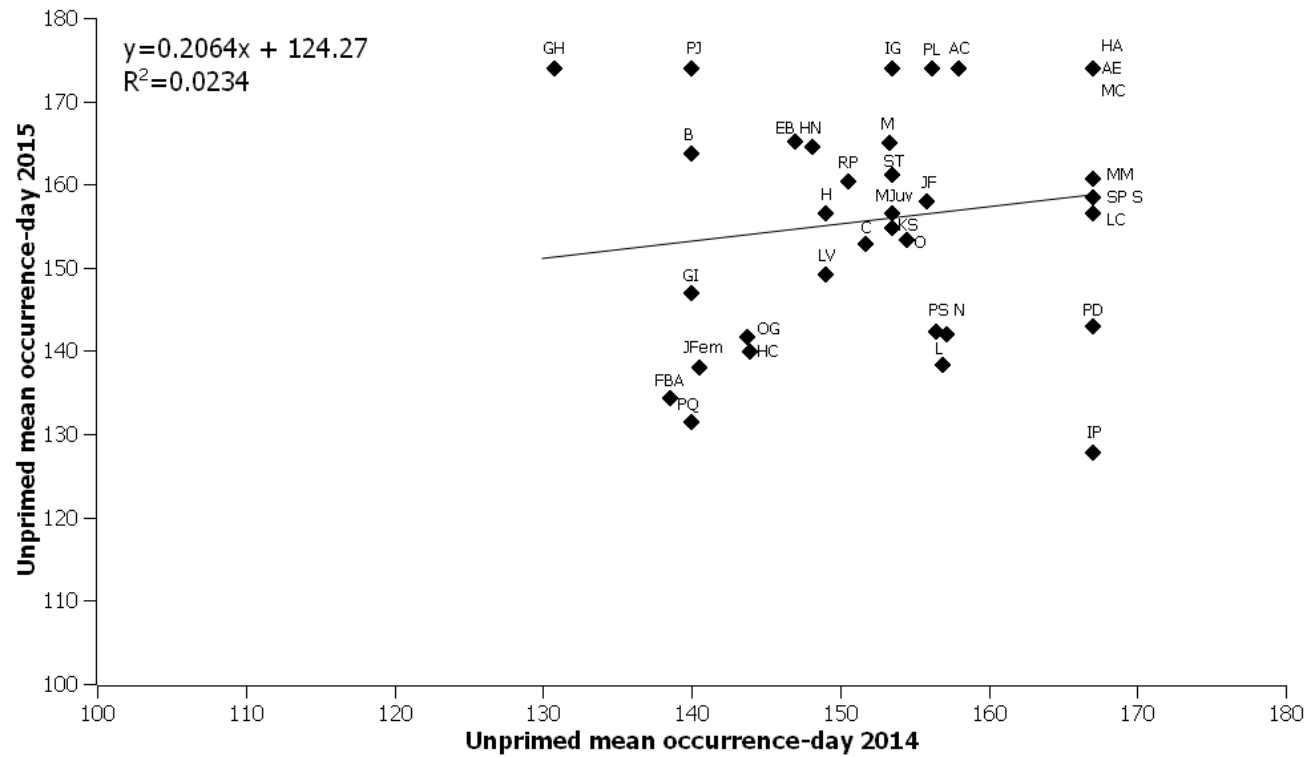


Fig. 3.5b. The relationship between mean day of year occurrences for all shared species in 2014 and 2015 on unprimed samples.

Legend: GH: *Gammarellus homari*; FBA: filamentous brown algae sp; GI: *Gammarus insensibilis*; B: *Balanus* sp.; PQ: *Polycera quadrilineata*; .; PJ: *Parajassa pelagica*; JFem: *Jassa* sp. female; OG: *Obelia geniculata*; HC: Harpacticoid Copepods; EB: *Eusyllis blomstrandii*; HN: *Hardametopa nasuta*; LV: *Lacuna vincta*; H: *Harmothoe* sp.; RP: *Rissoa parva*; C: *Ceramiales* sp; M: *Myrianida* sp.; ST: *Spirobranchus triqueter*; IG: *Idotea granulosa*; MJuv: *Mytilus* sp. juvenile; KS: *Kellia suborbicularis*; O: Ostracoda indent; JF: *Jassa falcata*; PL: *Pisidia longicornis*; PS: *Polysiphonia* sp; L: *Laminariales* juvenile.; N: Nematoda indent.; AC: *Acari* sp.; SP: *Saccorhiza polyschides*; LC: *Lomentaria clavellosa*; MM: *Membranipora membranacea*; PD: *Platynereis dumerilii*; S: Sipuncula indent.; IP: *Idotea pelagica*; MC: *Musculus costulatus*; AE: *Anomia eippium*; HA: *Hiatella arctica*

3.4 Discussion

3.4.1 Community composition and development of epibionts on primed *Alaria esculenta* 10 cm holdfast sections and unprimed sections.

Diverse assemblages developed on both primed *Alaria esculenta* sections and unprimed sections. The assemblage developed from unfouled material at deployment to 63 individual taxa sampled on the primed treatment and 54 taxa sampled on the unprimed treatment in 2015 which had higher species richness than 2014. This general build-up of species over sampling months followed a seasonal pattern of development from time of deployment in February (Year 1) and December (Year 2) until harvest in June for both growing seasons. The species identified on our primed samples have all been previously recorded on wild or cultivated kelp holdfasts (Jones 1971, Christie et al. 2003, Blight & Thompson 2008, Tuya et al. 2011, Schaal et al. 2012, Walls et al. 2016). Although we did not record species abundance directly, the dominant faunal groups based on species occurrence in our primed samples were amphipod crustaceans, polychaetes and molluscs. This agrees with previous studies of wild kelp holdfasts from European waters including Blight & Thompson (2008), Christie et al. (2003) and Walls et al. (2016), the latter of which is the only study of cultivated holdfast assemblages to our knowledge.

Functional diversity was dominated by suspension feeders, omnivores and detritivores for both our primed and unprimed samples. However, algal diversity was higher on unprimed samples with 4 of the 5 unique species on unprimed samples being algae, e.g. the kelps *Saccorhiza polyschides* and *Saccharina latissima*, the green algae *Ulva* sp., and *Desmarestia viridis* a filamentous brown alga. Consistent higher species richness in primed samples during early sampling suggests that habitat availability is very important for the colonization of species during early successional stages. The importance of habitat availability decreases as communities develop, with less consistency between the treatments with higher species richness. The rapid colonisation of this novel habitat suggests that the species have either planktonic larval settlement or if they have direct development, species are highly mobile (Walls et al. 2016). Fouling epibionts such as bryozoans, hydroids, molluscs and crustaceans begin to settle in spring and early summer, which coincides with deployment and the cultivation period for kelps (Walls et al.

2017). The sources of these colonizing species remains unknown. The nearest wild kelp populations are between 250 m (mouth of Ventry Harbour opening up to Dingle Bay) and 1 km (northeast direction from farm in Ventry Harbour) away from the farm site. Cultivation practices are not harmonized within the sector or even between years at the same site, however at Ventry Harbour over the duration of this study, the header and dropper ropes were taken in from sea and cleaned after each harvest season, also anchor chains were cleaned *in situ* by divers at irregular intervals. As a result, fauna inhabiting the farm infrastructure are unlikely to be a source for colonisation of the growing kelp.

Analysis of the community composition of primed and unprimed treatments revealed several important patterns, which remained constant between seasons. Assemblages were clearly distinct between treatments and also between sampling dates, with primed samples showing more separation during community development than unprimed samples. The dissimilarity results imply that species are not simply accumulated over time; there are compositional differences between early and later samples. Change in community composition was through addition of new species and replacement of early colonisers. This pattern was more evident in primed samples; with the species filamentous brown algae sp., showing higher occurrence in April samples with much reduced occurrence in later May and June samples.

Algal species were revealed to be the main cause of variation between community composition of treatments from the test of species influence. Filamentous brown algae sp., *Ceramiales* sp., *Laminariales* juveniles, *Polysiphonia* sp. and *Lomentaria clavellosa* were more closely associated with the unprimed treatment. The presence of *A. esculenta* from the beginning of colonisation may pre-empt other algal species from settling and dominating the primed droppers. Benedetti-Cecchi (2000) studied the effect of disturbance on turf and canopy-forming algae in Italy. He found that canopy-forming algae dominated cleared patches of substratum during their main recruitment period; even though turf-forming algae were initially present they were replaced by canopy-forming algae. However, turf-forming algae would characterise early stages of colonisation and mature assemblages in patches that were cleared outside of the main recruitment period of the canopy-forming algae. In our study, unprimed samples were dominated by filamentous and ephemeral algae species, which seemed unable to colonise primed samples potentially because of the presence of *A. esculenta*.

Furthermore, the bryozoan *Electra pilosa* was only present on primed samples late in the sampling period with very low occurrence in the unprimed treatment. *E. pilosa* settles in early spring (Ryland & Hayward 1977) and is found to be out competed by *Membranipora membranacea* on kelp fronds (Ryland 1962), however because *M. membranacea* is highly selective in habitat it does not thrive on other parts of the kelp thus *E. pilosa* is the dominant bryozoan on holdfast samples. Additionally, in the absence of *M. membranacea*, *E. pilosa* is also selective and was unable to settle on the unprimed samples. The polychaete *Harmothoe* sp., the amphipod *Gammarellus homari*, and the bivalves *Anomia ephippium* and *Hiatella arctica*, were all more closely associated with the primed treatment and have all been previously recorded on kelp holdfasts (Christie et al. 2003, Blight & Thompson 2008, Walls et al. 2016) and are suggested to utilise the crevices provided by the structurally complex holdfast morphology.

3.4.2 Predictability of primed and unprimed communities from one year to the next

Between growing seasons commonly occurring species for primed samples were found to be predictable using the central tendency method and mean occurrence-day (Table 3.6, Fig. 3.5a) This consistent pattern for primed samples was significant despite variation in deployment date and sampling date between years and factors such as water temperature, turbidity, irradiance and salinity presumably also varying from one year to the next (Walls et al. 2017). This pattern was not replicated for shared species on unprimed samples between years (Table 3.6, Fig. 3.5b). However, the colonisation of shared species between treatments within the same year was shown to be predictable (Table 3.6, Fig. B1a and B1b Appendix B). This informs us that treatment did not affect arrival and colonisation of shared epibionts within years and suggests that primed *A. esculenta* ropes are habitat to a specific assemblage whereas the unprimed habitat had more loosely associated assemblages. Interestingly, a predictable pattern was also observed between shared frond epibionts on cultivated *A. esculenta*, but patterns of mobile fauna from the same site sampled at the same time were not predictable (Chapter 5). Walls et al. (2017) suggested that the predictability of organism arrival times observed on their frond samples could be attributed to flushing times within the bay affecting local larval pools; this could also be a probable explanation for our primed holdfast assemblages (Herben 2005, Jessopp et al. 2007). Ecological priming with juvenile *A. esculenta* sporophytes provides a biological platform that

influences the development of predictable communities whereas the unprimed substratum leads to the development of unpredictable communities. This is an important consideration from a management perspective and the ability to understand the timing of occurrence of organisms and predict their arrival has significant benefits for the seaweed cultivation industry and management (Walls et al. 2017). With this knowledge, seaweed farmers can exert some control over the quality of their crop by being able to decide on a date-by-site basis when the optimum time to harvest is to avoid detrimental fouling species attaching to their crop. This study was only conducted over a two-year period and analysis of communities over longer durations would be required before definitive conclusions can be made.

Ecological priming using kelp sporophytes provides the complex physical structure that is the holdfast which has many interstitial spaces for epibionts to colonise. The holdfast also offers protection from predators and adverse environmental conditions (Norderhaug et al. 2002), accumulates food sources (Moore 1972) and increases the area of substratum and volume of habitable space available for colonisation (Ojeda & Santelices 1984, Teagle et al. 2017). Cultivated kelp holdfasts likely provide similar resources to colonising communities as wild kelps (Walls et al 2016). Hauser et al. (2006) experimentally altered the complexity of artificial holdfast mimics and found significantly lower diversity on low complexity mimics in comparison to those with higher complexity. The organisms inhabiting low complexity habitats need to be highly mobile to escape predation as there is less physical structure for refuge and food may be more difficult to find as it is not concentrated within the structure (Hauser et al. 2006). Hauser et al. (2006) also suggests that higher complexity habitats offer a greater surface area for attachment of species, in addition to providing a larger surface area to catch organisms floating in that water column. The latter point is especially interesting in the context of our droppers which are suspended within the water column, and thus are more likely to attract larvae and pelagic organisms drifting in the water. As a consequence of ecological priming our primed samples are more predictable than unprimed samples potentially due to the foundational structure provided by kelp holdfasts.

3.4.3 Succession of epibiont assemblages of primed *Alaria esculenta* holdfasts

There appear to be no published descriptions of succession on cultivated holdfasts so comparisons must be drawn from wild holdfast studies and successional studies from alternative systems. Kelp successional studies used holdfast volume rather than holdfast age to analyse succession, due to difficulties in determining the age of holdfasts partly because of the indistinct nature of growth rings and the lack of comparative data using age rather than volume in other studies (Smith et al. 1996). Interestingly, several studies suggest that successional processes do not involve species replacement but rather an additive progression (Ojeda & Santelices 1984, Smith et al. 1996, Smith 2000, Teagle et al. 2017). Smith et al. (1996) found that while early colonists on *Ecklonia radiata* holdfasts generally had a shift in dominance in larger holdfasts, all species that were recorded in smaller holdfasts were also present in larger samples. This was evident in *Macrocystis pyrifera* holdfasts that had a shift in dominance of polychaetes in smaller samples to a more diverse community in larger samples in which echinoids and decapod crustaceans were dominant (Ojeda & Santelices 1984). Ojeda & Santelices (1984) suggested that this form of succession may be more characteristic of habitats that grow, such as corals and sponges. This type of successional process is dissimilar to many other habitats where succession has been studied, including our cultivated kelp holdfasts, in which community change involved the replacement of early colonists with later species (Connell & Slatyer 1977, Dean & Connell 1987, Platt & Connell 2003, Cifuentes et al. 2010). In rocky shore and artificial habitats, the timing of disturbance or the creation of free-space can influence richness and abundance of initial colonizers which in turn affects succession (Sousa 1979, Dayton et al. 1984, Benedetti-Cecchi & Cinelli 1993, Underwood & Chapman 2006, Cifuentes et al. 2010). This is partly due to seasonality in organisms' reproductive patterns and/or growth and seasonal variation in environmental conditions (Jenkins & Martins 2010). This effect of timing was observed in the initial differences between early primed samples. However, as clearly evident from our primed samples and a number of other successional studies, varying successional trajectories subsequently converge towards a local climax community (Underwood & Chapman 2006, Cifuentes et al. 2010, Antoniadou 2014, Evans et al. 2016, Walls et al. 2017). One such study, Cifuentes et al. (2010), proposed that initial and intermediate successional stages can be highly variable, while late stages are highly deterministic if a dominant species is present that uses the available energy efficiently. This leads to a

convergence of communities with different start points. As our study followed succession over the first 4-7 months of development of primed and unprimed communities we do not know if these communities will converge into one climax state dominated by a superior competitor(s), either within the individual treatments or between treatments. However, from previously conducted studies it is highly probable that they could converge to similar end-point communities

3.4.4 Outlook

This study identifies a unique habitat provided by the ecological priming of droppers with *A. esculenta* sporophytes, creating a complex physical structure with a distinct community when compared to unprimed ropes. This distinct community may be attracted to the habitat and refuge provided by the interstitial spaces between the holdfast haptera and its ability to accumulate food. The effect of primed ropes may reflect suppression of algal species that would otherwise colonize suspended ropes, and the facilitation of species that have a particular association with kelps. The restoration of kelp forests (Carney et al. 2005, Yu et al. 2012) and the transplantation of habitat forming species (Perkol-Finkel et al. 2012, Ferrario et al. 2016) onto artificial structures has gained increased interest recently in attempts to mitigate the potential negative anthropogenic impacts of ocean sprawl (Airoldi & Beck 2007, Firth et al. 2016a,b). Deployment of seaweed lines may aid in habitat restoration by supplying spores and gametophytes to wild kelp beds that have been damaged by anthropogenic impacts, or by transplanting seeded kelp juveniles directly onto artificial structures. Ecologically priming the substratum with kelp seems likely to lead to the development of particular predictable associated communities. Colonisation onto kelps can occur from settlement of larvae or migration by mobile fauna (Walls et al. 2016, 2017). The duration of the ‘seeding’ effect remains to be defined. Communities may become more similar over time (depending on successional processes and dominant species), or the influence of a kelp-dominated habitat may increase the longer the longlines are left in the water column. The cultivation practices for kelps are subject to change and development. Harvesting practices may be adjusted so that holdfasts, stipes and small fronds remain in place for more than one growing season, however the applicability of these techniques depends on culture species. The importance of priming effects may depend on changes to cultivation practice and there is a need for further research to fully understand the novelty of habitats created by seaweed cultivation.

Acknowledgements

The authors acknowledge Dr. Benoît Quéguineur, Mr. David Moran and Mr. Brendan Walls for their assistance in field sampling. In addition, they thank Dr. Adrian Patterson and Dr. Jack O'Carroll from NUI Galway's Benthic Ecology Laboratory for taxonomic support and Mr. Michael Murphy and Mr. Paul Flannery of Dingle Bay Seaweed for boat work and assistance at Ventry Harbour and staff at Dingle Oceanworld Aquarium for providing laboratory space. This work was supported by the Energetic Algae project (EU Interreg IVB NWE Strategic Initiative; www.enalgae.eu). A.M.W. is funded by the Dr. Tony Ryan Research Trust, NUI Galway.

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Chapter 4

Successional changes of epibiont fouling communities of the cultivated kelp *Alaria esculenta*: predictability and influences

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Published: Aquaculture Environment Interactions 9 (2017) 55-69

Role: Lead author, responsible for overall sampling (preparation and collection), data processing, data analysis and writing of manuscript. Mark Johnson, Maeve Edwards and Louise Firth contributed to the conception and design of the sampling regime, analysis and interpretation of the data, and comments on the manuscript.

Abstract

There has been an increase in commercial-scale kelp cultivation in Europe, with fouling of cultivated kelp fronds presenting a major challenge to the growth and development of the industry. The presence of epibionts decreases productivity and impacts the commercial value of the crop. Several abiotic and biotic factors may influence the occurrence and degree of fouling of wild and cultivated fronds. Using a commercial kelp farm on the southwest coast of Ireland, we studied the development of fouling communities on cultivated *Alaria esculenta* fronds over 2 typical growing seasons. The predictability of community development was assessed by comparing mean occurrence-day. Hypotheses that depth, kelp biomass, position within the farm and the hydrodynamic environment affect the fouling communities were tested using species richness and community composition. Artificial kelp mimics were used to test whether local frond density could affect the fouling communities. Species richness increased over time during both years, and species composition was consistent over years with early successional communities converging into later communities (no significant differences between June 2014 and June 2015 communities). The timing of species occurrences was predictable across years for all shared species. Variations in biomass, depth and position within the farm had no significant effect on species richness and composition. Results from artificial kelp mimics suggest possible hydrodynamic effects. The ability to understand succession and the timing of occurrences of fouling organisms and predict their arrival has significant benefits for the seaweed cultivation industry.

Keywords: *Alaria esculenta*; Macroalgae; Aquaculture; Frond; Fouling; Epibiont; Predictability; Influences

4.1 Introduction

Traditionally, the cultivation of kelp has primarily been restricted to Asia (Murata & Nakazoe 2001) with comparatively lower biomass cultivated in European waters. However, over the last few years interest in kelp cultivation in Europe has increased, supported by feasibility studies (e.g. Bruton et al. 2009) and experimental farms which are being set up to begin to industrialise the industry and advance the cultivation of kelps native to this region (including several research projects, e.g. At~Sea 2015, www.atseaproject.eu; EnAlgae 2015, www.enalgae.eu; and MAB3 2015, www.mab3.dk). This interest includes Ireland with the establishment of Dingle Bay Seaweed in Ventry Harbour, County Kerry, in 2009 as one of the larger commercial kelp farms (18 ha) in Europe (M. D. Edwards pers. obs.).

A major challenge to the development and growth of the seaweed sector is the undesirable attachment of fouling organisms on kelp fronds. Here, when we refer to fouling organisms we include both mobile invertebrates and sessile species. These organisms will be referred to collectively as epibionts, with the terms epifauna and epiphytes referring to animal and alga epibionts, respectively (Wahl 1997). The presence of epibionts decreases the productivity of farms (Fletcher 1995, Peteiro & Freire 2012) and impacts the commercial value of the crop (Park & Hwang 2012). The negative impacts of fouling on seaweeds have been well documented for both wild and cultivated fronds. These include a potential loss of biomass, as fouling organisms can affect the flexible nature of kelp, resulting in increasing breakage of fronds (Dixon et al. 1981, Krumhansl et al. 2011). Individuals can be torn from substrata due to increased drag from epibionts (Dayton 1985, D'Antonio 1985). Frond fouling can also lead to necrosis of kelp tissue (Fletcher 1995, Peteiro & Freire 2013b) and the inhibition of reproduction by preventing spore release (D'Antonio 1985, Saier & Chapman 2004). Photosynthesis can be decreased as fouling organisms can create a barrier to nutrient uptake (Hurd et al. 2000), can block the surface area of the frond (Hepburn et al. 2006) and restrict light availability (Cancino et al. 1987). In addition, heavily fouled fronds have a lower market value as they are considered to be unsuitable for human consumption due to a degradation of taste and quality (Park & Hwang 2012, Peteiro & Freire 2013b, Getachew et al. 2015). Lower-value fronds are instead used for animal feed (Bruton et al. 2009) and severely fouled fronds are discarded (Peteiro & Freire 2013b).

Outside Europe, research has been conducted on the fouling organisms associated with cultivated seaweeds for many decades. For example, studies of *Gracilaria* sp. farming in Chile, which mainly focused on epiphytes (Buschmann & Gómez 1993, Fletcher 1995, Leonardi et al. 2006) and kelp farming in Asia, with a focus on fouling hydroids and copepods (Park et al. 1990, 2008, Park & Hwang 2012). However, due to the slower growth of the cultivation industry in Europe, research on associated epibionts has only begun more recently (Peteiro & Freire 2013b, Førde et al. 2016, Walls et al. 2016). Similar species have been found to be responsible for fouling on both wild and cultivated kelp fronds, including bryozoans (Førde et al. 2016), hydroids, amphipods, harpacticoid copepods and gastropods (Norton et al. 1977, Seed & Harris 1980, Park et al. 2008, Park & Hwang 2012, Peteiro & Freire 2013b). Among the few published studies that exist, often only an investigation of fouling communities at the end-point of the cultivation period has been conducted (Peteiro & Freire 2013b). In other cases, the development of a single group or a fouling species that may have a major negative effect on the fronds has been followed in isolation over the growing season (Park et al. 2008, Park & Hwang 2012, Førde et al. 2016).

Both abiotic and biotic factors are known to influence the occurrence and degree of fouling on the fronds of both wild and cultivated kelps (Vairappan 2006, Peteiro & Freire 2013b). Abiotic factors include seasonal variation of environmental parameters (temperature, light and salinity), location, hydrodynamics and depth. Cultivated kelps are generally deployed in winter and harvested in early summer to match the natural growing season of their wild kelp counterparts, and this coincides with temperature and light increases which also bolster the growth of epibiotic organisms. Vairappan (2006) found that outbreaks of epiphytes on farmed *Gracilaria* sp. were correlated with drastic changes in seawater temperature and salinity. Increasing water temperatures have also been associated with hydroid and copepod infestations on cultivated kelps in Korea (Park et al. 2008, Park & Hwang 2012). Peteiro & Freire (2013b) found that within a bay on the northwest coast of Spain, higher fouling occurred in a sheltered area of the bay than in a more exposed area. On a smaller spatial scale, location within the farm may also have an effect, as the outer areas of the farm may receive greater water flow and correspondingly exhibit less fouling than in the sheltered middle area of the farm (Peteiro & Freire 2013b). The hydrodynamics around fronds may be influenced by frond density, with different water movement occurring around

densely growing fronds compared with fronds growing at lower densities, which in turn will affect frond fouling (Peteiro & Freire 2013b, Peteiro et al. 2016). The position of fronds in the water column has also been found to have an impact on fouling organisms. Førde et al. (2016) found that bryozoan population densities decreased with increasing depth of cultivated kelp in Norway.

Biotic factors found to have an influence on frond fouling include kelp age, frond morphology, secondary metabolite production and interactions between fouling organisms. Kelp fronds grow from the meristematic region at the base of the frond, so the tip or distal end of the frond is the oldest part of the frond and can have more fouling than younger parts of the frond (Jennings & Steinberg 1997, Park et al. 2008, Park & Hwang 2012, Zagal et al. 2013). This may simply be a reflection of accumulation and growth of epibionts over time (Jennings & Steinberg 1997) or reduced physiological activity within the older tissue (Park & Hwang 2012) allowing greater colonisation by organisms. Frond morphology, such as surface roughness and thickness, has also been found to affect fouling (Jennings & Steinberg 1997, Peteiro & Freire 2013b). Fletcher & Day (1983) found no significant difference in epifaunal abundance between smooth and crinkled *Ecklonia radiata* fronds; however, diversity was greater on the more complex habitat of the crinkled fronds than on the smooth fronds. Many large brown algae, including kelps, produce antifoulants, which may hinder growth and settlement of fouling organisms (Al-Ogily & Knight-Jones 1977) and may be significant in determining epibiont abundance and species richness (Blight & Thompson 2008, but see Jennings & Steinberg 1997). Interactions between fouling organisms such as grazer abundance and predation can in turn affect the abundance and distribution of other fouling organisms. Marzinelli et al. (2011) modelled the presence of artificial habitats such as pilings on sea urchin abundance and found that a decrease in the abundance of sea urchins caused an increase in the cover of fouling bryozoans on kelp. In addition, competition between species can have an effect. For example, Førde et al. (2016) studied 2 bryozoan species on cultivated *Saccharina latissima* in Norway and found that competition between the 2 species occurred through overgrowing and habitat selection (Ryland 1962, Seed & O'Connor 1981).

The kelp *Alaria esculenta* (Linnaeus) Greville is an economically valuable seaweed which is mainly cultivated for human consumption as it is rich in sugars, vitamins and protein (Guiry & Blunden 1991). Also known as 'Atlantic Wakame',

it is similar to the true ‘Wakame’ *Undaria pinnatifida* which is extensively cultivated in Japan, eastern China and South Korea (Yamanaka & Akiyama 1993). *A. esculenta* has been cultivated in Ireland for the last 15 years (Kraan & Guiry 2001) and is an economically attractive species due to its high growth rate of up to 10 cm d⁻¹ (Druehl et al. 1988), as well as being native to Irish coastal waters (Kraan & Guiry 2001). Other uses of *A. esculenta* include animal fodder, biochemical extracts used in cosmetic products and the production of alginates, as it contains up to 42 % alginic acid (Kraan & Guiry 2001). *A. esculenta* is already one of the most cultivated species in Ireland (M. D. Edwards pers. comm.) and worldwide demand for cultivated kelp is expected to continue to expand (FAO 2016). There is a paucity of literature on the ecology of fauna associated with wild *A. esculenta*, possibly due to difficulties in sampling the wave-exposed rocky shores where this species is typically found (Kraan et al. 2000). Future expansion of *A. esculenta* cultivation requires an understanding of the associated epibionts, particularly to inform seaweed farmers on the best time to harvest their crop to reduce losses of biomass as a result of the presence of fouling organisms.

Given the importance of fouling to commercial kelp production and the gaps in knowledge of the communities associated with cultivated *A. esculenta*, the main aim of this study was to describe the development of fouling communities on the fronds of cultivated *A. esculenta* over a typical growing season. This was conducted by identifying all associated epibionts during different times of the cultivation period to track changes in community composition and development. We tested the predictability of epibiont communities from one growing season to the next by comparing their mean occurrence-day. If the fouling sequence is predictable, this would allow farmers to estimate the optimal time for harvesting before fouling decreases the value of the crop. Alternative hypotheses that depth, kelp biomass, position within the farm and the hydrodynamic environment have effects on fouling communities were tested using the species richness and community composition of fouling communities on cultivated fronds. Artificial kelp mimics were used to test whether local frond density could affect the fouling communities, with the null hypothesis being that local (frond scale) changes in current speeds associated with frond density are not sufficient to modify fouling assemblages.

4.2 Materials and Methods

4.2.1 Study site

This study was conducted in the southwest coast of Ireland in Ventry Harbour, County Kerry ($52^{\circ} 06' 49.45''$ N, $10^{\circ} 21' 20.17''$ W; Fig. 4.1), at the largest operating commercial seaweed farm in Ireland (18 ha site). Ventry Harbour is a moderately sheltered and shallow embayment orientated towards the southeast, approximately 2.5×1.5 km (3.75 km²) with a wide mouth opening into Dingle Bay. *Zostera marina* (seagrass) is extensively distributed throughout the sandy seabed, leading to a rocky boulder reef towards the mouth of the bay. The licensed seaweed farm is orientated northwest to southeast, and located to the westerly side of Ventry Harbour (Fig. 4.1). The depth underneath the farm is approximately 6 m at the northwestern end before gently sloping to 20 m at the eastern edge of the farm at mean low water spring tide (MLWS). The tidal range in Ventry Harbour is between 0.6 and 4.0 m. Irradiance values, obtained from nearby Valentia weather observatory ($51^{\circ} 56' 23''$ N, $10^{\circ} 14' 40''$ W), ranged from 5447 to 63 823 J cm² for 2014. Sea surface temperature data was obtained from the M3 offshore weather buoy located approximately 56 km southwest of Mizen head ($51^{\circ} 13' 0''$ N, $10^{\circ} 33' 0''$ W), and ranged from 10.1 to 17.6°C for 2014. Although offshore values are less extreme than inshore values, Ventry Harbour is a well flushed bay, so values are broadly representative. The longline structure is similar to the set-up in Walls et al. (2016) (Fig. 2.3 Chapter 2); however, the farm in Ventry consists of 3 parallel units of 280 m linear longlines suspended approximately 1.5 m below the sea surface, and the dropper ropes used in this study were 1 m in length. The longlines were kept in position by buoys attached to the header rope and by 1500 kg anchor blocks at either end of the lines. The farm cultivates the kelps *Alaria esculenta* and *Saccharina latissima* for human consumption, animal feed and use in cosmetic products.

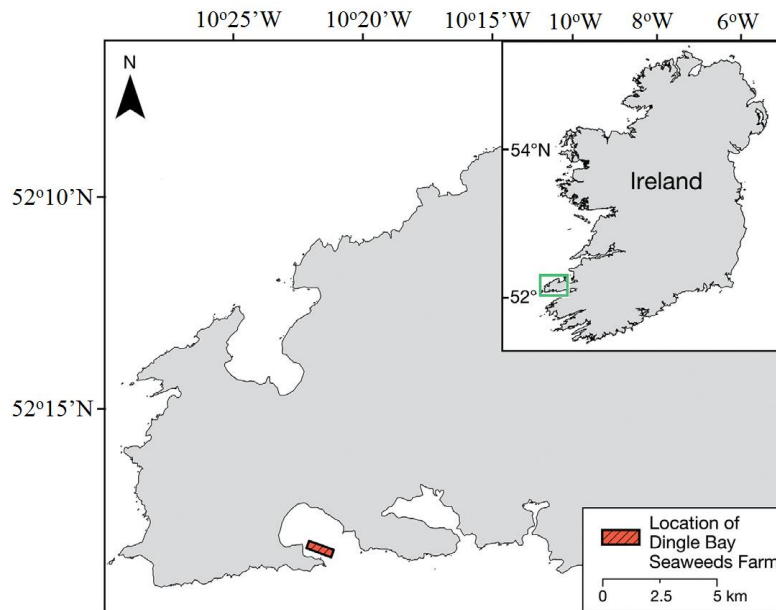


Fig. 4.1. Dingle Bay Seaweeds farm and sampling site at Ventry Harbour, County Kerry, Ireland

4.2.2 Experimental set-up

Experimental treatments were based on 1 m poly propylene dropper ropes (10 mm diameter) with 2 initial set-ups. Treatment 1 consisted of droppers sprayed directly with juvenile *A. esculenta* sporophytes that had been developed from gametophyte cultures held in the seaweed hatchery at the NUIG Carna Research Station (County Galway), following standard industry protocols (Fig. 4.2a). The seeded dropper ropes were left to develop under controlled growth conditions (Edwards & Watson 2011) for 5 to 12 weeks. There was a longer duration until deployment in Year 1 due to considerable winter storms that occurred in early 2014. Sporophytes were approximately 10 mm at time of deployment. Treatment 2 dropper ropes supported an artificial seaweed habitat (Fig. 4.2b). This included individual *A. esculenta* frond-shaped structures measuring approximately 1.5 m in length and 10 cm width, using 1200-gauge Visqueen damp proof membrane plastic. The size of these plastic fronds were representative of the size of individuals measured in June at the Ventry Harbour site, averaged over a number of cultivation seasons. The plastic fronds were inserted into the 10 mm polypropylene rope and secured. Untwisted and frayed polypropylene rope was then wrapped around the dropper rope to create artificial holdfasts, mimicking the morphology of cultivated *A. esculenta* holdfasts by creating many interstitial spaces between the rope strands. Artificial substrate droppers were deployed at 2 different densities: high density droppers consisted of 60 sporophytes m^{-1} and low

density of droppers consisted of 20 sporophytes m^{-1} . Assemblages on artificial kelp mimics were compared to seeded kelp fronds to determine if they represented a good analogue for living kelp. Treatment 2 (artificial seaweed) droppers were submerged in tanks of seawater under the same laboratory conditions as the seeded droppers for the same length of time prior to deployment. At deployment, all dropper replicates had a 1 kg concrete weight attached to the end of the rope and were deployed vertically on the longline header rope, spaced 1.5 m apart to mitigate against rubbing and tangling (Walls et al. 2016). Dropper ropes were suspended at a depth of 1.5 to 2.5 m below the surface, which is a depth range experienced at commercial seaweed farms. Each dropper was randomly assigned to a location on the longline header rope prior to deployment. Seeded ($n = 35$) and artificial (high, $n = 10$ and low, $n = 10$ density) droppers were deployed on 18 February 2014 for the 2013/2014 growing season (Year 1); deployment was delayed due to winter storms in early 2014. The experiment was repeated for the 2014/2015 growing season (Year 2), when the seeded ($n = 35$) treatment was deployed on 15 December 2014.

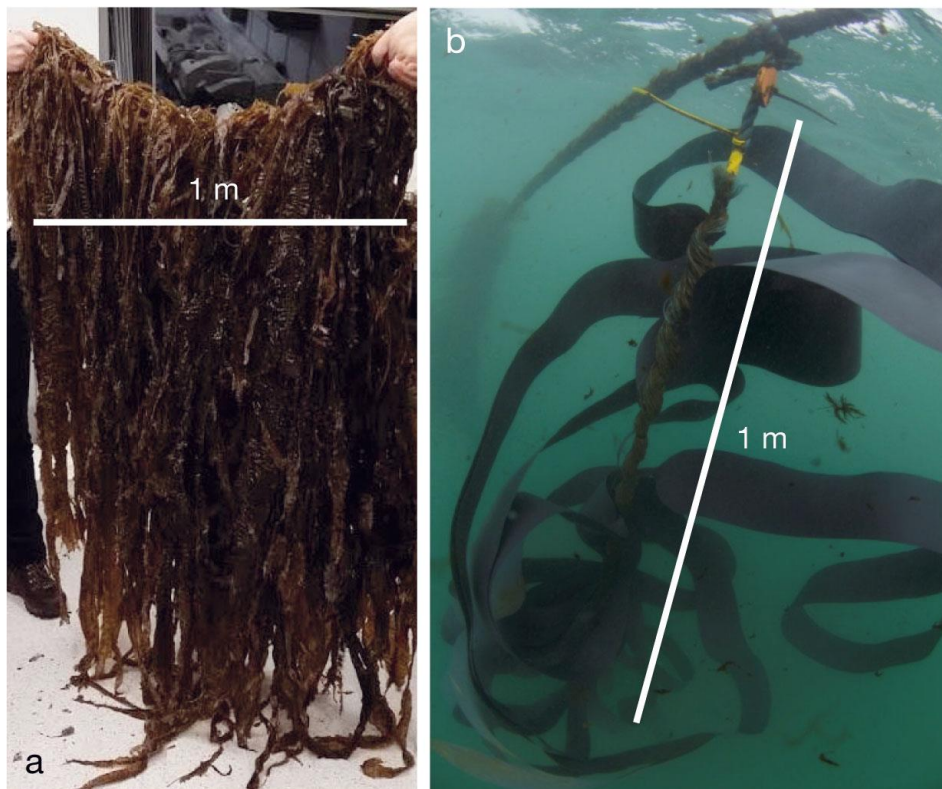


Fig. 4.2. (a) Seeded *Alaria esculenta* dropper in May 2015 (6 month deployment), and (b) artificial low density *A. esculenta* dropper in May 2014 (3 month deployment). Each dropper is 1 m in length

4.2.3 Sampling protocol for seeded and artificial *A. esculenta* droppers

All samples were collected by SCUBA divers. In April, May and June 2014 and 2015, 5 droppers were randomly chosen and collected from the seeded treatment using open-ended mesh bags (150 × 55 cm, 0.5 mm mesh size). If the dropper was not uniformly covered in developing sporophytes (i.e. showed evidence of rubbing or entanglement), another dropper replicate was selected. The mesh bag was carefully slipped over the dropper and tightly secured at top and bottom (just above the weight) using cable ties, enclosing the entire 1 m dropper and kelp biomass. In June 2014, 5 artificial high density droppers and 5 artificial low density droppers were sampled using the mesh bags as described above. Unfortunately, it was not possible to isolate and sample only the stipe and frond material, as this would involve cutting fronds from suspended holdfasts *in situ*, potentially dislodging and losing mobile or loosely attached fauna (Walls et al. 2016). Frond fauna are dominated by sessile organisms (e.g. bryozoans and hydroids) and less mobile species (e.g. molluscs) and thus are less likely to move and contaminate neighbouring samples (Park et al. 2008, Peteiro & Freire 2013b, Førde et al. 2016). All samples were processed within 6 h. The mesh bags were untied before randomly pre-selected 10 cm sections (n = 3) of each dropper were excised from the 1 m dropper (Fig. 4.2a). In the rare event that any randomly selected section of seeded dropper was not entirely covered in sporophytes, an alternative section was chosen. The fronds and stipes of seeded and artificial samples were cut just above the holdfast and stored in sealed plastic bags containing 100 % ethanol. Holdfasts still attached to the section of dropper were stored in additional sealed plastic bags containing 100 % ethanol.

4.2.4 Sample processing

The stipe and frond samples from each 10 cm replicate section of dropper were removed from the plastic bags and rehydrated in freshwater for at least 1 hrs to allow the material to soften so they could be handled without breakage. From a preliminary study we calculated species accumulation curves, and found that processing 60 g of frond material per 10 cm section was sufficient to sample the majority of fauna present for each sampling month. Processing 60 g of frond material from each section in each month allowed uniform comparison across seeded sampling months. 60 g of frond material represented approximately 20 – 50 % of total sample biomass. For the artificial droppers we found that processing 1

frond per sample was sufficient to encapsulate the majority of species present for both the high and low density droppers. Both the front and back of seeded and artificial fronds were analysed during processing.

After the preliminary study had been concluded, further sample processing was carried out. Presence-absence quantification of species richness was conducted on samples rehydrated in freshwater. Fronds were randomly selected from a tray and observed under the microscope for 2 min intervals; species present were identified and the weight of frond sample was recorded. This was repeated until the pre-defined weight of 60 g was achieved. The weight of the excess frond material not processed was recorded to obtain the total biomass per sample. All epifauna were identified to species level where possible using Hayward (1988) and Hayward & Ryland (2002), with taxonomy cross-checked using web resources (WoRMS Editorial Board 2016). Epiphytes were identified using Bunker et al. (2012) and taxonomic specialist confirmation from the Irish Seaweed Research Group.

4.2.5 Statistical analysis

The data were analysed as a 4-factor nested design with section, dropper, month and year nested within each other. This design was used to test the differences between species richness and sampling years and months for seeded samples using fully nested analysis of variance (ANOVA). Fully nested ANOVA was also employed to test for differences between total sample weight and sampling years and months for seeded samples. All data were checked for normality by visual inspection of the residuals, and assumptions for ANOVA were met. The differences between faunal assemblage composition of frond samples from different months and years for the seeded treatments and high and low density treatments for the artificial samples were compared using multivariate tests. A Simpson's dissimilarity matrix was generated from the presence-absence data for the 6 seeded sampling dates (April, May and June, 2014 and 2015) and the 2 artificial treatments separately using the PopTools (Hood 2014) add-on in Excel. Simpson's dissimilarity has the advantage that it only measures the turnover of species and is not affected by changes in species richness between samples (Baselga 2010). This makes dissimilarities measured by Simpson's index easier to interpret than indices that mix turnover and species richness components of dissimilarity (e.g. Sørensen's index). Multidimensional scaling (MDS; Shepard 1962, Kruskal 1964a,b) ordination was carried out using PRIMER V6® (Clarke &

Gorley 2006), giving the position of each frond sample in two-dimensional (2D) space based on its species composition.

Where significant differences between sampling dates and treatments were detected, a variation on SIMPER analysis was conducted to highlight the species that contributed most to the observed differences. The SIMPER programme in PRIMER could not be used as Simpson's dissimilarity is not compatible with the algorithm used in the programme. Instead, we conducted a SIMPER-like analysis, hereafter referred to as a test of species influence, by comparing the observed dissimilarity within and between samples when all species were included to the dissimilarities generated by excluding each species individually. The average between-group and within-group dissimilarities were compared to determine whether omitting a species made the groups appear more or less similar. In summary, if a species makes groups more similar when excluded from the matrix, it suggests that the species makes a contribution to the observed differences between groups. Ratios of between- to within-group dissimilarities were normalized to facilitate comparisons. Any species >1 SD of the mean normalized score was identified as having an above average contribution to the overall dissimilarity between the groups.

To test the predictability of the fouling of shared species from Year 1 (2014) to Year 2 (2015), we used day of year to calculate the central tendency of species occurrence. This central tendency is the average day when a species was observed, weighted by occurrence when observed. Day of year is the number assigned to a whole solar day that starts at 1 on 1 January and finishes at 365 on 31 December (non-leap year). The central tendency method as described by Colebrook (1979) is sensitive to changes in the timing of seasonal cycles (Edwards & Richardson 2004, Moore et al. 2011). A regression of year 2014 and 2015 mean species occurrence against each other for all commonly occurring or shared species is expected to be significant with a slope close to 1 if the temporal pattern of fouling is similar from year to year.

Tests of association were used to examine the relationships between fouling and different biotic and abiotic factors. The relationship between total sample biomass and the square root of species richness was assessed using Pearson's product moment correlation. Inspection of residual plots revealed that square root transformation was appropriate. The effect of total seeded biomass on

community structure within individual months was investigated using multivariate tests. The RELATE procedure in the PRIMER community analysis programme (Clarke & Warwick 1994) was used to test if samples with larger total biomass had different community composition within individual months. We compared a Simpson's dissimilarity matrix of species presence-absence data with a matrix of similarities based on Euclidean distances for total sample weight. Pearson's product moment correlation was used to identify any correlations between sample depth along the dropper and square root-transformed species richness. We again conducted a RELATE test to assess the relationship between sample depth and species composition. The location of dropper position in sequence along the longline was assessed as another possible factor affecting species richness (square root) using Pearson's product moment correlation. Additionally, the effect of dropper location on species composition was assessed using exploratory MDS ordinations in PRIMER to visually assess the potential for gradients across the sample site. This was considered more robust than analysing a directional gradient (e.g. east–west) as there was no hypothesis for the type of spatial pattern expected. Potential patterns included a clustering of east and west samples to either edge of the plot with middle samples in between, or east and west samples forming a circle around the middle samples.

4.3 Results

A total of 44 fouling species were recorded on seeded and artificial *Alaria esculenta* fronds; 22 species were recorded on 2014 seeded samples, 32 species on 2015 seeded samples and 35 species on artificial frond samples from June 2014. Species richness for seeded samples in 2014 ranged from 4 species in April to 17 in June, and from 9 in April to 22 in June for 2015 samples. In total, 30 species were recorded from the low density artificial fronds and 27 species were recorded from the high density artificial fronds. Crustaceans were the most species-rich group in all samples. A full species list with occurrence per sample is supplied in Appendix C Table C1.

4.3.1 Community composition and development of fouling organisms on seeded *A. esculenta* fronds over the growing season

Species richness increased after the replicate treatments were deployed at the site in Ventry (Fig. 4.3). Significant differences in species richness were recorded between sampling months (April, May and June) and droppers from the

fully nested ANOVA (Fig. 4.3, Table 4.1); however, the factor year was not significant. A variance component analysis showed that 86 % of the variance was between months with 4.04 % variance between droppers; year did not account for any of the variance, and the remaining 9.94 % was explained by the section replicates. The 2015 samples may have had more species in a pairwise comparison, but the ANOVA design tested whether the effect of years was greater than the seasonal effects; it was not.

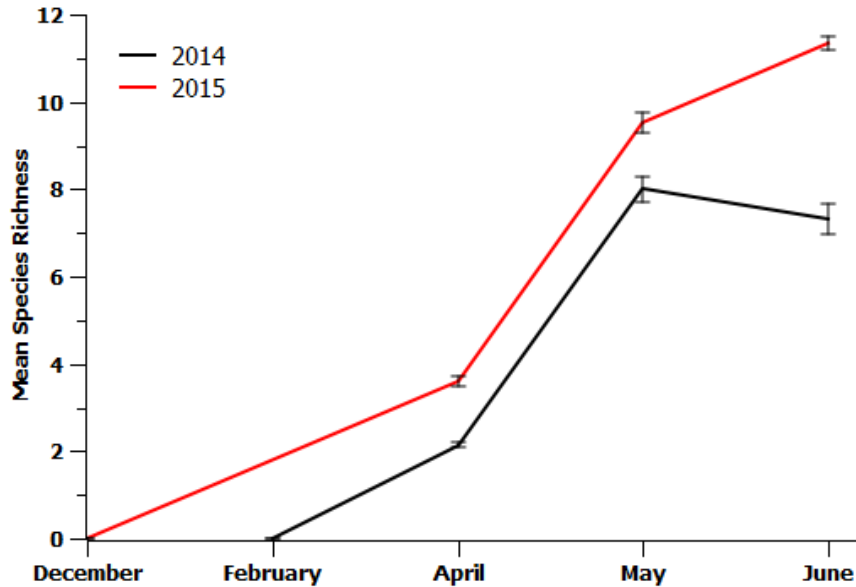


Fig. 4.3. Species richness (mean \pm SE) on seeded *Alaria esculenta* fronds sampled in April, May and June 2014 and 2015. Species richness represents the number of taxa identified on 60 g subsamples from each 10 cm section ($n = 3$) from each dropper ($n = 5$)

Table 4.1. Fully nested ANOVA of species richness with dropper (1 to 5) nested in month (April, May and June) nested in year (2014 and 2015). Significant difference at * $p < 0.05$

Source	<i>df</i>	SS	MS	<i>F</i>	<i>p</i>
Year	1	132	132	0.647	0.466
Month	4	815	203	59.299	<0.001*
Dropper	24	82	3	2.227	0.006*
Error	60	92	1		
Total	89	1122			

Significant differences in total sample biomass were recorded between sampling months (April, May and June) from the fully nested ANOVA (Table 4.2); again, year was not a significant factor. A variance component analysis showed that 16.09 % of the variance was between months with 15.91 % variance between

droppers and <1 % explained by year; the remaining 67.83 % was explained by the section replicates. Some separation and clustering of fouling assemblages of *A. esculenta* seeded fronds can be seen between months in the MDS plot (Fig. 4.4). A stress value of 0.15 indicates a reliable 2D representation of the data (Clarke & Warwick 1994). The pattern shows clear clustering of communities into distinct early April communities to later May and June communities, which show little separation. There is no clear separation of community assemblages between 2014 and 2015, indicating similar communities were present over both years.

Table 4.2. Fully nested ANOVA for total sample biomass at different times: sampling year (2014 and 2015) and sampling months (April, May and June). Significant difference at * $p < 0.05$

Source	df	SS	MS	F	p
Year	1	134776	134776	1.022	0.369
Month	4	527663	527663	3.088	0.035*
Dropper	24	1.02510 x 10 ⁶	42712	1.704	0.049*
Error	60	1.50421 x 10 ⁶	25070		
Total	89	3.19175 x 10 ⁶			

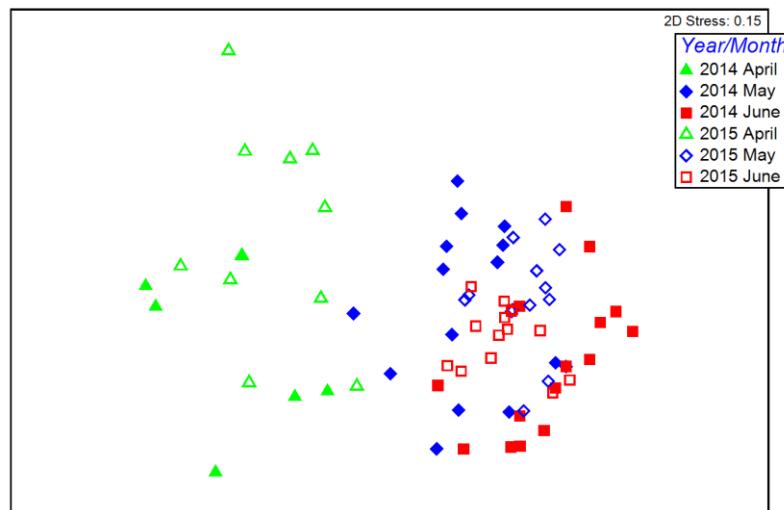


Fig. 4.4. Two-dimensional MDS plot of 89 *Alaria esculenta* frond sections: 44 from the 2014 sections (one April section had no species present so was removed) and 45 from the 2015 sample, based on presence-absence Simpson's dissimilarity matrix of species collected from each frond section (stress = 0.15)

Differences in assemblage composition among different sampling months and years were found to be significant (ANOSIM; $R = 0.628$, $p < 0.001$). All pairwise combinations of dates indicated significant differences except June 2014

and May 2015 ($R = 0.142$, $p > 0.05$) and June 2014 and June 2015 ($R = -0.184$, $p > 0.05$).

A test of species influence analysis was conducted to determine which taxa were the most influential in causing differences in assemblage structure of *A. esculenta* fronds between sampling months and years (Table 4.3). Filamentous brown alga sp. was a major contributor to the observed differences between April and May samples in 2014 and 2015. Occurrence of filamentous brown algae sp. was at its maximum in April and was low in May (between 0 and 2 samples). The bryozoan *Membranipora membranacea* was a driver of the dissimilarity between May and June 2014 samples, being present in 6 samples in May and 15 in June. The bivalve mollusc *Anomia ephippium* and the polychaete *Eulalia viridis* were important discriminatory samples between May and June 2015. Comparing months from different years, *Obelia geniculata* accounted for differences between April 2014 and 2015 frond samples. The polychaetes *Myrianida* sp. and *Spirobranchus triqueter* and the gastropod *Lacuna vineta* were important contributors to the observed differences between May 2014 and 2015 samples. From the dissimilarity scores in Table 4.3, May and June 2014 and 2015 samples were less dissimilar than April and May 2014 and 2015 samples, and May 2014 and 2015 samples were less dissimilar than April 2014 and 2015 samples showing not only do the samples converge over months but they also converge over different years, resulting in the June 2014 and 2015 community assemblages not being significantly different (ANOSIM).

Table 4.3. Test of species influence to determine the epifaunal species contributing to observed differences in the structure of *Alaria esculenta* frond assemblages between months and years. Dissimilarity scores are the ratio of average between-group dissimilarities to within-group dissimilarities for each pairwise comparison. Normalized score is the reduction in dissimilarity score when excluding the species of interest, normalized to mean = 0, SD = 1 using the mean and standard deviation of all individual species' scores. A higher loss in dissimilarity indicates that a species is important in distinguishing the dates compared

Months	Dissimilarity score all species included	Species responsible for dissimilarity	Normalized score	Species occurrence (max. 15 mo ⁻¹)
April & May 2014	3.82	Filamentous brown algae sp.	2.64	April: 14 ^a , May: 2
May & June 2014	1.60	<i>Membranipora membranacea</i>	3.90	May: 6, June: 5
April & May 2015	3.01	Filamentous brown algae sp.	3.11	April: 15, May 0
May & June 2015	1.40	<i>Eulalia viridis</i>	2.21	May: 3, June: 0
		<i>Anomia ehippium</i>	2.98	May: 0, June: 13
April 2014 & April 2015	2.02	<i>Obelia geniculata</i>	2.28	April (2014) ^a :11, April (2015): 2
May 2014 & May 2015	1.65	<i>Myrianida</i> sp.	2.05	May (2014): 8, May (2015): 3
		<i>Spirobranchus triqueter</i>	2.11	May (2014): 0, May (2015): 12
		<i>Lacuna vincta</i>	2.11	May (2014): 9, May (2015): 3

^aIn April 2014, the maximum occurrence was 14, as 1 sample had no species and was removed from all analyses

4.3.2 Predictability of fouling organisms on seeded *A. esculenta* fronds

The timing of species occurrences was consistent across years for all shared species in 2014 and 2015. The regression slope relating mean day of year species occurrence in 2014 to mean day of year species occurrence in 2015 (Fig. 4.5) was significant, with $R^2 = 0.7703$, slope = 1.14 (± 0.151 SE) and intercept = -20.24 (± 22.958 SE). This describes a predictable occurrence pattern of shared species between 2014 and 2015.

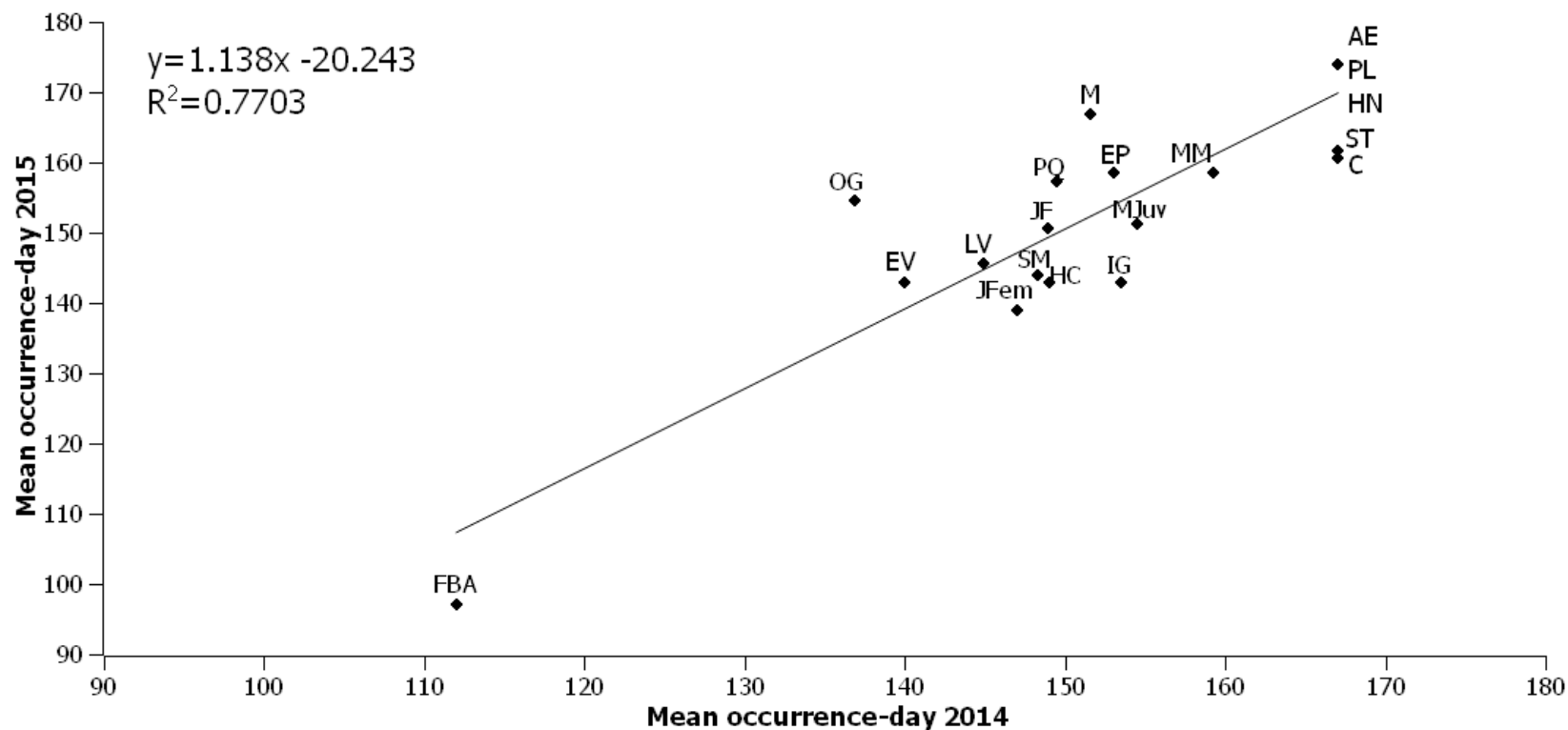


Fig. 4.5. Relationship between mean day of year occurrences for all shared species in 2014 and 2015 on the fronds of seeded *Alaria esculenta*. FBA: filamentous brown algae sp.; OG: *Obelia geniculata*; EV: *Eulalia viridis*; LV: *Lacuna vincta*; JFem: *Jassa* sp. female; SM: *Stenothoe monoculoides*; HC: harpacticoid copepods; JF: *Jassa falcata*; PQ: *Polycera quadrilineata*; IG: *Idotea granulosa*; MJuv: *Mytilus* sp. juvenile; EP: *Electra pilosa*; MM: *Membranipora membranacea*; M: *Myrianida* sp.; ST: *Spirobranchus triqueter*; C: *Ceramium* sp.; AE: *Anomia ehippium*; PL: *Pisidia longicornis*; HN: *Hardametopa nasuta*

4.3.3 Factors affecting community structure and species richness of seeded *A. esculenta* fronds

Differences in fouling species richness and community composition did not seem to reflect replicate-specific variation in biomass, depth and dropper position. The relationship between total sample weight and the square root of species richness was not significant for all within-month correlations ($p > 0.05$), except the April 2014 sample which had a significant negative correlation ($r = -0.613$, $p = 0.015$). The mean (\pm SE) Pearson's correlation for 6 correlations conducted was 0.051 ± 0.1391 . There was no relationship between Simpson's dissimilarity matrix for species composition and Euclidean distance matrix for total sample weight for replicates within individual sampling months. (RELATE test; number of tests = 6, maximum $r = 0.158$, minimum $p > 0.1$). Depth of dropper sections did not have a significant effect on species richness when tested using Pearson's product moment correlation on the square root of species number for all sampling months (April, May, and June 2014/ 2015) ($p > 0.1$), with mean Pearson's correlation 0.091 ± 0.0845 . There was no relationship between Simpson's dissimilarity matrix for species composition and Euclidean distance matrix for sample depth for replicates within individual sampling months (RELATE test; number of tests = 6, maximum $r = 0.157$, minimum $p > 0.1$). Dropper position on the longline and square root of species richness was not significant for all months ($p > 0.05$), except for the May 2015 samples which had significant negative correlation ($r = -0.563$, $p = 0.029$; mean \pm SE Pearson's correlation: -0.2083 ± 0.1163). The effect of dropper position on the longline was also assessed as a possible factor causing species compositional differences by visually examining 2D MDS plots (Fig. C1a-f Appendix C). However, no indication of a relationship was found, e.g. no clustering of either east or west samples.

4.3.4 Comparison of seeded and artificial *A. esculenta* frond communities

Some separation in assemblage composition between seeded and artificial high and artificial low density treatments sampled in June 2014 can be seen from the MDS plot (Fig. 4.6). A stress value of 0.25 indicated that the data are only partially represented by a 2D plot (Clarke & Warwick 1994), and were better represented by a 3D plot (stress = 0.18). Although Fig. 4.6 helps to illustrate this separation, it does not fully encapsulate the structure of the data. Subsequent nested ANOSIM results indicated a significant difference in assemblage composition ($R =$

0.176, $p < 0.05$). No significant differences were observed between seeded and artificial low density treatments ($R = 0.268$, $p > 0.05$) or between seeded and artificial high density treatments ($R = -0.136$, $p > 0.05$); however, significant differences were observed between artificial low and high treatments ($R = 0.46$, $p < 0.01$) from the resulting pairwise tests.

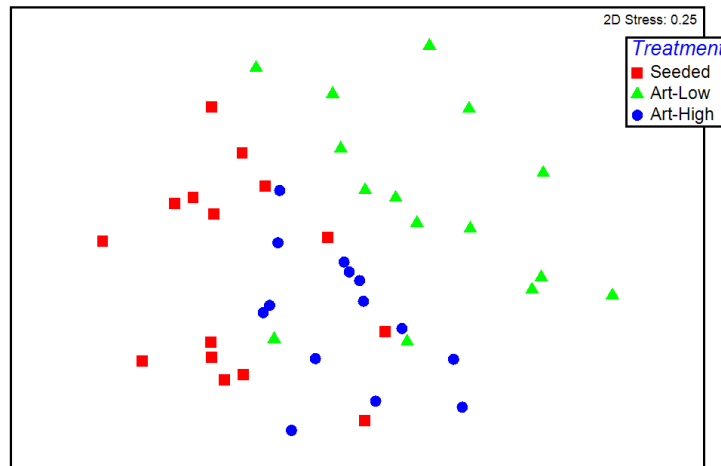


Fig. 4.6. Two-dimensional MDS plot of 45 *Alaria esculenta* frond sections, 15 from seeded samples, 15 from artificial low samples and 15 from artificial high samples, based on Simpson's dissimilarity matrix of species occurrence from each frond section sampled (stress = 0.25)

As only the artificial treatments showed significant differences in community composition, a test of species influence was conducted to determine which taxa were the major contributors to this observed dissimilarity (Table 4.4). The red alga *Ceramium* sp., the nudibranch *Polycera quadrilineata* and the crustacean *Necora puber* were major contributors to the observed differences between treatments. *Ceramium* sp. and *N. puber* had higher occurrence on the low density fronds, whereas *P. quadrilineata* was more associated with the high density fronds.

Table 4.4. Test of species influence to determine the epifaunal species contributing to observed differences in the structure of *Alaria esculenta* frond assemblages between artificial treatments. Dissimilarity and normalized scores are as described for Table 4.3. A higher loss in dissimilarity indicates that a species is important in distinguishing the treatments compared

Treatment	Dissimilarity score all species included	Species responsible for dissimilarity	Normalized score and distance from mean	Species occurrence (max. 15 per mo⁻¹)
Artificial low (AL)	1.55	<i>Ceramium</i> sp.	3.23	AL: 11; AH: 1
and artificial high (AH)	1.55	<i>Polycera quadrilineata</i>	3.92	AL:3; AH: 15
	1.55	<i>Necora puber</i>	1.15	AL: 7; AH: 0

4.4 Discussion

4.4.1 Community composition and development of fouling organisms on seeded *Alaria esculenta* fronds over the growing season

Fouling assemblages sampled from the fronds of cultivated *Alaria esculenta* followed a seasonal pattern, with a build-up of species over sampling months from zero species at time of deployment to over 20 species at the end of the study. The identified species have all been previously recorded as kelp fouling organisms from wild or cultivated kelp (Seed & Harris 1980, Christie et al. 2003, Park et al. 2008, Park & Hwang 2012, Peteiro & Freire 2013b, Førde et al. 2016). Species richness of seeded kelp fronds in Ventry Harbour is within the range found from other studies of the fauna on wild kelp fronds (Sloane et al. 1957, Norton et al. 1977, Seed & Harris 1980, Christie et al. 2003, Włodarska-Kowalczyk et al. 2009). The accumulation of fouling species richness on *A. esculenta* fronds probably reflects seasonal factors influencing reproduction, dispersal and settlement. Fouling epibionts such as bryozoans, hydroids, molluscs and crustaceans begin to settle in spring and early summer (Hayward & Ryland 2002, Park et al. 2008, Park & Hwang 2012, Førde et al. 2016). We found a significant increase in species richness with each sampling month. Species richness in standardised samples was not linked with total sample biomass.

The multivariate analysis of assemblage development on *A. esculenta* fronds showed that species are not simply accumulated over time; there are compositional differences between early and later samples. Change in community composition was through addition of new species and replacement of early colonisers. April and May communities between 2014 and 2015 were significantly different, possibly due to differences in deployment time. From the test of species influence, these communities were distinguished by filamentous brown algae sp., which was dominant in April and dramatically decreased in May of both years, i.e. the species were replaced. May and June 2014 communities were differentiated by the bryozoan *Membranipora membranacea*, which settles later in the summer (Ryland & Hayward 1977). The presence of the bryozoan *Electra pilosa* was higher early in the sampling season (May) of both years, but decreased as bryozoan coverage increased; *M. membranacea* was dominant in later sampling months (June). This development may be due to species' preference in substrate selection (Ryland 1962) and differing growth rates (Seed & Harris 1980, Yorke & Metaxas 2011). Our findings are consistent with the Førde et al. (2016) study of bryozoans

on the fronds of cultivated *Saccharina latissima* in Norway and are examples of species replacement.

The convergence of the epibiont communities over different years is shown in the MDS plot (Fig. 4.4) and reflected in pair-wise tests that showed no significant differences between June 2014 and May 2015 samples and June 2014 and 2015 samples, even with a 2 month difference in deployment time. April 2014 and 2015 samples and May 2014 and 2015 samples were shown to be significantly different due to species occurrence, which may reflect variations in larval settlement times between years.

4.4.2 Predictability of communities from one year to the next

The convergence of fouling assemblages between different years suggests that species colonise fronds at similar times. The mean occurrence-day of fouling organisms on the fronds of seeded *A. esculenta* cultured in Ventry Harbour was predictable from one year to the next (Fig. 4.5), despite variations in deployment date and sampling date between years, and factors such as water temperature, turbidity, irradiance and salinity presumably also varying from one year to the next. It would be reasonable to expect that the degree of predictability would decline with increasing intervals between years or when comparing communities in different locations. Differences in shoreline topography such as headlands and embayments can modify local hydrodynamic processes, creating eddies and fronts that affect the dispersion of zooplankton (Alldredge & Hamner 1980, McCulloch & Shanks 2003). Local larval pools can regulate the diversity of species potentially able to settle at a site by setting an upper limit of species available (Herben 2005). Thus, the retention or export of larvae within or out of bays may influence the predictability and consistency of the fouling sequence within and between bays. Only highly mobile organisms or those with pelagic larval dispersal will be able to colonise and establish on suspended kelps (Walls et al. 2016). Using flushing times as a variable to describe the likelihood that larvae are retained in bays, Jessopp et al. (2007) investigated the effect of coastline configuration on larval assemblages. The authors found clear evidence that the physical characteristics of bays influence the local larval pool. Species richness was found to be higher in bays with longer flushing times, and conversely, species richness was lower in bays with shorter flushing times. Ventry Harbour is a shallow, medium sized embayment

(approximately 3.75 km²) with a wide opening into Dingle Bay, and is likely to have a short flushing and thus lower species richness than more enclosed bays.

4.4.3 Factors affecting fouling of seeded *A. esculenta* fronds

None of the factors tested had a large influence on seeded fouling communities. Total sample biomass did not have an effect on species composition or species richness (exception: April 2014, possible Type I error), meaning that the effect of biomass was not a strong influence compared to other factors such as larval settlement and environmental conditions.

Depth of sections also had no relationship with species composition or species richness. One explanation could be that droppers were 1 m long and only suspended 1.5 to 2.5 m below the water surface, and this variation in depth was not large enough to cause an effect. Førde et al. (2016) found significantly less bryozoan cover with increasing depths up to 15 m on the fronds of cultivated *S. latissima*. However, droppers of 15 m length are not practical in commercial seaweed farms in Ireland where licensed sites are often located in depths less than 15 m. Kelp growth is also affected by depth; however, optimum cultivation depth varies among latitudes (Buschmann et al. 2008, Handå et al. 2013, Peteiro et al. 2016). Thus, in addition to practical limitations, there is a probable trade-off between fouling and farm productivity linked to cultivation depth.

The position of droppers within the farm did not have an effect on community structure or species richness (exception: May 2015, possible Type I error). Droppers were positioned along a 280 m header rope; this distance may not have had great variations in environmental parameters such as exposure and turbidity. Larger-scale site differences may have an effect; e.g. Peteiro & Freire (2013b) found that in a bay in northwest Spain the abundance of epibionts was lower in an exposed site than in a sheltered site.

4.4.4 Comparison of seeded and artificial *A. esculenta* frond communities

Multivariate tests showed no significant differences between the seeded treatment and the artificial high and low density treatments. Interestingly, even though frond tissue type and size were different, the community assemblages were not. However, when the seeded treatment factor was removed, there was a significant difference between artificial density treatments, which suggests a

density effect. We calculated a surface area to weight relationship for *A. esculenta* to assess how representative the artificial density treatments were. Using separate *A. esculenta* morphometric data sampled from Ventry in 2013 and 2014, we estimated frond surface area (length multiplied by maximum width of frond) and correlated this with frond weight. The regression slope relating frond surface area to frond weight was significant, with $R^2 = 0.7792$ and slope = $0.017 (\pm 0.0004 \text{ SE})$. This allowed us to calculate seeded frond surface area per 10 cm section from our total sample biomass data per section. The results, presented in Table 4.5, show that seeded surface area lies between artificial low and artificial high surface area for 10 cm sections. The differences between fouling communities (*A. esculenta* intermediate between high and low density artificial treatments) are therefore consistent with a frond density effect. Peteiro & Freire (2013a), and Peteiro et al. (2016 and references therein) have indicated that high densities of fronds in a reduced space (e.g. dropper rope) can modify the environment via reduction in currents. Thus, the observed density effect probably altered the hydrodynamic environment experienced by the frond communities i.e. high density frond communities experience less disturbance with low density communities experiencing more hydrodynamic activity. However, some caution is needed before generalising the effect of density, as the different treatment substrates may have specific surface chemistries and specific micro environments (Michael et al. 2008) which could be responsible for the differences in epibiont communities.

Table 4.5. Surface area (cm^2) and standard error for seeded, artificial low and artificial high treatments per 10 cm section. na: not applicable

Treatment	Surface area (cm^2) per 10 cm section	Standard error
Seeded	4889.44	1379.18
Artificial low	3000	na
Artificial high	9000	na

Alterations in hydrodynamic environment could explain the species compositional differences and variations in species occurrence between artificial treatments. For example, increased hydrodynamic conditions may prevent the settlement of fouling organisms such as hydroids and bryozoans on algal fronds (Fraschetti et al. 2006, Saunders & Metaxas 2008, Peteiro & Freire 2013b). The nudibranch *Polycera quadrilineata* had higher occurrence on high density fronds, with the sheltered conditions providing a favourable habitat for itself and its prey

species (bryozoans). The opposite pattern was true for the decapod *Necora puber* and the red algae *Ceramium* sp., which could be attributed to increased water currents circulating more nutrients and food particles over the low density fronds. As this tests the impact of small-scale hydrodynamics, multivariate differences in frond fouling communities on seaweed farms caused by hydrodynamic differences over larger spatial scales need to be researched (Peteiro & Freire 2013b).

These results support the use of artificial droppers as experimental mimics of cultivated fronds for use at potential seaweed farm sites. This will enable an assessment of the potential fouling communities likely to affect cultivated fronds without using seeded tissue, which can be harvested and sold, while also controlling factors such as biomass, productivity and algal anti-fouling.

4.4.5 Succession of fouling kelp communities

No comparison can be made between succession on our seeded fouling kelp communities and other kelp studies as no published descriptions of succession in fouling assemblages were found using an International Scientific Index (ISI) search for succession, kelp, epibionts and other variants of these keywords. This lack of information is probably due to the focus in the cultivation industry on increasing production and not ecological implications. Studies of succession are, of course, common in other systems, such as rocky shores and artificial substrates. The timing of disturbance or the creation of new substrata can have an enormous impact on succession (Dayton et al. 1984, Benedetti-Cecchi & Cinelli 1993, Underwood & Chapman 2006) because of seasonality in organisms' reproductive patterns and/or growth and seasonal variation in environmental conditions (Jenkins & Martins 2010). This effect of timing was apparent in the initial differences in fouling on seeded *A. esculenta* between years. However, initial differences may not persist as communities transition to similar end-points (Underwood & Chapman 2006, Jenkins & Martins 2010). A number of studies suggest that varying successional trajectories ultimately converge towards the local climax communities (Underwood & Chapman 2006, Antoniadou 2014, Evans et al. 2016). Our epibiotic fouling communities followed this pattern of convergence from different start points to similar end-points.

4.4.6 Implications for the kelp cultivation industry

The ability to understand the timing of occurrences of fouling organisms and predict their arrival has significant benefits for the seaweed cultivation industry. With this knowledge, seaweed farmers can exert some control over the quality of their crop by being able to decide on a date-by-site basis when is the optimum time to deploy in order to achieve a harvest of optimal quality and yield. Earlier harvesting of fronds could avoid the period when the most severe fouling occurs (late May to June in southwest Ireland); this approach has been very effective for kelp producing farms in Asia (Park et al. 2008, Park & Hwang 2012). Early deployment of juvenile kelp seedlings to sea would allow for a longer cultivation period in the cooler winter months, leading to increased growth of fronds while fouling rates are low (Peteiro & Freire 2009). The crop would then reach harvestable size earlier in the season, before an increase in seawater temperature and associated fouling organisms (Park et al. 2008, Peteiro & Freire 2009, 2013b, Park & Hwang 2012, Førde et al. 2016).

Acknowledgements

The authors acknowledge Dr. Benoît Quéguineur, Mr. David Moran and Mr. Brendan Walls for their assistance in field sampling. In addition, we thank Dr. Adrian Patterson and Dr. Jack O'Carroll from NUI Galway's Benthic Ecology Laboratory for taxonomic support, Mr. Michael Murphy and Mr. Paul Flannery of Dingle Bay Seaweed for boat work and assistance at Ventry Harbour, and staff at Dingle Oceanworld Aquarium for providing laboratory space. This work was supported by the Energetic Algae project (EU Interreg IVB NWE Strategic Initiative; www.enalgae.eu). The authors also acknowledge the 2 anonymous reviewers and editor Symon Dworjanyn for their comments that have helped to improve the manuscript. A.M.W. is currently funded by the Dr. Tony Ryan Research Trust, NUI Galway.

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Chapter 5

Aquaculture structures of kelp farms: assessment of artificial habitat availability for mobile fauna

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Abstract

Recent growth trends of the global aquaculture industry show a continued upward trajectory, naturally resulting in the proliferation of associated structures deployed in freshwater and at sea. Observations of seaweed farms (infrastructure and biomass) suggest that the whole farm functions as a unit, providing space for associated species such as amphipods, molluscs and fish, arriving as larvae or colonizing as adults. To date, most of the research has focused on fouling epibionts on the fronds of cultivated kelp species. Using a commercial seaweed farm on the southwest coast of Ireland, we studied the development of mobile assemblages associated with cultivated *Alaria esculenta* over two growing seasons. Comparison of assemblages were made with unseeded rope droppers analogous to farm infrastructure. Species richness increased over time during two separate cultivation seasons however, there was no clear distinction of assemblage composition between treatments; rather the development of a common mobile farm assemblage. Treatment did not affect the arrival and colonisation of shared mobile species between seeded and rope samples for the same year. However, assemblages were not predictable between years for seeded and rope treatments separately. This study showed that a lack of specificity and high mobility rates of fauna were likely causes for the general farm fauna and habitat complexity did not cause differences in assemblage structure between treatments. Seaweed farms as a whole (not just the growing kelp biomass) need to be considered as providers of ecosystem services above and beyond those associated with other structures such as, mooring ropes.

Keywords: *Alaria esculenta*; Macroalgae; Kelp; Aquaculture; Artificial structures; Mobile Fauna; Succession;

5.1 Introduction

The last few decades have seen huge growth in the development of artificial structures in the marine environment (Firth et al. 2016a), enabling the exploitation of the ocean's energy and food resources. The term 'ocean sprawl' has been used to describe this expansion of coastal and marine infrastructure (Firth et al. 2016a, b) and is gaining recognition as one of the biggest threats to marine ecosystems (Airoldi & Beck 2007, Firth et al. 2013, Dafforn et al. 2015). In particular, the aquaculture industry has grown dramatically over the last 50 years to an all-time high of 131.4 million tonnes live weight of aquatic animals and plants in 2014 (FAO 2016). This growth has been driven by the demand of the increasing human population for the provision of food. Depending on the design and location, artificial structures can provide important ecosystem services such as habitat, protection for species of commercial or conservation importance (Inger et al. 2009, García-Gómez et al. 2014, Firth et al. 2015). Equally however, artificial structures have also been found to support lower biodiversity (Moschella et al. 2005, Firth et al. 2013), different communities (Chapman 2003, Evans et al. 2016) and promote opportunistic and non-native species (Airoldi & Bulleri 2011, Firth & Hawkins 2011) compared to natural habitats. Even with the surge of interest in understanding community ecology and services associated with artificial structures, research into aquaculture structures (particularly macroalgal cultivation) and their ecosystem services is lacking (Firth et al. 2016a). Within the aquaculture industry, the cultivation of aquatic plants (predominantly macroalgae), is expanding rapidly; increasing by almost 8 % per year over the past decade (FAO 2016). Over a third of the 27.3 million tonnes of global annual aquatic plant production came from just two kelp species (*Laminaria japonica* and *Undaria pinnatifida* (FAO 2016).

The ecological and economic importance of wild kelp has been highlighted in previous chapters (Chapter 2, 3 and 4; Walls et al. 2016, 2017) and comprehensively discussed in many studies and reviews (Guiry & Blunden 1991, Christie et al. 2003, Smit 2004, Smale et al. 2013, Peteiro et al. 2016, Teagle et al. 2017). The practice of cultivating kelp species is beginning to expand from traditional locations in Asia to European waters. This growth in the sector has been identified in Chapter 4 'Successional changes of epibiont fouling communities of the cultivated kelp *Alaria esculenta*: Predictability and influences' (Walls et al. 2017). In addition to providing macroalgal biomass to supply the many traditional (e.g. food) and expanding uses (e.g. biofuels) of kelp (Guiry 1989, Walls et al.

2016). Observations of the artificial infrastructure associated with seaweed farms and the kelp biomass itself suggest that farms provide important ecosystem services such as habitat provision (Park et al. 1990, Peteiro & Freire 2013, Førde et al. 2016, Walls et al. 2016, 2017), protection from predators and may act as nursery grounds for juvenile fish and invertebrates similar to that of wild kelp forests (Smale et al. 2013, Walls et al. 2016).

It is not known how early colonisers arrive to the suspended seaweed farm, but they can potentially come from two different sources, recruitment by larvae or immigration by adults (Chapman 2002). In marine habitats, many invertebrates have larval dispersal (Chapman 2002), and the potential to disperse great distances from their parents (Scheltema 1986). This is especially true in inshore coastal habitats where water-movement can be turbulent (Scheltema 1986). Intuitively, all species with dispersive larvae (or dispersive propagules, e.g. algal spores) have the potential to colonize new habitat (Chapman 2002). Multiple settlement and post-settlement factors interact to determine patterns of recruitment into new patches of habitat (Keough & Downes 1982, Underwood 1999, Knights et al. 2012). Colonization by adults or juvenile marine invertebrates with direct development may be more difficult but they do colonize new patches of habitat, e.g. algal fronds and holdfasts (Peteiro & Freire 2013, Førde et al. 2016, Walls et al. 2016). This may occur via active processes, such as crawling or swimming from nearby habitats, or via passive processes, such as drifting in the water column (Martel & Chia 1991, Clarkin et al. 2012). Highly mobile organisms and those with pelagic larval dispersal will have a greater opportunity to inhabit and establish on suspended kelp farms than species with direct development and more restricted movements (Walls et al. 2016). The development of diverse invertebrate communities will in turn attract mobile animals such as fish, birds and other larger predators (e.g. seals). These farms also create suitable habitats for some fish species to spawn, shelter for juvenile fish and can function as nursery grounds so may thereby enhance locally the recruitment of fish stocks under pressure (Eklöf et al. 2006) and benefit local fishers. Understanding early colonization of a new patch of habitat can have important effects on later patterns of succession, through processes such as facilitation, interference or pre-emption of space (Sousa 1979, Anderson & Underwood 1997, Chapman 2002).

Given the predicted increase in aquaculture structures, including seaweed farms, and their possible impact on local biodiversity and lack of knowledge of

their associated mobile assemblages investigation is urgently required. In this study, we focus on the mobile assemblages (i.e. mobile invertebrates and fish assemblages) that develop on suspended dropper ropes seeded with *Alaria esculenta* individuals. Farm-associated fauna were surveyed at different times during the cultivation period (late winter – early summer) to track changes in assemblage composition and development. We compared mobile faunal assemblages that developed on polypropylene ropes seeded with juvenile *A. esculenta* with those assemblages that developed around unseeded polypropylene rope which represents the farm infrastructure. The seeded treatments were deployed with juvenile *A. esculenta* providing structure which increases in complexity as the kelp biomass grows. The rope droppers had a very simple structure and comparatively less biomass with only the attachment of epiphytes during deployment to provide structure. We tested the predictability of mobile assemblages for both treatments from one growing season to the next by comparing mean occurrence-day. The comparison of seeded and rope treatments allowed for an estimation of the effect of structure provided by the kelp. If seeded mobile assemblages are found to have distinct assemblages when compared to rope treatments, this suggests possible ecosystem services provided by seaweed farms above and beyond those associated with other deployed structures such as mooring ropes.

5.2 Materials and Methods

This study was conducted at the seaweed farm located in Ventry Harbour, County Kerry (see Fig. 3.1; Fig. 1 in Walls et al (2017)). Descriptions of the study site and the farm set-up can be found in previous chapters, Chapter 3 ‘Ecological priming of artificial aquaculture structures: Kelp farms as an example’ and Chapter 4 ‘Successional changes of epibiont fouling communities of the cultivated kelp *Alaria esculenta*: Predictability and influences’ Walls et al. (2017).

5.2.1 Experimental set-up

Experimental treatments were based on 1 m polypropylene dropper ropes (10 mm diameter) with two initial set-ups. Treatment 1 “seeded droppers” consisted of droppers seeded directly with juvenile *Alaria esculenta* sporophytes. The development and deployment details of these droppers can be found in Chapter 4. Treatment 2 dropper ropes consisted of 1 m polypropylene rope and will be referred to as “rope droppers” hereafter. The deployment details of these droppers

are described in Chapter 4. These rope droppers represent the unstructured habitat for comparison with the structured seeded droppers.

5.2.2 Sampling protocol and processing for seeded *A. esculenta* droppers and rope droppers

All sampling was conducted in April, May and June of 2014 and 2015 as described in Chapters 3 and 4 and samples were transferred to Dingle Oceanworld Aquarium and processed within 6 hrs. The mesh bags were untied and the dropper inside was removed and sampled for frond, holdfast or rope assessments. The mesh bags were carefully washed down with freshwater to remove all contents and sieved through a 0.5 mm sieve. All samples were stored in sealed plastic bags containing 100 % ethanol until processing in the laboratory. All mobile fauna collected were identified species level where possible using Hayward (1988) and Hayward & Ryland (2002) with taxonomy cross-checked using web resources (WoRMS Editorial Board 2016).

5.2.3 Statistical analysis

Univariate analysis of variance (ANOVA) was used to examine differences between species richness among seeded and rope treatment droppers (fixed factor) crossed with month (fixed factor) crossed with year (fixed factor). All data was checked for normality by visual inspection of the residuals and assumptions for ANOVA met. The differences between faunal assemblage composition of mobile species from different months and years for the seeded and rope treatments were compared using multivariate tests. A Simpson's dissimilarity matrix was generated from the presence-absence data for the six seeded and six rope treatments and different sampling dates (April, May and June, 2014 and 2015). This coefficient is 0 when assemblages from separate droppers are identical and scaled to 100 when there are no species in common between separate samples. Simpson's dissimilarity has the advantage that it only measures the compositional turnover and is not affected by changes in species richness between samples (Baselga 2010). This makes dissimilarities measured by Simpson's index easier to interpret than in the case for indices that mix turnover and species richness components of dissimilarity (e.g. Sørensen's index). The Primer software used for multivariate analysis does not calculate Simpson's dissimilarities, so these were calculated from species presence-absence data in EXCEL using the PopTools (Hood 2014) add-on and

dissimilarity matrices were subsequently imported into PRIMER. Samples were ordinated using a multidimensional scaling plot (MDS) (Shepard 1962, Kruskal 1964a, b) using PRIMER V6 ® (Clarke & Gorley 2006), giving the position of each dropper sample in two-dimensional space based on its species composition.

Permutational multivariate analysis of variance (PERMANOVA, Anderson et al. 2008) was used to test for differences in multivariate species assemblages among seeded and rope treatments (fixed factor) with month (fixed factor) crossed with year (fixed factor), based on 9999 unrestricted permutations of raw data. However, this design returned a Negative pseudo-F value for treatment factor and treatment crossed with year. This is sometimes caused when levels of one factor which are completely contained within the other factor level, e.g. mean of one level are closer to the mean of the other level than they are to their own mean. We then analysed a number of different PERMANOVA designs which avoid the term with no result or negative associated with it. The suitable design removed the year factor and had sampling date as a fixed factor with 6 levels (April-14, May-14, June-14, April-15, May-15 and June-15) and treatment as fixed factor with 2 levels. Species assemblages between treatment groups within different months were compared using pair-wise tests, depending on significant interactions between the two factors. Permutational multivariate dispersion (PERMDISP) routine revealed that the multivariate dispersion (around the centroid) was not significant ($P > 0.5$) for all factors (year, month and treatment).

To test the predictability of the presence of mobile fauna on seeded and rope droppers from Year 1 (2014) to Year 2 (2015), we used day of year to calculate the central tendency of species occurrence. This central tendency is the average day when a species was observed, weighted by occurrence when observed. Day of year is the number assigned to a whole solar day that starts at 1 on the 1st of January and finishes at 365 on 31st of December (non-leap year). The central tendency method as described by Colebrook (1979) can identify changes in the timing of seasonal cycles (Edwards & Richardson 2004, Moore et al. 2011). To test if species arrival times were similar between the different treatment droppers within the same year the mean species arrival times were compared between treatments within years 2014 and 2015 separately. Regression analysis was conducted using Minitab v16 to test if arrival times of commonly occurring species were significant. Significance is expected with a slope close to 1 if the temporal pattern of species presence is similar from year to year.

5.3 Results

In total, we recorded 47 mobile species sampled from 1 m dropper lines. 37 species were recorded from 2014 seeded and rope samples and 40 species were recorded from 2015 seeded and rope treatments. Species richness for seeded samples in 2014 ranged from 5 in April to 13 in June and 12 in April to 18 in June for year 2015. Species richness for rope samples in 2014 ranged from 4 in April to 13 in June and 10 in April to 17 in June for year 2015. In all samples crustaceans were the most species rich group followed by annelids and molluscs. A full species list is supplied in Appendix D Table D1.

5.3.1 Assemblage composition and development of mobile fauna on from seeded and rope droppers.

Species richness of mobile fauna for both treatments increased after the replicate droppers were placed out at the site in Ventry (Fig. 5.1). Significant differences in species richness were recorded between years, months treatment and year crossed with months from the ANOVA (Fig. 5.1, Table 5.1), however, and interaction between treatment and or year and month were not significant. Species richness within Year 1 is higher on seeded treatments during each sampling month, however overall species richness declines between May and June sampling dates. Species richness is similar for both treatments early in sampling during year 2, but seeded species richness is higher than rope in June. 2015 samples may have had more species in a pairwise comparison, but the ANOVA design was testing whether the effect of years was greater than the seasonal or treatment effects.

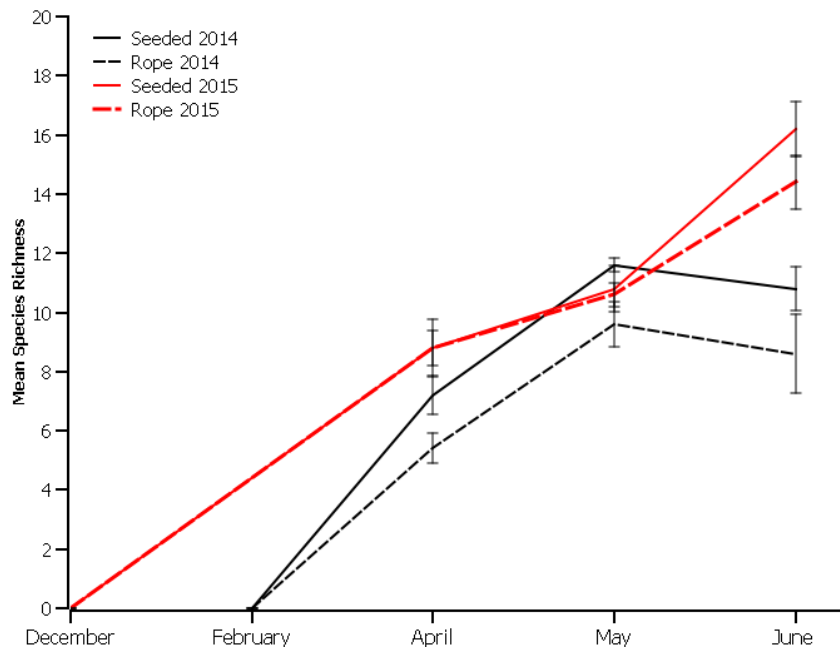


Fig. 5.1. Species richness (mean \pm SE) of mobile fauna on seeded *Alaria esculenta* and rope droppers sampled in April, May and June 2014 and 2015. Species richness represents the number of taxa identified from 1 m droppers ($n = 5$).

Table 5.1. ANOVA of species richness with month (April, May and June) nested in year (2014 and 2015) crossed with treatment (seeded and rope). Significant difference at * $p < 0.05$, ** $p < 0.01$

Source	<i>df</i>	SS	MS	<i>F</i>	<i>p</i>
Year	1	112.07	112.07	36.35	0.000**
Month	2	250.23	125.12	40.58	0.000**
Treatment	1	26.67	26.67	8.65	0.005**
Year x Month	2	76.03	38.02	12.33	0.000**
Year x Treatment	1	6.67	6.67	2.16	0.148
Month x Treatment	2	3.43	1.72	0.56	0.58
Year x Month x Treatment	2	1.63	0.817	0.26	0.77
Error	48	148	3.08		
Total	59	624.73			

Patterns of differences among assemblages from different years, months and treatments were difficult to interpret because the high stress value of the MDS plot (0.24, Fig. 5.2a). A stress value of 0.24 indicates the data are only partially represented by the two-dimensional plot and little reliance should be placed on the finer detail of the plot (Clarke & Warwick 1994). However, the broad-scale pattern shows a separation of early April assemblages to later May and June assemblages which show little separation. There is no clear separation of assemblages between

years or treatments, indicating similar assemblages were present over both years and treatments.

Species assemblage composition differed between years, months and treatments from the PERMANOVA analysis using the full design; (Table 5.2, $p < 0.001$). However, this design returned a ‘Negative’ pseudo-F value for treatment factor and year crossed with treatment. This may be caused by one of the levels of our treatment factor being contained within the other factor level, this pattern can be seen from an MDS of our treatment factor (Fig. 5.2b).

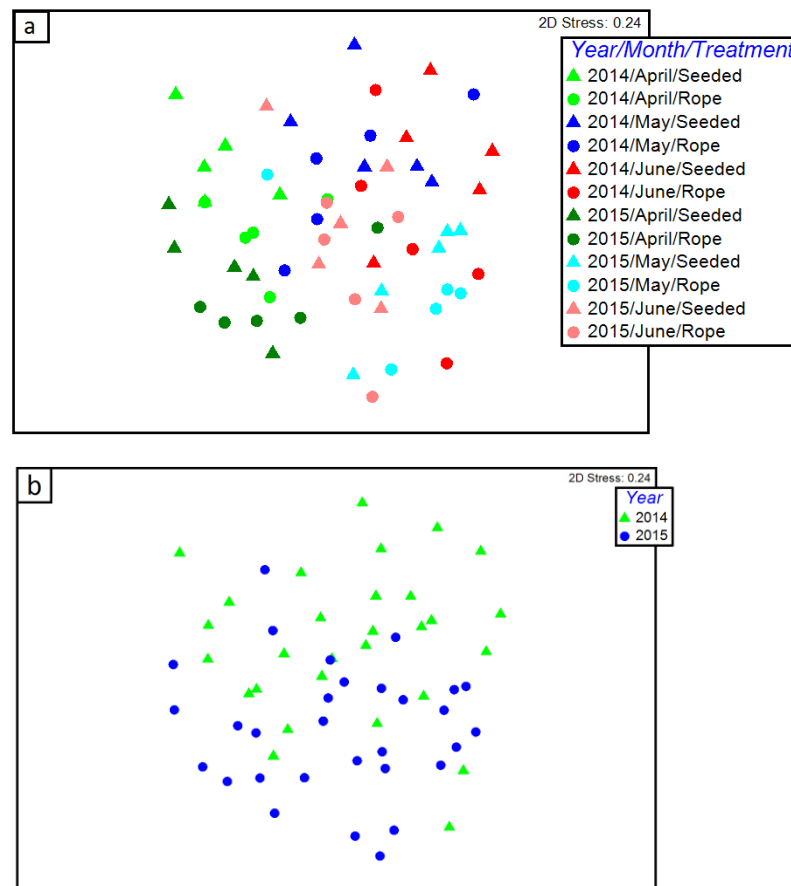


Fig. 5.2a. Two-dimensional MDS plot comparing assemblages of 60 mobile fauna samples: 30 from year 2014 sections and 30 from year 2015 sections based on presence-absence Simpson’s dissimilarity matrix of species collected from each mobile sample (stress = 0.24). Fig. 5.2b. Two-dimensional MDS plot of 60 mobile fauna samples: 30 from seeded treatment and 30 from rope treatment, based on presence-absence Simpson’s dissimilarity matrix of species collected from each mobile sample (stress = 0.24), showing how treatment may be the cause of the ‘Negative’ pseudo-F value.

Table 5.2. Permutational multivariate analyses of variance based on Simpsons dissimilarity matrix based on presence-absence data for mobile fauna sampled during year (2014 and 2015) over months (April, May and June) on different treatments (seeded and rope). All tests were conducted using unrestricted permutation of raw data with 9999 permutations. Significant difference at * $p < 0.05$, ** $p < 0.01$

Source	<i>df</i>	SS	MS	<i>F</i>	P (perm)
Year	1	0.367	0.367	9.851	0.0001**
Month	2	1.134	0.567	15.228	0.0001**
Treatment	1	0.180	0.180	4.831	0.0018**
Year x Month	2	7.538 x10 ²	3.769 x10 ²	1.013	0.463
Year x Treatment	1	-1.669x10 ²	-1.669x10 ²	NEGATIVE	
Month x Treatment	2	0.133	6.654x10 ²	1.789	0.106
Year X Month x Treatment	2	0.258	0.129	3.465	0.0017**
Residuals	48	1.787	3.722x10 ²		
Total	59	3.916			

To overcome the problem of negative pseudo-F values, we analysed a number of alternative PERMANOVA designs and found the results to be stable across these designs. The most suitable design removed the year factor and had sampling date as a random factor with 6 levels (April-14, May-14, June-14, April-15, May-15 and June-15) and year as fixed factor with 2 levels. This design enabled analysis of the year factor and its interactions without a negative result in the PERMANOVA table. The results from this updated design showed, sampling date and treatment and the interaction of sampling date and treatment were significant ($p < 0.05$), ($p > 0.05$; Table 5.3). A comparison of the treatment values sums of squares and mean squares between the two outputs from alternative designs (Table 5.2 and 5.3) are the same (0.180), confirming that the negative output was not causing unusual results.

Table 5.3. Permutational multivariate analyses of variance based on Simpsons dissimilarity matrix based on presence-absence data of mobile fauna for sampling date (April-14, May-14, June-14, April-15, May-15 and June-15) and treatment (seeded and rope). All tests were conducted using unrestricted permutation of raw data with 9999 permutations. Significant difference at * $p < 0.05$, ** $p < 0.01$

Source	<i>df</i>	SS	MS	<i>F</i>	P (perm)
Sampling Date	5	1.576	0.315	8.466	0.0001**
Treatment	1	0.180	0.180	4.831	0.0017**
Sampling Date x Treatment	5	0.374	7.486x10 ²	2.011	0.0137*
Residuals	48	1.787	3.722x10 ²		
Total	59	3.916			

From the significant result of sampling date crossed with treatment we compared treatment levels within each sampling date using pair-wise tests. Results showed significant differences between seeded and rope treatments for June 2014, April 2015 and May 2015 ($p < 0.05$), the other months showed no significant difference between treatments (Table 5.4).

Table 5.4. Multivariate pair-wise comparisons of mobile assemblages sampled from different treatments (S=Seeded and R=Rope) during months April, May and June over years 2014 and 2015. *t* multivariate t-statistic, Significant difference at * $p < 0.05$, ** $p < 0.01$

Comparison	<i>t</i>	P (perm)
April-2014 S & R	0.780	0.586
May-2014 S & R	0.887	0.556
June-2014 S & R	2.072	0.009**
April-2015 S & R	1.822	0.030*
May-2015 S & R	2.169	0.009**
June-2015 S & R	1.0705	0.372

5.3.2 Predictability of mobile assemblages on seeded and rope treatments

The temporal pattern of shared mobile species presence on seeded and rope treatments were not consistent between years. Comparisons of mean species arrival times for seeded species between 2014 and 2015 and mean species arrival times for rope species between 2014 and 2015 were not significant from the regression analysis ($p > 0.05$; Table 5.5, Fig. D1a and D1b Appendix D). The timing of

species occurrence was consistent between treatments within the same year for both years 2014 and 2015. The regression slope relating mean day of year of shared species occurrence in 2014 for seeded and rope samples was significant ($p < 0.05$; Table 5.5, Fig. 5.4a) and the regression slope relating mean day of year of shared species occurrence in 2015 for seeded and rope samples was also significant ($p < 0.05$; Table 5.5, Fig. 5.4b). This describes a predictable pattern of colonisation of shared species between treatments within the same year; however, the temporal pattern for the same treatment between years was not predictable.

Table 5.5. Comparisons of mean species occurrence between treatments and years. Significant difference at $*p < 0.05$

Comparisons	R²	Slope & Std Error	Intercept & Std Error	Regression P-value
Seeded-2014 vs. 2015	0.0025	0.052 (± 0.2343)	137.949 (± 33.3173)	0.829
Rope-2014 vs. 2015	0.0192	0.135 (± 0.2100)	122.294 (± 31.0300)	0.529
2014 -Seeded vs. Rope	0.3773	0.665 (± 0.1961)	50.083 (± 27.9322)	0.003*
2015-Seeded vs. Rope	0.2104	0.431 (± 0.1739)	87.676 (± 26.0016)	0.021*

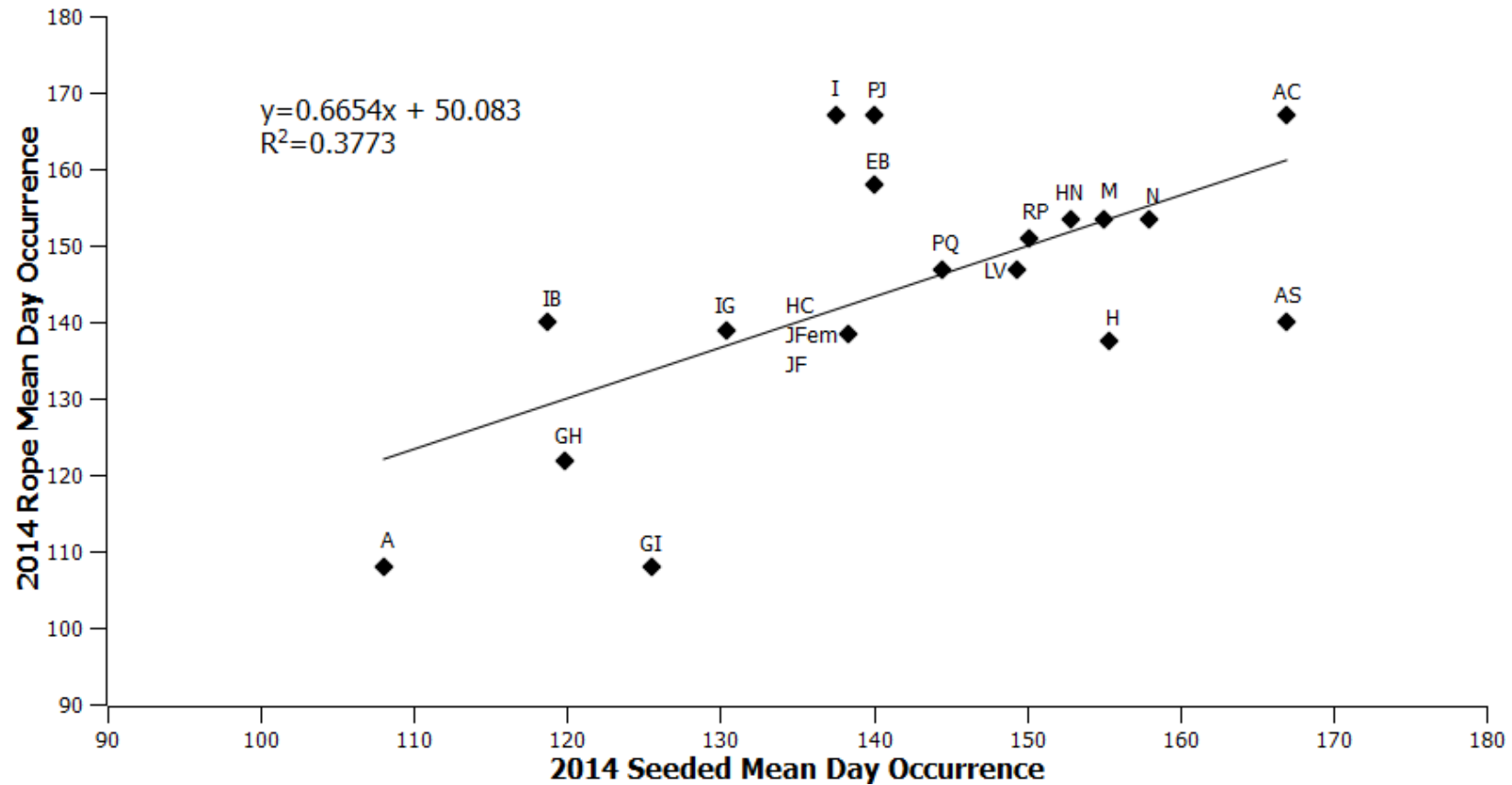


Fig. 5.4a. The relationship between mean day of year occurrences for all shared species sampled on seeded and rope treatments in year 2014.

Legend: A: *Aora* sp.; IB: *Idotea baltica*; GH: *Gammarellus homari*; GI: *Gammarus insensibilis*; IG: *Idotea granulosa*; JFem: *Jassa* sp. female; HC: Harpacticoid Copepods; JF: *Jassa falcata*; I- *Idotea* sp.; PJ: *Parajassa pelagica*; EB: *Eusyllis blomstrandii*; PQ: *Polycera quadrilineata*; LV: *Lacuna vincta*; RP: *Rissoa parva*; HN: *Hardametopa nasuta*; M: *Myrianida* sp.; H: *Harmothoe* sp.; N: Nematoda indent.; AC: *Acari* sp.; AS: *Asterias* sp.

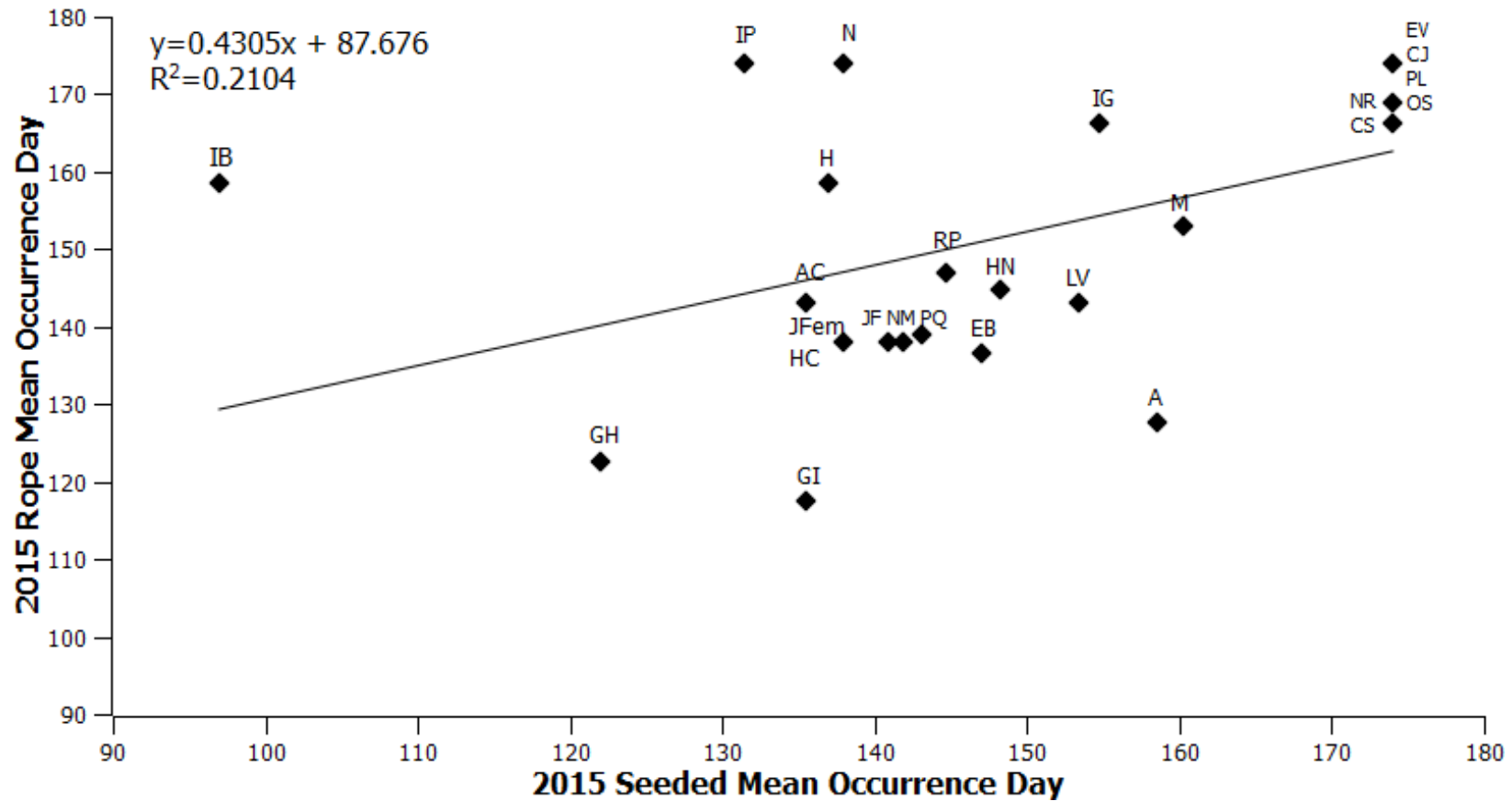


Fig. 5.4b. The relationship between mean day of year occurrences for all shared species sampled on seeded and rope treatments in year 2015.

Legend: IB: *Idotea baltica*; GH: *Gammarellus homari*; IP: *Idotea pelagica*; GI: *Gammarus insensibilis*; AC: *Acari* sp.; H: *Harmothoe* sp.; NM: Nemertea indent.; JFem: *Jassa* sp. female; HC: Harpacticoid Copepods; JF: *Jassa falcata*; N: Nematoda indent.; PQ: *Polycera quadrilineata*; RP: *Rissoa parva*; EB: *Eusyllis blomstrandii*; HN: *Hardametopa nasuta*; LV: *Lacuna vincta*; IG: *Idotea granulosa*; A: *Aora* sp.; M: *Myrianida*; EV: *Eulalia viridis*; NR: *Nereiphylla rubiginosa*; CS: *Caprella septentrionalis*; CJ: *Caprella juv.*; PL: *Pisidia longicornis*; OS: *Onoba semicostata*.

5.4 Discussion

5.4.1 Assemblage composition and development of mobile fauna on seeded and rope droppers.

After a deployment time of 4 to 6 months at sea, mobile faunal assemblages developed on both seeded and rope treatments. The assemblages developed from zero species at time of deployment to over 18 on seeded treatment and 17 on rope treatment. This general build-up of species over sampling months followed a seasonal pattern of development from time of deployment in February (Year 1) and December (Year 2) until harvest in June for both growing seasons, however species richness decreased from May to June in Year 1 2014. None of the species identified on either the seeded or rope treatments were unexpected and all have previously been identified as being associated with either wild or cultivated kelp species (Seed & Harris 1980, Christie et al. 2003, 2009, Park et al. 2008, Peteiro & Freire 2013). We found significant differences in species richness for each sampling month, however there was no effect of treatment or year on species richness. The life histories of sampled species represented organisms with both direct development and planktonic larval stages. The species with planktonic larvae included echinoderms, polychaete worms, gastropod molluscs, some crustacean species and two fish species (*Pollachius pollachius* and *Gaidropsarus vulgaris*). The accumulation of these species probably follows seasonal factors influencing reproduction, dispersal and settlement, before they metamorphosize and colonise the new habitat. They may also have colonised from nearby habitats as adults. Species with direct development included, crustaceans (amphipods, caprellids and isopods) and two fish species (*Cyclopterus lumpus* and *Pholis gunnellus*). The pattern of colonisation for these species probably also follows seasonal patterns in reproduction, in addition to the time needed for species to travel and colonize the farm either by passively drifting in the water column or by actively swimming (Clarkin et al. 2012).

Looking more closely at species composition, the multivariate analysis showed that there was no clear distinction of mobile assemblages between seeded and rope samples (MDS; Fig. 5.2). However, the interaction between sampling date and treatment from the updated PERMANOVA design (Table 5.3) returned a significant value, but treatment itself was not significant. These results are difficult to interpret as from the subsequent pair-wise test (Table 5.4), treatments were significantly different for June 2014 and for April and May 2015 samples but not

for the other months. These results are complicated and do not suggest any predictable pattern. To try to understand these patterns we assessed the species present within months and between treatments. Overall, there was no obvious differences in species between treatments, with no cohort of species being present on one treatment and absent on the other. Some individual species were only found on one treatment; these species were typically rare and found in low occurrence i.e. present in one sample during one month. These rare species were generally found to be cosmopolitan species and were not restricted to specific habitats. However, the lumpfish *Cylopterus lumpus* was only found on seeded droppers which is logical as adults lay their eggs on kelp and once hatched juveniles attach to the kelp and use it for protection and as a nursery ground. *C. lumpus* have sampled on wild kelp in the North Atlantic (Christie et al. 2003, Norderhaug et al. 2005).

5.4.2 Predictability of seeded and rope assemblages from one year to the next

Within the same year the mean occurrence-day of shared species on seeded and rope droppers was predictable (Fig. 5.4a and 5.4b). Within individual years, 2014 and 2015, the mean difference in occurrence-day between seeded and rope treatments was ($16.9 \pm \text{SE } 3.91$) and ($16.2 \pm \text{SE } 3.38$) respectively. Thus, treatment did not affect the arrival and colonization of shared mobile species. However, somewhat expected from the multivariate results, the mean occurrence-day of mobile fauna on seeded droppers and separately on rope droppers were not predictable from one year to the next (Fig. D1a and D1b Appendix D). The mean difference in occurrence-day for individual treatments, seeded and rope, between years 2014 and 2015 was ($9.5 \pm \text{SE } 2.39$) and ($11.5 \pm \text{SE } 3.14$) respectively. Interestingly, this was in contrast to the pattern observed of fouling epiphytes on the fronds of *Alaria esculenta* (Walls et al. 2017) sampled from the same site over the same two-year period. The frond fouling community was dominated by sessile animals (e.g. bryozoans and hydroids) with strong habitat selection and mainly larval settlement, whereas the present study was dominated by fauna with high mobility rates and direct development.

There are a number of possible reasons for the development of a general mobile assemblage rather than distinct seeded and rope assemblages and for the lack of predictability of assemblages between years. The main factors that likely combine and contribute to the pattern that we have identified, include habitat specificity and mobility rates of our sampled fauna.

Low habitat specificity of mobile species could contribute to the formation of a general mobile assemblage with no distinction between treatments. In similar terrestrial plant assemblages, much of the associated diversity comes from herbivorous insects, many of which have restricted host ranges (Haddad et al. 2001). In this study, amphipods were the most dominant group of mobile invertebrates found associated with both seeded and rope treatments. While there are some examples of herbivorous amphipod species being specialised to macroalgae (Poore et al. 2008), interactions between our amphipod assemblages are unlikely to be specific to the *A. esculenta* tissue dominant on the seeded droppers or the multiple fouling macroalgae species (e.g. *Saccorhiza polyschides*, *Polysiphonia* sp., *Ceramiales* sp.) present on the rope droppers. Kelp is not a suitable food source, for many organisms, before it has been released as particulate organic matter (POM) and degraded by bacteria (Norderhaug et al. 2003). However, a few species are known to directly graze on fresh kelp tissue including the gastropods *Patella pellucida* and *Lacuna vincta* and the sea urchins *Strongylocentrotus droebachiensis* and *Paracentrotus lividus* (Steneck et al. 2002, Molis et al. 2010, Leclerc et al. 2013). a few species Norderhaug et al. (2003) found that none of the tested amphipod species (including *Jassa falcata*) in their study were able to live or grow on fresh kelp tissue, however the gastropod *Rissoa parva* did survive. Detritivores dominated the feeding type of the mobile fauna sampled on both treatments accounting for over 45 %, presumably feeding on kelp and other epiphytic algal detritus. Carnivores represented 30 % of the sampled fauna, likely feeding on juveniles and opportunistically scavenging other animals. 23 % of the species were omnivores consuming a variety of plant and animal material. The herbivorous gastropod *Lacuna vincta* accounted for the final 2 %, *L. vincta* is known to graze on fresh kelp tissue (Molis et al. 2010) and was the only exclusively herbivorous species sampled from our mobile fauna. With the food resource of the dominant mobile fauna likely to be degraded macroalgae and POM, then specialised associations between mobile fauna and fresh macroalgae tissue growing on the dropper ropes are unlikely. Kelp detritus and POM are probably transported via current flow around the farm thus food availability is not restricted leading to low habitat specificity.

Christie et al. (2009) found that the most abundant faunal taxa associated with macrophytes are highly mobile. They move freely between individuals (Norderhaug et al. 2002) and exhibit high dispersal out of macrophyte beds

(Waage-Nielsen et al. 2003, Jørgensen & Christie 2003). High faunal mobility enables rapid colonisation and utilisation of new habitats (Norderhaug et al. 2002). We sampled fauna with varying degrees of mobility, such as amphipods and other crustaceans that have high relative mobility and gastropods and polychaetes which had lower relative mobility rates. As the seaweed farm is between 250 m and 1 km away from natural kelp populations and associated faunal assemblages, swimming and drifting in the water column must be an important method for dispersal. Amphipods, isopods and caprellids can swim, however, drifting with tidal currents and wave action is probably a more efficient method (Jørgensen & Christie 2003). Gastropod species in kelp forests have also been reported to drift, using mucous thread to increase buoyancy (Vahl 1983, Martel & Chia 1991, Jørgensen & Christie 2003) and the gastropod *L. vincta* can use its foot to help propel itself while drifting in the water column (pers. obs.).

The difference in structural complexity between the treatments did not cause different assemblages to develop. This was an unexpected result as seeded droppers were deployed with juvenile *A. esculenta* individuals, which although small, had a more complex structure than the rope droppers. Both treatments increased in complexity throughout the experiment as macrophytes settled, established and grew (pers. obs.). However, the seeded droppers formed a much more complex habitat than the rope droppers. Norderhaug (2004) has shown that kelp-associated fauna select habitat according to the architectural structure and complexity and not according to food value. Thus, habitat is mainly important for protection from predators and not as a food source (Christie et al. 2009). Interesting, this selectivity was not seen in our study, a possible explanation could be the distance between our treatment replicates. The different treatment droppers were deployed randomly along the header rope and spaced 1.5 m apart. This distance may have been small enough that species could travel easily between droppers allowing the formation of this general farm mobile faunal assemblage. Jørgensen & Christie (2003) found that artificial structures placed within a kelp forests were rapidly colonised both horizontally and vertically. The distances colonised by these species was up to 7 m horizontally from the edge of the kelp forest and 1.6 m vertically above the kelp forest (Jørgensen & Christie 2003). This shows that migration between treatments spaced 1.5 m apart is possible and such distances are typical of the set-up at commercial seaweed farms.

5.4.3 Implications

This study identifies a habitat provided by cultivated kelp farms for mobile fauna. However, there was no difference in assemblage structure between the habitat provided by the seeded and rope droppers. We found that a common mobile faunal assemblage developed around the farm regardless of kelp biomass. These assemblages develop from both the settlement of larvae and immigration by adults. The effect of habitat complexity was not evident between seeded and rope assemblages. This lack of habitat specificity coupled with the high mobility rates of fauna were the most likely causes of the homogenous assemblages between treatments. Regardless, the presence of kelp detritus as a food source within the farm is probably a very important reason for the assemblages' presence. In this study, the rope droppers could be considered analogous to other ropes of the farm infrastructure (e.g. anchor and header ropes), supporting similar species richness and assemblage composition as seeded kelp droppers. This implies that artificial kelp aquaculture sites are not depleted in species and suggests that an increase in these types of structures may not negatively impact on biodiversity as much as other artificial structures such as seawalls have been found to (Firth et al. 2016b). The development of assemblages, even if only present for a short time, attracts predators and thus is a source for secondary production and enhancement of local biodiversity within the area (Smale et al. 2013). Cultivation practices for farmed kelps are subject to change and development, depending on site practices and biomass requirements. Harvesting techniques may be adjusted so that holdfasts, stipes, a basal portion of the frond and smaller fronds remain in place for more than one growing season, extending the survival of the mobile assemblages, which may depend on kelp detritus as a food source.

Acknowledgements

The authors acknowledge Dr. Benoît Quéguineur, Mr. David Moran and Mr. Brendan Walls for their assistance in field sampling. In addition, they would like to thank Dr. Adrian Patterson and Dr. Jack O'Carroll from NUI Galway's Benthic Ecology Laboratory for taxonomic support and Mr. Michael Murphy and Mr. Paul Flannery of Dingle Bay Seaweed Ltd. for boat work and assistance at Ventry Harbour and staff at Dingle Oceanworld Aquarium for providing laboratory space. This work was also supported by the Energetic Algae project (EU Interreg IVB NWE Strategic Initiative; www.enalgae.eu). A.M.W. is funded by the Dr. Tony Ryan Research Trust, NUI Galway.

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Chapter 6

Incorporation of cultivated kelp (*Alaria esculenta* and *Saccharina latissima*) into the diet of the blue mussel *Mytilus edulis*: a stable isotope study

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Abstract

Wild kelp forests represent one of the most productive habitats in temperate coastal areas. Much of this productivity enters the food web as detritus, which may represent one of the main carbon source for consumers in these environments. With an anticipated increase in commercial-scale kelp cultivation in Europe, the contribution of detrital kelp from cultivated sources has been overlooked. Here, we examine the diet of the suspension-feeding mussel *Mytilus edulis* at a commercial kelp farm growing *Alaria esculenta* and *Saccharina latissima* on the southwest coast of Ireland. We assessed all *M. edulis* putative food sources using stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) to determine if cultivated kelp is incorporated into their diet. We also analysed *M. edulis* and its food sources at a nearby rocky shore (control) site to compare with our farm site results. Bayesian based SIAR modelling (stable isotope analyses in R) of the contribution of putative food sources revealed a shift in importance of phytoplankton and zooplankton to the diet of mussels at the control site compared to macroalgal sources at the farm site. These results suggest that when there is an abundance of good quality kelp detritus mussels will decrease their dependence on planktonic food sources and increase consumption of kelp detritus. This study shows that cultivated kelp detritus is incorporated into the food web and seaweed farms provide additional ecosystem services above the production of biomass. This result has wider implications for the application of seaweed farms in habitat and food provision.

Keywords: Kelp cultivation; *Alaria esculenta*; *Saccharina latissima*; Food webs; Detritus; *Mytilus edulis*; Suspension-feeding; Stable isotopes; Integrated Multi-Trophic Aquaculture (IMTA)

6.1 Introduction

The global cultivation of aquatic plants, dominated by macroalgae, is growing rapidly by almost 8 % per year in the past decade (FAO 2016). Over 33 % of the 27.3 million tonnes of annual global aquatic plant produced in 2014 was from the two kelp species *Laminaria japonica* and *Undaria pinnatifida* (FAO 2016). China, Indonesia and the Philippines have traditionally been the dominant producers of cultivated macroalgae including kelp. However, over the last 15 years, kelp cultivation in European waters has received increasing interest (Kraan et al. 2000, Kerrison et al. 2015, Peteiro et al. 2016). This attention has been supported by feasibility studies (Bruton et al. 2009) and pilot-scale farms (Sanderson et al. 2012, Marinho et al. 2015) being set up to begin to develop the industry and advance cultivation techniques of kelps native to European waters (e.g. Edwards & Watson 2011). In addition to the supply of biomass for the many traditional (e.g. food, fertilizers) and expanding (e.g. pharmaceutical and cosmetic products, biofuel & bioactive compound production) uses, macroalgal farms also provide ecological functions in the form of ecosystem services. Cultivated kelp create novel habitats for species to attach to (Peteiro & Freire 2013, Førde et al. 2016, Walls et al. 2016, 2017), mitigate environmental impacts through bio-filtration (Neori et al. 2004, Troell et al. 2009) and supply a source of carbon in the form of primary productivity which may enter the food web through both direct and detrital pathways (Uchida 1996, Yoshikawa et al. 2001).

Wild kelp forests represent some of the most productive habitats on Earth (Mann 1973, 2000, Reed et al. 2008) and are a major source of primary productivity in temperate zones (Steneck et al. 2002). Around coastlines of the temperate Atlantic Ocean, kelp primary production can exceed $1,000 \text{ g C m}^{-2} \text{ year}^{-1}$ (Mann 1973, Smale et al. 2013), while primary production from phytoplankton in the same regions is typically between 100 and $300 \text{ g C m}^{-2} \text{ year}^{-1}$ (Mann 2000). In contrast to wild kelp ecosystems, the total amount of production from cultivated kelps is likely to be lower as harvest of cultivated kelp biomass usually occurs in early summer and hence there is no further production of kelp occurring once it has been harvested (Yoshikawa et al. 2001). Kelp primary productivity enters the food web through two routes; direct grazing on kelp tissue or via detrital pathways. Relatively few species are known to directly graze on fresh kelp tissue including the gastropods *Patella pellucida* and *Lacuna vineta* (Molis et al. 2010, Leclerc et al. 2013a). Due to the high C:N ratios of fresh kelp tissue, degradation by bacteria

needs to occur before energy from kelp biomass is available to most consumers (Duggins et al. 1989, Norderhaug et al. 2003). As a result, much of the energy transfer of temperate kelp production into food webs is primarily through particulate (POM) and dissolved organic matter (DOM), with estimates of wild kelp production entering the food webs in these forms higher than 80 % (Krumhansl & Scheibling 2012).

Kelp detritus can range in size from small particles to whole thalli depending on the processes involved in biomass removal. Tissue can be lost in three main ways: 1) Whole thalli are removed from breakage at the stipe or when the holdfast becomes detached from its substratum, which includes rocks or boulders in wild kelp forests or rope substratum at cultivated sites; 2) Parts of the frond can break off removing large pieces from the frond; 3) Erosion of the distal ends of fronds can occur as tissue is continually lost through decay (Zhang et al. 2012, Krumhansl & Scheibling 2012). DOM is also released during this process and is estimated to account for 16 – 35 % of annual energy production in kelps (Krumhansl & Scheibling 2012 and references therein). Kelp tissue loss and the production of detritus is caused by wave and current driven motion (Witman 1987, Scheibling & Gagnon 2009, Filbee-Dexter & Scheibling 2012), damage by grazing and epiphytic organisms (Duggins et al. 2001, Park et al. 2008, Krumhansl et al. 2011) and natural senescence during the kelp life cycle (Lüning 1979). As cultivated kelps do not complete their full natural life cycle at sea and are harvested at peak growth in early summer to avoid tissue degradation by epiphytes and environmental conditions, the importance of the latter two factors is likely to be less for cultivated kelps than for wild kelps.

The use of stable isotope ratios has provided ecologists with a powerful molecular tool in a wide range of ecological contexts. One of the most commonly used applications of stable isotope analysis is in determining trophic linkages between consumers and their putative prey and thereby establishing energy flow in food webs (Fry & Sherr 1984, Doucett et al. 1996). In this regard, stable isotopes have considerable advantages over more traditional approaches such as dietary (gut contents) analysis (Grey 2006). Stable isotope signatures reflect the food assimilated by the consumer rather than simply ingested (Rosenfeld & Roff 1992) and, importantly, gut contents only provide a limited “snapshot” of the diet (Gearing 1991, Pinnegar & Polunin 1999, Grey 2001) whereas stable isotope ratios provide a time-integrated indication of diet (Hesslein et al. 1993). Generally, there

is enrichment of the heavier isotope forms (^{13}C , ^{15}N) in consumers relative to their diet, as the lighter isotopes (^{12}C , ^{14}N) are preferentially utilised during metabolism and respiration (Dubois et al. 2007a). $\delta^{13}\text{C}$ isotopes identify the food source of consumers and $\delta^{15}\text{N}$ isotopes identify the trophic position of organisms in the food web. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have been useful in estimating the contribution of kelp-derived carbon to associated and adjacent food webs in wild kelp forests (Duggins et al. 1989, Bustamante & Branch 1996, Fredriksen 2003, Schaal et al. 2010, Leclerc et al. 2013a). This has mainly been studied in suspension feeders, as they are usually the dominant trophic group in these marine habitats (Miller & Page 2012) and because these animals filter tiny particles from the water, gut content analysis is very difficult (Pasquaud et al. 2007). These studies have shown that kelp detritus is often an important constituent of suspended POM, thus an important source of carbon to suspension feeders. However, it has been suggested in a recent review (Miller & Page 2012), that the extent of detrital kelp contribution to food webs may be overestimated due to the use of proxy estimates for phytoplankton $\delta^{13}\text{C}$ ratios (e.g. offshore POM, diatom cultures), rather than using phytoplankton itself.

It is expected that cultivated kelp farms supply detrital material to the surrounding waters in much the same way as wild kelp forests. This material may be incorporated into the food web via suspension-feeding organisms. In the present study, we aim to analyse stable isotope ratios of the suspension-feeding mollusc *Mytilus edulis* and its putative food sources at a control and a kelp aquaculture site to assess if cultivated kelp detritus is incorporated into their diet. To our knowledge, stable isotope signatures of cultivated kelps have not before been analysed and this is the first study to attempt to directly quantify the importance of cultivated kelp in trophic food webs.

6.2 Materials and Methods

6.2.1 Study site

This study was conducted on the southwest coast of Ireland in Ventry Harbour, County Kerry ($52^{\circ} 06' 49.45''$ N, $-10^{\circ} 21'20.17''$ W; Fig. 6.1) at the largest operating commercial seaweed farm in Ireland (18 ha site). Ventry Harbour is a moderately sheltered and shallow embayment orientated towards the southeast, approximately 2.5×1.5 km (3.75 km²) in area, with a wide mouth opening into Dingle Bay. The predominant substrate is sand, with *Zostera marina* (seagrass)

extensively distributed throughout, leading to a rocky boulder reef towards the mouth of the bay. The licensed seaweed farm is orientated northwest to southeast, and located to the westerly side of Ventry Harbour (Fig. 6.1). The depth underneath the farm is approximately 6 m at the northwestern end before gently sloping to 20 m at the eastern edge of the farm at mean low water spring tide (MLWS). The tidal range in Ventry Harbour is between 0.6 - 4.0 m. Irradiance values obtained from nearby Valentia weather observatory (51° 56' 23" N, -10° 14' 40" W) ranged from 5,447 to 63,823 J cm⁻² (mean daily maximum value per month) for 2014. Sea surface temperature data was obtained from the M3 offshore weather buoy located approximately 56 km southwest of Mizen head (51° 13' 0" N, -10° 33' 0" W), and ranged from 10.1 to 17.6 °C for 2014. Although offshore values are less extreme than inshore values, Ventry Harbour is a well flushed bay so values are broadly representative. The longline structure is similar to the set-up described in the Fig. 3 of Walls' study (Walls et al. (2017); Chapter 4 Fig. 4.3), however, the farm in Ventry consists of 3 parallel units of 280 m linear longlines suspended approximately 1.5 m below the sea surface. The longlines were kept in position by buoys attached to the header rope and by 1500 kg anchor blocks at either end of the lines. The farm cultivates the kelps *Alaria esculenta* and *Saccharina latissima* for human consumption, animal feed and use in cosmetic products.



Fig. 6.1. Control and farm sampling sites at Ventry Harbour, County Kerry, Ireland. F = Farm Sampling Site and C = Control Sampling Site.

6.2.2 Sample collection and processing

Sampling was carried out in May 2016 at two sites (Fig. 6.1); one site was located within the farm at (52° 06' 52.34" N, -10° 21'20.44" W) and the second, the control site, was located 200 m away on the southerly shore (52° 06' 38.96" N, -10° 21'10.23" W). The control site consisted of a rocky shore with sandy inlets interspersed between bedrock. The location of the site on the southwest shore means it is very sheltered from wave action. At both farm and control sites, the most abundant putative food sources and the target consumer *Mytilus edulis* were sampled. At each site, two replicates of seawater (5 L) were collected using a Niskin bottle below the surface (1 m depth) to sample the suspended particulate organic matter (POM) and phytoplankton and zooplankton samples were collected by net tows (mesh size 100 µm and 200 µm, respectively; mesh diameter 0.5 m and 1 m, respectively) pulled for approximately 2.5 km at approximately 1 m below the water surface. Dominant macroalgal species (*Ascophyllum nodosum*, *Fucus spiralis* and *Ulva* sp.) and *M. edulis* individuals were collected at low tide along the intertidal zone of the control site and added to labelled sample bags. At the farm site, cultivated macroalgae (*Alaria esculenta* and *Saccharina latissima*) and *M. edulis* were sampled from the header rope on the longline structure and *Z. marina* seagrass was sampled from below the farm using SCUBA divers, samples were added to labelled sample bags. All samples were transferred back to the laboratory in cooler boxes. See Table 6.1a and b for samples collected and their replicate number.

In the laboratory, all samples (excluding POM samples) were immediately frozen at -20 °C until preparation and analysis. Seawater samples (POM) were pre-filtered by 100 µm and 200 µm mesh to remove phytoplankton, zooplankton and larger detrital particles. The filtrate from each sample was then divided in two and filtered on pre-combusted (4 hrs, 520 °C) Whatman® GF/F filters (0.7 µm). As any inorganic component contained within the POM sample can impact the carbon isotope ratios of the POM sample but is not assimilated by consumers, thus one half of each of the POM samples were briefly acidified with 1M HCl and then rinsed with distilled water according to the protocol described by Jacob et al. (2005). The second half of each of the POM samples were analysed to determine the nitrogen stable isotope ratios of the POM unaffected by the acid washing procedure. All POM samples were then rinsed thoroughly with distilled water before being dried in oven (24 hrs, 60 °C) and stored in desiccator until further

analysis. Macroalgal and macrophyte samples were defrosted, cleaned of any epiphytes and rinsed using distilled water to remove any sediment. Macroalgal basal and distal tissue sections, *Z. marina* fresh (green) and decaying (brown) leaves, were scraped with a scalpel to remove epiphytes and washed with distilled water. All samples were added to labelled Eppendorf tubes and dried at 60 °C for 48 hrs. Dried samples were ground into a fine homogenous powder using a pestle and mortar and stored in pre-combusted glass vials until further processing. The *M. edulis* samples were defrosted and the mantle tissue was extracted, rinsed with distilled water and dried (48 hrs, 60 °C). The samples were then ground using a pestle and mortar. Lipids from the mantle tissue were removed using a methanol-chloroform (2:1 by volume) solution. Lipid extraction was performed because lipids are depleted in ¹³C compared with whole organisms and the lipid content of animal tissue samples is variable (Post 2002, Fry 2006, Redmond et al. 2010). The methanol-chloroform solution was added to the mantle tissue sample, agitated and allowed to settle for 30 min before the supernatant was removed. This process was repeated until the solution was clear and samples were then re-dried (48 hrs, 60 °C) and stored in pre-combusted glass vials until further processing. Phytoplankton and zooplankton samples were cleaned under a microscope removing any particles of macroalgal or macrophyte detritus, dried (48 hrs, 60 °C), ground and stored until further analysis. The lipids from the zooplankton samples were removed as described above for the mussel mantle tissue. All samples were then weighed into tin capsules in quantities of 0.7- 2.0 mg, according to specifications related to their nitrogen content, and sent to Washington State Laboratory for analysis of their isotopic content.

6.2.3 Isotopic analysis

Carbon and nitrogen isotope ratios were determined using an elemental analyser (ECS 4010, Costech Analytical) coupled with a continuous flow isotope ratio mass spectrometer (Dekta Plus XP, Thermofinnigan). Data are expressed in the standard δ unit.

$$\delta X = [(R_{\text{sample}} / R_{\text{reference}}) - 1] \times 10^3$$

where X is the element in question, $R = {}^{13}\text{C}/{}^{12}\text{C}$ for carbon and ${}^{15}\text{N}/{}^{14}\text{N}$ for nitrogen. These ratios were calculated in relation to the certified reference materials Vienna Pee Dee Belemnite-limestone (V-PDB) and atmospheric dinitrogen (at-air), for carbon and nitrogen, respectively. The VPDB and at-air scales were achieved using

in-house protein standards, calibrated against NBS-19 and IAEA N3 reference materials. The standard deviation of repeated measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of a laboratory standard was 0.10 ‰ *versus* V-PDB and 0.05 ‰ *versus* air, respectively.

6.2.4 Suspension feeder diet analysis

The relative importance of each of the putative prey items in the diet of *Mytilus edulis* was determined using the `siarmcmcdirichletv4` model function in the programme SIAR, with each of the models run with 50,000 iterations and a burnin of 50,000 iterations (Stable Isotope Analyses in R) (Moore & Semmens 2008, Parnell et al. 2008, Jackson et al. 2009) in R (R Development Core Team 2007). This approach provides robust estimates of the proportion of energy derived from various sources in consumers with the model allowing for errors associated with source values and trophic fractionation coefficients to be incorporated into the model calculation, and produces probability density functions for the contribution of each putative prey resource.

For the model of the control site we ran POM, phytoplankton, zooplankton, *Ulva* sp., *A. nodosum* and *F. spiralis* samples. For the farm site POM, phytoplankton, zooplankton, *A. esculenta* (basal and distal), *S. latissima* (basal and distal) and *Z. marina* (fresh and decaying leaves) samples were run in the model. Basal and distal samples for each of the kelps *A. esculenta* and *S. latissima* and fresh and decaying leaves for *Z. marina* at the farm site and *A. nodosum* and *F. spiralis* from the control site were pooled for analysis within the model due to high similarity between the samples as the model is unable to differentiate between samples with very high similarity. The stable isotope results for all putative food sources and those samples included in the models can be seen in Table 6.1a and b. The similarity between samples which were pooled before inclusion in the models is clearly evident from the mean and standard deviation stable isotope results.

Table 6.1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean and standard deviation of the main putative food sources in Ventry Harbour County Kerry at (a) control site and (b) farm site.

(a)	Control Site					
	Sources	<i>n</i>	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
			Mean	Sd	Mean	Sd
Particulate Organic Matter (POM)	1	-25.43	n/a	5.66	n/a	
Zooplankton	2	-16.04	0.25	7.4	0.28	
Phytoplankton	2	-20.18	0.08	7.69	0.05	
<i>Ulva</i> sp.	6	-16.31	0.34	13.15	0.65	
<i>Ascophyllum nodosum/ Fucus spiralis</i>	12	-18.71	0.24	6.01	0.16	
<i>Ascophyllum nodosum</i> *	6	-18.91	0.82	5.66	0.15	
<i>Fucus spiralis</i> *	6	-18.52	0.87	6.35	0.58	

*Samples pooled before inclusion in the model

(b)	Farm Site					
	Sources	<i>n</i>	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
			Mean	Sd	Mean	Sd
Particulate Organic Matter (POM)	1	-25.38	n/a	7.15	n/a	
Zooplankton	2	-17.20	0.05	8.02	0.12	
Phytoplankton	2	-20.46	0.04	7.64	0.17	
<i>Alaria esculenta</i>	12	-19.36	0.82	6.12	0.98	
<i>Alaria esculenta</i> Basal*	6	-19.61	0.74	6.88	0.24	
<i>Alaria esculenta</i> Distal*	6	-19.11	0.89	5.37	0.85	
<i>Saccharina latissima</i>	12	-17.56	0.58	9.72	1.60	
<i>Saccharina latissima</i> Basal*	6	-17.50	0.43	10.04	1.35	
<i>Saccharina latissima</i> Distal*	6	-17.61	0.74	9.41	1.89	
<i>Zostera marina</i>	12	-14.17	1.00	5.79	0.42	
<i>Zostera marina</i> Fresh Leaves*	6	-13.25	0.19	5.91	0.30	
<i>Zostera marina</i> Decaying Leaves*	6	-14.62	0.80	5.60	0.47	

*Samples pooled before inclusion in the model

Isotopic step fractionation is no longer considered a constant value, especially regarding trophic-step enrichment from primary producers to primary consumers. Numerous factors such as species, tissue type, age, sex and sample treatment are known to possibly affect discrimination processes in organism's tissues (Del Rio et al. 2009). Among the fractionation values in the literature we

chose to use 2.2 ‰ for $\delta^{13}\text{C}$ and 3.8 ‰ for $\delta^{15}\text{N}$, since these values were specifically calculated using feeding experiments for the suspension feeder *M. edulis* (Dubois et al. 2007a). However, Dubois et al. (2007a) did not remove lipids from the samples prior to analysis and ^{13}C can be depleted in lipids by as much as 12 ‰ when compared to non-fatty tissues (DeNiro & Epstein 1977, Ricca et al. 2007). Ricca et al. (2007) found that lipid extraction significantly increased $\delta^{13}\text{C}$ values by an average of 0.6 ‰ in *M. edulis* but no changes were observed for $\delta^{15}\text{N}$ values. Thus, we adjusted Dubois et al. (2007b) values with Ricca's (2007) values and chose a trophic step fractionation of 1.6 ‰ (± 0.259) for $\delta^{13}\text{C}$ and 3.8 ‰ (± 0.243) for $\delta^{15}\text{N}$.

6.3 Results

6.3.1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of putative food sources

The results of the isotopic characterisation of the putative food sources for control and farm sites are presented in Table 6.1a (control) and Table 6.1b (farm) respectively. In control and farm sites, putative food sources were more discriminated by $\delta^{13}\text{C}$ than by $\delta^{15}\text{N}$ ($\delta^{13}\text{C}$ range = 9.1 ‰ and 11.2 ‰ and $\delta^{15}\text{N}$ range = 7.2 ‰ and 3.9 ‰ for control and farm, respectively). The $\delta^{13}\text{C}$ of POM was consistent between sites at -25 ‰, however the $\delta^{15}\text{N}$ ratio varied between locations with a signature of 5.66 ‰ at the control site and 7.15 ‰ at the farm site. Site variations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios for zooplankton were observed ($\delta^{13}\text{C}$ -16.04 ‰ and $\delta^{15}\text{N}$ 7.4 ‰ at control site and $\delta^{13}\text{C}$ -17.20 ‰ and $\delta^{15}\text{N}$ 8.02 ‰ at farm site). Phytoplankton samples were largely similar ($\delta^{13}\text{C}$ -20.18 ‰ and $\delta^{15}\text{N}$ 7.69 ‰ at control site and $\delta^{13}\text{C}$ -20.46 ‰ and $\delta^{15}\text{N}$ 7.64 ‰ at farm site). Variation was observed between the isotopic signatures of the brown algal species sampled at both sites. *Ascophyllum nodosum* and *Fucus spiralis* pooled samples had a $\delta^{13}\text{C}$ ratio of -18.71 ‰ and $\delta^{15}\text{N}$ of 6.01 ‰. These signatures lie in between those of the kelps *Alaria esculenta* and *Saccharina latissima* sampled at the farm site ($\delta^{13}\text{C}$ -19.36 ‰ and $\delta^{15}\text{N}$ 6.12 ‰ for *A. esculenta* and $\delta^{13}\text{C}$ -17.56 ‰ and $\delta^{15}\text{N}$ 9.72 ‰ for *S. latissima*). The seagrass *Zostera marina* was ^{13}C -enriched ($\delta^{13}\text{C}$ -14.17 ‰) and ^{15}N -depleted ($\delta^{15}\text{N}$ 5.79 ‰). Lastly, the green alga *Ulva* sp. was enriched in both ^{13}C ($\delta^{13}\text{C}$ -16.31‰) and in ^{15}N ($\delta^{15}\text{N}$ 13.15‰). This enrichment of the ^{15}N value is very unexpected for a primary producer, however all of the replicate samples were between $\delta^{15}\text{N}$ 12.30 – 13.96 ‰ and may be unusually high due to an external factor which we could not identify.

6.3.2 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Mytilus edulis*

In both control and farm sites, *M. edulis* samples were differentiated from each other by their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures. Variation in $\delta^{13}\text{C}$ of 0.7 ‰ and 0.86 ‰ and a variation of 0.63 ‰ and 0.98 ‰ in $\delta^{15}\text{N}$ ratios were observed in *M. edulis* samples at the control and farm sites, respectively. Differences between the mean isotopic signatures of mussels sampled at control (^{13}C -16.94 ‰ \pm 0.24 and $\delta^{15}\text{N}$ 8.75 ‰ \pm 0.24) and farm ($\delta^{13}\text{C}$ -17.34 ‰ \pm 0.36 and $\delta^{15}\text{N}$ 7.72 ‰ \pm 0.41) sites suggest they are feeding on different food sources. The potential contribution of food sources to the diet of the suspension-feeding *M. edulis* can be observed from the biplots Fig. 6.2a (control) and Fig. 6.2b (farm).

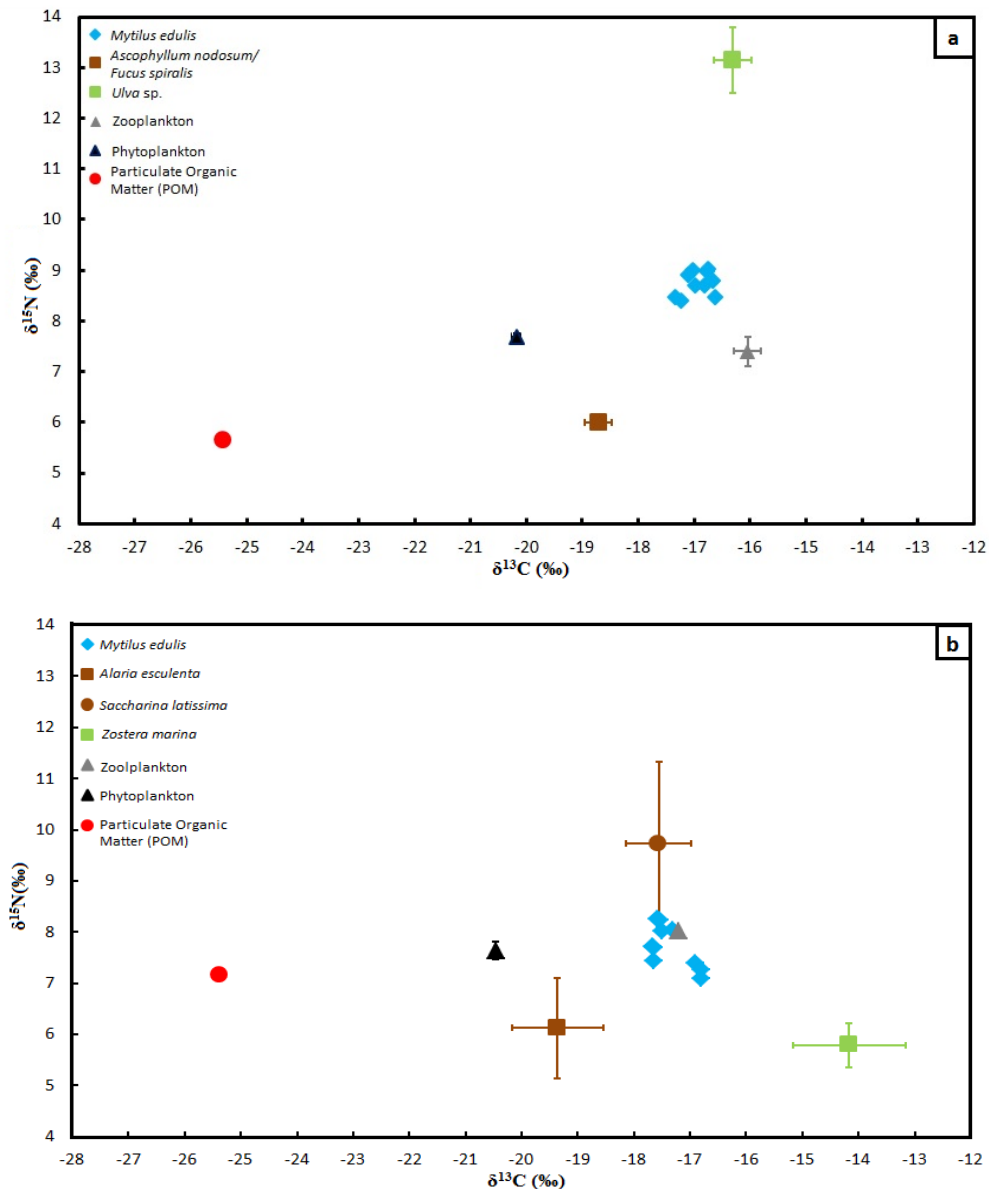


Fig. 6.2. Mean (\pm SD) $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) ratios of the putative food sources and *Mytilus edulis* sampled at Ventry Harbour, County Kerry at (a) the control site and (b) the farm site.

6.3.3 Bayesian mixing model results

The results of SIAR Bayesian modelling for the diet of *M. edulis* showed variation in the importance of putative diet between sites (Fig. 6.3a (control) and Fig. 6.3b (farm)). There is an increase in the amount of material assimilated from kelp at the farm site (Fig. 6.3b) relative to the control site with a concomitant decrease in the relative importance of zooplankton and phytoplankton. The contribution of zooplankton and phytoplankton to the diets of mussels at the control site (Fig. 6.3a) is 59 % with the contribution of these sources at the farm site decreasing to 31 %. The macroalgae at the control site, *Ascophyllum nodosum* and *Fucus spiralis* contribute 26 % and *Ulva* sp. just 4 % of the diet of the mussels. By comparison, at the farm site the macroalgae *Alaria esculenta* and *Saccharina latissima* contribute 21 % and 10 % respectively, and the seagrass *Zostera marina* contributes 21 % to the diet of *M. edulis*. Thus, the importance of macroalgal sources in the energy assimilated by *M. edulis* at the farm site is considerably higher than for mussels at the control site.

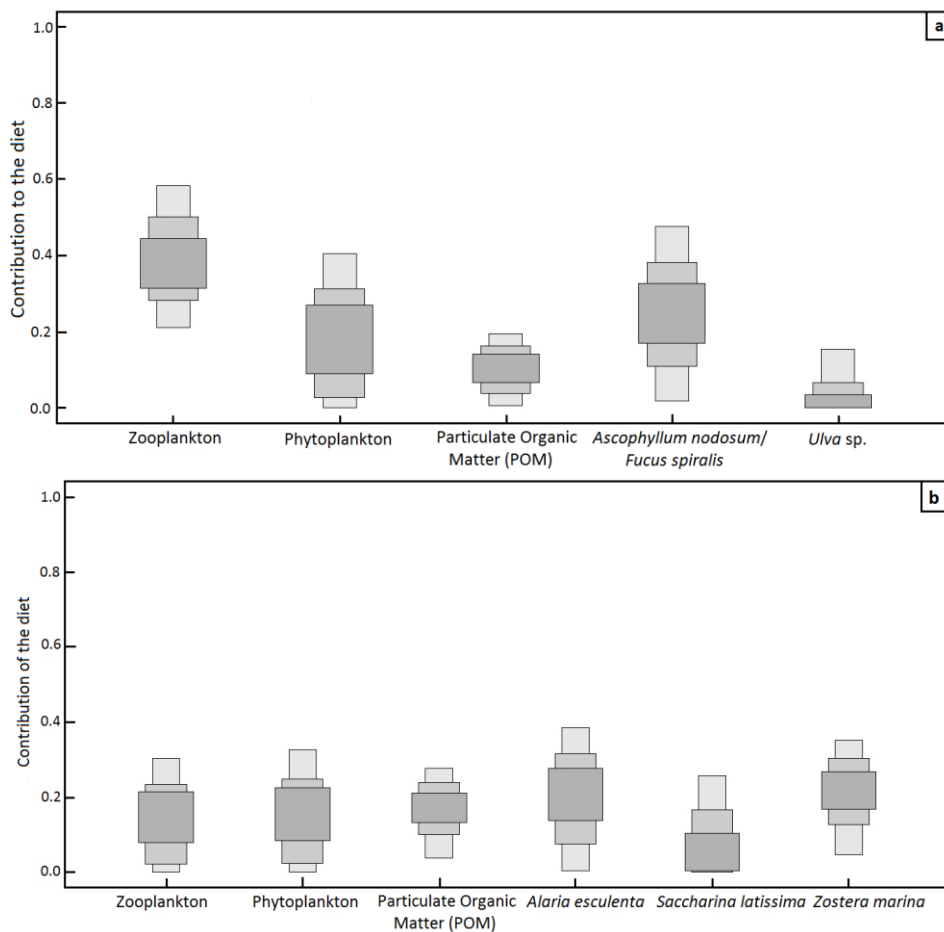


Fig. 6.3. Boxplots of the relative contributions of potential food sources to the diet of *Mytilus edulis* at (a) the control site and (b) the farm site in Ventry Harbour, County Kerry, as determined using SIAR Bayesian based modelling. Confidence intervals (CI): CI_{50%}=dark grey, CI_{75%}= medium grey and CI_{95%}= light grey.

6.4 Discussion

The primary aim of this study was to assess the importance of cultivated kelp in the diet of associated fauna. Results show that within the extent of the seaweed farm, cultivated kelp makes up a significant component of the diet of the suspension-feeding mussel *Mytilus edulis*. We observed a shift in the importance from the contribution of zooplankton and phytoplankton to the diet of mussels at the control site to macroalgal sources at the farm site. These results suggest that when there is an abundance of good quality kelp detritus available to *M. edulis* they decreased their dependence on planktonic food sources (zooplankton and phytoplankton) and increased the consumption of kelp. Although the importance of wild kelp detritus to the diet of suspension-feeding organisms has been identified (Duggins et al. 1990, Fredriksen 2003, Schaal et al. 2012, Leclerc et al. 2013b), this is the first time that the significance of cultivated kelp detritus to these organisms and the incorporation of this primary productivity source into the food web has been observed.

The contribution of the cultivated kelps *Alaria esculenta* and *Saccharina latissima* to the diet of mussels at our farm site was approximately 31 % from SIAR modelling (Fig. 6.3b). This value compares well with values for the contribution of wild kelp detritus to suspension feeders calculated through stable isotope analysis. Duggins et al. (1989) showed that kelp detritus accounted for between 25-35 % of the diet of *M. edulis* on the Alaskan coast. More recently, Schaal et al. (2010) estimated that suspension feeders derive at least between 35-50 % of their diet from macroalgal derived organic matter, with *M. edulis* deriving 46-66 %. However, caution must be used with some of these results as Miller and Page (2012) have challenged these estimates in a review of isotope-based meta-analysis and suggest that the contribution of kelp to suspension feeders diets has been overestimated in the literature due to difficulties associated with assessing the isotopic composition of coastal phytoplankton. Instead, the use of phytoplankton proxies (e.g. offshore POM, diatom cultures) have been used rather than phytoplankton itself, thus creating uncertainty in mixing models. In the present study, we collected our phytoplankton very close to our sample sites using net

trawls. By ‘cleaning’ the samples to remove any detrital material as described in the materials and methods we believe this process minimises any potential for overestimation of the contribution of kelp to the diet of suspension feeders.

The suspension feeder *Mytilus edulis* generates a current with beating latero-frontal cilia situated on the gills and are used to sieve and collect particles (Riisgård et al. 2000). They are also capable of complex pre-investigation selection using their labial palps (Beninger et al. 1995) and have 100 % retention efficiency of particles approximately 2-3 μm in size. Bracken et al. (2012) found using stable isotope analysis that *Mytilus* species in Oregon and New Zealand selectively consumed high-quality food sources. Farm and control *M. edulis* samples were significantly different in both their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios. SIAR modelling provides some insights into the cause for the variation between sites. For example, phytoplankton and zooplankton were found to be important constituents to the diet of control mussels, however, this importance decreased from 59 % at the control site to 31 % at the farm site with a switch to macroalgal sources, suggesting a shift in the reliance of food sources. However, the quality (nutritional value) of the macroalgal detritus will greatly affect selectivity, digestion efficiency and absorption of the material by suspension feeders (Tenore & Hanson 1980, Lopez & Cheng 1982, Findlay 1982, Uchida 1996).

Isotope analyses of wild kelp suggested that they become enriched with heavy isotopes with age (Leclerc et al. 2013a). Fredriksen (2003) described a similar pattern for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and attributed it to different biochemical reactions during the growth cycle. In the present study, we found no variations in basal and distal samples from the cultivated kelps *A. esculenta* and *S. latissima* for $\delta^{13}\text{C}$. Schaal et al. (2010) also analysed both basal and distal parts of the kelp frond and found intra-individual $\delta^{13}\text{C}$ variability observed in wild *Lamnaria digitata* was relatively low. In contrast, it has been reported that $\delta^{15}\text{N}$ can be very variable among individuals and even within an individual frond (Stephenson et al. 1986). While we also observed variations in $\delta^{15}\text{N}$ values between our kelp samples, this variation was not large enough for the SIAR model to distinguish between the samples thus the basal and distal samples were pooled.

Fresh macroalgae is available to organisms at the farm however it is assumed due to the high C:N ratios of fresh kelp tissue (Norderhaug et al. 2003) that detrital kelp is consumed preferentially over fresh kelp. The degradation of

kelp detritus has been shown to be associated with nitrogen-enrichment and a loss of herbivore-deterrent phenolic compounds (Norderhaug et al. 2003). This results in a nutritive enrichment of the detritus leading to its assimilation by suspension feeders. The $\delta^{13}\text{C}$ of fresh macroalgae was used as a proxy for detritus in this study. The modification of macroalgae isotopic signatures during decomposition is not a clearly identified pattern (Schaal et al. 2010). However, the stable isotope signature is known to alter when degradation by microbial breakdown occurs (Stephenson et al. 1986, Hill & McQuaid 2009). Yet, although $\delta^{15}\text{N}$ values can be modified during degradation, previous experimental studies assessing the effects of bacterial degradation on algae isotopic signature found little to no effect on $\delta^{13}\text{C}$ values (Stephenson et al. 1986, Schaal et al. 2010). As our sources had variation in their $\delta^{13}\text{C}$ values, the use of fresh tissue as a proxy for detritus appears to be relevant and unlikely to affect the outputs of contribution of food source modelling.

For future studies, it would be useful to go further and measure potential links of cultivated kelp detritus to higher trophic levels in the food chain as was conducted by Fredriksen (2003) in wild kelp forests in Norway, who linked *Laminaria hyperborea* detritus to fish and seabird species. Additionally, analysis of temporal changes in the diet of mussels would enable testing of the hypothesis that mussels at seaweed farms potentially selectively switch food sources between kelp detritus and zooplankton and phytoplankton when naturally available food quality and/or availability is low, as suggested by Bracken et al. (2012). The current study showed that suspension-feeding mussels do incorporate cultivated kelp detritus into their diet. However, an analysis of the quality (i.e. the state of degradation) and quantity of available detritus also needs to be assessed to understand if changes in the diet of mussels are associated with the changing structure of the growing and senescing seaweeds as well as the impact of removal of this detrital source at harvest time.

Wider implications of this study indicate that seaweed farms provide an additional ecosystem service in the form of a contribution to food webs, as well as a reservoir for biodiversity, habitat provision (Walls et al. 2016, 2017) and nutrient assimilation in Integrated Multi-Trophic Aquaculture (IMTA) scenarios (Neori et al. 2004, Troell et al. 2009). The value of extractive organisms at lower trophic levels in these IMTA systems are often considered most commonly from only a nutrient mitigation perspective for organisms at higher trophic levels (Holdt & Edwards 2014) with energy flow described through the system from top to bottom

levels. The provision of an additional energy (food) supply available from seaweed to a commercially important species, such as *Mytilus edulis*, may assist in the production of these dual crops at more farm sites in the future. This would enable both aquaculture sectors to diversify their production while the associated ecosystem services provide added value to the local ecosystem that increases the worth of these activities far beyond the market value of the primary crops. Nonetheless, further research into the quality of kelp detritus and the selectivity of mussels is required before the benefits of cultivated kelp detritus as a food source can be fully understood and appreciated.

Acknowledgements

The authors acknowledge Mr. David Moran and Mr. Brendan Walls for their assistance in field sampling and Mr. Michael Murphy and Mr. Paul Flannery of Dingle Bay Seaweed for boat work and assistance at Ventry Harbour. In addition, we thank Ms. Patricia Brown for specialist knowledge, Dr. Liam Morrison for laboratory facilities and Dr. Louise Firth for assistance in attaining funding for this study. This work was supported by the Thomas Crawford Hayes Research Trust, NUI Galway. A.M.W. is funded by the Dr. Tony Ryan Research Trust, NUI Galway.

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Chapter 7

Impact of kelp cultivation on the Ecological Status of benthic habitats and *Zostera marina* seagrass biomass

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Accepted: Marine Pollution Bulletin

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Abstract

The Ecological Status of subtidal benthic communities within a commercial kelp farm on the southwest coast of Ireland was not impacted by macroalgal cultivation. Additionally, there was no effect on the biomass of *Zostera marina*, a key habitat under the EU Habitats Directive and OSPAR Commission. However, sediment grain size and total organic matter were influenced by abiotic and biotic aspects of the farm. A temporal effect on univariate and multivariate species data, Infaunal Quality Index (IQI) and *Z. marina* biomass was observed. This effect was likely a community response to high storm disturbance in winter 2013/14.

The use of IQI to assess the impact of macroalgal cultivation on benthic communities is a novel approach. This study supports a view that environmental impacts of macroalgal cultivation are relatively benign compared to other forms of aquaculture. Further research must be conducted to understand all interactions between aquaculture activities and the environment.

Keywords: Kelp cultivation; Ecological impact; Macrobenthic community composition; Ecological Status assessment; Infaunal Quality Index (IQI); *Zostera marina* biomass

7.1 Introduction

World aquaculture production continues to grow year on year with approximately 131.4 million tonnes of fish, aquatic animals and plants produced in 2014 (FAO 2016). It has long been established that cultivation methods can impact on the benthic environment; these impacts include organic loading of the sediments and associated biogeochemical changes caused by the bio-deposition of faeces and pseudofaeces at culture sites (Crawford et al. 2003, Kalantzi & Karakassis 2006, Forde et al. 2015, O'Carroll et al. 2016). However, many of these studies have focused on finfish (Silvert & Sowles 1996, Kalantzi & Karakassis 2006) and shellfish (Stenton-Dozey et al. 1999, Crawford et al. 2003, Dubois, Marin-Léal, et al. 2007, O'Carroll et al. 2016) aquaculture. Assessments of the impacts of macroalgal cultivation has so far focused on tropical macroalgal species (Johnstone & Olafsson 1995, Ólafsson et al. 1995, Eklöf et al. 2005) or their impact when combined with shellfish cultivation in integrated multi-trophic aquaculture (IMTA) systems (Zhang et al. 2009, Ren et al. 2014, Ning et al. 2016).

Seaweed aquaculture farms are generally situated in nearshore coastal environments with average water depth ranging from 6 – 20 m. Semi-exposed sites with good current flow and shelter from the open ocean are ideal to provide the nutrients required for biomass growth without damage of the crop and infrastructure during storms. Typical farm set-up consists of a header ropes suspended approximately 1 m below the surface by buoys and kept in position by anchor ropes and weights, vertical ropes called dropper ropes (approximately 3 m in length) are sometimes added to increase the surface area of the farm (Edwards & Watson 2011, Peteiro et al. 2016, Walls et al. 2016, 2017). Seaweed cultivation is an extractive cultivation method meaning it assimilates nutrients required for growth from the environment with no need for the addition of supplementary feed or nutrients (Chopin & Sawhney 2009). As a consequence seaweed farms are assumed to have a more benign impact on the benthos when compared to finfish or shellfish aquaculture (Soto 2009, Roberts & Upham 2012). However, possible impacts include organic enrichment from loss of kelp biomass to the seabed and surrounding environment (Zhang et al. 2012 and discussed in more detail below) and from faeces and pseudofaeces released from fouling organisms (e.g. bivalves, polychaetes and amphipods) which use kelp as a habitat (Walls et al. 2016, 2017). In addition, the infrastructure of the farm and the biomass could have baffling

effects and possible wave attenuation altering local hydrodynamics similar to that of wild kelp forests (Mork 1996, Lovas & Torum 2001, Rosman et al. 2007).

Over 33 % of the 27.3 million tonnes of global annual aquatic plant production in 2014 came from just 2 kelp species *Laminaria japonica* and *Undaria pinnatifida* (FAO 2016). Kelps are among the largest sources of primary productivity in marine habitats (Mann 1973, Reed et al. 2008) and this primary productivity enters the food web through two routes; direct grazing on kelp tissue or detrital pathways. Much of the standing stock in temperate kelp beds is released either as particulate organic matter (POM) also called detritus or as dissolved organic matter (DOM). Krumhansl and Scheibling (2012) estimate that > 80 % of kelp production enters the carbon cycle as POM or DOM. Kelp detritus can range in size from small particles to whole thalli depending on how the biomass was removed. There are three main ways tissue can be lost. 1-Whole thalli are removed from breakage at the stipe or when the holdfast becomes detached from its substratum, either rocks or boulders in wild kelp forests or suspended rope substratum at cultivated sites. 2- Parts of the frond can break off removing large pieces from the frond. 3- Erosion of the distal ends of fronds can occur as tissue is continually lost through decay and natural senescence (Zhang et al. 2012, Krumhansl & Scheibling 2012). The impacts of detrital deposition from macroalgal cultivation on the benthos could be analogous to the impacts caused by the bio-deposition of faeces and pseudofaeces from finfish and shellfish aquaculture on benthic communities.

Over the last few years, interest in kelp cultivation in Europe has increased, supported by feasibility studies (e.g. Bruton et al. 2009) and experimental farms which are being set up to begin to industrialise the industry and advance the cultivation of kelps native to this region. This interest includes Ireland, with the establishment of Dingle Bay Seaweed in Ventry Harbour, County Kerry in 2009 as one of the larger commercial kelp farms (18 ha) in Europe (M.D. Edwards pers. comm.). With an increase in demand for kelp biomass to supply traditional (e.g. food) and expanding uses (e.g. biofuels) of kelp (Guiry 1989, Walls et al. 2016), the industry is set to expand and investigation into the possible impacts of this cultivation method on the local environment is essential.

The aim of this study was to assess any potential impacts on infaunal community structure at a commercial macroalgal farm at Ventry Harbour, County

Kerry on the southwest coast of Ireland over a two-year period. This was conducted by using an asymmetrical before after control impact (BACI) design to test for differences between control and impact stations in terms of univariate and multivariate faunal distributions and biotic indices including Infaunal Quality Index (IQI). IQI has been used to successfully discriminate the responses of macrobenthic communities to a wide range of natural and anthropogenic environmental impacts including aquaculture, in both coastal and transitional waters. However, many of the studies investigating aquaculture impacts using AMBI (part of IQI) based indicators have only focused on finfish and shellfish aquaculture and not macroalgal cultivation. Additionally, we assessed particle grain size and total organic matter to investigate if the kelp farm had an impact on sediment characteristics. Lastly, the farm site in Ventry Harbour is located above a *Zostera marina* seagrass bed, which is recognised as an important habitat as they provide ecosystem services such as substratum stabilisation, shelter and substrate for associated organisms, nursery grounds for fish, and are hugely productive (Davidson & Hughes 1998, OSPAR Commission 2008, Herkül & Kotta 2009, Bertelli & Unsworth 2014). As a result of the supply these important ecosystem services *Zostera marina* beds are recognised as a characteristic component of five Annex I habitats in the EU habitats Directive (92/43/EEC). Additionally in 2004, OSPAR produced descriptions of habitats on the Initial List of OSPAR Threatened and/or Declining Species and Habitats, which outlined 14 habitat types considered to be a cause for concern and included *Zostera* seagrass beds (OSPAR Commission 2008). Given the importance of *Zostera* habitats we conducted analyses to test the trends of *Z. marina* biomass at our impacted and control sites over the duration of the study.

7.2 Materials and Methods

7.2.1 Study site

This study was conducted in the southwest coast of Ireland in Ventry Harbour, County Kerry (52° 06' 49.45" N, -10° 21'20.17" W; Fig. 7.1) at the largest operating commercial seaweed farm in Ireland (18 ha site). Ventry Harbour is a moderately sheltered and shallow embayment orientated towards the southeast, approximately 2.5 x 1.5 km (3.75 km²) with a wide mouth opening into Dingle Bay. *Zostera marina* (seagrass) is extensively distributed throughout the sandy seabed, leading to a rocky boulder reef towards the mouth of the bay. The licensed seaweed farm is orientated northwest to southeast, and located to the westerly side

of Ventry Harbour (Fig. 7.1). The depth underneath the farm is approximately 6 m at the northwestern end before gently sloping to 20 m at the eastern edge of the farm at mean low water spring tide (MLWS). The tidal range in Ventry Harbour is between 0.6 and 4.0 m. Irradiance values obtained from nearby Valentia weather observatory (51° 56' 23" N, -10° 14' 40" W) ranged from 5,447 to 63,823 J cm⁻² (mean daily maximum value per month) for 2014. Sea surface temperature data was obtained from the M3 offshore weather buoy located approximately 56 km southwest of Mizen head (51° 13' 0" N, -10° 33' 0" W), and ranged from 10.1 to 17.6 °C for 2014. Although offshore values are less extreme than inshore values, Ventry Harbour is a well flushed bay so values are broadly representative. The longline structure is similar to the set-up in Fig. 3 of Walls' study (Walls et al. (2016); Fig. 2.3 Chapter 2); however, the farm in Ventry consists of 3 parallel units of 280 m linear longlines suspended approximately 1.5 m below the sea surface. The longlines were kept in position by buoys attached to the header rope and by 1500 kg anchor blocks at either end of the lines. The farm cultivates the kelps *Alaria esculenta* and *Saccharina latissima* for human consumption, animal feed and use in cosmetic products.

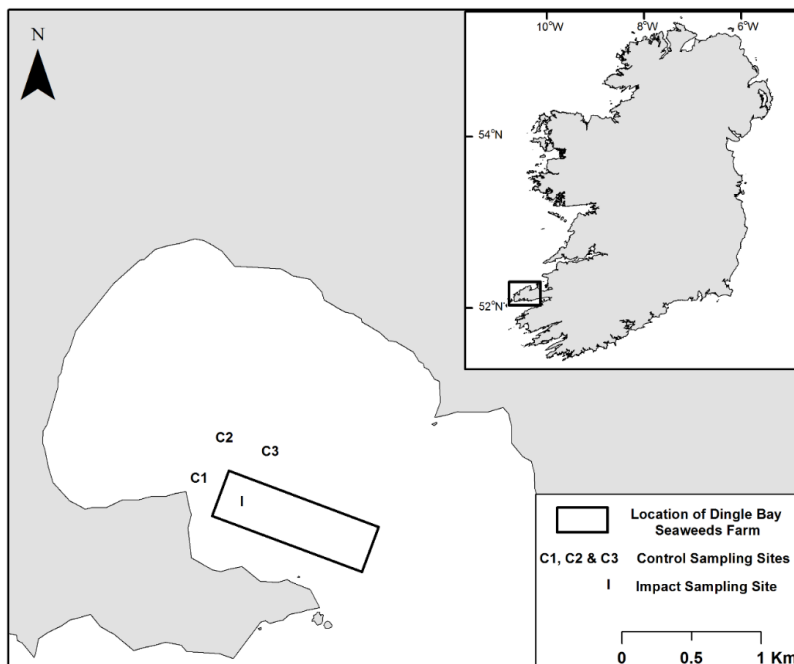


Fig. 7.1. Dingle Bay Seaweeds farm and sampling sites at Ventry Harbour, County Kerry, Ireland. I = Impacted Treatment Site; C1 = Control Treatment Site 1; C2 = Control Treatment Site 2; C3 = Control Treatment Site 3.

7.2.2 Sampling design

In this study, an asymmetrical distribution of control versus impact stations was used in a BACI (before after control impact) experiment *sensu* (Underwood 1994). Sampling was conducted during 6 dates over a two-year period between 2014 and 2016 (including May '14 and '15, September '14 and '15 and February '15 and '16). Sampling in September was before any impact from deployment of seaweed lines and February and May were after impact i.e. seaweed was deployed and growing at the farm. 4 sites were sampled during each sampling date; 1 treatment site corresponded to potential impacts associated with cultivation activities underneath the farm (designated the Impacted Treatment, 52° 06' 54.418"N, -10° 21'23.724"W) and 3 treatment sites located outside of the farms footprint and not subject to any known anthropogenic activity were selected as control sites (designated as Control Treatment 1; 52° 06' 56.459"N, -10° 21'27.719"W, Control Treatment 2; 52° 06' 59.46"N, -10° 21'25.499"W and Control Treatment 3; 52° 06' 59.339"N, -10° 21'21.24"W) (Fig.7.1). The sites were selected as homogeneous patches of sandy sediment, at a depth of 7 to 10 m, with *Zostera marina* distributed throughout all sites.

Within each treatment site a 20 x 20 m area was identified and 5 cores were sampled using a 0.01 m² diameter corer modified for use by SCUBA divers. The sampling locations were chosen using pre-determined random numbers within each site. Once all 5 samples were collected the cores were raised to the boat and the samples were transferred to labelled buckets. One additional sediment sample was collected for sediment analysis in a labelled Ziploc® bag at each of the 4 treatment sites. The samples were transferred back to the laboratory in cooler boxes and sediment samples were frozen at -20 °C pending analyses. Faunal core samples were sieved through a 500 µm mesh and any retained material was stored separately in labelled buckets and fixed in buffered formalin for 48 hrs pending laboratory analyses.

7.2.3 Sample processing

In the laboratory, formalin fixed macrofaunal samples were washed in running freshwater over a 500 µm sieve to remove formalin and excess sediment and the retained material was stored in 70 % ethanol. The macrofauna were stained using Eosin-Biebrich scarlet dye, sorted, enumerated and identified to species level using standard keys. Fauna were checked for nomenclatural inconsistencies and

synonyms using online Taxon Match tool in the World Register of Marine Species (WoRMS Editorial Board, 2016; <http://www.marinespecies.org/aphia.php?p=match>).

Sediment samples from each sampling site were removed from the freezer and allowed to defrost. Sediment granulometry was determined for each sample using laser particle sizing (LPS). It was not necessary to carry out wet-dry sieving as none of the sediment samples were seen to contain particles > 2 mm upon visual inspection. For each sample, 3 replicate aliquots of material were added to the Hydro-G dispersion unit of a Malvern Mastersizer 2000 until obscuration reached between 15 % and 18 % (Forde et al. 2012). For each aliquot the measurement cycle was 5 x 30,000 scans. The LPS distribution data was expressed as percentage weight within full Phi classes ranging between 4 and -2 Phi units. These data were processed using GRADISTAT (Blott & Pye 2001) software to derive sediment type classification, distribution modality and sediment particle graphic mean (Mz; Folk & Ward 1957). Mz is a parameter used to describe the mean particle size of a distribution and is analogous to the graphic mean employed with the normal distribution in conventional statistics (Forde et al. 2012). Here, Mz values can be used with confidence as 95 % of sediment samples exhibited unimodal distributions.

Sediment samples from each sampling station were dried to constant weight at 100 °C. For each dried sample, total organic material (TOM) was measured by loss of ignition (LOI). LOI allows TOM to be calculated through the combustion of 5 g of sediment in a furnace at 450 °C after 6 hrs (McIntyre & Eleftheriou 2005). Any organic material will have been oxidised within this time. TOM values were determined by expressing as a percentage the sediment weight loss following combustion over the initial weight of the dried sediment (Dean 1974).

The ash-free dry weight (AFDW) of *Zostera marina* within each core sample was determined. *Z. marina* was removed from the core samples and added to a pre-weighed labelled tin container. The samples were dried in the oven at 105 °C for 24 hrs (or until constant weight was attained). Each dried sample was then added to pre-weighed labelled crucibles and combusted in a muffle furnace at 450 °C for 16 hrs to oxidise all organic material. Samples were left to cool and were weighed again. AFDW was calculated as dry weight minus the weight of the

inorganic material which remained after combustion. Note there were no samples for all treatments in May 2014 and only 4 replicates for February 2015 Control 3 treatment.

7.2.4 Statistical analysis

Statistical analyses was carried out using the PRIMER v6® (Clarke & Gorley 2006) with PERMANOVA+ add-on (Anderson et al. 2008) packages and MINITAB v 17. One-way similarity percentages (SIMPER) analysis in PRIMER v6® (Clarke 1993) was used to determine the major taxa characterising the replicate cores within each treatment for each sampling date. The analysis was carried out on square-root transformed faunal abundance data. The SIMPER routine listed taxa characterising the faunal community within each treatment area in decreasing order of contribution to similarity between replicate cores ($n = 5$) (Clarke & Gorley 2006).

Using untransformed faunal data, the DIVERSE routine in PRIMER was used to calculate a range of diversity measures for each replicate faunal core. The diversity measures calculated included the total number of taxa (S), total number of individuals (N), Shannon diversity index (H' (Log_e)) and Simpson's evenness diversity index ($1 - \lambda'$). Replicate core diversity measures were used to calculate mean values for each treatment area. Changes in faunal distributions in relation to natural or anthropogenic disturbances can be effectively assessed using multiple metrics to describe different aspects of community structure (Reiss & Kröncke 2005, Borja et al. 2011).

A multidimensional scaling (MDS) (Shepard 1962, Kruskal 1964a, b) ordination was carried out using PRIMER v6®, giving the position of each core sample in two-dimensional spaces based on Bray-Curtis similarity matrix (Bray & Curtis 1957) of square-root transformed species composition data.

PERMDISP routine was conducted to observe if any significant variations in multivariate dispersion (around the centroid) were present among treatments. Permutational analysis of variance (PERMANOVA (Anderson et al. 2008)) was used to analyse community structure. An asymmetrical BACI design was employed (Underwood 1991, 1994). The fixed factor treatment had 2 levels: control (C) and impact (I). The fixed factor before/after had 2 levels: before (B)

and after (A). The random factor sampling date was nested within before/after and had 6 levels: May-14, September-14, February-15, May-15, September-15 and February-16. The PERMANOVA design was applied to zero adjusted Bray-Curtis similarity matrix (Bray & Curtis 1957) using square-root transformed faunal abundance data. P-values were determined by 9999 permutations of raw data.

To test the effect of factors treatment, before-after and sampling date on total organic carbon levels an analysis of covariance (ANCOVA) was run using MINITAB v17 to assess if an interaction is present. TOM correlates with sediment particle size with fine-grained sediments typically containing higher levels of organic matter than coarse sediments. To overcome any potential confounding effects of variation in particle size distributions between samples Mz was added as a covariate.

IQI was calculated for each faunal core using IQI version 4 (freely available at: <http://www.wfduk.org/resources%20/coastal-and-transitional-waters-benthic-invertebrate-fauna>) of the propriety tool in Microsoft Excel developed by the UK Environment Agency (Phillips et al. 2014). The IQI calculation involves truncation of the species list, spelling and synonym standardisation. The IQI Ecological Quality Ratio (EQR), a continuous variable between 0 and 1, is calculated by Eq. (1).

$$IQI = \frac{\left(0.38 \times \frac{(1-AMBI/7)}{(1-AMBI/7)_{ref}}\right) + \left(0.08 \times \frac{(1-\lambda')}{(1-\lambda')_{ref}}\right) + \left(0.54 \times \frac{S^{0.1}}{S^{0.1}_{ref}}\right) - 0.4}{0.6} \quad (1)$$

Where:

- AMBI is the AZTI Marine Biotic Index (Borja et al. 2000),
- $1-\lambda'$ is Simpson's Evenness Index,
- $S^{0.1}$ is number of taxa (S) raised to the power of 0.1,
- Ref. parameters are the maximum reference values for the habitat

The IQI tool sets reference conditions for each component which must be described regarding the physicochemical and hydromorphological quality elements of the metric based on local environmental parameters including sediment particle distribution and salinity classification. Environmental parameters can be entered into the tool as continuous or categorical variables. For each core, salinity classification was entered as "coastal" while sediment parameters were entered as wt % values across full Phi classes ranging between 4 and -2 units. Within the IQI

tool AMBI value is a continuous variable based on the proportions of five ecological groups to which the species are allocated depending on their tolerance to disturbance (Borja et al. 2000, Muxika et al. 2007). Group allocation per animal is based on extensive literature describing North Atlantic species in relation to disturbance and expert knowledge (Teixeira et al. 2010). Based on AMBI index values benthic communities are classified as undisturbed, slightly disturbed, moderately disturbed, heavily disturbed or extremely disturbed. Other metrics used in the calculation of IQI include Simpson's evenness diversity index ($1 - \lambda'$) and the number of invertebrate taxa (S). IQI EQR values are converted to Ecological Status (ES) classes using the following class boundary values; Good–High, 0.75; Moderate–Good, 0.64; Poor–Moderate, 0.44; Bad–Poor, 0.24. These indices have been developed in response to the European Water Framework Directive (WFD; (Directive 2000)). The WFD provides a framework for the improvement and protection of inland ground and surface waters as well as transitional coastal waters within all EU member states. The final objective was for all water bodies to achieve at least a good ES by 2015 (now extended to 2025). Under the Water Framework Directive (WFD) model management of ES focuses on the “Moderate–Good” critical boundary with remedial management actions required to restore areas classified as Moderate (or worse) to Good (or better).

An analysis of variance (ANOVA) general linear model was run using MINITAB v17 on the IQI EQR values to assess if an interaction exists between the fixed factors treatment (I and C), before/after (B and A) and the random factor sampling date nested within before-after.

To test for the effect of factors treatment, before-after and sampling date on *Zostera marina* biomass an ANOVA general linear model was run using MINITAB v17 on the AFDW of *Z. marina* data to assess if an interaction is present.

7.3 Results

7.3.1 Community characteristics

A total of 131 benthic invertebrate species were sampled during this survey from May 2014 to February 2016. See Table E1 Appendix E for full list of species sampled and their abundances. In general, the benthic communities present at each treatment area were comprised of polychaetes, amphipods, bivalves and

gastropods. The biotope that the communities present conform to is a sublittoral seagrass *Zostera marina* dominated community on medium to fine sediment (SS.SMP.SSgr) (Connor et al. 2004). These communities are generally found in shallow sublittoral sediments, depth range 0 – 10 m, in sheltered to extremely sheltered embayments, marine inlets, estuaries and lagoons, with very weak tidal currents and variable salinity (Connor et al. 2004). While commonly found on mud and muddy sands this biotope may also occur in coarser sediments, in particular marine examples of *Zostera* communities similar to the communities present at our sites (Connor et al. 2004).

The characterising species of each site remained largely similar over sampling dates. The polychaete *Chaetozone gibber*, *Owenia fusiformis*, nematodes and the bivalves *Tellina tenuis* and *Kurtiella bidentata* were generally the species contributing the highest percentage to group similarity within treatment sites. The taxa characterising the communities of SIMPER analysis at each treatment site during each sampling date are included in Table E2 Appendix E. Average between-group dissimilarity across all sampling dates for impact vs. control (1, 2 and 3) sites was 43.64 % (± 1.73) and within control (1, 2 and 3) sites was 42.68 % (± 1.47).

Mean species richness and mean number of individuals follows a pattern of low values in May 2014 which steadily increase over the duration of the study for all treatment sites, with February 2016 having the highest diversity and abundance. However, mean Simpson's evenness index and the mean Shannon diversity index was consistent across sampling dates (Table 7.1). This biotope is very spatially and temporally variable which is also reflected in the multivariate results below.

Table 7.1. Summary of mean community diversity, sediment characteristics and *Zostera marina* biomass at treatment sites (Impacted, Control 1, 2 and 3) sampled over 6 sampling dates at Ventry Harbour, County Kerry.

Community characteristics include: S: total number of taxa; N: total number of individuals; H' Log_e: Shannon diversity index; 1-λ': Simpson's evenness index. Mean values are based on replicate cores (n = 5) recovered within each Treatment site (standard error of means are included in parenthesis)

Sediment characteristics include; TOM %: total organic matter; Mz: graphic mean. Only one sample was taken per site for sediment and organic content analysis (n = 1). *

No data for February 2016 Control 2

Zostera marina biomass data includes Ash-free dry weight (g) **No data for May 2014 all treatments; *** Only

4 replicates for February 2015 Control 3

Sampling Date	Treatment	Community characteristics				Sediment characteristics		<i>Zostera marina</i> biomass
		S	N	H' (Log _e)	1-λ'	TOM %	Mz	Ash-free dry weight
May 2014	Impacted	19.8 (1.59)	44.6 (3.54)	2.65 (0.11)	0.92 (0.02)	3.22	1.85	**
	Control 1	16.8 (2.01)	43.8 (3.31)	2.36 (0.22)	0.85 (0.06)	3.49	2.14	**
	Control 2	19.6 (1.25)	59.2 (6.22)	2.53 (0.11)	0.89 (0.03)	3.34	2.04	**
	Control 3	23.6 (1.69)	72.4 (8.33)	2.76 (0.05)	0.92 (0.01)	3.28	1.72	**
September 2014	Impacted	30.8 (2.82)	155.8 (21.98)	2.80 (0.07)	0.91 (0.01)	2.76	1.80	0.36 (0.10)
	Control 1	24.2 (0.86)	99 (3.62)	2.57 (0.05)	0.88 (0.01)	3.27	2.23	0.28 (0.02)
	Control 2	27.8 (1.85)	94.6 (16.48)	2.81 (0.09)	0.91 (0.02)	3.2	1.83	0.24 (0.03)
	Control 3	28.8 (1.28)	93.6 (5.35)	2.85 (0.11)	0.91 (0.02)	3.18	1.74	0.22 (0.02)
February 2015	Impacted	23.6 (1.72)	94.8 (16.33)	2.41 (0.11)	0.84 (0.02)	2.56	1.56	0.30 (0.09)
	Control 1	20.8 (1.39)	102.2 (18.06)	2.28 (0.11)	0.83 (0.02)	2.81	2.58	0.15 (0.07)
	Control 2	29.2 (2.52)	170.4 (33.08)	2.63 (0.09)	0.88 (0.01)	3.18	1.95	0.39 (0.06)
	Control 3	25.6 (3.71)	143 (29.57)	2.63 (0.07)	0.91 (0.01)	3.26	1.87	0.59 (0.15) ***

Table 7.1. Continued

Sampling Date	Treatment	Community characteristics				Sediment characteristics		<i>Zostera marina</i> biomass
		S	N	H' (Log _e)	1-λ'	TOM %	Mz	Ash-free dry weight
May 2015	Impacted	29.4 (2.04)	170.6 (14.31)	2.4 (0.07)	0.83 (0.02)	2.93	1.82	0.96 (0.16)
	Control 1	27.2 (3.06)	190.6 (32.96)	2.25 (0.17)	0.8 (0.06)	3.47	2.64	0.52 (0.21)
	Control 2	29.6 (2.29)	185.4 (11.79)	2.6 (0.08)	0.88 (0.01)	3.42	2.18	0.60 (0.12)
	Control 3	24 (1.55)	133.4 (16.96)	2.54 (0.07)	0.89 (0.01)	3.09	1.77	0.50 (0.06)
September 2015	Impacted	37.6 (2.32)	253 (55.55)	2.88 (0.04)	0.92 (0.01)	2.59	1.63	0.54 (0.03)
	Control 1	33.2 (3.06)	198.8 (26.95)	2.69 (0.14)	0.89 (0.02)	3.94	2.77	0.44 (0.04)
	Control 2	32.8 (2.52)	196 (17.46)	2.74 (0.13)	0.9 (0.02)	3.10	2.01	0.36 (0.04)
	Control 3	31.2 (1.66)	152.6 (18.07)	2.71 (0.1)	0.89 (0.02)	2.68	1.59	0.28 (0.06)
February 2016	Impacted	30.6 (2.18)	164.2 (21.97)	2.7 (0.08)	0.89 (0.01)	2.70	1.76	0.54 (0.09)
	Control 1	33.4 (2.27)	220.0 (25.4)	2.53 (0.13)	0.85 (0.03)	*	*	0.52 (0.07)
	Control 2	29.6 (1.57)	176.8 (24.16)	2.58 (0.05)	0.87 (0.01)	2.91	2.24	0.39 (0.03)
	Control 3	31.2 (2.2)	143.4 (6.85)	2.8 (0.09)	0.91 (0.01)	2.66	2.71	0.44 (0.04)

7.3.2 Effect of treatment on community structure

Some separation and clustering of benthic invertebrates can be seen between sampling dates and before/after factor in the MDS plot (Fig. 7.2), however little separation can be seen between treatments (I and C). A stress value of 0.24 indicates the data are only partially represented by the two-dimensional plot and little reliance should be placed on the finer detail of the plot (Clarke & Warwick 2001). However, the broad scale pattern shows little separation between treatments and some separation of May 2014, September 2014 and February 2015 and clustering of the remaining sampling dates May 2015, September 2015 and February 2016.

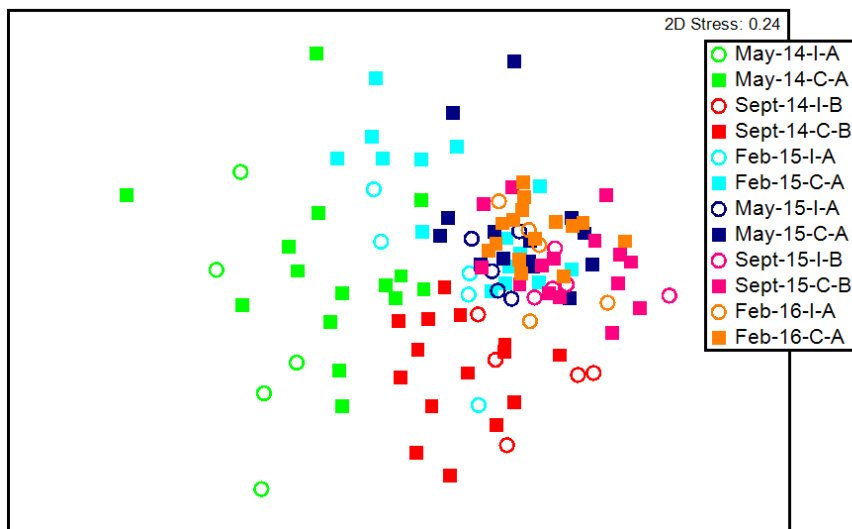


Fig. 7.2. Two-dimensional MDS plot of 120 core samples: $n = 5$ cores from each treatment site (I = Impacted- open circles and C= Control- closed squares, A= After and B= Before) during each sampling month, based on square-root of species abundance Bray-Curtis similarity matrix of species sampled from each core (stress = 0.24)

PERMDISP routine revealed that the variation in multivariate dispersion (around the centroid) was significant ($P < 0.01$) for all factors (Sampling date $P = 0.001$; Treatment $P = 0.014$; and Sampling Date*Treatment $P = 0.002$). From the PERMDISP output significant result for sampling date can be attributed to higher dispersion early in sampling dates (May 2014), which decreases as sampling dates continue to lowest dispersion values for February 2016. The significant results for differences between treatment seem to be random and are not consistent with the impact versus control results. All PERMDISP mean and standard error values for within-group dispersion can be found in Table E3a-c Appendix E.

Results from the PERMANOVA analysis on community structure are shown in Table 7.2. P (perm) results showed no significant difference on community structure between control and impact treatment sites. Pair-wise test show that within and between-group similarity between Impact and Control treatments are very similar (I-I: 51.26 %; C-C: 52.59 % and I-C: 51.3 %). There was no significant effect of time before or after seaweed farming activities. Sampling date nested within before/after factor was significant ($P < 0.05$). Pair-wise tests indicate that within and between sampling date before and after factors were always significant. Average similarity between- and within-groups increases with time, e.g. within-group similarity for May-14 was 47 % whereas for February-16 within-group similarity was 64.5 %. This result is visible in the MDS (Fig. 7.2) and the diversity and abundance univariate data in Table 7.1 and confirms a change in species composition over time.

Table 7.2. Permutational multivariate analyses of variance based on Bray-Curtis similarity matrix based on square-root transformed abundance data for benthic invertebrates sampled during 6 sampling dates at 2 treatments. All tests were conducted using unrestricted permutation of raw data with 9999 permutations. *df*: degrees of freedom; Significant difference at ** $p < 0.001$

Source	<i>df</i>	SS	MS	Pseudo-F	P (perm)	Unique Perms
Treatment	1	2391.4	2391.4	2.160	0.067	9926
Before/After	1	5514.6	5514.6	0.982	0.334	15
Sampling Date (Before/After)	4	22470	5617.5	6.118	< 0.001**	9842
Treatment x Before/After	1	1873	1873	1.691	0.135	9935
Treatment x Sampling Date (Before/After)	4	4429.4	1107.3	1.206	0.126	9826
Residuals	108	99159	918.14			
Total	119	1.4269 x10 ⁵				

7.3.3 Effect of treatment on sediment characteristics

Sediment total organic matter (TOM) values at the treatment sites were very similar ranging between 2.56 % and 3.94 % (Table 7.1). Similarly, sediment Mz (Phi) values at the treatment sites did not vary widely, ranging between a minimum of 1.56 and maximum of 2.77. Results from the analysis of covariance (ANCOVA) general linear model with Mz as a covariate indicated that TOM was significantly affected by treatment. However, before/after, sampling date nested within before/after and treatment interactions were not significant. From analysis

of the data, TOM % values for impact treatment are consistently lower or equated to the lowest value in the range of values for the control treatment. The impacted site also has low Mz values, this does not conform with the typical pattern observed in the literature that fine-grained sediments contain higher levels of organic matter than coarse sediments. These differences between treatment sites are reflected in the ANCOVA (Table 7.3).

Table 7.3. ANCOVA of TOM % with treatment, before/after and sampling date factors and Mz as a covariate. Significant difference at * $p < 0.05$. Note there was no data from February 2016 control 2 treatment.

Source	<i>df</i>	SS	MS	F	p
Treatment	1	0.19790	0.197899	5.32	0.043*
Before/After	1	0.00003	0.000034	0.00	0.989
Sampling Date(Before/After)	4	0.67359	0.168398	6.52	0.053
Treatment*Before/After	1	0.10205	0.102048	4.09	0.125
Treatment*Sampling Date (Before/After)	4	0.10510	0.026276	0.35	0.841
Mz	1	0.32190	0.321897	4.23	0.067
Error	11	0.76095	0.076095		
Total	22	2.62226			

7.3.4 Effect of treatment on Infaunal Quality Index Ecological Status (IQI ES) classification

All sites had an Ecological Status (ES) of either ‘good’ or ‘high’ from the IQI results, the ES and mean IQI EQR values for each site are given in Table E4 Appendix E. Using ANOVA general linear model, we tested the effect of treatment, before/after and sampling date on IQI EQR values. Sampling date nested within before/after had a significant effect on IQI EQR values (Table 7.4). Analysis of the mean IQI EQR values suggest a general trend of increasing values as the study continued, e.g. IQI EQR values at start of the study in May 2014 for Impacted site was 0.713 and Control sites 1,2 and 3 were 0.69-0.722 and at end of the study in February 2016 for Impacted site was 0.755 and Control sites 1,2 and 3 were 0.75-0.803. This long-term pattern has been seen above in our species diversity and abundances (Table 7.1) and the multivariate PERMANOVA results (Table 7.2).

Table 7.4. General linear model of Infaunal Quality Index Ecological Quality Ratio (IQI EQR) values with treatment, before/after and sampling date factors. Significant difference at ** p < 0.001

Source	df	SS	MS	F	p
Treatment	1	0.000001	0.000001	0.00	0.978
Before/After	1	0.003481	0.003481	0.30	0.613
Sampling Date (Before/After)	4	0.046337	0.011584	17.00	0.009**
Treatment*Before/After	1	0.000516	0.000516	0.76	0.433
Treatment*Sampling Date (Before/After)	4	0.002725	0.000681	0.82	0.515
Error	108	0.089712	0.000831		
Total	119	0.171754			

7.3.5 Effect of treatment on *Zostera marina* biomass

Observations of the *Z. marina* AFDW data show a slight increase with sampling data with a peak in May 2014 at the impacted site (Fig. 7.3). Generally, impact and control sites weight are quite similar. Results from the ANOVA general linear model indicate no significant effect of treatment, before/after or sampling date on *Z. marina* AFDW samples (Table 7.5).

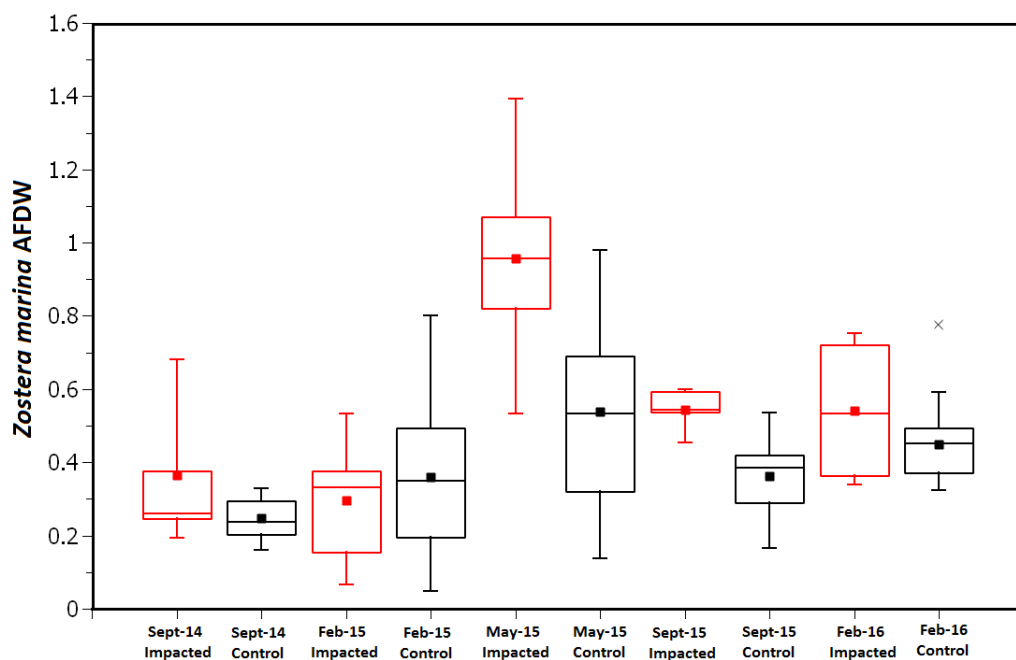


Fig. 7.3. Box plot showing ash-free dry weight (AFDW) of *Zostera marina* at impacted (I = red) and control (C = black) treatment sites over sampling dates. The horizontal line and solid square within each box mark the median and means of the data, respectively. The boxes encompass 50 % of the data, and whiskers show the range between the 5 % (bottom) and 95 % (top) portions of the data. Outliers are represented by crosses ($n=5$ samples for the impacted sites and $n=15$ samples for the control site; note $n=14$ for February)

Table 7.5. General linear model of *Zostera marina* ash-free dry weight (AFDW) with treatment, before/after and sampling date factors. Note there was no data for May 2014 sampling date and only 14 replicates for February 2015 control treatment.

Source	df	SS	MS	F	p
Treatment	1	0.00280	0.00280	1.98	0.254
Before/After	1	0.00928	0.00928	2.55	0.208
Sampling Date (Before/After)	3	0.01091	0.00364	2.57	0.229
Treatment*Before/After	1	0.00002	0.00002	0.01	0.911
Treatment*Sampling Date (Before/After)	3	0.00424	0.00141	0.83	0.507
Error	10	0.01703	0.00170		
Total	19	0.05025			

7.4 Discussion

Given the predicted increase in demand for kelp biomass and the expansion of the industry to novel waters in Europe and Ireland, there is significant potential for environmental impacts. Although macroalgal cultivation is an extractive form of aquaculture and not a fed form such as finfish aquaculture, it still provides a source of organic matter to the marine environment through the release of particulate organic matter (POM) detritus (Zhang et al. 2009). By increasing the natural levels of organic material to benthic habitats, kelp aquaculture could cause significant organic enrichment of benthic habitats and changes in macrofaunal assemblages.

In the current study, we observed a general trend of long-term change evident from our univariate and multivariate results attributed to sampling date and not any impact versus control treatment effect. Species richness and diversity increased temporally, however Shannon diversity and Simpson's evenness indices remained relatively constant. Thus, the increase in species and abundance was not due to influx of opportunistic or stress tolerant species as described by Pearson and Rosenberg's (1978) succession model which outlines how benthic infaunal community structure changes along a gradient of increasing organic enrichment and oxygen depletion. The main characterising species were similar for each site and no highly opportunistic stress tolerant species were sampled (SIMPER; Table E2 Appendix E). The polychaete *Owenia fusiformis* contributed a high percentage to overall similarity within treatment sites and is a disturbance-sensitive species from the AMBI classification species list (Borja et al. 2000). This temporal shift in community structure is also obvious from our multivariate results including the

MDS ordination plot (Fig. 7.2) and PERMDISP results (Table E3a-c Appendix E). The higher dispersion rates early in sampling dates (May-14, September-14 and February-15) are confirmed by the spread of the same sampling dates from the MDS with a clustering of samples and a decrease in dispersion for later sampling dates (May-15, September-15 and February-16). Also, the PERMANOVA returned a significant result for sampling date nested within before/after (Table 7.2), with within-group similarity increasing temporally for sampling dates.

This temporal shift in community structure can be explained through analysis of the properties of the biotope and the biotic environment. The biotope present at all our treatment sites is a sublittoral *Zostera marina* dominated community (Connor et al. 2004). This biotope is highly variable both spatially and temporally (Davidson & Hughes 1998, Unsworth et al. 2014). The use of an asymmetrical BACI (before after control impact) design (Underwood 1991, 1994) here was essential and provided a robust design with multiple controls to handle the inherent variability within sites. In addition to being highly variable this *Zostera* biotope is also very sensitive to disturbance, in particular storm disturbance (Davidson & Hughes 1998). During the period of December 2013 to February 2014, Ireland and the UK were subject to a number of winter storms as a consequence of low pressure, tidal surges and record wave heights. These events resulted in considerable damage to coastal infrastructure, caused persistent flooding and significant erosion events (Kendon & McCarthy 2015). Smale and Vance (2015) also discovered that this storm season significantly altered mixed kelp canopies on the south coast of the UK, due to a decrease in some kelp species which are less resistant to storm disturbance. Our study site at Ventry Harbour was also subject to these storms with large (> 1 tonne) blocks pulled from the harbour wall during this period (pers. obs.). Storms and high wave activity have been observed to remove large amounts of *Zostera marina* (Orth & Moore 1983, Den Hartog 1987, Olesen & Sandjensen 1994). Sediments and infaunal communities are likely to be affected by this disturbance as *Zostera* rhizomes and root networks bind together the substratum reducing erosion and allowing oxygen to penetrate the sediment (Davidson & Hughes 1998, Herkül & Kotta 2009). It is likely that the temporal changes we observed were a response to the severe storm activity experienced at this site. Again, the use of the asymmetrical BACI design allowed us to account for the natural variability and other studies should employ this design when conducting environmental impact studies of this nature.

We observed a slight trend in the data of increasing *Z. marina* biomass temporally which was evident in both the impact and control treatment sites which again could be in response to the winter storms on 2013/14, as mentioned above. However, despite this trend and the larger weights recorded for May 2015 at the impacted site, none of the factors tested in this study influenced *Z. marina* ash-free dry weight values. Numerous factors are likely to affect the degree of sensitivity of *Zostera* habitats to physical disturbances such as storms. Rasheed et al. (2014) found that seagrass at deeper depths recovered quicker than shallower species. Although the Rasheed et al. (2014) study was conducted on tropical species it is possible that the depth of our *Z. marina* beds (7-10 m) lessened the degree of disturbance experienced at the site and increased the rate of recovery.

In the current study, significant effects of kelp aquaculture were detected on sediment TOM across treatment sites. Also, the TOM % and Mz values show that values were lower at the impacted site, this pattern conflicts with the frequently observed pattern in the literature that higher levels of organic matter are found in fine-grained sediments when compared to sediments with larger grain size. Potential baffling effects of the seaweed farm could be the cause of the small particle size of the sediments at the impacted site due to larger particles being inhibited from settling. Little is known about the baffling effects of cultivated seaweed structures; the role of wild kelp forests in coastal protection has been investigated, however a lot of work still needs to be conducted (Firth et al. 2016). Kelp forests protect coastlines and adjacent sedimentary habitats by attenuating wave energy, buffering against storm surges, and preventing the movement of sediment from adjacent beaches (Mork 1996, Lovas & Torum 2001, Rosman et al. 2007). Baffling from the cultivated kelp may cause large particles of sediment and detritus to be exported away from the farm allowing only the settlement of smaller particles underneath the farm. Another explanation for the low TOM % at the impact site could be the consumption of detritus by fouling organisms attached to the kelp which already acts as a habitat for these species (Walls et al. 2016, 2017). Stable isotope studies have shown the importance of kelp detritus in marine food webs (Fredriksen 2003, Schaal et al. 2012, Leclerc et al. 2013). Fouling organisms such as filter-feeding bivalve molluscs and deposit feeding polychaetes may consume detritus at the farm site and thus less organic matter is deposited to the seabed. However, there are many factors that need to be considered, such as quality of organic matter, alternative food sources, organism selectivity and bio-deposition of faeces and pseudofaeces before this hypothesis can be properly tested. Few

studies have assessed TOM % underneath macroalgal farm, yet, a preliminary environmental study at a 21 ha pilot farm site of *Macrocystis pyrifera*, in Chile indicated organic matter under the culture site did not show significant trends of increase over time (Buschmann et al. 2014).

Infaunal Quality Index (IQI) was identified as an appropriate tool, as like most Water Framework Directive (WFD) benthic multimetrics, it has been developed in subtidal systems as a means of assessing, and comparing anthropogenic impacts across a range of sediment types and in different locations (Borja et al. 2009). EQR values have been shown to be suitable monitoring tools within highly variable sedimentary habitats (Forde et al. 2015). The IQI classification of the sites in this study revealed that the ES of all our treatment sites were either 'good' or 'high' status. A significant effect of sampling date nested within before/after on EQR values was identified. Detailed analysis of the EQR values revealed that the ecological classification of sites was improving as the study continued. This pattern parallels with the temporal change we identified for the univariate and multivariate species data, which reflects the macrofaunal data which is incorporated in the calculation of IQI EQR values. The significant result from the ANOVA detected the long-term temporal response to the storm disturbance during winter 2013/14.

From this primary study, we found that the impact of macroalgal kelp cultivation on the benthic environment studied over a 2-year period was minimal. We hypothesise that the farm created a baffling effect within the water column which was detected by a response of sediment TOM % and Mz. Studies on the hydrodynamic environment experienced by particles with seaweed farms are required before any definitive conclusions can be made on this baffling effect. Additionally, the wider ecosystem services of the farm i.e. the provision of food to attached fauna, could be an explanation for lower TOM % values at the impacted site. If this process is found to occur and cultivated kelp detritus is incorporated into the food web, then kelp farms provide additional ecosystem benefits beyond the supply of commercial crop. However, the quality of the organic material supplied needs to be measured (e.g. sediment traps) and the selectivity and preference of the fouling organisms for kelp detritus needs to be understood.

An intriguing result which was not anticipated, was the influence of disturbance from storm activity in winter 2013/14 (Kendon & McCarthy 2015,

Smale & Vance 2015) which was detected on benthic communities, the Ecological Status and *Zostera marina* biomass. We do not know if the detected change is a return to pre-disturbance levels or if it is to a new altered state. An understanding of the functioning of the benthic environment pre-disturbance events (storm and aquaculture) would be optimal, but, in a dynamic and spatially and temporally variable environment this would require an extensive time series to be established.

This is the one of the first studies to assess the impacts of kelp cultivation on the benthic environment. The data collected suggest that seaweed farms may be a benign form of aquaculture and have little impact on the local environment. Further research is required over larger spatial scales, varying locations and longer temporal scales to properly understand the interactions between seaweed farms and the benthos. Additionally, kelp farms may also cause shading on the benthos and changes to the local hydrodynamics which have potential to impact the benthic communities and further research is also required into these areas.

With demand for cultivated kelp set to continue and a focus on government bodies and stakeholders to develop the industry, the licensing of new sites for seaweed aquaculture is ongoing. The apparent resilience of *Zostera* beds to severe storm disturbance and the minimal impact of kelp farming identified in this study may indicate that subtidal seagrass habitats situated in semi-sheltered embayments represent favourable habitats for the positioning of new kelp farms. This point should be taken with caution as research into this area is only beginning and other characteristics of *Zostera* beds such as shoot and root density, reproductive rates and epiphytes may be impacted and need to be studied.

Acknowledgements

The authors acknowledge Mr. David Moran and Mr. Brendan Walls for their assistance in field sampling. In addition, we thank Dr. Adrian Patterson and Dr. Jack O'Carroll from NUI Galway's Benthic Ecology Laboratory for taxonomic support, Mr. Michael Murphy and Mr. Paul Flannery of Dingle Bay Seaweed for boat work and assistance at Ventry Harbour. This work was supported by the Energetic Algae project (EU Interreg IVB NWE Strategic Initiative; www.enalgae.eu). A.M.W. is funded by the Dr. Tony Ryan Research Trust, NUI Galway.

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Chapter 8

Discussion

8.1 Overview of key findings

The main aims of this thesis were to investigate potential ecosystem services provided by kelp farms, specifically their role in habitat provision and food web subsidy in addition to examining the potential negative effects caused by loss of primary productivity (biomass) from the farm. The site at Ventry Harbour, Co. Kerry provided an ideal location to explore the ecological interactions between a commercial-scale kelp aquaculture site and the surrounding ecosystem. Prior to this study there was a paucity of literature on the ecological role played by kelp farms (and indeed macroalgal cultivation sites in general) within the environment where they are sited. Previous studies on kelp farming have adopted a more commercially driven outlook by researching methods to increase biomass production from farms and increase efficiencies within the cultivation process, with the main aim to advance cultivation techniques to expand the industry (Peteiro & Freire 2013a, Buschmann et al. 2014, Peteiro et al. 2014, Marinho et al. 2015). Additionally, potential impacts from the loss of cultivated detrital kelp to the environment is only beginning to be studied and understood mainly in Asian countries (Yoshikawa et al. 2001, Zhang et al. 2012). This thesis is one of the first to take a detailed look at the interactions between the growing kelp and the artificial farm infrastructure with associated organisms (but see Peteiro & Freire 2013b, Førde et al. 2016), while also providing greater insight into the potential environmental impacts of kelp farming from a European perspective.

The most noteworthy results of this study include:

- Cultivated (suspended) *Laminaria digitata* holdfasts provide a novel habitat which is distinct from their wild (benthic) counterparts.
- Ecological priming of seaweed droppers with *Alaria esculenta* sporophytes can lead to the development of distinct and predictable community assemblages on ‘primed’ compared to ‘unprimed’ treatments.
- The successional development of fouling communities on the fronds of cultivated *A. esculenta* were predictable between growing seasons.
- A general farm mobile faunal community was found to develop with a lack of habitat specificity for either kelp biomass or farm infrastructure (e.g. ropes) as a habitat.
- Cultivated kelp species (*A. esculenta* and *Saccharina latissima*) are incorporated into the diet of the suspension-feeding mollusc *Mytilus edulis*,

thus demonstrating that cultivated kelp detritus contributes directly to the food webs of associated farm organisms.

- The Ecological Status of benthic communities within the kelp farm were not impacted by the presence of a seaweed farm, particularly from potential detrital deposition from macroalgal cultivation. Additionally, there was no effect of detrital deposition detected on the biomass of *Zostera marina*, a protected habitat under the EU Habitats Directive and OSPAR Commission.

The results presented here have clearly established that commercial-scale kelp farms provide supplementary ecosystem services in addition to those primarily associated with biomass production (e.g. food provision, economic benefits). This thesis has also shown these artificial aquaculture sites are not depleted in species and suggests that an increase in such types of structures may not negatively impact on biodiversity as many other artificial structures have been found to (Firth et al. 2016a, Wood et al. 2017). These results represent an opportunity for the industry to incorporate these findings in the support documentation when applying for foreshore and aquaculture licences relating to kelp cultivation. Equally, the results may demonstrate an environmentally sustainable production method which may be of benefit to the industry in their pursuit of a premium price for their product.

8.2 Kelp farms as a habitat

It has been shown repeatedly that mature wild kelp sporophytes act as an important habitat for associated species (for recent comprehensive review see Teagle et al. 2017); this thesis has shown that cultivated kelp also act as a habitat in much the same way (Chapter 2, 3, 4 and 5, Walls et al. 2016, 2017). Initially, we established if there was a difference between the role of wild (benthic) and cultivated (suspended) kelp in habitat provision. Chapter 2 (Walls et al. 2016) identified a habitat value associated with cultivated kelp holdfasts that was not simply an expansion of existing wild kelp habitats but could clearly be regarded as a novel habitat. Cultivated holdfasts contained higher species richness than wild holdfasts and different faunal assemblages were found between the two holdfast types. The species richness of both our holdfast types fell within the range observed from other comparable studies of varying kelp species sampled from different locations. While we could not determine the specific mechanisms underlying the differences in species richness and assemblage structure between

the holdfasts, three main factors of habitat (1), age (2) and morphology (3) were suggested to help explain the much of the variation in species richness and community composition. 1) The most obvious difference between wild and cultivated kelp is the alteration in habitat state from benthic to suspended growth substrates which may influence associated kelp fauna. This could affect the ability of fauna (particularly less mobile species) that normally inhabit benthic kelps to migrate to cultivated kelps which are suspended above the seabed. Additionally, alterations in abiotic conditions such as hydrodynamic environment (Moore 1972), sedimentation rates (Schaal et al. 2012) and depth (Coleman et al. 2007) etc., may also be factors which could alter ecological processes and thus cause changes in species richness and community composition. 2) The age of our wild and cultivated holdfasts varied considerably. Depending on cultivation practices, kelp may be seeded fresh to ropes for each growing season, so cultivated holdfasts are younger on average than those from wild stands. Sheppard et al. (1980) found species richness increased as holdfasts aged in wild populations of *L. hyperborea* from the UK. 3) Morphologies of cultivated kelp holdfasts differ to wild kelp and morphology (Fig. 2.1a and 2.1b Chapter 2) has been found to be the best physical predictor to explain variation in epifaunal assemblage structure between holdfasts of differing morphologies (Tuya et al. 2011). The presence of a unique epifaunal assemblage on cultivated kelp holdfasts suggested possible ecosystem services provided by seaweed farms in addition to the production of biomass. This finding lead to the extensive study at the commercial-scale kelp farm in Ventry Harbour, Co. Kerry.

In addition to revealing the presence of the unique epifaunal assemblage the comparison between suspended and benthic holdfasts also highlighted complications in the measurement of space within the holdfasts. As detailed in Chapter 2 (Walls et al. 2016) there are two methods for measuring habitat volume, the algebraic method described by Jones (1971) and the displacement method described by Sheppard et al. (1980) and used more recently by Blight & Thompson (2008). Comparison of these methods revealed a methodological miscalculation when calculating tissue volume; the tissue weight should be *divided* by specific gravity and not multiplied as stated in original texts. Also, we found that method standardization is needed between either holdfast volume or habitat volume to allow ease of comparison between future studies. Habitable volume, the space available for colonisation by fauna within the holdfast, is our preferred method as it avoids possible issues in measurements of holdfast metrics which are needed for

holdfast volume. Also, the habitable volume method is a more reasonable measure to use, as the indirect nature of holdfast volume as an estimate of habitat can cause problems when comparing holdfasts of different morphologies. Further, this study has identified Jones' (1971) miscalculation for tissue volume with the hope that the formula will be corrected for use in future studies.

As previously known from studies conducted on wild kelp, most recently reviewed by Teagle et al. (2017), distinct parts of cultivated kelp (holdfast, stipe and frond) similarly represent different habitats for associated flora and fauna (Walls et al. 2016, 2017). In addition, the artificial structures provided by seaweed farms interact with associated communities in an unexpected and unique way to similar artificial structures (e.g. energy infrastructure, coastal defence and finfish and shellfish aquaculture) in coastal environments. In Chapter 3, we detailed the association of organisms with both cultivated holdfasts and the farm infrastructure. While comparing droppers primed with *Alaria esculenta* and clean unprimed dropper ropes we tested the presence of an ecological priming effect. We defined the process of 'ecological priming' as the practice of providing a biological platform that influences the successional development of specific communities. Results showed that species richness increased over time during two growing seasons for both treatments, thus cultivated holdfasts (primed) and farm ropes (unprimed) provided habitat for species, however distinct communities developed on primed and unprimed droppers revealing the presence of a priming effect. Droppers primed with *A. esculenta* sporophytes suppressed some algal species while potentially facilitating other faunal species. We hypothesised that the presence of *A. esculenta* from the beginning of colonisation pre-empts other algal species from settling and dominating the primed droppers and alters the recruitment process as the kelp grows. A similar process was found by Benedetti-Cecchi (2000) when studying the effect of disturbance on turf and canopy forming algae in Italy. He found that canopy-forming algae dominated cleared patches of substratum during their main recruitment period, however outside of this period turf-forming algae dominated. Another explanation for facilitation of specific species by the presence of *A. esculenta* is the habitat preference of particular species to kelp e.g. the bryozoan *Membranipora membranacea* is highly selective to kelp (Ryland & Hayward 1977).

In a commercial context, the fronds of kelp are the most valuable part of the whole individual and it forms the bulk of the biomass that is sold and utilised in

production (Park et al. 1990, 2008, Peteiro & Freire 2013b, Førde et al. 2016). Previous studies have detailed the presence of frond fouling by epibionts as a major challenge for the industry, however, these studies often only investigate fouling communities at the end-point of the cultivation period (Peteiro & Freire 2013b) or only follow a single nuisance species in isolation over the growing period (Park et al. 2008, Park & Hwang 2012, Førde et al. 2016). In Chapter 4 (Walls et al. 2017), we investigated all fouling organisms associated with the fronds of *A. esculenta* during each cultivation period over two-years, and found that kelp fronds represent a favourable habitat for mostly sessile communities with species richness increasing over time. Species composition was consistent between years with early successional communities converging into later communities. High and low density artificial frond treatments were used to test the effect of local hydrodynamics on frond fouling. Variation in species occurrence between treatments suggested that local frond density affects the hydrodynamic environment experienced by frond communities (Peteiro & Freire 2013a, Peteiro et al. 2016, Walls et al. 2017). The ability to understand occurrence of epibiont communities and the factors causing variation within these communities can be used to devise better techniques for the industry to lessen the negative effects of fouling on algal biomass and increase the economic value of the crop (Walls et al. 2017).

Lastly, we discovered the presence of a general farm mobile faunal community by comparing the mobile assemblages associated with droppers seeded with *A. esculenta* and those associated with clean polypropylene rope (representing artificial farm infrastructure) (Chapter 5). Our study revealed there was no distinction of communities between seeded and rope treatments; we determined that high mobility rates and a lack of habitat specificity among sampled fauna were the likely cause for the development of a general farm fauna. Christie et al. (2009) found that the most abundant faunal taxa associated with macrophytes are highly mobile. They move freely between kelp sporophytes (Norderhaug et al. 2002) and exhibit high dispersal out of macrophyte beds (Waage-Nielsen et al. 2003, Jørgensen & Christie 2003). The lack of habitat selectivity in our study could be explained by the distance between treatment droppers which was only 1.5 m. Jørgensen & Christie (2003) found that artificial structures placed within kelp forests were rapidly colonised by species of distances up to 7 m horizontally from the edge of the kelp forest and 1.6 m vertically above the kelp forest. Results from Chapter 5 imply that artificial kelp aquaculture sites are not depleted in species and suggests that an increase in these types of artificial structures may not negatively

impact on biodiversity. This contrasts with other hard artificial structures which have been found to decrease biodiversity in an area (Firth et al. 2016a). With the rapid proliferation of artificial structures, termed ocean sprawl (Airoldi & Beck 2007, Firth et al. 2016b), in the marine environment, wider implications of this study suggest kelp aquaculture sites may be less damaging to the local ecosystem than artificial structures such as, coastal defence structures, offshore oil and gas and renewable energy installations.

8.2.1 Predictability of communities

The different communities associated with the distinct parts of the kelp (holdfast and frond) and the farm infrastructure all result from many ecological processes and interactions occurring within the wider environment. Hence, it is very difficult to compare these communities and the mechanisms underpinning them. However, one process which was tested within each community was the predictability of communities between different growing seasons. By calculating the mean occurrence-day of associated organisms and using regression analysis between years, we tested the predictability of these communities. We found that a hierarchy of predictability exists between frond (Chapter 4), holdfast (Chapter 3) and mobile (Chapter 5) communities, with frond communities showing high predictability, primed holdfast communities were predictable but unprimed communities were not and no predictability was evident between years for mobile assemblages. The predictable pattern of species arrival was clearly evident in fouling frond communities and droppers primed with *A. esculenta* sporophytes. This was despite variations in deployment date and sampling date between years, and factors such as water temperature, turbidity, irradiance and salinity presumably also varying from one year to the next. The communities sampled were mainly sessile organisms (in the case of the frond fauna) with pelagic larval dispersal. Only highly mobile species or those with pelagic larval dispersal can colonise suspended kelp (Walls et al. 2016). This predictability of species arrival times was attributed to the physical characteristics and flushing times within Ventry Harbour effecting the local larval pools available to colonise the suspended kelp (Herben 2005, Jessopp et al. 2007). Thus, the retention or export of larvae within or out of bays may influence the predictability and consistency of the fouling sequence within and between bays. The predictable communities of primed droppers have important implications for habitat restoration. Deployment of seaweed lines may aid in habitat restoration by supplying spores and gametophytes to wild kelp beds

that have been damaged by anthropogenic impacts, or by transplanting of seeded kelp juveniles directly onto artificial structures. Ecologically priming the substrate with kelp seems likely to lead to the development of particular predictable associated communities. It would be reasonable to expect that the degree of predictability would decline with increasing intervals between years or when comparing communities from different locations (Walls et al. 2017). However, the pattern of community predictability was not replicated for unprimed rope sections, which is further evidence for the presence of an ecological priming effect. Mobile assemblages associated with seeded and rope droppers were also not predictable from one year to the next. The probable cause is the life history of the mobile fauna sampled and their lack of habitat selectivity or preference to specific habitats. Our results showed that communities with a higher association and dependence on a substrate and pelagic larval development such as, sessile fauna on kelp fronds, are more predictable than mobile fauna with low habitat specificity, high mobility rates and direct development.

8.3 Fate and impact of kelp primary productivity

Wild kelp forests represent some of the most productive habitats on Earth (Mann 1973, 2000, Reed et al. 2008) and are a major source of primary productivity in temperate zones (Steneck et al. 2002). In contrast to wild kelp, total production from cultivated kelp is likely to be lower as harvest of cultivated biomass usually occurs in early summer and hence there is no further production of kelp once it has been harvested (Yoshikawa et al. 2001). Kelp primary productivity enters the food chain through two routes; direct grazing on kelp tissue or via detrital pathways (Krumhansl & Scheibling 2012, Miller & Page 2012). The ecological interactions and impacts potentially caused by the loss of primary productivity (detrital kelp) and its fate within the local footprint of the farm remain to be fully understood (Zhang et al. 2009, 2012, Buschmann et al. 2014, Ning et al. 2016). Studies using stable isotope analysis have shown that wild kelp detritus is often an important constituent of suspended particulate organic matter (POM), thus is an important source of carbon to suspension feeders (Fredriksen 2003, Schaal et al. 2012, Leclerc et al. 2013). Stable isotope analysis was used in Chapter 6 to detail the role of cultivated kelp in food web subsidy. The results showed that cultivated kelp makes up a significant component of the diet of the suspension-feeding mussel *Mytilus edulis*. We observed a shift in the diet of *M. edulis* from zooplankton and phytoplankton at the control site to a macroalgal dominated diet at

the farm site. These results suggest that when there is an abundance of good quality kelp detritus, *M. edulis* will decrease the importance of planktonic food sources (zooplankton and phytoplankton) and increase consumption of kelp. This study (Chapter 6) has important implications for the food web subsidy provided by cultivated kelp to not only the species attached to the farm infrastructure but potentially to nearby species also. However, an analysis of the quality and quantity of the available detritus is needed to understand if the changes in the diet of mussels are associated with the changing structure of the growing kelp. Wider implications of this study indicate that seaweed farms may provide a high-quality food source which could increase secondary production in the area by attracting organisms to the farm in much the same way as wild kelp forests support magnified secondary productivity (Mann 2000, Steneck et al. 2002, Smale et al. 2013). Additionally, an increase in suspension-feeding organisms could aid in nutrient assimilation in Integrated Multi-Trophic Aquaculture (IMTA) scenarios (Neori et al. 2004, Troell et al. 2009) due to suspension feeders' role in biofiltration.

Macroalgal cultivation is an extractive form of aquaculture meaning the biomass assimilates nutrients required for growth from the environment with no need for the addition of supplementary feed or nutrients (Chopin & Sawhney 2009). So, in comparison to feed forms of aquaculture such as, finfish aquaculture, the impacts on the benthos are assumed to be benign (Soto 2009, Roberts & Upham 2012). However, kelp cultivation still provides a source of organic matter to the marine environment through the release of detrital POM (Zhang et al. 2009, Ren et al. 2014, Ning et al. 2016). By increasing the natural levels of organic material in the environment, kelp aquaculture could cause significant organic enrichment of benthic habitats and changes in macrofaunal assemblages inhabiting the benthos. Our study, detailed in Chapter 7, revealed that the Ecological Status of subtidal benthic communities were not negatively impacted by the farm. In addition, the *Zostera marina* beds below the farm which are noted as a key habitat under the EU Habitats Directive and OSPAR Commission were not found to be effected by potential increase in detrital deposition. While a temporal effect on Infaunal Quality Index (IQI) and *Z. marina* biomass was observed, it was likely a community response to the high storm disturbance experienced in winter 13/14 and not the presence of the seaweed farm. These results are in stark contrast to impacts on the benthic environment such as, organic loading of the sediments and associated biogeochemical changes caused by the bio-deposition of faeces and pseudofaeces, from finfish and shellfish aquaculture (Crawford et al. 2003; Forde

et al. 2015; Kalantzi and Karakassis, 2006; O'Carroll et al. 2016). With demand for cultivated kelp set to continue and a focus on government bodies and stakeholders to develop the industry, the licensing of new sites for seaweed aquaculture is ongoing. The minimal impact of kelp farming observed in this study indicates that the development and growth of the sector will not have detrimental effects on the environment (at the scale tested) and thus, expansion is unlikely to be limited by environmental impacts, as with other forms of aquaculture e.g. finfish. However, this work is preliminary and more studies with farms of varying scales in different locations are required.

8.4 Management implications

Although developing new cultivation techniques and methods to improve the seaweed cultivation sector was not an objective of this thesis, over the course of this research we identified a number of approaches which could benefit the sector. The ability to predict the arrival time of fouling species, especially on the valuable frond, could assist farmers in getting the highest market value for their crop. Seaweed farmers can exert control over the quality of their crop by being able to decide on a date-by-site basis when is the best time to deploy in order to achieve a harvest of optimum quality and yield. Early deployment of juvenile kelp seedlings to sea would allow for a longer cultivation period in the cooler winter months leading to increased growth rates of kelp while fouling rates are low (Peteiro & Friere 2009). Additionally, early harvesting of fronds could avoid the period when the most severe fouling occurs (Walls et al. 2017). This period is in late May to June in southwest Ireland but will vary depending on latitude i.e. June to July in Norway and April to May in Spain. The crop would then reach harvestable size earlier in the season before an increase in seawater temperature, irradiance and associated fouling organisms occurs. Additionally, results from Chapter 4 suggest that altering the density of sporophytes seeded onto the ropes or orientating the farm so to achieve a more favourable hydrodynamic environment around the farm could make a significant difference to fouling rates (Walls et al. 2017). Peteiro et al. (2016) and Peteiro and Freire (2013b) have indicated that high density fronds can modify the environment via and fouling rates via a reduction in current and that fouling on fronds at sheltered sites is higher than fouling rates at more exposed sites. These recommendations are simple modifications to already established techniques which are relatively easy to implement and could provide dramatic benefits to the quality of the crop and increase productivity of the industry.

8.5 Future work

While this thesis has made great advancements in understanding the role of kelp farms in habitat provision and food web subsidy, there are many other ecosystem services potentially provided by seaweed farms such as coastal defence, habitat restoration and biofiltration of associated organisms, that remain to be examined. Global climate change and sea-level rise due to burning of fossil fuels are expected to lead to more extreme climactic events including increased storm intensity and frequency (Intergovernmental Panel on Climate Change (IPCC) 2014). This has led to a proliferation of hard structures to defend the coastline (Firth, et al. 2016b). Seaweed farms are located in inshore waters close to land and could help in dissipating the intensity of wave action. Wild kelp forests have been shown to provide a buffer against storms by wave dampening and attenuation thus reducing the velocity of breaking waves and altering water motion (Lovas & Torum 2001). However, very little is known about wave attenuation in wild kelp forests (Firth et al. 2016b) with no work, to my knowledge, conducted on wave attenuation by seaweed farms. Modelling and theoretical knowledge of the hydrodynamic environment around farm structures would be required for a study into the role of seaweed farms in coastal defence scenarios. If farms were shown to defend the coast from increased storminess then licenses for new farm sites may be more easily attained, due to this multifunctional use of the farm as an added benefit and thus an assessment of this potential ecosystem service could assist in the expansion of the sector.

In attempts to mitigate the negative impacts of ocean sprawl (Airoldi & Beck 2007, Firth et al. 2016a, b), the restoration of kelp forests (Carney et al. 2005, Yu et al. 2012) and the transplantation of habitat forming species (Perkol-Finkel et al. 2012, Ferrario et al. 2016) onto artificial structures has gained increased interest recently. Habitat restoration of wild kelp beds that have been damaged by anthropogenic impacts may occur through the provision of spores and gametophytes from specifically deployed seaweed lines, or by transplanting seeded kelp juveniles directly onto artificial structures. Transplantation of kelp individuals has been shown to work in many situations and locations including, Australia where the popular “Operation Crayweed” project (www.operationcrayweed.com) has successfully transplanted the macroalgae *Phyllospora comosa* along the Sydney coastline. By restoring this key habitat-forming species associated biodiversity has increased as communities establish on the seaweed (Marzinelli et

al. 2016). This restoration technique is not limited in its use to kelp ecosystems and has also been used to re-establish coral reefs. The ‘gardening’ concept described by Rinkevich (2015) is a two-step process which includes a nursery and a transplantation phase. The nursery phase involves development of large stocks of coral colonies in mid-water floating nurseries and the second step involves transplantation of these nursery-farmed corals (Rinkevich 2015). This process has enhanced the ability of coral reef organisms to adapt and respond to climate change and avoid further degradation (Rinkevich 2015). Ecologically priming the substrate as discussed in Chapter 3 seems likely to lead to the development of particular and predictable associated communities. Seaweed longlines could act as a nursery for juvenile individuals and associated organism which could later be transplanted. Alternatively, spores and gametophytes from macroalgae and juvenile faunal individuals and larvae could be supplied to benthic habitats from longlines suspended above the seabed. The extent of such ‘seeding’ influences will increase with the time longlines are left in the water. Harvesting practices may be adjusted so that the biomass remains in place for more than one growing season. The practicalities and ecological processes behind the use of cultivated kelp farms in habitat restoration remain to be understood and targeted *in situ* experimental studies would be required.

In contrast to habitat restoration which represents a potential positive impact of seaweed farming, there is increasing concern that artificial structures are acting as ‘stepping stones’ between regions and facilitate the spread of invasive species (Airoldi et al. 2015). Although, we found no invasive species over the course of this study at our farm site in Ventry Harbour, Co. Kerry, this does not mean that they are not there or indeed, may not arrive in the future. Akin to the naturally occurring anthropogenic disturbance that occur in other habitats such as, rocky shores, artificial structures can create free-space and facilitate the establishment of invasive species (Clark & Johnston 2011, Bracewell et al. 2013). By ecologically priming seaweed lines with *A. esculenta* individuals there is less free-space available, which not only leads to a particular predictable community (Chapter 3), but may also help to stop or depress the chance of invasive species arrival. Also, by ecologically priming artificial structures not only is the diversity on these structures enhanced but the resilience of the assemblages on these structures to biotic invasion by non-native species is increased (Stachowicz et al. 2002, Arenas et al. 2006). This idea is supported by the diversity resistance hypothesis that states that diverse communities are highly competitive and

therefore more resistant to invasion (Elton 1958, Stachowicz et al. 2002). However, other parts of the farm infrastructure are not ‘primed’ with kelp and represent free-space that invasive species may be able to occupy. Cultivation practices are in no way harmonized across the sector and depend on the site characteristics and the operator. For example, some, but not all producers remove header ropes and longline structures from the sea once the biomass growing season has concluded. This process may reduce the chance of invasive species occurrence on such artificial infrastructures. The mechanisms behind artificial structures and invasive species are poorly understood and this remains an important knowledge gap within the field of coastal ecology (Firth, et al. 2016b). More specifically, the role and interactions of artificial kelp farms and invasive species must be investigated.

While the potential impact of organic enrichment of the sediment by increased settlement of detrital kelp primary productivity was the focus of Chapter 7; results revealed minimal impacts, which is of great benefit to the growth of the industry. There are other potential impacts of seaweed aquaculture which should be studied before a definitive conclusion can be made that seaweed farming is a benign form of aquaculture, particularly under constantly changing environmental and climatic conditions. Impacts from kelp farms may not be isolated to the immediate footprint of the farm, there could be wider reaching implications to the area where farms are situated. As seaweeds take up nutrients to grow (Chopin & Sawhney 2009), depending on the site and the scale of the farm, this process could have positive or negative effects on nutrient availability within the area. Integration of seaweed farms in Integrated Multi-Trophic Aquaculture (IMTA) could help mitigate the effect of excess nutrients produced by finfish and shellfish aquaculture (Chopin 2013). In addition, with increased fertilizer use required for agricultural intensification and the urbanization of growing populations, nutrient concentrations and areas of eutrophication are increasing in our coastal waters (Withers et al. 2014). Seaweed farms could be used to ‘mop up’ these excess nutrients in coastal environments where they occur. However, the reverse situation could occur with competition for nutrients with other photosynthesising organisms occurring in the vicinity (Aldridge et al. 2012). The effects of excessive nutrient removal by cultivated kelp would be the reverse of the symptoms of eutrophication i.e. lower nitrogen and phosphate concentrations in the water, decreased productivity and energy fluxes through pelagic systems, decreased flux of organic material to the seabed and subtle alterations in community structure (Aldridge et al. 2012). The nutrient availability and carrying capacity of potential large-scale seaweed

aquaculture sites should be considered before licenses are granted. This will aid in understanding the requirements of a particular site whether excess nutrients are available due to eutrophication or nutrient concentrations are limited within the area, which could also vary seasonally.

In this thesis, we have shown that seaweed farms (biomass and infrastructure) are habitat for species (Chapter 3, 4 and 5) and that kelp detritus is incorporated into the food web (Chapter 6). What we have not explored is the potential role of associated fauna in biofiltration and mitigation of increased nutrients from anthropogenic sources in the water column. Mussels are important suspension-feeders and dominant organisms on the farm (pers. obs.). They provide an important biofiltration service and can influence particulate and dissolved organic matter concentrations by removing toxins and particulates and prevent phytoplankton blooms while improving the overall water quality (Wilkinson et al. 1996). Tank experiments with feeding trials and measurements of water nutrient concentrations would be required to assess this potential service. If organisms are found to have a significant impact on nutrient concentrations this supports the role of seaweeds in IMTA and IMTA as a standard practice in traditional aquaculture set-ups.

While our study site at Ventry Harbour, Co. Kerry is one of Europe's largest functioning seaweed farms, at 18 hectares, it is of insignificant size when compared to the world seaweed industry. Figures for the footprint of seaweed farms in China are difficult to attain, but satellite imagery shows farm (macroalgae and shellfish) infrastructure filling entire bays. China is the world's top producer of aquatic plants, producing 12.8 million tonnes totalling to a 54 % share in total world aquatic plant production (FAO 2014). An assessment of the ecological interactions and environmental impacts of large-scale commercial farms would be vital to thoroughly understand the potential implications. Although, it is unlikely that the industry in Europe will expand to the size of the Asian seaweed industry for several reasons, such as site suitability, marine spatial planning constraints, i.e. competition for space with other marine activities or market constraints, e.g. the existence of demand for seaweed aquaculture products necessary to maintain a profit margin (Duarte et al. 2017). However, the industry in Europe is set to continue to expand within many countries, particularly in northwest Europe with active seaweed cultivation currently underway e.g. Norway, Denmark, Scotland (SAMS), France, Spain, Portugal at research or commercial-scale aquaculture sites.

The kelp *Alaria esculenta* has the longest history of cultivation in Ireland, however it does not feature as a highly-cultivated species when viewing the statistics from the world stage. The kelp species *Laminaria japonica* and *Undaria pinnatifida* are principally cultivated in Asian countries are the top cultivated kelps in the worlds accounting for over 33 % of total aquatic plant production in 2014 (FAO 2016). Arnold et al. (2016) studied the epibiotic assemblages associated with wild *U. pinnatifida* and found when compared to native macroalgae species this invasive kelp supported a structurally dissimilar and less diverse assemblage than native perennial kelp species. It would be useful to study the role of these cultivated species in habitat provision and compare the results to wild studies (Arnold et al. 2016, Chapter 2) and results from this thesis (Chapter 2, 3,4 and 5). Unfortunately, these species are not native to European waters and thus were beyond the scope of this study.

One of the knowledge gaps which we were unable to tackle during completion of this thesis was a comparative study between different commercial-scale aquaculture sites. The site at Ventry Harbour, is Ireland's largest commercial seaweed farm. When we started this work in 2013 there were and still are no farms of comparable size. There are only experimental-scale research sites (e.g. Bantry Marine Research Station, Bantry Bay, Co. Cork and Queens University Marine Laboratory, Strangford Lough, Co. Down) with approximately two to three 100 m longlines, cultivating various seaweed species. Due to a mixture of scale and location effect, comparisons between our site at Ventry to these small-scale sites would not have provided conclusive results and it is likely that an assessment of their ecological impacts would not have shown any influences if our large scale-site showed little impact. In an ideal situation, comparison of our results to large commercial sites across Europe would have provided a more robust study. Interestingly, a study with this goal now forms part of a new EU Horizon 2020 project called GENIALG (2017-2020). The 19 project partners (including The National University of Ireland, Galway) will focus on the cultivation of the kelp *Saccharina latissima* and the green alga *Ulva* sp., investigating their supply chains from culture to product, with a substantial study on ecosystem service provision.

While one of the main aims of this thesis was to identify ecosystem services associated with seaweed farms we were unable to quantify or value the identified services. Ecosystem services are being used as ways to document the values humans place on ecosystems and to evaluate benefits derived from natural

resources (De Groot et al. 2002, Millennium Ecosystem Assessment 2005). Identifying the services provided by seaweed farms is the first step before quantifying the services. Quantification then allows the services to be valued, a crucial step, as services with a direct monetary value are more likely to be considered within management strategies (Beaumont et al. 2008). Without monetary valuation, less visible services such as nutrient cycling can be overlooked or thought of as 'free' and not considered in management decisions (Beaumont et al. 2008). To explain the practice of identifying, quantifying and valuing ecosystem services, I will use a practical example from this thesis. We identified through Chapter 3 that seaweed farms provide habitat for species e.g. the mussel *Mytilus edulis*. To quantify this service e.g. the amount of habitat available, we evaluate the number of *M. edulis* present and scale-up for the whole farm. Thus, we could say that X Kg of kelp biomass or meters of seeded rope supports X amount of *M. edulis*. Lastly, a valuation of the habitat provided to mussels needs to be conducted. If mussels are harvested from the farm site there is a market-price value linked to their sale, or if mussels aid in biofiltration and mitigate the effects of eutrophication there is a value associated as resources are not impacted by eutrophication e.g. fishing, tourism etc. Policy makers are increasingly recognising the role of environmental valuation to guide and support the management and conservation of ecosystem services (Beaumont et al. 2008). This thesis is the first step on the ladder to valuing the ecosystem services associated with kelp farms.

8.6 Challenges to the global seaweed cultivation industry

The global seaweed aquaculture industry is undergoing a rapid expansion, with most up to date figures showing the industry is worth US\$ 6.4 billion and cultivated seaweeds are supplying 96 % of the global biomass (FAO 2014, 2016). Aquatic plant production which is overwhelmingly dominated by seaweeds has expanded at a rate of 8 % per year over the past decade, up from 6.2 % in the previous decade with output more than doubling in this period (FAO 2016). This growth over the last decade in Asian countries, the largest producers, has mainly been driven by a demand for contaminant-free edible seaweeds. With growth in European waters being directed by high-tech developments and advancements within the commercial sector requiring seaweed-derived products for pharmaceutical, antimicrobial and biotechnological applications, these countries generally have little traditional interest in seaweeds for both aquaculture or consumption (Mazarrasa et al. 2014, Cottier-Cook et al. 2016). In addition, the

continued progress of including seaweed cultivation in IMTA has resulted in further expansion of the industry (Chopin 2013). While we have begun to investigate some of the impacts of kelp cultivation, the rapid expansion of the industry can result in a multitude of unforeseen ecological consequences which can include disease outbreaks, introduction of non-native pests and pathogens and a reduction in the genetic diversity of native seaweed stocks (Loureiro et al. 2015, Cottier-Cook et al. 2016). Seaweed cultivation has been actively promoted by government initiatives, particularly in developing countries because it is widely perceived as one of the most environmentally benign aquaculture forms (Cottier-Cook et al. 2016). Also, the direct and indirect benefits of seaweed farm ecosystem services have made macroalgal cultivation a favourable aquaculture form. Based on the findings from this thesis, we have confirmed these beliefs that seaweed cultivation, certainly on the scale we have tested (18 ha. site), is a benign form of aquaculture with no evidence of negative impacts on the local environment and provides many ecosystem services, some of which we have examined here.

Certainly, there is a need to adopt robust marine spatial planning (MSP) legislation. The European Commission recently adopted legislation to create a common framework for MSP implementation, reasoning that competition for maritime space (e.g. renewable energy, aquaculture, fisheries etc.) required more efficient management practices to avoid potential conflicts and to create synergies between different stakeholders (Katsanevakis et al. 2011, Cottier-Cook et al. 2016). The integration of seaweed aquaculture into MSP should not be too difficult a matter as we have shown that seaweed farms do not negatively impact on the environment and provide benefits to the local ecosystem. Seaweed farms are a less visible form of infrastructure compared to finfish aquaculture cages and tidal and wind energy installations and thus should be more acceptable to other users of the coast. The integration of seaweed farming with finfish aquaculture in IMTA makes better use of the 3D space beneath the farm. In addition, the increased biodiversity and secondary production associated with seaweed farms may increase fish densities (Eklöf et al. 2006) keeping local fishers satisfied. The Marine Institute, Ireland's governing body concerning the marine environment have recognised the importance of MSP in an important policy document "Harnessing our Ocean Wealth" (Government of Ireland 2012) and more recently with a tender to investigate Ireland's MSP needs. This policy document supports the development of seaweed aquaculture and the resolution of licencing issues (delay in granting licenses) for aquaculture sites. As recently, as May 2017 the Department of

Agriculture Food and the Marine have released a review of the aquaculture licensing process (DAFM 2017) in Ireland. The report recommends a wait of no more than six months for licencing decisions to be made for all new licence applications. Thus, the growth of the sector in Ireland and further afield in European and Asian waters is likely to continue to flourish (FAO 2016). Results from this thesis provide some very valuable information to aid licence, planning and decision making. However, this is one of the first studies of its kind to identify and assess the ecosystem services and impacts of commercial seaweed (kelp) farms in Europe. Many more factors such as farm scale, seaweed species, site location and hydrodynamics need to be researched to fully understand the ecological interactions and impacts (positive or negative) of commercial seaweed cultivation and before development and expansion of the sector can be fully supported.

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Appendix A: Potential novel habitat created by holdfasts from cultivated *Laminaria digitata*: assessing the macroinvertebrate assemblages

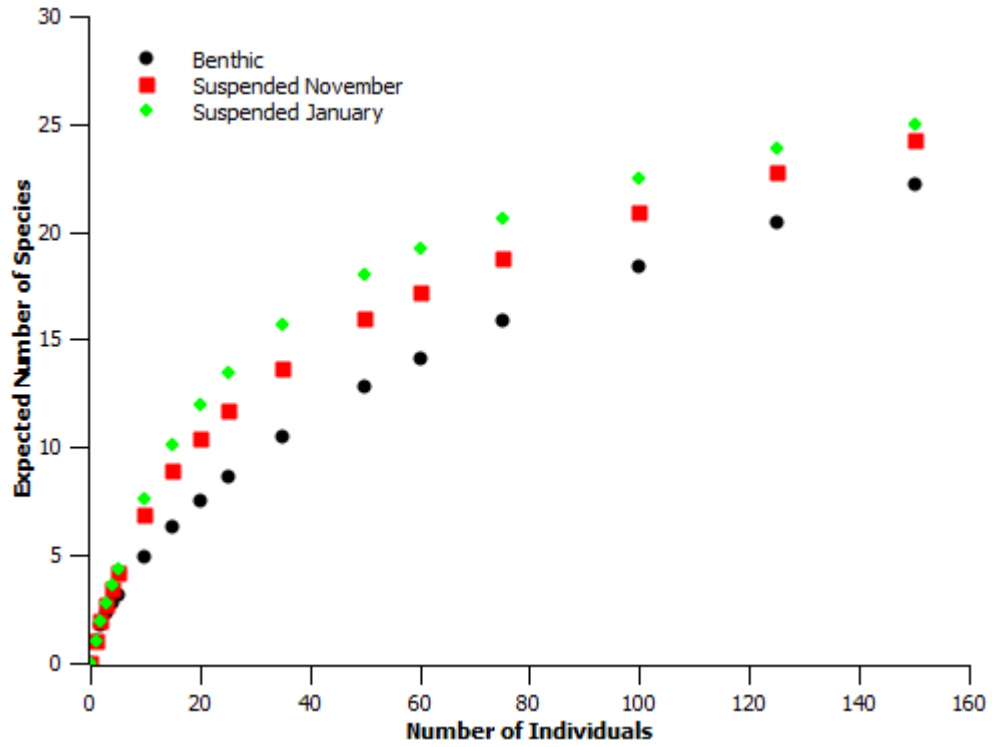


Fig. A1. Rarefaction plot for benthic and suspended (November and January) holdfasts

Table A1. Species list and number of individuals present in *Laminaria digitata* benthic, suspended November and suspended January holdfast samples.

Species	Benthic Holdfast Samples	Suspended November Holdfast Samples	Suspended January Holdfast Samples
Phylum Porifera			
Class Calcarea			
<i>Grantia compressa</i>	6	1	2
<i>Sycon ciliate</i>	96	5	3
Class Demospongiae			
<i>Halichondria panacea</i>	5	0	0
<i>Hymeniacidon perleve</i>	12	0	0
Phylum Nematoda			
Nematoda indent.	897	51	14
Phylum Annelida			
Class Polychaeta			
Polynoidae indent.	5	31	0
<i>Harmothoe impar</i>	1	0	2
<i>Lepidonotus clava</i>	2	1	0
<i>Eulalia viridis</i>	5	0	1
<i>Eumida sanguinea</i>	2	2	0
<i>Psamathe fusca</i>	1	1	0
<i>Nereimyra punctata</i>	0	5	0
<i>Syllis</i> sp.	16	17	8
<i>Syllis prolifera</i>	20	14	9
<i>Eusyllis</i> sp.	28	2	2
<i>Odontosyllis</i> sp.	6	0	18
<i>Nereiss</i> sp.	6	0	5
<i>Nereis pelagica</i>	1	1	0
<i>Platynereis dumerilii</i>	2	6	4

Species	Benthic Holdfast Samples	Suspended November Holdfast Samples	Suspended January Holdfast Samples
<i>Prionospio multibranchiata</i>	47	0	0
<i>Dipolydora caeca</i>	5	0	1
<i>Aphelochaetasp.</i>	38	11	18
<i>Capitella capitata</i>	1	0	0
<i>Dasybranchus spp.</i>	0	1	7
<i>Fabricia stellaris</i>	275	0	1
<i>Hydroides norvegicus</i>	0	0	2
<i>Spirobranchus sp.</i>	1	0	0
Phylum Crustacea			
Class Malacostraca			
Order Cumacea			
<i>Pseudocuma longicorne</i>	1	0	0
Order Isopoda			
<i>Anthura gracilis</i>	2	0	0
<i>Janira maculosa</i>	3	0	0
Order Amphipoda			
<i>Apherusa jurinei</i>	1	15	26
<i>Iphimedia minuta</i>	3	0	24
<i>Liljeborgia pallida</i>	2	0	0
<i>Bathyporeia pelagica</i>	0	2	0
<i>Echinogammarus olivii</i>	0	1	0
<i>Cheirocratus sundevallii</i>	7	1	6
<i>Ampithoe gammaroides</i>	36	39	24
<i>Jassa falcata</i>	19	9	28
Aoridae indent.	3	9	4
<i>Phthisica marina</i>	0	0	1

Species	Benthic Holdfast Samples	Suspended November Holdfast Samples	Suspended January Holdfast Samples
Order Decapoda			
Decapoda indent.	2	55	10
<i>Hippolyte varians</i>	0	15	20
Order Acarina			
Halacaridae indent.	2	0	0
Phylum Mollusca			
Class Polyplacophora			
<i>Acanthochitona crinite</i>	2	0	0
Class Gastropoda			
<i>Patella pellucida</i>	16	1	0
<i>Patella vulgate</i>	3	0	0
<i>Gibbula</i> sp.	1	0	0
<i>Bittium reticulatum</i>	1	0	0
<i>Lacuna parva</i>	7	0	0
<i>Rissoa parva</i>	107	3	0
<i>Onoba semicostata</i>	6	0	0
Class Bivalvia			
<i>Mytilus edulis</i>	11	3	0
<i>Chlamys</i>	4	2	0
<i>Anomia ephippium</i>	21	2	0
<i>Kellia suborbicularis</i>	2	2	0
<i>Lasaea adansoni</i>	1	1	3
<i>Hiatella arctica</i>	47	1	1
Phylum Echinodermata			
<i>Ophiothrix fragilis</i>	4	0	0
Phylum Chordata			

Species	Benthic Holdfast Samples	Suspended November Holdfast Samples	Suspended January Holdfast Samples
Class Ascidiacea			
<i>Clavelina lepadiformis</i>	8	0	0
<i>Ascidia</i> sp.	0	63	16
<i>Molgula</i> sp.	1	0	0
Total	1801	373	260

Appendix B: Ecological priming of artificial aquaculture structures: kelp farms as an example

Table B1. Full species list of flora and fauna sampled from April, May and June primed (P) and unprimed (UP) dropper sections for 2014 and 2015 and their occurrence per month (max. occurrence per month is 15)

Species	April 2014 P	April 2014 UP	May 2014 P	May 2014 UP	June 2014 P	June 2014 UP	April 2015 P	April 2015 UP	May 2015 P	May 2015 UP	June 2015 P	June 2015 UP
Phylum Ochrophyta												
Filamentous brown algae sp.	7	10	1	14	4	10	12	15		14	1	11
<i>Laminariales</i> juvenile				9		15		12		14	5	12
<i>Saccorhiza polyschides</i>						3				4		4
<i>Saccharina latissima</i>										4		4
<i>Desmarestia viridis</i>												2
Phylum Rhodophyta												
<i>Polysiphonia</i> sp.				9	3	14	12	10	14	15	5	14
<i>Ceramium</i> sp.		2	2	12	3	15	4	3	7	14	4	15
<i>Lomentaria clavellosa</i>						1	2	1	3	6	1	8
Phylum Chlorophyta												
<i>Ulva</i> sp.										1		2
Phylum Cnidaria												
Class Hydrozoa												
<i>Obelia geniculata</i>		1	13	15	4	4	8	7	15	14	11	9
<i>Laomedea flexuosa</i>	2								9	6		5
<i>Ectopleura larynx</i>									7	4	7	5
<i>Dynamena</i> sp.								1	1			
<i>Kirchenpaueria pinnata</i>											1	
<i>Sarsia</i> sp.											1	

Species	April 2014 P	April 2014 UP	May 2014 P	May 2014 UP	June 2014 P	June 2014 UP	April 2015 P	April 2015 UP	May 2015 P	May 2015 UP	June 2015 P	June 2015 UP
Phylum Bryozoa												
Class Gymnolaemata												
<i>Electra pilosa</i>			3		12				15	1	15	8
<i>Membranipora membranacea</i>						3			2	3	2	4
<i>Vesicularia spinosa</i>					4						6	2
Class Stenolaemata												
<i>Crisia</i> sp.									8	1	2	
Phylum Echinodermata												
Class Asteroidea												
<i>Asterias</i> juvenile.			5	1	6				5		2	
Class Ophiuroidea												
<i>Ophiuroidea</i> sp.					1							
<i>Amphipholis squamata</i>									1			
Class Crinoidea												
<i>Crinoidea</i> sp.											1	
Phylum Nematoda												
Nematoda indent.			5	8	4	14	5	11	15	15	15	15
Phylum Annelida												
Class Polychaete												
<i>Myrianida</i> sp.	1	1	7	11	10	14	2	1	9	1	14	10
<i>Eusyllis blomstrandii</i>		1	2	3	5	3	3		11	2	11	5
<i>Polygordius appendiculatus</i>					2							
<i>Eunidae</i> sp.					1							
<i>Platynereis dumerilii</i>			2		1	1				5		
<i>Harmothoe</i> sp.	5		6	2	8	1		1	15	2	15	5
<i>Spirobranchus triqueter</i>				2	2	2			15	5	15	7

Species	April 2014 P	April 2014 UP	May 2014 P	May 2014 UP	June 2014 P	June 2014 UP	April 2015 P	April 2015 UP	May 2015 P	May 2015 UP	June 2015 P	June 2015 UP
<i>Eulalia viridis</i>			2	3	2						9	4
<i>Nereiphylla rubiginosa</i>				1	1				4	1	9	3
<i>Nereimyra punctata</i>					6				1		4	
<i>Cossura</i> sp.					1							
<i>Pseudopotamilla reniformis</i>											1	
Class Oligochaeta												
<i>Oligochaeta</i> indent.									1	3		4
Phylum Sipuncula												
<i>Sipuncula</i> indent.					1	1				2		2
Phylum Nemertea												
Nemertea indent.							3	1	3	4	13	6
Phylum Arthropoda												
<i>Acari</i> sp.						2	2	1	1			
<i>Pycnogonida</i> indent.			1	1			3		2		3	
Diptera larvae										2		
<i>Anurida maritima</i>											1	
Phylum Crustacea												
Order Amphipoda												
<i>Jassa</i> fem.	15	12	15	15	15	15	15	15	15	15	15	15
<i>Jassa falcata</i>	3		5	7	9	10	9	1	13	10	15	13
<i>Parajassa pelagica</i>	1		1	1	3	1	1				2	1
<i>Gammarellus homari</i>	4	2		1	1		10		5		2	1
<i>Gammarus insensibilis</i>	3			2			3	1	1	1	5	2
<i>Aora gracilis</i>			1						1			
<i>Aora</i> sp.			1					1	1			3
<i>Hardametopa nasuta</i>		1	3	2	5	3	6		11	4	12	9

Species	April 2014 P	April 2014 UP	May 2014 P	May 2014 UP	June 2014 P	June 2014 UP	April 2015 P	April 2015 UP	May 2015 P	May 2015 UP	June 2015 P	June 2015 UP
<i>Elasmopus rapax</i>	1		1									
<i>Dexaminidae</i> sp.					1							
<i>Gitana sarsi</i>					1				2		5	
Suborder Caprellidea												
<i>Caprella septentrionalis</i>											9	5
<i>Caprella penantis</i>					1							
<i>Caprella</i> juvenile											8	4
Order Isopoda												
<i>Idotea granulosa</i>	2		2	1	2	1	2		1		5	1
<i>Idotea balthica</i>							2					
<i>Idotea pelagica</i>			1		6	2				2		1
<i>Isopod</i> sp.					1				1			
Order Decapoda												
<i>Pisidia longicornis</i>			2	2	6	3			12		12	3
<i>Necora puber</i>											9	
Megalopore larvae											4	3
Class Copepoda												
Harpactocoida indent.	8	8	15	15	3	15	13	13	14	15	14	15
Order Cumacea												
<i>Cumacea</i> indent.	1	2	1	1								
Class Ostrocooda												
<i>Ostracoda</i> indent.			2	6	1	7				2	2	1
Class Cirripedia												
<i>Balanus</i> sp.			2	1	3					1	2	2

Species	April 2014 P	April 2014 UP	May 2014 P	May 2014 UP	June 2014 P	June 2014 UP	April 2015 P	April 2015 UP	May 2015 P	May 2015 UP	June 2015 P	June 2015 UP
Phylum Mollusca												
Class Gastropoda												
<i>Rissoa parav</i>	1	1	13	10	11	9		1	10	5	15	11
<i>Lacuna vincta</i>			6	8	2	4			3	4	2	1
<i>Patella pellucida</i>						1						
<i>Onoba semicostata</i>												
Class Bivalvia												
<i>Mytilus</i> sp.			15	15	15	15	8	1	15	15	15	15
<i>Musculus costulatus</i>						1	3	2	3	1	3	1
<i>Kellia suborbicularis</i>				3	4	3		1	2		14	3
<i>Anomia epipphium</i>					5	1					15	1
<i>Hiatella arctica</i>					1	1	1		2		15	2
<i>Turtonia minuta</i>	2											
<i>Lasaea adansoni</i>			1									
<i>Chlamys</i> sp.												
Order Nudibranchia												
<i>Polycera quadrilineata</i>			7	4			6	7	15	6	11	4
Phylum Chordata												
Class Ascidiacea												
<i>Ciona intestinalis</i>											6	
Class Actinoptergii												
<i>Cyclopterus lumpus</i>	1						1					
Total number of species	16	11	31	31	42	33	25	22	42	38	52	49

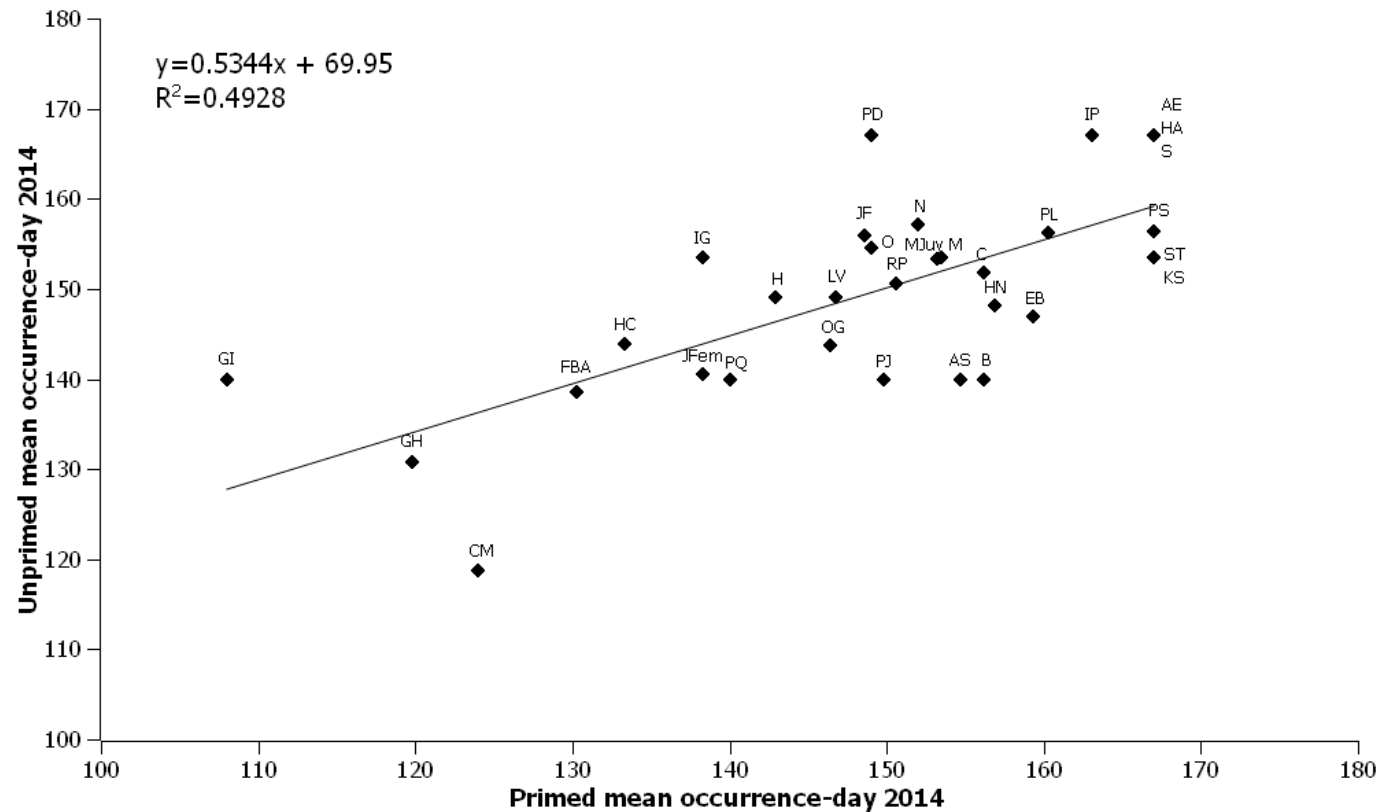


Fig. B1a. The relationship between mean day of year occurrences for all shared species in year 2014 for primed and unprimed treatments.

Legend: GI: *Gammarus insensibilis*; GH: *Gammarellus homari*; CM: Cumacea indent.; FBA: filamentous brown algae sp; HC: Harpacticoid Copepods; JFem: *Jassa* sp. female; IG: *Idotea granulosa*; PQ: *Polycera quadrilineata*; H: *Harmothoe* sp.; OG: *Obelia geniculata*; LV: *Lacuna vincta*; PJ: *Parajassa pelagica*; RP: *Rissoa parva*; JF: *Jassa falcata*; O: Ostracoda indent.; PD: *Platynereis dumerilii*; N: Nematoda indent.; MJuv- *Mytilus* sp. juvenile; M: *Myrianida* sp.; AS: *Asterias* sp.; B: *Balanus* sp.; C: *Ceramiales* sp; HN: *Hardametopa nasuta*; EB: *Eusyllis blomstrandii*; PL: *Pisidia longicornis*; IP: *Idotea pelagica*; AE: *Anomia eippium*; HA: *Hiatella arctica*; S: Sipuncula indent.; PS: *Polysiphonia* sp; ST: *Spirobranchus triqueter*; KS: *Kellia suborbicularis*

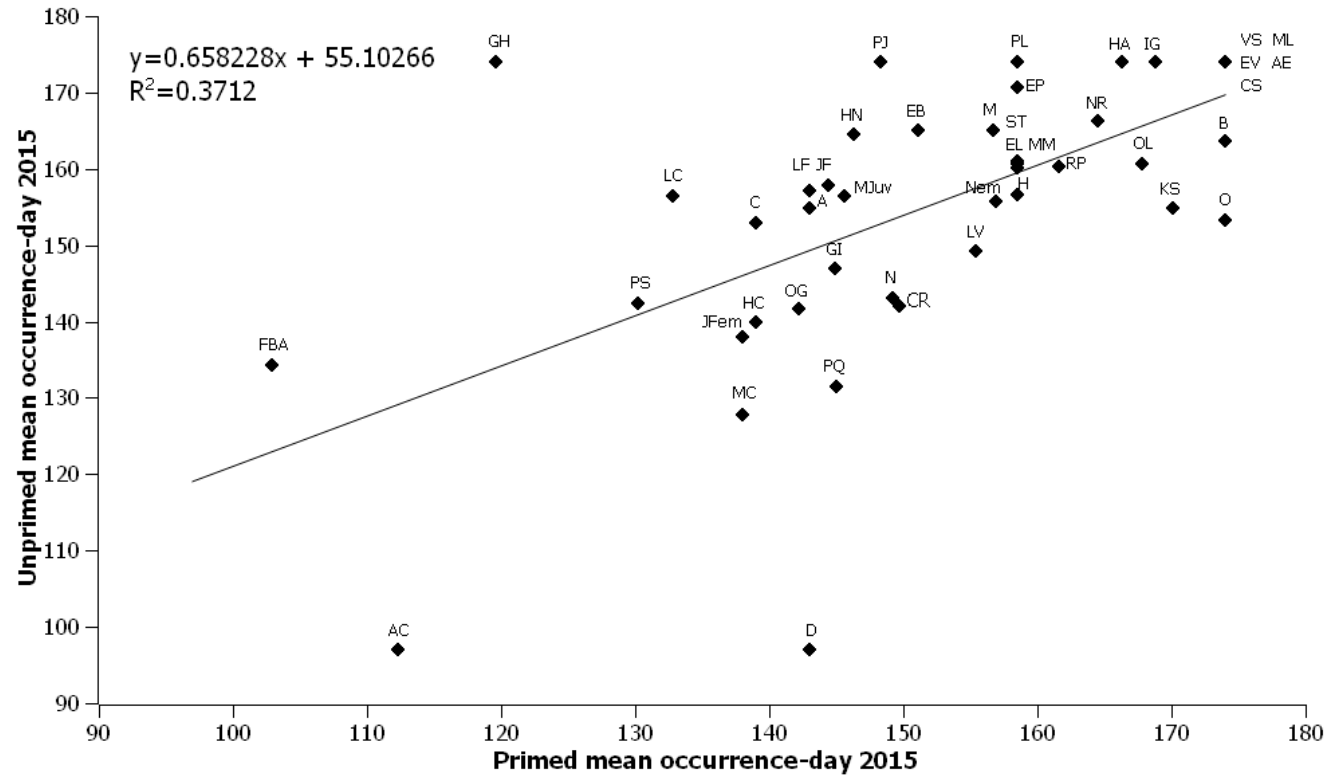


Fig. B1b. The relationship between mean day of year occurrences for all shared species in year 2015 for primed and unprimed treatments.

Legend: FBA: filamentous brown algae sp.; AC: *Acari* sp.; GH: *Gammarellus homari*; PS: *Polysiphonia* sp.; LC: *Lomentaria clavellosa*; JFem: *Jassa* sp. female; HC: Harpacticoid Copepods; OG: *Obelia geniculata*; MC: *Musculus costulatus*; C: *Ceramiales* sp.; PQ: *Polycera quadrilineata*; D: *Dynamena* sp.; LF: *Laomedea flexuosa*; GI: *Gammarus insensibilis*; JF: *Jassa falcata*; A: *Aora* sp.; MJuv: *Mytilus* sp. juvenile; HN: *Hardametopa nasuta*; PJ: *Parajassa pelagica*; EB: *Eusyllis blomstrandii*; N: Nematoda indent.; C: *Crisia* sp.; LV: *Lacuna vincta*; Nem: Nemertea indent.; H: *Harmothoe* sp.; M: *Myrianida* sp.; ST: *Spirobranchus triqueter*; MM: *Membranipora membranacea*; EL: *Ectopleura larynx*; EP: *Electra pilosa*; PL: *Pisidia longicornis*; RP: *Rissoa parva*; NR: *Nereiphylla rubiginosa*; HA: *Hiatella arctica*; IG: *Idotea granulosa*; OL: *Oligochaeta* indent.; KS: *Kellia suborbicularis*; O: Ostracoda indent.; B: *Balanus* sp.; VS: *Vesicularia spinosa*; EV: *Eulalia viridis*; AE: *Anomia epiphium*; ML: Megalopore larvae; CS: *Caprella septentrionalis*

Appendix C: Successional changes of epibiont fouling communities of the cultivated kelp *Alaria esculenta*: predictability and influences

Table C1. Full species list of epibionts from April, May and June *Alaria esculenta* fronds for 2014 and 2015 and Artificial Low and High June 2014 samples and their occurrence per month (max. occurrence per month is 15).

Species	April 2014	May 2014	June 2014	April 2015	May 2015	June 2015	Artificial-Low	Artificial-High
Phylum Ochrophyta								
Filamentous brown algae sp.	14	2		15			15	15
Encrusting brown algae sp.					1		15	15
<i>Laminariales</i> juv.							13	13
Phylum Rhodophyta								
<i>Polysiphonia</i> sp.				1		3	13	13
<i>Ceramium</i> sp.			3		2	3	11	1
Phylum Cnidaria								
Class Hydrozoa								
<i>Kirchenpaueria pinnata</i>								1
<i>Obelia geniculata</i>	11	15	9	2	15	15	13	15
Phylum Bryozoa								
Class Gymnolaemata								
<i>Electra pilosa</i>		15	14		15	15	11	15
<i>Membranipora membranacea</i>		6	15		15	15	9	13
Class Stenolaemata								
<i>Crisia</i> sp.						2		
Phylum Echinodermata								
Class Asteroidea								
<i>Asterias rubens</i>							5	4
Class Ophiuroidea								
<i>Ophiuroidea</i> sp.						1		

Species	April 2014	May 2014	June 2014	April 2015	May 2015	June 2015	Artificial-Low	Artificial-High
Phylum Annelida								
<i>Myrianida</i> sp.		8	6		3	10	10	15
<i>Eulalia viridis</i>		1			3			
<i>Eusyllis blomstrandii</i>						3		
<i>Harmothoe</i> sp.					1		6	2
<i>Spirobranchus triqueter</i>			3		12	15	12	12
<i>Platynereis dumerilii</i>							2	1
<i>Phyllodoce lamelligera</i>							1	
Phylum Sipuncula								
Sipuncula indent.								2
Phylum Nemertea								
Nemertea indent.						1		
Phylum Arthropoda								
Pycnogonida indet.							1	1
Phylum Crustacea								
Order Amphipoda								
<i>Jassa</i> fem.	5	15	15	14	15	15	15	15
<i>Jassa falcata</i>	2	9	9	5	11	15	12	11
<i>Gammarellus homari</i>				4			4	
<i>Gammarus insensibilis</i>					1			
<i>Stenothoe monoculoides</i>		2	1		1		11	12
<i>Hardametopa nasuta</i>			2			1		
Order Isopoda								
<i>Idotea granulosa</i>		1	1		1		3	
<i>Sphaeromatidae</i> juv.		1						
Suborder Caprellidea								
<i>Caprella septentrionalis</i>						1		

Species	April 2014	May 2014	June 2014	April 2015	May 2015	June 2015	Artificial-Low	Artificial-High
<i>Caprella penantis</i>						2		
Order Decapoda								
<i>Pisidia longicornis</i>			2			2	4	
<i>Necora puber</i>							7	
Megalopore larvae							3	
Class Copepoda								
Harpacticoida indent.		9	4	8	13	13	15	15
Class Cirripedia								
<i>Balanus</i> sp.		2					8	12
Phylum Mollusca								
Class Bivalvia								
<i>Mytilus</i> sp.		13	15	4	15	15	15	15
<i>Anomia ephippium</i>			2			13	11	14
<i>Hiatella arctica</i>					1			
Class Gastropoda								
<i>Rissoa parva</i>							10	11
<i>Lacuna vineta</i>		9	2	1	3	2	11	12
<i>Patella pellucida</i>								1
Order Nudibranchia								
<i>Polycera quadrilineata</i>		13	7		15	13	3	15
Total	4	16	17	9	19	22	30	27

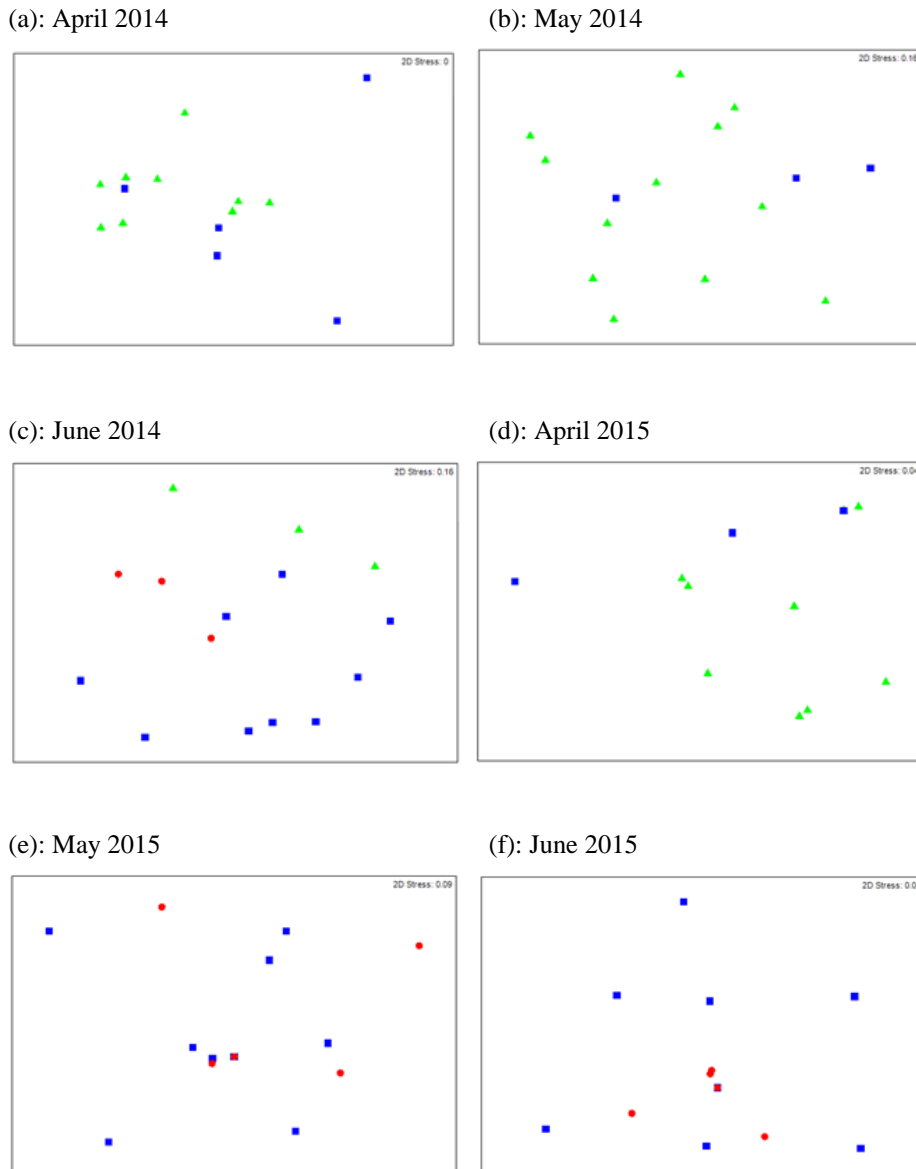


Fig. C1(a-f). Two-dimensional MDS plot of 15 frond sections sampled in (a) April 2014 (note one frond sample had no epibionts present); (b) May 2014; (c) June 2014; (d) April 2015; (e) May 2015; (f) June 2015: Position on the line; Green triangles- western end of longline, first 30 m approximately; Blue squares- middle of longline, middle 30 m approximately; Red circles- eastern end of longline, last 30 m approximately Based on presence-absence Simpson's dissimilarity matrix of species collected on each frond section. No patterns in the plots were observed and differences between treatments are as great as differences between all samples. Potential patterns included a clustering of east and west samples to either edge of the plot with the middle samples in between or east and west samples forming a circle around the middle samples.

Appendix D: Aquaculture structures of kelp farms: assessment of artificial habitat availability for mobile fauna

Table D1. Full species list of mobile fauna from April, May and June seeded and rope treatments sampled in 2014 and 2015.

n=5 droppers were sampled for each treatment on each sampling date. Max occurrence per sample month 5.

Species	April 2014 Seeded	April 2014 Rope	May 2014 Seeded	May-2014 Rope	June 2014 Seeded	June 2014 Rope	April 2014 Seeded	April 2014 Rope	May 2014 Seeded	May 2014 Rope	June-2014 Seeded	June 2014 Rope
Phylum Echinodermata												
Class Asteroidea												
<i>Asterias</i> sp.				1	3							
<i>Asterias gibbosa</i>											1	
Phylum Nematoda												
Nematoda indent.			1	3	2	3	3	5	5	5	4	5
Phylum Annelida												
<i>Myrianida</i> sp.			4	4	5	4		1	4	5	5	5
<i>Eulalia viridis</i>				1							1	1
<i>Harmothoe</i> sp.		1	3		4	1	2		1	3	2	3
<i>Eusyllis blomstrandii</i>			1	1		2	1	3	1	1	2	3
<i>Nereiphylla rubiginosa</i>										1	3	3
<i>Platynereis dumerilii</i>						1		1		3		
Class Oligochaeta												
<i>Oligochaeta</i> indent.										1		
Phylum Sipuncula												
Sipuncula indent.						1				2		
Phylum Nemertea												
Nemertea indent.							1		1		1	1
Phylum Arthropoda												
<i>Acari</i> sp.					1	1	2			1	2	
<i>Pycnogonida</i> indent.			3	1		2			5			
<i>Anurida maritima</i>											2	

Species	April 2014 Seeded	April 2014 Rope	May 2014 Seeded	May-2014 Rope	June 2014 Seeded	June 2014 Rope	April 2014 Seeded	April 2014 Rope	May 2014 Seeded	May 2014 Rope	June-2014 Seeded	June 2014 Rope
Phylum Crustacea												
Order Amphipoda												
<i>Jassa</i> sp.	5	5	5	5	5	5	5	5	5	5	5	5
<i>Jassa falcata</i>	5	5	5	5	4	5	4	5	5	5	5	5
<i>Parajassa pelagica</i>			2			2		1				
<i>Gammarellus homari</i>	4	4		3	1		5	4	1		2	2
<i>Gammarus insensibilis</i>	4	3	2		1		3	4		1	3	1
<i>Aora</i> sp.	1	2						2	1	1	1	1
<i>Hardametopa nasuta</i>	1		2	2	5	2	2	3	5	1	5	5
<i>Ampithoe ramondi</i>			1									
<i>Dexamine thea</i>			1									
<i>Nototropis swammerdamei</i>			1									
<i>Gitana sarsi</i>				1								
Suborder Caprellidea												
<i>Caprella septentrionalis</i>						1					4	5
<i>Caprella penantis</i>						1			1		2	
<i>Caprella</i> juv.											2	5
Order Isopoda												
<i>Idotea granulosa</i>	5	2	4	4	2	2	1			1	3	3
<i>Idotea balthica</i>	2		1	1			4			1		1
<i>Idotea pelagica</i>					3		1		3			2
<i>Idotea</i> sp.	1				1	1				1		
Order Cumacea												
<i>Cumacea</i>	1							1				

Species	April 2014 Seeded	April 2014 Rope	May 2014 Seeded	May- 2014 Rope	June 2014 Seeded	June 2014 Rope	April 2014 Seeded	April 2014 Rope	May 2014 Seeded	May 2014 Rope	June- 204 Seeded	June 2014 Rope
Order Decapoda												
<i>Pisidia longicornis</i>			1		3						2	3
Megalopa larvae											3	
Class Ostrocooda												
<i>Ostracoda</i> indent.				1								
Order Tanidacea												
<i>Tanaidacea</i> indent.									1			
Order Harpacticoida												
Harpacticoida indent.	5	5	5	5	5	5	5	5	5	5	5	5
Phylum Mollusca												
Class Gastropoda												
<i>Rissoa parva</i>			5	3	3	2	3	2	2	2	5	4
<i>Lacuna vineta</i>	1		5	3	5	1			4	2	2	
<i>Onoba semicostata</i>											2	1
Order Nudibranchia												
<i>Polycera quadrilineata</i>			5	3	1	1	2	3	5	5	3	3
Phylum Chordata												
<i>Cyclopterus lumpus</i>	1										1	
<i>Pholis gunnellus</i>				1								
<i>Pollachius pollachius</i>			1								2	
<i>Gaidropsarus vulgaris</i>											1	
Total	13	8	21	19	18	20	16	15	18	21	30	23

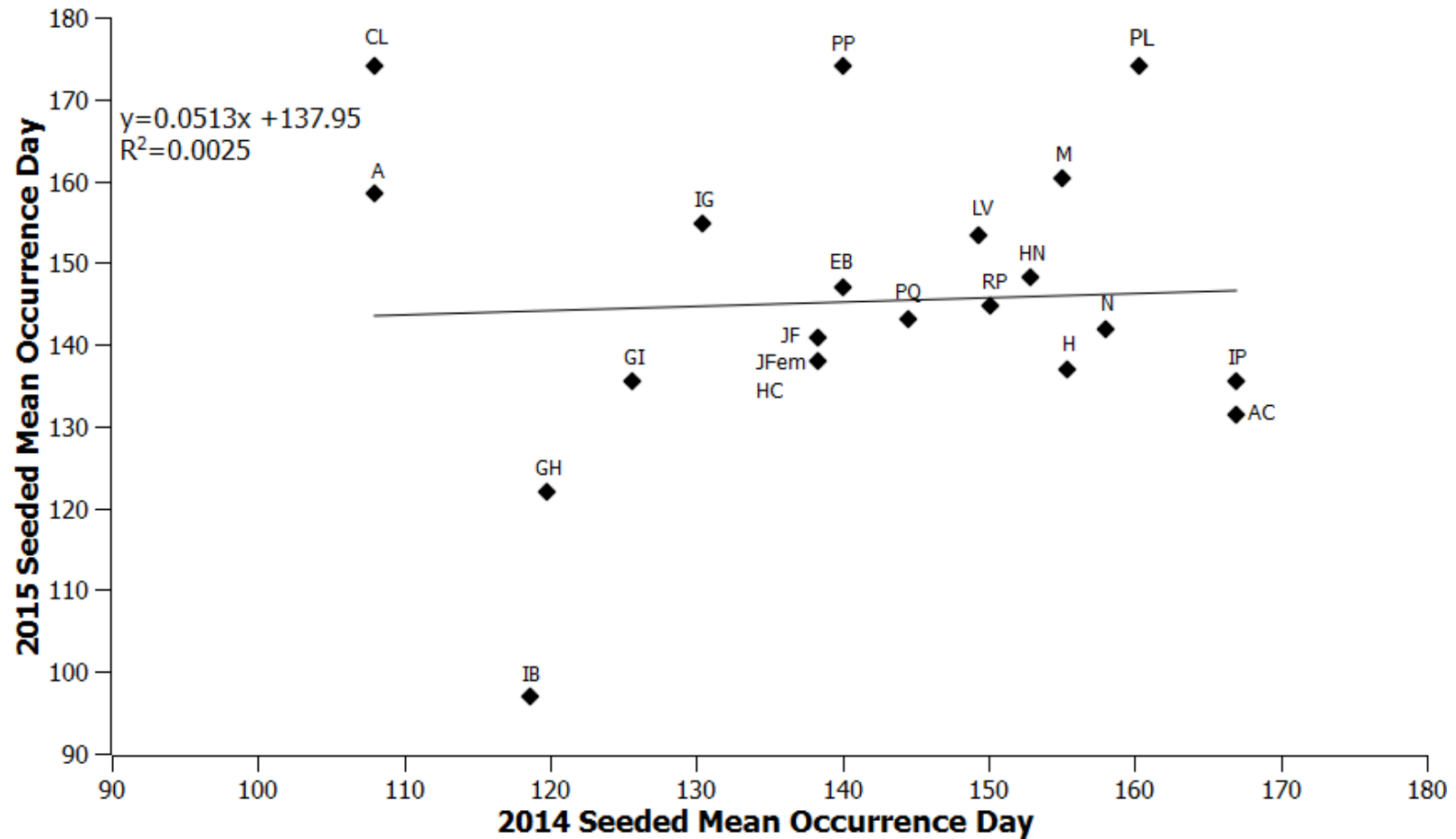


Fig. D1a. The relationship between mean day of year occurrences for all shared species in year 2014 and 2015 sampled on seeded dropper treatment. Legend: A: *Aora* sp.; CL: *Cyclopterus lumpus*; IB: *Idotea baltica*; GH: *Gammarellus homari*; GI: *Gammarus insensibilis*; IG: *Idotea granulosa*; JFem: *Jassa* sp. female; HC: Harpacticoid Copepods; JF: *Jassa falcata*; PP: *Pollachius pollachius*; EB: *Eusyllis blomstrandii*; PQ: *Polycera quadrilineata*; LV: *Lacuna vineta*; RP: *Rissoa parva*; HN: *Hardametopa nasuta*; M: *Myrianida* sp.; H: *Harmothoe* sp.; N: Nematoda indent.; PL: *Pisidia longicornis*; IP: *Idotea pelagica*; AC: *Acari* sp.

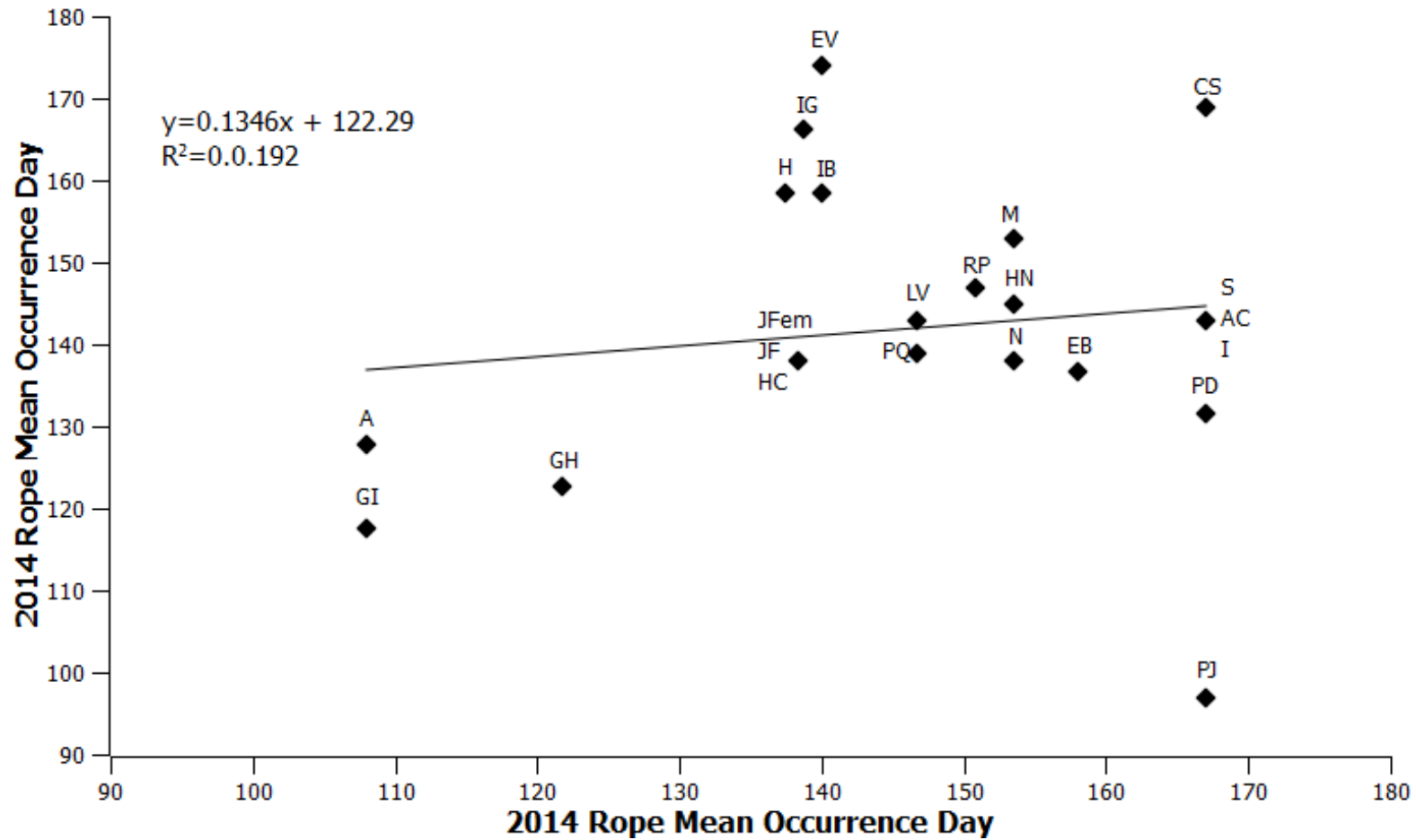


Fig. D1b. The relationship between mean day of year occurrences for all shared species in year 2014 and 2015 sampled on rope dropper treatment. Legend: GI: *Gammarus insensibilis*; A: *Aora* sp.; GH: *Gammarellus homari*; H: *Harmothoe* sp.; HC: Harpacticoid Copepods; JFem: *Jassa* sp. female; JF: *Jassa falcata*; IG: *Idotea granulosa*; EV: *Eulalia viridis*; IB: *Idotea baltica*; LV: *Lacuna vincta*; PQ: *Polycera quadrilineata*; RP: *Rissoa parva*; N: Nematoda indent.; M: *Myrianida* sp.; HN: *Hardametopa nasuta*; EB: *Eusyllis blomstrandii*; PD: *Platynereis dumerilii*; S: *Sipuncula* indent.; AC: *Acari* sp.; PJ: *Parajassa pelagica*; CS: *Caprella septrionalis*; I: *Idotea* sp.

Appendix E: Impact of kelp cultivation on the Ecological Status of benthic habitats and *Zostera marina* seagrass biomass

Table E1. Full species list of benthic macrofaunal and their abundance sampled from impacted and control treatment sites over six sampling dates.

Species	May 2014	September 2014	February 2015	May 2015	September 2015	February 2016
Phylum Cnidaria						
<i>Anemonia viridis</i>					1	4
<i>Edwardsia sp.</i>	1	1	1		1	1
<i>Lucernaria sp.</i>					1	
Phylum Echinodermata						
Class Ophiuroidea						
<i>Acrocnida brachiata</i>	11	10	13	11	24	6
<i>Amphiura filiformis</i>	23	51	36	29	44	41
<i>Amphipholis squamata</i>	12	13	46	51	16	55
<i>Ophiura albida</i>	2	3	2	4	4	9
<i>Ophiothrix sp.</i>	2					
Class Echinoidea						
<i>Echinocardium cordatum</i>	1				3	1
<i>Echinocyamus pusillus</i>				3	7	
<i>Strongylocentrotidae sp.</i>	1	4	1		1	2
Class Asteroidea						
<i>Asterias sp.</i>					1	
Class Holothuroidea						
<i>Leptosynapta sp.</i>				2		
<i>Labidoplax digitata</i>					1	
Phylum Nematoda						
Nematoda indent.	104	289	425	529	560	881

Species	May 2014	September 2014	February 2015	May 2015	September 2015	February 2016
Phylum Annelida						
<i>Polynoidae</i> sp.	9	31	10	17	9	6
<i>Pholoe baltica</i>	42	88	76	54	81	67
<i>Magelona minuta</i>	29	19	107	84	87	72
<i>Chaetozone gibber</i>	95	414	560	773	686	626
<i>Aricidea minuta</i>	3	18	35	25	45	60
<i>Glycera tridactyla</i>	8	14	9	2	13	9
<i>Goniada maculata</i>		1	3		2	2
<i>Glycera</i> sp.		2	6	9	8	9
<i>Diplocirrus glaucus</i>	5	3	3	11	16	22
<i>Terrellides</i> sp.	3	5		3	0	1
<i>Clymenura</i> sp.	9	65	57	57	60	53
<i>Etone flava</i>	4	1	2			
<i>Phyllodoce laminosa</i>	3	1	2	1	3	
<i>Phyllodoce lamelligera</i>		2			2	3
<i>Phyllodoce</i> sp.	3	9			3	
<i>Owenia fusiformis</i>	77	120	176	204	277	169
<i>Galathowenia oculata</i>	24	68	71	40	19	21
<i>Lumbrineris gracilis</i>	5	6	3	2	5	2
<i>Eusyllis</i> sp.	1				1	3
<i>Sigalion squamosus</i>	2	2		5	6	3
<i>Sthenelais boa</i>			1			
<i>Sigalion mathildae</i>				1		
<i>Podarkeopsis capensis</i>	1	1	4		5	10
<i>Lanice conchilega</i>	1	2	5	5	0	10
<i>Nephtys hombergii</i>	2	3	1	2	1	

Species	May 2014	September 2014	February 2015	May 2015	September 2015	February 2016
<i>Nereis pelagica</i>	2	13	8	2	3	
<i>Paraonides</i> sp.						1
<i>Exogone longicirris</i>		1	1	3	10	41
<i>Arabella iricolor</i>				1		
<i>Scalibregma inflatum</i>					1	
<i>Pectinaria auricoma</i>			1		6	2
<i>Petta pusilla</i>			1			1
Class Oligochaeta						
Oligochaeta indent.		1	1			
Phylum Nemertea						
Nemertea indent.	5	2	13	13	11	16
Phylum Sipuncula						
Sipuncula indent.		2	1			
Phylum Arthropoda						
Pycnogonidae indent.				1		
<i>Anoplodactylus petiolatus</i>	3	3	1	3	1	7
<i>Achelia echinata</i>	1	1				3
<i>Nymphon brevistre</i>						1
<i>Collembolasp.</i>			1			
Phylum Crustacea						
Order Amphipoda						
<i>Pontocrates altamarinus</i>	8	6	27	11	11	16
<i>Perioculodes longimanus</i>	16	39	19	33	54	33
<i>Pontocratessp.</i>	1		1			
<i>Urothoe elegans</i>	17	63	25	13	27	47
<i>Ampelisca brevicornis</i>	5	9	11	7	7	6

Species	May 2014	September 2014	February 2015	May 2015	September 2015	February 2016
<i>Aora gracilis</i>	2	51	4	2	2	
<i>Leptocheirus pectinatus</i>		4			2	
<i>Stenothoe monoculoides</i>	6	29	2	11	41	18
<i>Erichthonius punctatus</i>	4	6	4	1	28	
<i>Erichthonius difformis</i>		1		3		4
<i>Erichthonius</i> sp.		12	3	2	13	
<i>Jassa falcata</i>			2	1		
<i>Jassa</i> sp.	1	5	1	4	2	4
<i>Dexamine spinosa</i>		9				
<i>Dexamine thea</i>		9	5	1	31	9
<i>Nototropis swammerdami</i>	8		19	14	2	34
<i>Abludoelita obtusata</i>	6	2		1	11	15
<i>Animoceradocus semiserratus</i>		3	3	2		
<i>Cheirocratus sundevalli</i>		2	6	7	19	3
<i>Microprotopus maculatus</i>	1	5		7	18	1
<i>Gitana sarsi</i>			1		1	
<i>Harpinia crenulata</i>		1		1		
<i>Leucothoe procera</i>						
<i>Leucothoe richardii</i>		1				
<i>Amphithoe ramondi</i>				4	1	
<i>Amphithoe rubricata</i>						
<i>Gammarus insensibilis</i>		1	1			
<i>Photis longicaudata</i>				2		
<i>Bathyporeia</i> sp.						2
<i>Ampithoe</i> sp.		1				1
<i>Melitidae</i> sp.						

Species	May 2014	September 2014	February 2015	May 2015	September 2015	February 2016
<i>Gammarus</i> sp.				1		
<i>Aora</i> sp.		24	1	28	112	9
<i>Amphilochoides</i> sp.		12	13	27	120	26
Suborder Caprellidae						
<i>Pariambus typicus</i>	56	58	43	86	504	74
Order Isopoda						
<i>Astacilla longicornis</i>		1			13	1
<i>Idotea pelagica</i>					1	
Order Decapoda						
<i>Crangon crangon</i>	1					
<i>Processa edulis crassipes</i>		8			3	1
<i>Mysideis</i> sp.		1				
Megalopore larvae					1	
<i>Pisidia longicornis</i>						1
<i>Macropodia</i> sp.						1
Order Harpacticoida						
Harpacticoida indent.	70	57	14	39	13	14
Order Ostracoda						
Ostracoda indent.	13	110	45	108	44	195
Order Tanaidacea						
Tanaidacea indent.	28	35	50	36	120	55
Order Cumacea						
<i>Diastylis</i> sp.	15	15	8	38	26	19
<i>Pseudocuma</i> sp.	15	11	16	33	26	131
Class Maxillopoda						
<i>Balanus</i> sp.				1		

Species	May 2014	September 2014	February 2015	May 2015	September 2015	February 2016
Phylum Mollusca						
Class Bivalvia						
<i>Lucinoma borealis</i>	22	30	19	28	25	26
<i>Kurtiella bidentata</i>	80	106	105	90	128	131
<i>Nucula nitidosa</i>	7	10	15	4	16	13
<i>Venus casina</i>	16	3	22	14	40	40
<i>Tellina tenuis</i>	160	108	300	631	284	205
<i>Phaxas pellucidus</i>	5	1	6	22	2	1
<i>Parvacardium scabrum</i>		6	1	1	1	
<i>Parvicardium</i> sp.	2			1		
<i>Thracia phaseolina</i>	13	36	40	88	113	78
<i>Thyasira flexuosa</i>	5	2	6	2	26	30
<i>Chamelea gallina</i>	1	5	2	2	4	2
<i>Corbula gibba</i>					3	1
<i>Mactra</i> sp.	1	4		5	2	3
<i>Spisula elliptica</i>				1		
<i>Hiatella arctica</i>		2	1	1	23	5
<i>Mytilus edulis</i>		3	2	2	2	2
<i>Anomia ephippium</i>					1	
Bivalvia Indent.	1	2				
Class Gastropods						
<i>Rissoa parva</i>	4	19	2		8	
<i>Lacuna vincta</i>	3	2		1		
<i>Retusa</i> sp.	7	15	13	35	42	42
<i>Skeneopsis planorbis</i>					3	
<i>Scaphander lignarius</i>			1			2

Species	May 2014	September 2014	February 2015	May 2015	September 2015	February 2016
<i>Euspira nitida</i>				1		1
<i>Acteon tornatilis</i>					1	
<i>Raphitoma</i> sp.						1
<i>Aplysia punctata</i>		1	1			
Total	1099	2214	2551	3400	3999	3518

Table E2. One-way Similarity Percentages (SIMPER) analysis of treatment sites (I=Impacted and C=Control) within sampling dates. Analysis conducted on Bray-Curtis Similarity matrix of square root transformed abundance data with 70 % cut-off for low contributions

Group May-14-I

Average similarity: 42.92

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Kurtiella bidentata</i>	2.50	7.05	3.76	16.42	16.42
<i>Tellina tenuis</i>	1.77	3.90	1.07	9.09	25.51
<i>Chaetozone gibber</i>	1.38	3.42	1.14	7.96	33.48
<i>Pholoe baltica</i>	1.43	3.36	1.13	7.82	41.30
<i>Owenia fusiformis</i>	1.44	3.10	1.02	7.22	48.52
Nematoda	1.18	2.85	1.08	6.64	55.16
<i>Amphiura filiformis</i>	1.23	2.62	1.09	6.10	61.26
<i>Urothoe elegans</i>	0.97	2.42	1.12	5.64	66.90
<i>Venus casina</i>	0.97	2.33	1.11	5.42	72.32

Group May-14-C

Average similarity: 50.18

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Tellina tenuis</i>	2.84	7.30	3.01	14.55	14.55
Nematoda	2.35	5.89	2.03	11.74	26.29
<i>Owenia fusiformis</i>	1.97	5.47	3.90	10.90	37.19
<i>Chaetozone gibber</i>	2.18	5.32	2.16	10.61	47.80
<i>Pariambus typicus</i>	1.41	2.90	1.17	5.78	53.58
<i>Harpacticoida</i>	1.49	2.62	1.15	5.23	58.81
<i>Magelona minuta</i>	1.09	2.24	1.21	4.46	63.27
<i>Pholoe baltica</i>	1.14	2.05	0.98	4.09	67.36
<i>Kurtiella bidentata</i>	1.31	1.92	0.81	3.82	71.19

Group September-14-I

Average similarity: 55.59

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Chaetozone gibber</i>	5.70	8.73	9.40	15.70	15.70
Nematoda	4.16	5.67	8.16	10.20	25.90
<i>Owenia fusiformis</i>	2.89	4.11	2.49	7.40	33.30
<i>Galathowenia oculata</i>	2.71	4.08	5.79	7.33	40.63
<i>Kurtiella bidentata</i>	2.91	3.60	2.09	6.47	47.10
<i>Tellina tenuis</i>	2.53	3.50	2.38	6.29	53.39
<i>Harpacticoida</i>	2.34	2.88	4.03	5.19	58.58
<i>Urothoe elegans</i>	1.74	2.32	3.39	4.17	62.75
<i>Amphiura filiformis</i>	1.57	2.22	4.52	4.00	66.74
Tanaidacea	1.52	1.60	1.15	2.87	69.62
<i>Pholoe baltica</i>	1.93	1.59	1.09	2.86	72.48

Group September-14-C
Average similarity: 55.49

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Nematoda	3.31	5.60	2.11	10.09	10.09
<i>Chaetozone gibber</i>	3.58	5.56	1.65	10.01	20.10
<i>Owenia fusiformis</i>	2.14	4.00	3.67	7.21	27.31
<i>Tellina tenuis</i>	2.11	3.97	2.86	7.16	34.48
Ostracoda	2.24	3.55	2.24	6.39	40.87
<i>Pholoe baltica</i>	1.83	3.28	1.63	5.92	46.79
<i>Clymenura sp.</i>	1.49	2.70	2.12	4.86	51.65
<i>Urothoe elegans</i>	1.48	2.11	1.17	3.79	55.44
<i>Pariambus typicus</i>	1.47	2.05	0.97	3.70	59.14
<i>Galathowenia oculata</i>	1.22	1.83	1.21	3.29	62.44
<i>Perioculodes longimanus</i>	1.21	1.77	1.20	3.19	65.63
<i>Thracia phaseolina</i>	1.14	1.70	1.23	3.06	68.69
<i>Harpacticoida</i>	1.09	1.60	1.22	2.88	71.57

Group February -15-I
Average similarity: 56.61

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Chaetozone gibber</i>	5.59	12.11	5.82	21.39	21.39
<i>Tellina tenuis</i>	2.63	5.86	7.16	10.35	31.74
Nematoda	2.91	5.29	4.14	9.35	41.09
<i>Owenia fusiformis</i>	2.40	4.97	4.80	8.78	49.87
<i>Kurtiella bidentata</i>	1.59	3.30	2.42	5.82	55.69
<i>Magelona minuta</i>	1.56	3.28	5.86	5.79	61.48
<i>Galathowenia oculata</i>	1.53	3.05	3.57	5.38	66.86
Ostracoda	1.08	2.60	7.29	4.59	71.45

Group February -15-C
Average similarity: 55.27

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Chaetozone gibber</i>	4.86	7.93	3.91	14.35	14.35
<i>Tellina tenuis</i>	4.04	7.23	3.23	13.08	27.43
Nematoda	4.58	6.81	3.33	12.33	39.76
<i>Magelona minuta</i>	2.35	3.90	2.64	7.06	46.81
<i>Owenia fusiformis</i>	2.75	3.72	2.21	6.74	53.55
<i>Galathowenia oculata</i>	1.78	2.75	3.30	4.98	58.53
<i>Kurtiella bidentata</i>	2.07	2.56	1.13	4.62	63.16
<i>Amphipholis squamata</i>	1.51	2.11	1.12	3.82	66.98
<i>Pholoe baltica</i>	1.69	1.87	1.14	3.37	70.35

Group May-15-I
Average similarity: 63.55

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Chaetozone gibber</i>	7.47	12.64	6.21	19.88	19.88
Nematoda	4.74	6.56	6.02	10.33	30.21
<i>Tellina tenuis</i>	4.43	6.40	5.37	10.07	40.27
<i>Owenia fusiformis</i>	3.11	4.97	4.52	7.83	48.10
<i>Kurtiella bidentata</i>	2.51	3.85	4.56	6.06	54.16
<i>Pholoe baltica</i>	1.85	2.66	7.18	4.19	58.34
Ostracoda	1.68	2.44	4.48	3.85	62.19
<i>Magelona minuta</i>	1.64	2.22	3.55	3.50	65.69
<i>Thracia phaseolina</i>	1.84	2.15	1.11	3.39	69.08
<i>Amphiura filiformis</i>	1.48	2.11	3.49	3.32	72.40

Group May-15-C
Average similarity: 61.29

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Tellina tenuis</i>	5.65	8.65	5.28	14.11	14.11
<i>Chaetozone gibber</i>	5.49	8.32	2.99	13.57	27.68
Nematoda	4.88	6.94	3.83	11.32	39.01
<i>Owenia fusiformis</i>	2.98	4.09	1.98	6.67	45.68
<i>Thracia phaseolina</i>	2.02	3.16	3.90	5.15	50.83
Ostracoda	2.28	3.05	2.69	4.97	55.80
<i>Magelona minuta</i>	1.91	2.56	1.41	4.18	59.98
<i>Clymenura sp.</i>	1.62	2.18	1.54	3.55	63.53
<i>Amphipholis squamata</i>	1.49	1.80	1.42	2.94	66.48
<i>Pariambus typicus</i>	1.82	1.79	1.00	2.92	69.40
<i>Kurtiella bidentata</i>	1.54	1.66	0.89	2.70	72.10

Group September-15-I
Average similarity: 65.65

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Chaetozone gibber</i>	6.09	7.32	7.85	11.15	11.15
Nematoda	4.99	5.77	6.79	8.79	19.93
<i>Owenia fusiformis</i>	4.44	5.20	3.70	7.92	27.85
<i>Tellina tenuis</i>	4.23	4.72	7.05	7.18	35.03
<i>Pariambus typicus</i>	5.48	4.54	2.83	6.91	41.94
<i>Kurtiella bidentata</i>	3.34	3.53	6.58	5.38	47.32
<i>Thracia phaseolina</i>	2.67	2.96	7.97	4.52	51.84
<i>Amphiura filiformis</i>	2.02	2.29	4.99	3.49	55.33
<i>Pholoe baltica</i>	2.28	2.23	3.56	3.40	58.73
<i>Clymenura sp.</i>	2.00	2.02	2.55	3.07	61.80
<i>Magelona minuta</i>	1.80	1.99	4.18	3.04	64.84
<i>Retusa sp.</i>	1.69	1.95	2.60	2.97	67.81
<i>Aora sp.</i>	2.36	1.85	2.05	2.81	70.62

Group September-15-C
Average similarity: 61.13

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Chaetozone gibber</i>	5.66	8.12	4.69	13.29	13.29
Nematoda	5.17	6.85	4.77	11.20	24.48
<i>Pariambus typicus</i>	4.26	5.22	3.38	8.53	33.02
<i>Owenia fusiformis</i>	3.37	4.82	4.09	7.89	40.90
<i>Tellina tenuis</i>	3.21	3.72	1.48	6.09	46.99
<i>Thracia phaseolina</i>	2.10	2.69	1.99	4.40	51.39
<i>Magelona minuta</i>	1.97	2.32	2.26	3.80	55.19
Tanaidacea	2.22	2.07	1.16	3.39	58.58
<i>Pholoe baltica</i>	1.71	1.99	2.16	3.26	61.84
<i>Kurtiella bidentata</i>	1.85	1.87	1.35	3.07	64.90
<i>Periculodes longimanus</i>	1.57	1.79	1.53	2.93	67.84
<i>Clymenura sp.</i>	1.47	1.77	1.98	2.90	70.74

Group February -16-I
Average similarity: 64.20

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Chaetozone gibber</i>	5.59	7.88	3.77	12.27	12.27
Nematoda	5.40	7.71	8.51	12.01	24.28
<i>Owenia fusiformis</i>	2.92	4.32	6.22	6.72	31.00
<i>Tellina tenuis</i>	3.24	4.23	2.82	6.58	37.58
<i>Kurtiella bidentata</i>	2.71	3.67	5.05	5.72	43.30
<i>Thracia phaseolina</i>	2.29	3.55	3.84	5.53	48.84
Ostracoda	2.25	3.12	5.01	4.86	53.69
<i>Clymenura sp.</i>	1.94	3.10	9.62	4.83	58.52
<i>Pseudocuma sp.</i>	1.97	3.04	3.80	4.73	63.25
<i>Aricidea minuta</i>	1.87	2.86	4.31	4.45	67.70
<i>Retusa sp.</i>	1.84	2.55	4.05	3.97	71.68

Group February-16-C
Average similarity: 65.57

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Nematoda	6.72	9.33	6.02	14.24	14.24
<i>Chaetozone gibber</i>	5.42	8.07	6.17	12.31	26.55
<i>Tellina tenuis</i>	3.02	4.12	4.74	6.28	32.83
<i>Owenia fusiformis</i>	2.82	4.10	4.39	6.26	39.09
Ostracoda	2.97	3.42	2.72	5.22	44.31
<i>Pseudocuma sp.</i>	2.57	3.37	2.90	5.14	49.44
<i>Kurtiella bidentata</i>	2.28	2.87	2.61	4.37	53.82
<i>Pariambus typicus</i>	1.96	2.54	3.45	3.88	57.69
<i>Magelona minuta</i>	1.85	2.37	1.61	3.62	61.32
<i>Clymenura sp.</i>	1.45	2.03	4.91	3.10	64.42
<i>Amphipholis squamata</i>	1.63	1.95	1.94	2.98	67.39
<i>Thracia phaseolina</i>	1.64	1.88	1.84	2.86	70.26

Table E3. PERMDISP results for (a) Sampling Date (b) Treatment and (c) Sampling Date*Treatment

(a) Sampling Date

Deviations from Centroid- F: 14.166; df1: 5; df2: 114 P (perm): 0.001

Mean and standard error (SE) for within-group dispersion

Group	Size	Mean	SE
May-14	20	36.144	1.5366
Sept-14	20	31.359	0.97174
Feb-15	20	31.281	1.0277
May-15	20	26.301	1.4451
Sept-15	20	26.274	0.99342
Feb-16	20	24.473	0.85689

(b) Treatment

Deviations from Centroid- F: 4.8023; df1: 3; df2: 116 P (perm): 0.014

Mean and standard error (SE) for within-group dispersion

Group	Size	Mean	SE
I	30	33.673	1.5448
C1	30	36.152	1.3547
C2	30	30.096	1.1255
C3	30	30.846	0.9594

(c) Sampling Date*Treatment

Deviations from Centroid- F: 4.0852; df1: 23; df2: 96 P (perm): 0.002

Mean and standard error (SE) for within-group dispersion

Group	Size	Mean	SE
May-14-I	5	35.883	3.5123
May-14-C1	5	34.854	3.3919
May-14-C2	5	27.601	0.86474
May-14-C3	5	27.477	2.0975
Sept-14-I	5	28.129	0.93798
Sept-14-C1	5	28.714	0.91174
Sept-14-C2	5	26.826	1.4835
Sept-14-C3	5	26.749	2.315
Feb-15-I	5	27.411	2.2013
Feb-15-C1	5	28.406	1.9572
Feb-15-C2	5	23.898	2.5637
Feb-15-C3	5	27.243	3.6761
May-15-I	5	23.106	0.47612
May-15-C1	5	27.373	3.7505
May-15-C2	5	20.128	0.61694
May-15-C3	5	21.885	2.6288
Sept-15-I	5	21.616	2.0255
Sept-15-C1	5	23.791	1.2467
Sept-15-C2	5	22.121	1.3235
Sept-15-C3	5	24.137	1.4895
Feb-16-I	5	22.487	2.4669
Feb-16-C1	5	21.246	1.1193
Feb-16-C2	5	18.806	0.43715
Feb-16-C3	5	20.714	1.1395

Table E4. List of Ecological Status and Infaunal Quality Index Ecological Quality Ratio (IQI EQR) values for treatment sites (Impacted, Control 1,2 and 3) from six sampling dates

Month-Year-Site	Ecological Status	Mean IQI EQR Value
May-14-Impacted	Good	0.713
May-14-Control 1	Good	0.690
May-14-Control-2	Good	0.684
May-14-Control-3	Good	0.722
Sep-14-Impacted	Good	0.738
Sep-14-Control-1	Good	0.706
Sep-14-Control-2	Good	0.729
Sep-14-Control-3	Good	0.736
Feb-15-Impacted	Good	0.712
Feb-15-Control-1	Good	0.700
Feb-15-Control-2	Good	0.739
Feb-15-Control-3	Good	0.721
May-15-Impacted	High	0.751
May-15-Control-1	High	0.801
May-15-Control-2	High	0.756
May-15-Control-3	Good	0.732
Sep-15-Impacted	High	0.765
Sep-15-Control-1	High	0.798
Sep-15-Control-2	Good	0.739
Sep-15-Control-3	Good	0.732
Feb-16-Impacted	High	0.755
Feb-16-Control-1	High	0.760
Feb-16-Control-2	High	0.750
Feb-16-Control-3	High	0.803

Acknowledgements

People say that a PhD is like a rollercoaster ride with lots of ups and down and like all students I have had my fair share. Without the continued support, encouragement and kindness I have received along the way from so many wonderful people I don't think I would have been able to complete the journey.

Firstly, I would like to thank my excellent supervisors. I could not have asked for a better team in my corner, each with their own strengths and ideas which helped me along the way. To Prof. Mark Johnson, I would like to express my sincere gratitude for your support and guidance during the course of my research. You have always been very patient over the years, especially while explaining the intricacies of some new complicated statistical analysis. This PhD was the brain child of Dr. Maeve Edwards many years ago. Over the almost 7 years I have been privileged to know Maeve, she has always been there for me whether it be for the many exhausting sampling trips down to Ventry-chatting all the way down and back, for some sewing/crafting tips and not least she was always there with a shoulder to cry on during stressful times. Your selflessness to put everyone else first always amazes me-you are some woman! To Dr. Louise Firth even though you were across the Irish Sea, you were always at the end of an email to review a piece of work or funding applications. You are brilliant at getting money from people-including funding for this PhD! Also, thank you for providing me with the opportunities network with so many leading researchers at conferences.

I would like to say a very heartfelt thank you to Dr. Richard Fitzgerald who is no longer with us. Richard was the first person that believed I had what it took to complete a PhD. He was always there to provide encouraging stories and words of wisdom (minus the bullshit!)-I loved that about you Richard.

May you Rest In Peace.

To Dr. Robert Kennedy you were always on hand for some sample design or statistical analysis query, thank you for all your help and use of sampling equipment.

To Dr. Conor Graham who introduced me to the dark art of stable isotope analysis, thank you for your patience and help with the processing, analysis and writing of the chapter.

Acknowledgements

To Dr. Adrian Patterson and Dr. Jack O'Carroll the lads in the benthic lab-I have learned so much about taxonomic identification, you guys were always very generous with your time, thank you for all your help.

To all of the staff at the Carna Research Station and the Ryan Institute, in particular Mr. Eoin McLoughlin and Mr. Colm Moriarty for much needed technical support.

To Mr. Mike Murphy and Mr. Paul Flannery from Dingle Bay Seaweeds without your co-operation this PhD simply would not have been able to take place.

To all at the Irish Seaweed Research Group (ISRG) thank you for welcoming me into the group and helping me along the way. JJ thank you for the tea and chats-were nearly there! Alex thank you for answering my stupid excel questions and always having the exact piece of equipment I needed. To Benoit thank you you're your help on sampling trips and for your photographic/videoing skills.

To my fellow post-grads, Annette, Sineád, Sarah and Sorcha thank you for all the Friday teas and cakes and for listening to me giving out about the 'bloody weather' while trying to get down to Ventry and for making me see that I am doing fine. To Martin thank you for your words of encouragement and showing me it can be done!

To my bestest friend Anna, you have always been there for me through thick and thin even when you have your own major stuff going on. You listened and encouraged me whether over the dinner table, while cleaning a tank in the aquarium or in Westport when I had to get away from the PhD. Your help with sampling and sorting samples was much appreciated. Your strength over your rollercoaster of a journey to get yourself 'fixed' is incredible-you are a star!

To the Morans, there are no words to describe your generosity and kindness, I am so grateful for everything over the last few years.

To Mam and Dad, I dedicate this thesis to you. Your support, encouragement and guidance throughout my life has provided me with the skills to complete this PhD for which I am grateful. I know it has not always been easy (especially over the last

Acknowledgements

few months, when I'm in tornado mood!) but you have really made the write-up process a lot easier. Mam thank you for always being at the other end of the phone and knowing when I needed you and when I just needed to get up and get on with it- (oh and for the soggy buns!) To Dad your practical help and no stress attitude with sampling was a godsend-could not have done it without you or grandads boat! We must take a trip to Dingle for chowder and a pint and to visit your favourite-the Rainbow Hostel! I really appreciate the last few months since I've been home getting to know you guys again on a more mature level-although sometimes I still act like a teenager!

To Dave, my fiancé my world! You have been there for it all, from sampling in Carna and Ventry to listening to me go on about my research our Prom walks when I'm trying to figure things out. Thank you for your belief in me, your calming words, your patience and your loving support. I cannot wait to start our new life together wherever Norwegian Air may take us!