Development of techniques for the restoration of temperate biogenic reefs

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Abstract

Biogenic reefs are of ecological importance due to the high levels of biodiversity they support and the valuable ecosystem services they provide. These reefs have limited distributions, are vulnerable to anthropogenic damage and their natural recovery has been estimated to be very slow. This project therefore aimed to develop restoration techniques that accelerate the natural recovery of biogenic reefs created by *Serpula vermicularis* (L.) (Polychaeta: Serpulidae), *Limaria hians* (G.) (Mollusca: Limacea) and *Modiolus modiolus* (L.) (Mollusca: Bivalvia) all three of which are of conservation importance in the North-East Atlantic. This aim was achieved through trials of novel restoration techniques to assess their potential for future larger scale restoration attempts.

The addition of hard substrate proved a reliable restoration technique for all three of the study species. In particular, substrates providing structural complexity supported the highest abundance of recruits. Other restoration techniques, including stock enhancement and substrate stabilisation were found to be less effective. The timing for the deployment for these substrates was also shown to effect the abundance of *S. vermicularis* recruits, with materials deployed in July having 61 % more colonists than materials deployed in November. The location of deployed substrates within the Loch Creran, Scotland were also shown to create differences in *S. vermicularis* recruitment, with sites away from existing reefs having 72 % more recruits than sites within existing reef areas. Differences in the effectiveness of restoration treatments between sites was also observed for *M. modiolus*, with Loch Creran and Scapa Flow sites having on average 1.15 and 1.03 juveniles per restoration unit respectively, compared to 70 juveniles per unit at the site north of Lleyn Peninsula, Wales. The project also highlights taxonomic problems with the identification of juvenile *M. modiolus*, before providing a robust method validated using DNA barcoding techniques to differentiation *M. modiolus* from other juvenile bivalves.

Whilst the project suggests that the successful restoration of these three biogenic reef-forming species is achievable, it also highlights that the first step in any restoration project must be the removal of pressures on that habitat. The substantial decline in the *L. hians* reef off Port Appin, Scotland from 40.5 hectares in 2006 to just 2.73 hectares in 2015 shows that without this first step any attempted restoration project would not succeed.

Acknowledgments

This PhD thesis would not have been possible without the time and commitment of many people and organisations. I'm especially grateful to the Esmée Fairburn Foundation for funding the project, and my supervisor's Dr Dan Harries and Dr Bill Sanderson for the conception of the project and their supervision and encouragement throughout.

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Chapter 1. General introduction

1.1 Biogenic reefs

Biogenic reefs represent some of the most spectacular and diverse ecosystems on earth, including ecosystems such as coral reefs which are commonly referred to as the "rainforests of the sea" (Wilkinson, 2004). Biogenic reefs are defined as "solid, massive structures created by accumulations of organisms" and "clearly forming a substantial, discrete community or habitat which is very different from the surrounding seabed" (Holt *et al.*, 1998). The organisms that create these biogenic reefs are often referred to as "ecosystem engineers" as they physically create, modify and maintain habitats (Jones *et al.*, 1997). In temperate waters biogenic reef-forming species commonly include polychaetes (e.g. sabellariids, serpulids) and bivalves (e.g. mytilids, ostreids) (Ayata *et al.*, 2009).

In UK inshore waters biogenic reef-forming species have been identified as *Sabellaria alveolata* (Linnaeus, 1767), *Sabellaria spinulosa* (Leuckart, 1849), *Mytilus edulis* (Linnaeus, 1758), *Modiolus* modiolus (Linnaeus, 1758) and *Serpula vermicularis* (Linnaeus, 1767) by Holt *et al.* (1998). All of these reef-forming species are of high conservation importance and are sensitive to anthropogenic and natural impacts (Holt *et al.*, 1998; OSPAR, 2005). Reefs formed by *Limaria hians* (Gmelin, 1791) were originally omitted from this list and were not considered an Annex I habitat under the Habitats Directive (Holt *et al.,* 1998). However since its publication and due to the work of several key researchers they are now considered a biogenic reef-forming species of conservation importance (Hall-Spencer and Moore, 2000a; Trigg *et al.*, 2011). Reefs created by the European oyster (*Ostrea edulis*, Linnaeus, 1758) reefs were also not considered an Annex I habitat under the Habitats Directive. This was because the known extant natural remaining reefs in Northern Europe is unknown due to the relaying of oysters from wild beds to nearshore and estuarine areas (Holt *et al.*, 1998; OSPAR Commission, 2009a). However they are listed as a threatened and/or declining habitat and are suggested for future inclusion as an Annex I habitat (OSPAR Commission, 2009a). This project focuses on the biogenic reefs created by *S. vermicularis*, *M. modiolus* and *L. hians* but also draws on knowledge from oyster restoration projects.

Biogenic reef-forming species are known as "ecosystem engineers" and have been cited as providing ecosystem services of global importance (Coen *et al.*, 2007; Beck *et al.*, 2011). Biogenic reefs formed by bivalves such as oysters have been shown to improve

water quality and remove suspended particulate matter therefore increasing water clarity. This has been seen to increase the abundance of aquatic vegetation and reduce the likelihood of toxic algal blooms (Coen *et al.*, 2007). Oyster reefs have also been shown to remove excessive nutrients from coastal bays, reducing the risk of eutrophication (Ulanowicz and Tuttle, 1992; Beck *et al.*, 2011). Another vital ecosystem service provided by coastal biogenic reefs is that of coastal protection. Oyster reefs are known to contribute to shoreline stabilisation, as their modification of the habitat entrains coarse material reducing wave and tidal energies, leading to reductions in lower marsh erosion (Meyer *et al.*, 1997; Piazza *et al.*, 2005; Scyphers *et al.*, 2011). Whilst the ecosystem services provided by the three study species are not well studied, the biogenic reefs formed by *M. modiolus* are credited with being important nursery grounds for commercially important species, providing substrate stabilisation and contributing to benthopelagic coupling (Jones, 1951; Navarro and Thompson, 1997; Fariñas Franco *et al.*, 2014). Of primary conservation importance is the biodiversity associated with the reefs created by the three study species (Holt *et al.*, 1998; Hall-Spencer and Moore, 2000a; OSPAR, 2005). The complex structures created by *M. modiolus, L. hians* and *S. vermicularis* reefs provide shelter for a diverse and abundant biotic community, creating local hotspots of biodiversity (Rees *et al.*, 2008; Chapman *et al.*, 2011; Trigg *et al.*, 2011).

Globally, temperate biogenic reefs are at risk with 85% of all oyster reefs having been lost, making them one of the most degraded marine ecosystems on the planet (Beck *et al.*, 2009). This global decline in oyster reefs is primarily attributed to over harvesting, with trawls and dredging leading to a loss of reef structure in addition to the direct removal of the oysters (Beck *et al.*, 2011). Whilst in UK waters many biogenic reef-forming species are not commercially exploited, anthropogenic disturbance has still been cited as the primary cause of reef loss (Holt *et al.*, 1998; Strain *et al.*, 2012; Cook *et al.*, 2013). Biogenic reefs are particularly vulnerable to anthropogenic disturbance as these stable habitats are not typically exposed to natural disturbances such as wave action (Watling and Norse, 1998). Habitats exposed to severe and frequent natural disturbance are more likely to withstand and recover from anthropogenic stressors, as only the most resistant and resilient organisms are present. The biotic community in stable habitats such as those created by reef-forming organisms tend to be long lived, slow growing and have low resilience or resistance to disturbance (Jennings and Kaiser, 1998; Watling and Norse, 1998; Collie and Hall, 2000). For example, *M. modiolus* reefs have proven to be very stable and persistent features. The reef off the Northern coast of the Lleyn Peninsula in

North Wales has been present for over 160 years, with very little change in extent or morphology recorded in the last 20 years (Lindenbaum *et al.*, 2008; Lindenbaum pers. comm. 2015). However they are particularly vulnerable to physical disturbance created by mobile fishing gear which frequently target the commercially important queen scallop (*Aequipecten opercularis*) which inhabit these reefs (OSPAR Commission, 2009b; Strain *et al.*, 2012; Cook *et al.*, 2013).

Serpula vermicularis reefs are also particularly vulnerable to anthropogenic disturbance due to their fragile structures (Moore *et al.*, 1998). In 2005 sidescan sonar identified dredge tracks through an area of healthy dense reef in Loch Creran, Western Scotland. Diver observations found the tracks consisted of broken reef rubble and uprooted boulders, whereas the surrounding reef remained intact. Over a 500 m stretch of coastline it was calculated that 11 % of the *S. vermicularis* reef had been converted to rubble (Moore *et al.*, 2009). The same survey also recorded significant losses of reef area due to mooring and aquaculture installations (Moore *et al.*, 2009). Significant declines in *Limaria hians* reefs have also been attributed to bottom towed fishing gear (Hall-Spencer and Moore, 2000a). In Loch Fyne the single pass of a scallop dredge was observed to rip apart and remove the reef material from the dredge path. Additionally it was observed that the remaining damaged *L. hians* attracted a dense aggregation of scavengers compounding the original losses (Hall-Spencer and Moore, 2000a).

A study into the impacts caused by the single pass of a scallop dredge on a *M. modiolus* reef found that the density of mussels was reduced and emergent clumps flattened (Cook *et al.*, 2013). The single pass of an otter trawl had a slightly different effect, creating furrows in the reef, damaging the organisms and reef structure in its path. The area between the pair of otter doors was also impacted by the ground rope and tickler chain of the trawl, causing a 90 % reduction of the emergent epifauna in this swept area (Cook *et al.*, 2013; Figure 1.1). These observations provided the clearest and most quantifiable effects of bottom towed fishing gear on a *M. modiolus* reef to date. The study also highlights the impacts caused by a single large disturbance event, rather than the cumulative impacts seen in many past studies (Service and Magorrian, 1997; Cranfield *et al.*, 2004; Strain *et al.*, 2012).

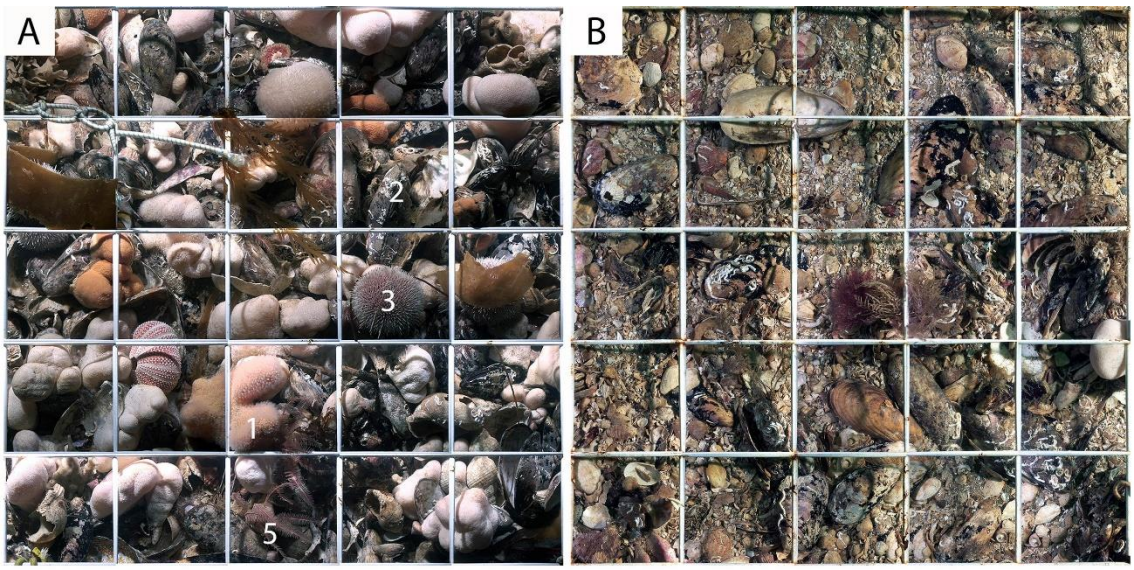

Figure 1.1. A fixed quadrat from the *M. modiolus* reef north of the Isle of Man in 2007 un-impacted (A) and 2009 impacted (B). Numbers indicate conspicuous epifauna: 1 *Alcyonium digitatum*, 2 *Modiolus modiolus*, 3 *Echinus esculentus*. From Cook *et al.* (2013).

There are several reasons why physical impacts from mobile fishing gear are so damaging to biogenic reefs. The initial action of the trawl or dredge abrades the seabed surface removing both a proportion of the reef-forming species and the epibiotic community associated with the reef (Lenihan and Peterson, 1998; Cook *et al.*, 2013). This damage creates a loss of reef structure, degrading the complex habitat created by the reef-forming species (Beck *et al.*, 2011; Cook *et al.*, 2013). This loss of the reef's complex structures leads to a decline in the biodiversity originally supported by the reef (Rees *et al.*, 2008; Cook *et al.*, 2013). For example, the byssus threads of adult *M. modiolus* and *L. hians* help support a complex structure and provide an important niche for juvenile mussels, protecting them from predation (Hall-Spencer and Moore, 2000a; Fariñas Franco *et al.*, 2013). It is thought that juvenile *M. modiolus* which live within the byssus threads of the larger adults have a much greater chance of survival because they are shielded from predation (Holt *et al.*, 1998). Without the reef's structure and the protection of the adult byssus threads, predation rates on juvenile mussels will increase, decreasing the chance of natural reef recovery (Holt *et al.*, 1998; Cook *et al.*, 2013; Fariñas Franco and Roberts, 2014). Oysters can, like many biogenic reef-forming species create elevated structures by binding together and building up from an uncohesive seabed. The loss of this cohesive element and reduction in reef height increases the risks of additional stressors, such as sedimentation and burial, anoxia (Lenihan and Peterson, 1998; Cook *et al.*, 2013). As a result physical disturbance creates multiple stressors on a reef and there are few examples

of oyster reef declines globally that can only be attributed to a single stressor (Beck *et al.*, 2011).

The natural recovery of biogenic reefs in UK waters following anthropogenic disturbance has not been intensively studied. In the limited studies that do exist the full recovery of a reef has not been seen. In Strangford Lough since the banning of commercial fishing gear in 2011 evidence is emerging that the extent and density of *M. modiolus* has continued to decline since the removal of physical disturbance (Strain *et al.*, 2012; Strong *et al.*, 2016). This perhaps indicates a longer term negative feed-back or destabilisation of the system resulting now in a loss of 87 % of the historical reef area (Strong *et al.*, 2016). Following the single pass of a trawl on the *M. modiolus* reef north of the Isle of Man there was also evidence of further structural degradation of the reef between 1 and 2 years post impact (Cook *et al.*, 2013). The recovery of a *L. hians* reef following experimental clearances has also been studied (Trigg and Moore, 2009). Whilst recovery was recorded in areas of cleared reef material over a 12 month period, the regrowth of reef material was limited in extent and thickness. The authors estimated based on recovery seen in the 12 month study, that the full recovery of an area of reef damaged by a scallop dredge would take 117 years (Trigg and Moore, 2009).

Many authors envisage the natural recovery of biogenic reefs to take tens to hundreds of years (Hall-Spencer and Moore, 2000b; Cranfield *et al.*, 2004; Kaiser *et al.*, 2006; Trigg and Moore, 2009; Cook *et al.*, 2013). This slow rate of natural recovery has led to habitat restoration being increasingly investigated as an option to decrease the recovery time of these impacted reefs.

1.2 Habitat restoration and rehabilitation

Ecological restoration has been defined as an "intentional activity that initiates or accelerates the recovery of an ecosystem with respect to its health, integrity and sustainability" (Clewell *et al.*, 2004). Terrestrial ecological restoration has been studied for many decades, but only in the last 20 years has restoration ecology become an academic field and entered peer reviewed literature (Young *et al.*, 2005). Marine ecological restoration has lagged behind that of terrestrial and freshwater restoration. This has primarily been attributed to not only the extensive scale of marine habitats, but also to issues surrounding their common ownership (Hawkins *et al.*, 2002). Despite these constraints an impressive volume of work on the restoration of coastal marine habitats

has emerged over the last 20 years (Edwards, 1999; Field, 1999; Frid and Clark, 1999; Miller, 2000).

Clewell *et al.*, (2004) addresses the question of what is "recovery" in ecological restoration. A restored ecosystem should be the aim of any new restoration projects, and is usually judged around returning the ecosystem to its pre-disturbed state (Frid and Clark, 1999; Clewell *et al.*, 2004). Additionally Clewell *et al.*, (2004) state that a recovered ecosystem "contains sufficient biotic and abiotic resources to continue its development without further assistance". However one of the problems for restoration ecology is finding a historical reference ecosystem to judge recovery against (Simenstad *et al.*, 2006; Jackson and Hobbs, 2009). This is particularly true of open marine systems, where determining the amount of change in a habitat, along with the scale of change for that habitat is particularly difficult (Hawkins *et al.*, 2002; Elliott *et al.*, 2007). This has led to the rise of habitat rehabilitation rather than restoration. Habitat rehabilitation can be a more pragmatic approach in returning a habitat to a specific stable state, rather than a complete return to a natural state (Edwards, 1999; Frid and Clark, 1999; Hawkins *et al.*, 2002). Several authors are of the opinion that the restoration of a marine habitat rarely, if ever, replaces a lost habitat, and that rehabilitation is a more achievable end point (Hawkins *et al.*, 2002; Elliott *et al.*, 2007). They also agree that the best practice for open systems is to identify and remove impacts so natural recovery can occur, although restoration might be attempted where biogenic structures are formed (Hawkins *et al.*, 2002; Elliott *et al.*, 2007), as the rate of natural recovery is likely to be decadal if at all (Trigg and Moore, 2009; Cook *et al.*, 2013). There have been many cases where the restoration of marine biogenic habitats has been successful, namely seagrass beds, mangrove forests, coral reefs and oyster reefs (see Turner and Lewis, 1996; Mann, 2000; Calumpong and Fonseca, 2001; Lewis III, 2005; Rinkevich, 2005; Schulte *et al.*, 2009 for reviews).

1.3 Temperate biogenic reef restoration

Unfortunately restoration often has more in common with engineering, as it is often hastily planned to exploit opportunities or respond to threats. As a result many restoration projects lack control and treatment areas, making restoration efforts impossible to quantify against natural change (Underwood, 1996; Hawkins *et al.*, 2002; Mann and Powell, 2007). However case studies from oyster reef restoration projects in America provide some important lessons for biogenic reef restoration projects in UK waters.

Coastal restoration in North America is well established with NOAA receiving $167 million to restore coastal habitats through the American Recovery and Reinvestment Act 2009. Even before this act NOAA had invested $30.6 million in Oyster reef restoration in Chesapeake Bay from 1997-2009 (NOAA, 2015). As a result of this investment it represents the greatest knowledge base for the restoration for biogenic reefs in temperate waters. The primary aim of this restoration work is generally to restore a lost ecosystem service (Brumbaugh *et al.*, 2006; Beck *et al.*, 2011). As such biogenic reef restoration is generally not the end point, but rather rehabilitation of the ecosystem to improve water quality or enhance a fishery. These restoration projects primarily aim to achieve these targets by direct intervention targeting two key areas; provision of habitat and stock enhancement (Brumbaugh *et al.*, 2006; Beck *et al.*, 2011).

On reefs where the overall abundance of shellfish is limited due to a legacy of overfishing for example, the addition of suitable substrate may be desirable. This can ensure greater recruitment of juveniles, and their increased survivorship (Coen and Luckenbach, 2000; Mann, 2000; Luckenbach *et al.*, 2005; Brumbaugh *et al.*, 2006; Schulte *et al.*, 2009). The provision of hard substrate is widely used in oyster reef restoration, and has been used to restore large areas of reef (Figure 1.2). Schulte *et al*., (2009) constructed oyster reefs of high relief and low relief over 9 protected sanctuaries, as well as establishing control areas. The authors recorded a 57 % increase in the oyster population in 5 years. They reported that high relief areas supported 67 % of the population and the low relief areas 32 % of the population. The authors attributed the success of the project to having the restored area protected from fishing pressure and the use of high relief reef areas. The high relief was speculated to provide optimal flow rates and therefore more favourable physiological performance, allowing for faster growth rates, increased disease resistance and decreased sedimentation.

Figure 1.2. Image from NOAA (2015), artificial reef block modules being placed in Alabama, note the second crane in the background showing the scale of the project.

The second key area for shellfish restoration by direct intervention aims to address recruitment limitations, which is achieved through stock enhancement (Brumbaugh *et al.*, 2006). This either utilises the addition of high densities of adult bivalves to improve the chance of successful spawning and reproductive success, or adult bivalves are used as brood stock in a hatchery based enhancement program (McCay *et al.*, 2003; Brumbaugh *et al.*, 2006). The transplanting of adult bivalves requires a detailed risk assessment to ensure the gains in the restoration area outweigh the loss in the donor area. This is particularly important if significant mortality in the stock is likely during translocation (McCay *et al.*, 2003). There are cases however when translocation from an area with high fisheries mortality to a protected area may be an appropriate technique (McCay *et al.*, 2003). This option however is generally regarded as a last resort and efforts would be more appropriately allocated to protecting the threatened reef, rather than attempting a translocation project, particularly if preserving biodiversity or fulfilling conservation objectives were the desired outcome (Hawkins *et al.*, 2002; Elliott *et al.*, 2007).

The use of hatchery reared juveniles to aid in oyster reef restoration projects has primarily been to improve the fisheries, and has been economically successful in a number of projects reviewed in Luckenbach *et al.*, (1999). There are several ecological and economic considerations that need to be addressed before undertaking a hatchery based restoration project. The larger the individuals released through seeding the lower the total abundances needed, due to lower predation rates. However increased time in a hatchery

substantially increases costs (NOAA, 2010). So a balance is needed between juvenile survivorship and increased costs, in addition to an understanding that some species have extremely high levels of juvenile mortality and restoration efforts would be better targeting other population "bottlenecks" (Caddy and Defeo, 2003).

1.4 Project introduction

The aim of this project is to investigate techniques supporting the restoration of temperate biogenic reefs. The project focuses on the reefs formed by *Serpula vermicularis*, *Limaria hians* and *Modiolus modiolus* which are of conservation importance in the UK (Holt *et al.*, 1998; Hall-Spencer and Moore, 2000a; OSPAR, 2005; Trigg *et al.*, 2011).

The Fan Worm (*S. vermicularis*) is widespread as isolated individuals throughout the Northeast Atlantic, however in certain enclosed water bodies they are known to form reefs. These reefs are rare and currently are only thought to exist in 4 locations, with the largest known reef area being found in Loch Creran, Scotland. Whilst there are few studies on the ecological importance of these reefs, they have been shown to support high levels of biodiversity and their limited distribution makes them highly vulnerable (Moore *et al.*, 1998; Chapman *et al.*, 2011). The second and third chapters of this thesis investigate the provision of hard substrate as a viable restoration technique. The study utilises, and builds on previous ecological studies on *S. vermicularis* (Moore *et al.*, 1998; Chapman, 2004; Chapman *et al.*, 2007; Hughes *et al.*, 2008). The two chapters focus on the significance of timing and substrate choice for potential restoration projects. The chapters also investigate spatial variations in the settlement of *S. vermicularis* around Loch Creran in relation to measured environmental variables.

The second study species the Flame Shell (*L. hians*), typically occurs on mixed sediments in rapid tidal currents, binding together the substrate with its byssus threads to create a dense turf several centimetres thick. Despite the limited knowledge of the ecosystem services they may provide, they have been shown to be biodiversity hotspots (Hall-Spencer and Moore, 2000a; Trigg *et al.*, 2011). Whilst these reefs are more prevalent than first thought (Moore *et al.*, 2013), their vulnerability to anthropogenic damage has also been noted in several locations (Hall-Spencer and Moore, 2000a; Trigg and Moore, 2009; Moore *et al.*, 2012). The fourth chapter builds upon the research into the natural recovery of *L. hians* reefs conducted by Trigg and Moore, (2009). The study also investigates substrate stabilisation techniques, the translocation of adults and juveniles as well as the provision of hard substrate as potential restoration techniques. The chapter also

documents the continued decline of the *L. hians* reef off Port Appin first recorded by Moore *et al.*, (2012).

The third study species the Horse Mussel (*Modiolus modiolus*) forms dense aggregations often in tide swept areas. Whilst individual *M. modiolus* are common, reefs with 30 % cover or more are rare (Holt *et al.*, 1998; OSPAR Commission, 2009b). These reefs support a diverse biotic community and are described as a biodiversity hotspot, in addition to providing ecosystem services such as substrate stabilisation and nutrient cycling (Jones, 1951; Navarro and Thompson, 1997; Rees *et al.*, 2008). The fifth chapter aims to build on the research already conducted on *M. modiolus* restoration in Strangford Lough (Roberts *et al.*, 2011; Fariñas Franco and Roberts, 2014). The study investigated the use of different substrates as potential restoration materials at three *M. modiolus* reefs. The three reefs were chosen to represent three different physical regimes that *M. modiolus* reefs are known to occur in; from a tidally swept exposed open coast location to a sheltered sea loch. Chapter 6 uses DNA barcoding techniques to investigate the validity of identifying juvenile *M. modiolus* from other juvenile Mytilidae species using external shell characteristics. This was necessary as the samples from Chapter 5 raised the possibility of the misidentification of other juvenile Mytilidae as *M. modiolus,* therefore confounding the results.

Each chapter is a self-contained study and introduces the background literature and species biology relevant to that study. Chapter 7 presents the conclusions and findings of each chapter and discusses the recommended restoration techniques that apply to all species and those that apply to each species individually. Chapter 7 also highlights the key areas where future research should be directed with the aim of improving the chances of successfully restoring these species.

Chapter 2. Developing successful techniques for the restoration of *Serpula vermicularis* reefs: effects of timing and location.

2.1 Introduction

Serpula vermicularis is common in the Northeast Atlantic and Mediterranean normally occurring as isolated individuals encrusting rock surfaces. In the UK *S. vermicularis* is present and widespread off most of the coast but is most abundant off Northwest Scotland (Tyler-Walters, 2008). *S. vermicularis* are able to form dense aggregations in enclosed water bodies. These aggregations have been classified as biogenic reefs (Holt *et al.*, 1998). The distribution of *S. vermicularis* reefs in the British Isles is extremely limited with records from Ardbear Lough & Killary Harbour in Ireland; and Loch Creran & Loch Teacuis in Scotland (Neff, 1969; Bosence, 1973; Minchin, 1987; Dodd *et al.*, 2009; Moore *et al.*, 2009). Current knowledge indicates that Loch Creran has the largest reef extent at 108 ha. Previous aggregations reported from Linne Mhuirich in Loch Sween disappeared during the 1990s (Lumb, 1986; Connor, 1990; Moore *et al.*, 1998, 2009; Poloczanska *et al.*, 2004; Dodd *et al.*, 2009).

Serpulid polychaete worms live in tubes constructed of a mixture of crystalline calcium carbonate and a mucopolysaccaride matrix (Neff, 1969). Of the approximately 300 described species of Serpulidae, around 10% are known to form aggregations. Fossil records also show that serpulid reef formations of up to 2 meters thick appear to have been common (Ten Hove and Van den Hurk, 1993). Extant serpulid reefs such as those made by *Ficopomatus enigmaticus* occur globally, usually in sheltered lagoonal conditions (Figure 2.1). Currently there is little literature on the ecological significance of these reefs (Leeder, 1973; Ten Hove, 1979; Ten Hove and Van den Hurk, 1993; Fornós *et al.*, 1997; Schwindt *et al.*, 2004).

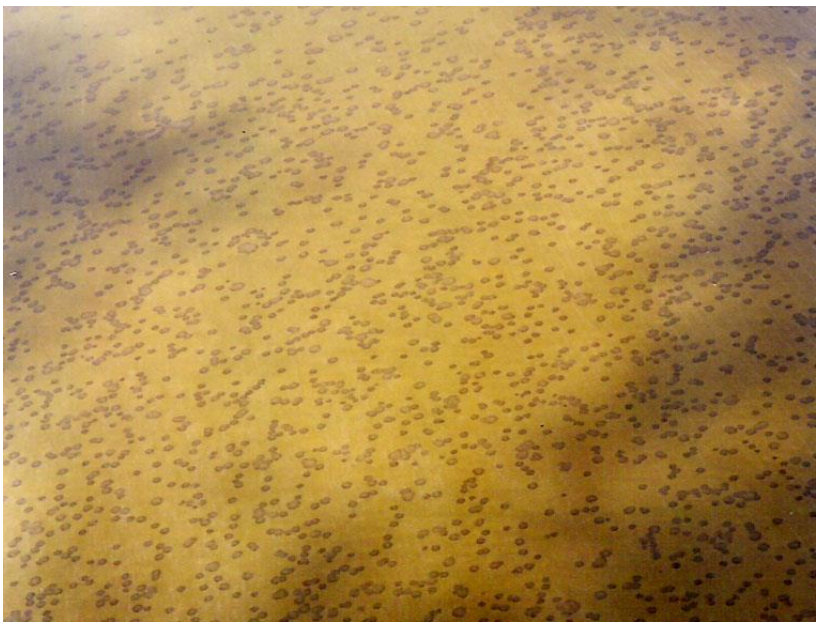

Figure 2.1. Aerial photograph of *Ficopomatus enigmaticus* reefs in Mar Chiquita lagoon Argentina, the average diameter of a reef is 2.5m. Photo: Alejandro Bortolus.

Serpula vermicularis reefs are a UK biodiversity action plan habitat and Loch Creran is designated as a Special Area of Conservation for its biogenic reefs formed by *S. vermicularis* and *Modiolus modiolus* under the EC Habitats Directive (Directive 92/43/EEC). Loch Creran is a Fjordic sea loch on the west coast of Scotland. It consists of two basins separated by a shallow (3 m deep at low water) narrow (100 m wide) sill (Almroth-Rosell and Tengberg, 2012). In the lower basin a belt of scattered reef runs around the loch on average between 2.7 and 9.3 metres depth. Reefs are less prevalent in the upper basin and occupy a narrower depth band, which averages between 2.6 and 6.6 meters depth (Moore *et al.*, 2009). Individual worms reach a maximum length of ~70 mm whereas the tubes are approximately 8 mm wide and 300 mm in length. The reefs stand up to 50 cm above the seabed and reach 60 cm in diameter. Colonies can encrust most hard substrates and reefs commonly originate from large bivalve shells or stones (Moore *et al.*, 1998, 2009) (Figure 2.2).

The distribution of *S. vermicularis* reefs in Loch Creran occurs primarily between 1 and 13 meters below chart datum, which is similar to the distribution reported from Ardbear Lough of 2 - 20 m (Bosence, 1979; Moore *et al.*, 2009). The lower limit of *S. vermicularis* at both locations is thought to be controlled by increasingly mud dominated substrate, increased suspended particulate matter, reduced flow and depleted oxygen levels in the case of Ardbear Lough. The upper limit is hypothesised to be controlled by wave action in Loch Creran, and low surface salinity and competition in Ardbear Lough (Bosence,

1979; Moore *et al.*, 1998). Ten Hove (1979) suggested increased food supply contributes to the mass occurrence of serpulids. Bosence (1979) also suggested high primary productivity as one of the reasons for serpulid reefs in Ardbear Lough. At least two-thirds of net primary production occurs above 8 m in Loch Creran (Tett and Wallis, 1978). *Serpula vermicularis* larvae have likely adapted to settle in this band of increased primary production (Tett and Wallis, 1978).

Figure 2.2. *Serpula vermicularis* reefs in Loch Creran. Reefs approximately 50 cm high.

There have been numerous studies on serpulid recruitment within the genus *Spirobranchus* which are regarded as a biofouling organisms (Meadows, 1969; Marsden, 1991; Qian, 1999). To date there are only two studies on the recruitment of *S. vermicularis,* both of which found that *S. vermicularis* recruitment peaked in late summer (Cotter *et al.*, 2003; Chapman *et al.*, 2007). Substrate roughness, orientation, colour and chemical constituency have all been cited as factors which may influence the settlement of *S. vermicularis* (Richmond and Seed, 1991; Brown, 2005; Chapman *et al.*, 2007). Chapman *et al*. (2007) found increased settlement on the lower surface of horizontally orientated *Pecten maximus* shells, compared to upper surface. This was hypothesised to be caused by negative phototactic behaviour which has been recorded in other serpulids (Young and Chia, 1982). This negative phototactic behaviour would favour the recruits as it reduces the effects of sedimentation, and decreased competition from other

colonisers such as algae or balanoids which prefer upper surfaces (Bosence, 1979; Cotter *et al.*, 2003; Chapman *et al.*, 2007).

Gregarious settlement behaviour has been suggested as a factor leading to reef formation. Such gregarious behaviour has been reported in other species of Serpulidae (Ten Hove, 1979; Toonen and Pawlik, 1994; Chan and Walker, 1998; Kupriyanova *et al.*, 2001). The occurrence of other serpulid species within the reef matrix may also influence any gregarious settlement cues, especially *Spirobranchus* species, which are the most numerically dominant species in the reef matrix (Moore *et al.*, 1998; Chapman *et al.*, 2011).

The vulnerability of *S. vermicularis* reefs to anthropogenic disturbance is evident given the fragile nature of their structures (Moore *et al.*, 1998, 2009). In Loch Creran the major threats are from mooring chains, dredging and aquaculture installations (Moore *et al.*, 2009). Figure 2.3 illustrates the damage that can be caused to reef structures through the sweeping action of single point mooring chains and dredge activity. Diver observations of these dredge tracks revealed scattered and broken reefs in an otherwise healthy reef area. This single pass of a dredge was estimated to have reduced 11 % of the reef in that area to rubble. The recovery of reefs after such events has not been observed, and the damage created by previous mooring installations is still evident within the Loch (Holt *et al.*, 1998; Moore *et al.*, 1998). In October 1996 an alginate factory on the shore of Loch Creran ceased the discharge of seaweed residue. Prior to this a 1 km area in front of the factory was devoid of serpulid reefs and a bacterial mat was present on the seafloor. In 2005 small *S. vermicularis* reefs were present in this area, although in a restricted band in shallow water (Moore *et al.*, 2006, 2009). The distribution of reefs in this area prior to the discharge is unknown, therefore making inferences about recovery unreliable. Nevertheless, the presence of small reefs shows that recolonisation or colonisation is possible in areas previously unsuitable for reef formation, and that it can occur in a relatively short time scale of < 10 years.

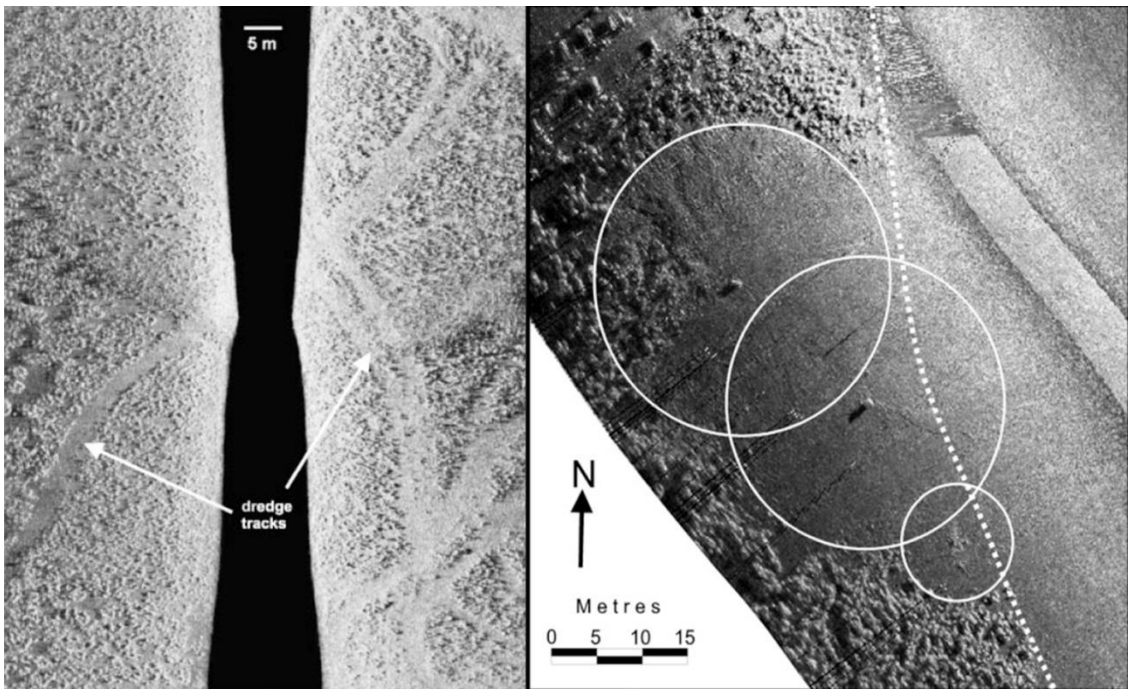

Figure 2.3. Sidescan sonar images from Moore *et al.*, (2009) the image on the left shows the damage to reef structures caused by a dredge. The image on the right shows the sweeping circular damage caused by three mooring chains.

To date there has only been one attempt to restore *S. vermicularis* reefs (Hughes *et al.*, 2008). The authors tested the feasibility of restoring the *S. vermicularis* colonies in Linne Mhuirich, by transplanting reef "clusters" from Loch Creran. Twenty clusters were transported to Linne Mhuirich from Loch Creran. All the transplanted clusters survived the translocation, but after 70 days only 11 clusters visibly contained worms. After 316 days this reduced to just 2 clusters, with 15 of the clusters missing from the pots they were mounted in. After ruling out human interference, due to the study site's remote location, the authors tentatively suggested that the most likely cause of missing clusters were Otters (*Lutra lutra*) which frequent the area. The experiment did show that *S. vermicularis* can tolerate translocation. As a second set of clusters were translocated within Loch Creran and after the same period only 1 cluster was missing and the remaining 19 were in a healthy condition, the study shows that translocation may prove an effective restoration technique. However, it also highlights the uncertainty and probable failure of restoration efforts in locations where the biotic and or abiotic factors that caused the initial loss of the habitat are unknown (Lumb, 1986; Hughes *et al.*, 2008).

Although the reefs within Loch Creran appear to have changed little in extent within recent years, the reefs in Linne Mhuirich have disappeared without any evident anthropogenic cause (Moore *et al.*, 1998; Hughes *et al.*, 2008). This unexplained decline

in Linne Mhuirich, and the lack of substantial deposits of reef debris in Loch Creran raises the possibility that *S. vermicularis* reefs are transient features within Scottish sea lochs (Hughes, 2011). *Serpula vermicularis* reefs were first recorded in Loch Creran in 1882, although the next record was not until 1989 with no evidence for reef presence between these dates (Anderson Smith, 1887; Connor, 1990), despite two surveys in 1967 and 1969 (Hughes, 2011), which both failed to record the presence of reefs at two locations that currently support reef aggregations. This raises the possibility that current *S. vermicularis* reefs in Loch Creran have developed since the 1960s, and the loss of the reefs in Linne Mhuirich may be part of a natural cycle (Hughes, 2011).

Given the limited distribution and sensitivity of *S. vermicularis* reefs, there is a pressing need to understand aspects of their ecology which would underpin any future restoration attempt. The provision of hard substrate has been suggested as an appropriate restoration technique, from previous work (Moore *et al.*, 1998; Chapman *et al.*, 2007). This technique has proven successful for restoration projects involving other marine invertebrate species (Seaman, 2007; Schulte *et al.*, 2009). The knowledge gained from this study should enable greater understanding of substrate colonisation and succession of deployed restoration materials in Loch Creran. These results might be used to inform future restoration efforts particularly in regards to targeting specific times and locations that would increase the success of a restoration project.

Aims and hypotheses

The aim of the study was to determine a deployment period that would maximise the settlement of *S. vermicularis* onto restoration materials. As well as examine the role location within Loch Creran has on the settlement of *S. vermicularis*. The null and alternative hypotheses being.

H_0: There will be equal abundances of *S. vermicularis* on restoration materials deployed at different times.

H_0: Restoration materials deployed at different locations in Loch Creran will have equal abundances of *S. vermicularis*.

H_1: Restoration materials deployed in late summer will have higher abundances of *S. vermicularis* than materials deployed at other times of the year.

H_2: Restoration materials deployed in areas of existing live reef would have higher abundances of *S. vermicularis* than areas without extant reefs.

2.2 Methods

Study sites

The main study site (Main Site) was located near the southern shore of Loch Creran on the West coast of Scotland (Figure 2.4). An additional three sites were spread around the lower basin of the loch with one further site in the upper basin of the loch (Figure 2.4). A further site (South Shian) located north of Rubha Mor was used to test the gregarious response of *S. vermicularis*. The coordinates for these sites are given in Table 2.1. Previous work by Chapman *et al.*, (2007) and Moore *et al.*, (2009), indicated settlement and reef density were greatest between 2 - 9 m below chart datum, therefore all sites were located to 6m below chart datum to ensure optimum settlement rates.

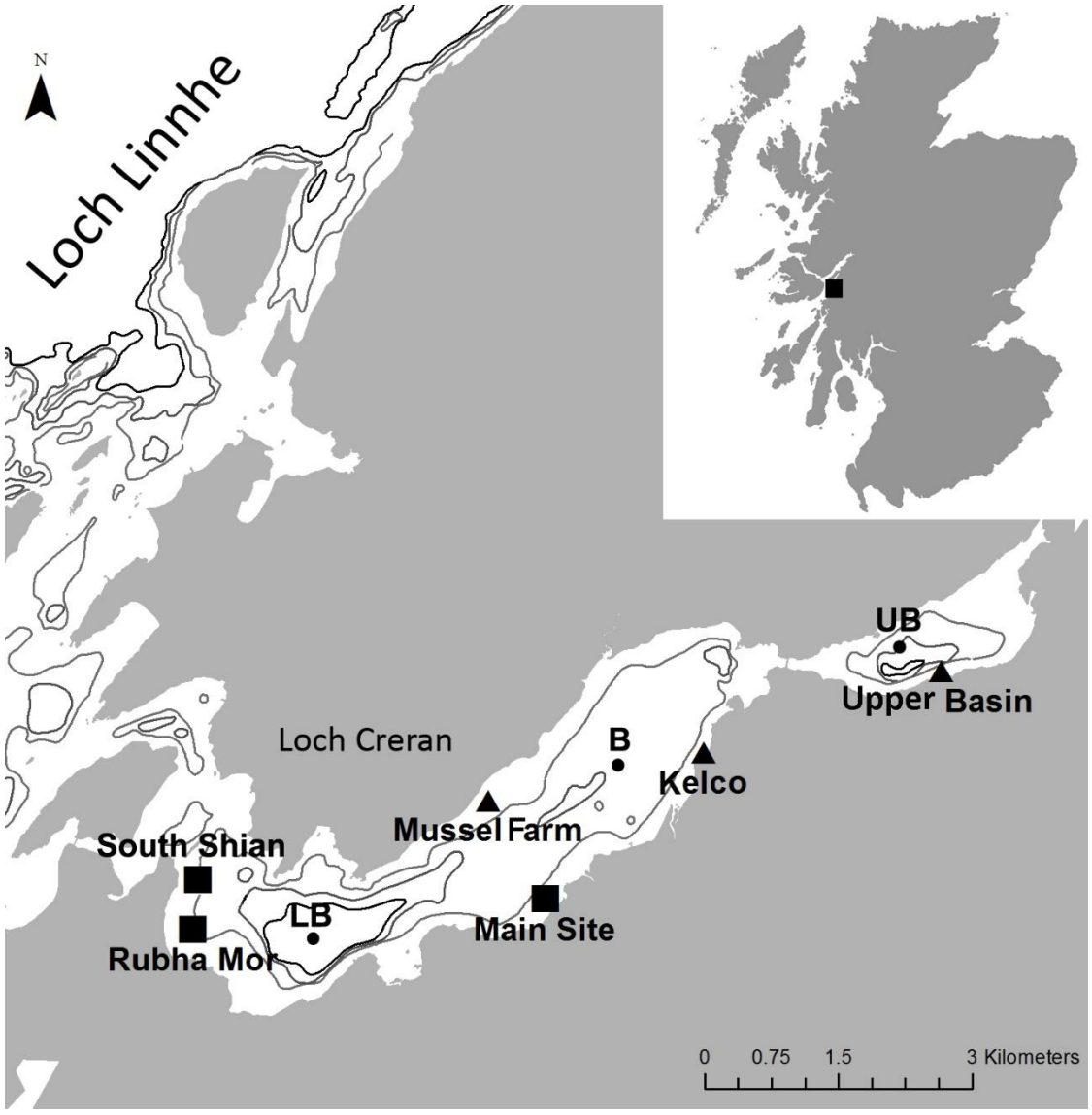

Figure 2.4. Loch Creran study site. Black squares indicate the location and names of the study sites with existing reefs, triangles indicate the non-reef sites. Small black circles indicate the three CTD sites, LB-Lower Basin, B-Barcaldine, UB-Upper Basin.

Table 2.1. Coordinates of all experimental sites in Loch Creran, all positions derived using the datum WGS1984.

Site	North	West
Main Site	56° 31.371	05° 19.989
Upper Basin	56° 32.821	05° 15.747
Kelco	56° 32.284	05° 18.315
Mussel Farm	56° 31.949	05° 20.654
Rubha Mor	56° 31.113	05° 23.832
South Shian	56° 31.447	05° 23.876

Temporal effects

Settlement tiles were used to test the effects of deployment timing. The tiles were 10 cm x 10 cm and made from quartzite, as this was used as it was similar in texture and colour to the rocks found locally. The tiles were attached vertically in pairs to canes pushed into the seabed at random locations within a homogenous area of seabed at the Main Site. There was a minimum spacing of 4 cm between tiles on a cane and a minimum of 1 m between canes. The tiles were positioned facing north to remove any effects relating to orientation (Figure 2.5). Using the power analysis formula $n \approx \frac{8 * s^2}{\delta^2}$ where δ is the difference you wish to detect, s^2 is the variance in the response and n = the number of replicates needed to reject the null hypothesis when β = 0.8 (Crawley, 2007), a sample size of 10 tiles per treatment was chosen to enable a 10 % change to be detected, using data obtained from Chapman, (2004).

Figure 2.5. Pair of settlement tiles on a cane deployed at the Main Site.

To investigate the effect deployment timing had on recruitment and post settlement survival, tiles were deployed at the Main Site every 2 months from January 2012 to November 2012; all tiles were then removed in November 2013. This gave deployment durations of between 12 and 22 months. The tiles are referred to throughout the manuscript by the month of their deployment.

Photographic monitoring of all the tiles was conducted approximately every 2 months, over the deployment period. This was conducted by divers to avoid disturbing the tiles. A Nikon D70s with a Nikon 40mm macro lens in a Seacam housing, with a single Sea & Sea flashgun was used to take the images. Images were corrected for colour and exposure when necessary in Adobe Lightroom 5.2, and viewed at 100 %. All taxa were identified to species level where possible. However, the use of photographic monitoring resulted in lower taxonomic resolutions than when the samples were analysed on recovery.

On recovery settlement tiles were transported and stored separately in ice pack chilled seawater. They were examined under a Leica MZ75 dissection microscope within 1 week of recovery. A 7% $MgCl_2$ solution was used to relax the samples, which facilitated viewing of the operculum on many serpulid individuals. Sessile fauna were identified to species level where possible. Small serpulids less than 2 mm in length were not readily identifiable and were recorded as Serpulidae spp.

Spatial effects

The effect of location within the Loch was tested by deploying 10 settlement tiles at 5 sites in February 2013 (Figure 2.4), and removed 12 months later. The tiles were again 10 cm x 10 cm made from quartzite and attached to canes randomly pushed into the seabed at each site. The tiles were also positioned facing north to remove any effects of orientation, and a minimum of 1 m spacing between canes was used. The sites were located to ensure a wide geographic spread around the Loch, and to represent areas with extant reefs (Main Site, Rubha Mor), and areas with no reefs (Mussel Farm, Upper Basin, Kelco) (Moore *et al.*, 2006). When recovered the tiles were stored and analysed under a dissection microscope, following the method outlined above for temporal effects.

Gregarious response

Two methods were used to test for a gregarious response of *S. vermicularis*. Firstly the distances from all settlement tiles deployed at the Main Site, to the nearest live reef were measured. This was done by divers using a surveyor's tape and recording to the nearest

centimetre. The random positioning of the settlement tiles around the site, allowed a range of distances to be recorded.

The second method aimed to test whether any sign of gregarious settlement was evident on tiles deployed directly on live *S. vermicularis* reefs. This was achieved by placing 16 tiles within the matrix of tubes on a living reef at the South Shian site (Figure 2.2, 2.4). A further 16 tiles on canes were deployed within 4 meters of this reef to act as a control, using the same methods outlined in section 2.2 Temporal Effects. The South Shian site had the greatest density of reefs aggregations of the 5 study sites. The dense reefs at this site attempted to ensure an adequate larval supply, increasing the probability of yielding sufficient settlement to test the effect of gregarious settlement. The tiles were deployed in April 2013 and were recovered in September 2014, giving deployment duration of 20 months. These tiles were stored and analysed following the methods outlined in section 2.2 Temporal effects.

Environmental data

Hydrolab MS5 minisondes fitted with salinity and temperature sensors were deployed on the seabed at the 5 experimental sites from February 2013 to February 2014. They recorded temperature and conductivity at hourly intervals. Salinity was calculated in parts per thousand (PPT) by the instruments using the algorithm outlined in Miller *et al.* (1988). Approximately every two months the sondes were collected from each site by divers. The sondes were cleaned of any fouling before being downloaded. The salinity sensors were re-calibrated using a specific conductance KCL solution to ensure accurate measurements. The batteries were also changed and the sondes redeployed within 24 hours. The sondes were rotated between sites over the year to remove any instrument based bias. Additionally, a Valeport model 602 CTD was deployed at 3 sites across the Loch approximately every 2 months (Table 2.2, Figure 2.4). The salinity and temperature data recorded at 6m below chart datum were used to confirm the validity of the data recorded by the sondes.

To characterise the sediment a single 5cm x 20cm core was taken in May 2012 from each of the 5 sites. The samples were dried until a constant weight was achieved, ~ 48 hours at 60 ^{0}C. They were then wet sieved with a 63µm sieve, using distilled water after soaking for 2 hours in 3 – 5 % sodium hexametaphosphate solution. The samples were then returned to the oven for another 48 hours, before being reweighed, to calculate the proportion of the sample less than 63 µm. The remaining sample was then dry sieved through a stack of sieves at 1 phi intervals from 4 (63 µm) to -4 (16 mm). The results

were converted into percentage of sediment in each size fraction, to allow comparison between sites.

Table 2.2. Coordinates of the 3 sites used for CTD measurements, all positions derived using the datum WGS1984.

Site	North	West
Lower Basin	56° 31.135	05° 22.950
Barcaldine	56° 32.066	05° 19.513
Upper Basin	56° 32.834	05° 16.350

Data analysis

Data analysis was conducted in R, with graphical interpretations conducted using the ggplot2 package (Wickham, 2009; R Core Team, 2015). Generalised Linear Models (GLMs) were used to test for spatial, temporal effects, and interactions with other key species. Negative binomial regression models were fitted using the lme4 package (Bates *et al.*, 2013), to account for the non-normal data and to control over dispersion in the model. These techniques proved to be the most appropriate for non-normally distributed count data (Ver Hoef and Boveng, 2007; Bolker *et al.*, 2009; O'Hara and Kotze, 2010). Null hypotheses were tested using an F test of deletion, by comparing the original model to a reduced model. F tests were used over Likelihood Ratio (LR) tests as they have proved more reliable for small sample sizes (Bolker *et al.*, 2009). Non parametric methods proved unreliable as the low means resulted in many ties in the data (Crawley, 2007). Pair wise analyses of categorical response variables following GLMs were conducted when a significant difference was detected. Testing for multiple comparisons between factors were made using the general linear hypothesis routine (glht) within the multcomp package (Hothorn *et al.*, 2008). A Generalised Linear Mixed Model (GLMM) was used to test for the effect of "reef presence" with the temporal dataset, using the lme4 package (Bates *et al.*, 2013). The model was fitted using a poisson error structure, to account for the non-normal count data (Bolker *et al.*, 2009; O'Hara and Kotze, 2010). Site was specified as a random effect within the model, and reef presence as the categorical fixed factor. Site was used as a random factor to account for the spatial pseudoreplication within the model (Millar and Anderson, 2004). The null hypotheses of no reef effect, was tested using an LR test of deletion, by comparing the original model to a reduced model. A GLMM using the MASS package (Venables and Ripley, 2002), was used to model the effect reef proximity had on the abundance of *S. vermicularis* on tiles deployed at the Main Site. The model was fitted using a quasi-poisson error structure, to account for the non-normal

count data. To account for variance created by differences in deployment duration and the pseudoreplication in the design, deployment month was used as a random variable, and a Wald test used to test the significance of reef proximity (Millar and Anderson, 2004).

Salinity and Temperature data were reviewed and outlying data points caused by instrumentation error such as low power or fouling were removed. These data were then averaged to give one reading per variable, per day, per site, they were also not normally distributed or conformed to any common distribution without transformation. Tests for differences in the salinity and temperature between sites were conducted using non-parametric Kruskal Wallis tests, if significant, pair wise comparisons were then conducted using a pair wise Wilcox test with a Bonferroni correction (Crawley, 2007).

The environmental data from the sondes, sediment data and location information were compiled to give 14 environmental variables and analysed in Primer v7 (Clarke and Gorley, 2015). The Environmental data were normalised to account for the fact the variables were measured with different scales. Euclidean distances were then calculated to create a resemblance matrix. A Bray-Curtis similarity matrix was then calculated using the abundance data of *S. vermicularis* and *S. triqueter* from each of the 5 sites, following a square root transformation. A Biological - Environmental and Stepwise Analysis (BVSTEP) using a permutation test with 999 permutations, was carried out to highlight the environmental variables that best explain the patterns in the biological data. This was visualised using a Multi-dimensional scaling plot (MDS) of the biological data, with the vectors of the key environmental variables overlain (Clarke and Gorley, 2015).

Additionally, a GLM was used to estimate the effect these 14 environmental variables had on the abundance of *S. vermicularis.* As the response variable was non normal count data negative binomial regression was used. Akaike's information criterion (AIC), was used to find the minimum adequate model and remove non-significant factors. The final model was then tested against a null model using an F test of deletion.

2.3 Results

Temporal effects

In total, 31 different species were recorded from the 60 tiles deployed bimonthly, including 4 species from the Serpulidae family. There was no significant relationship between duration tiles had been in Loch Creran for and the abundance of *S. vermicularis*

(Figure 2.6: F = 0.0185, P = 0.8869), deployment duration was only able to explain 0.03% of the variance in the abundance of *S. vermicularis*. There was however a significant difference in the abundance of *S. vermicularis* due to the month tiles were deployed in (Figure 2.7: F = 5.237, P >0.001). Pair wise tests found significantly more individuals on tiles deployed in July, compared to tiles deployed in January, March, September and November, with F always >3.001 and P always <0.03. Additionally, the pairwise tests found significantly more individuals on tiles deployed in May than November (F = 3.16, P = 0.01). Deployment month was able to explain 32.7 % of the deviance in the abundance of *S. vermicularis.*

The most abundant species recorded on the tiles was *Spirobranchus triqueter* (Linnaeus, 1758). A GLM revealed there was no significant interaction effect of *S. triqueter* on the abundance of *S. vermicularis* across the deployment length (F = 0.433, P = 0.823). A further GLM with *S. triqueter* abundance as the response variable, detected a significant difference in the abundance of *S. triqueter* due to deployment month (Figure 2.7: F = 3.402, P = 0.009). Pair wise tests found significantly higher abundances in May compared to January and September with Z always >3.5 and P always <0.005.

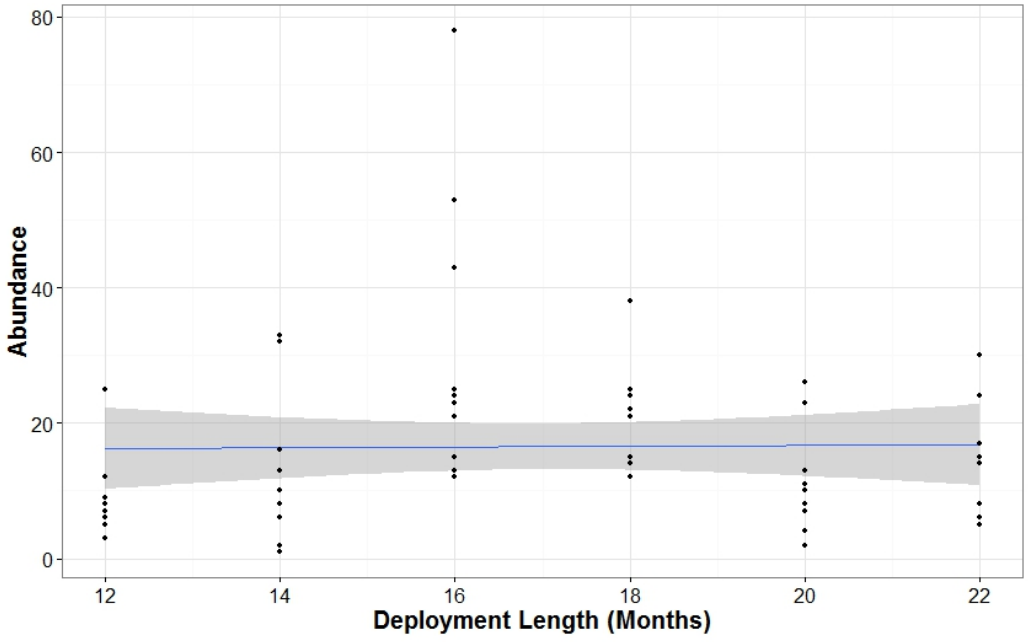

Figure 2.6. Abundance of *S. vermicularis* per settlement tile and deployment, from tiles deployed at the Main Site in Loch Creran. The line represents the fitted Generalised Linear Model (F = 0.0185, P = 0.8869), with the shaded area the standard error.

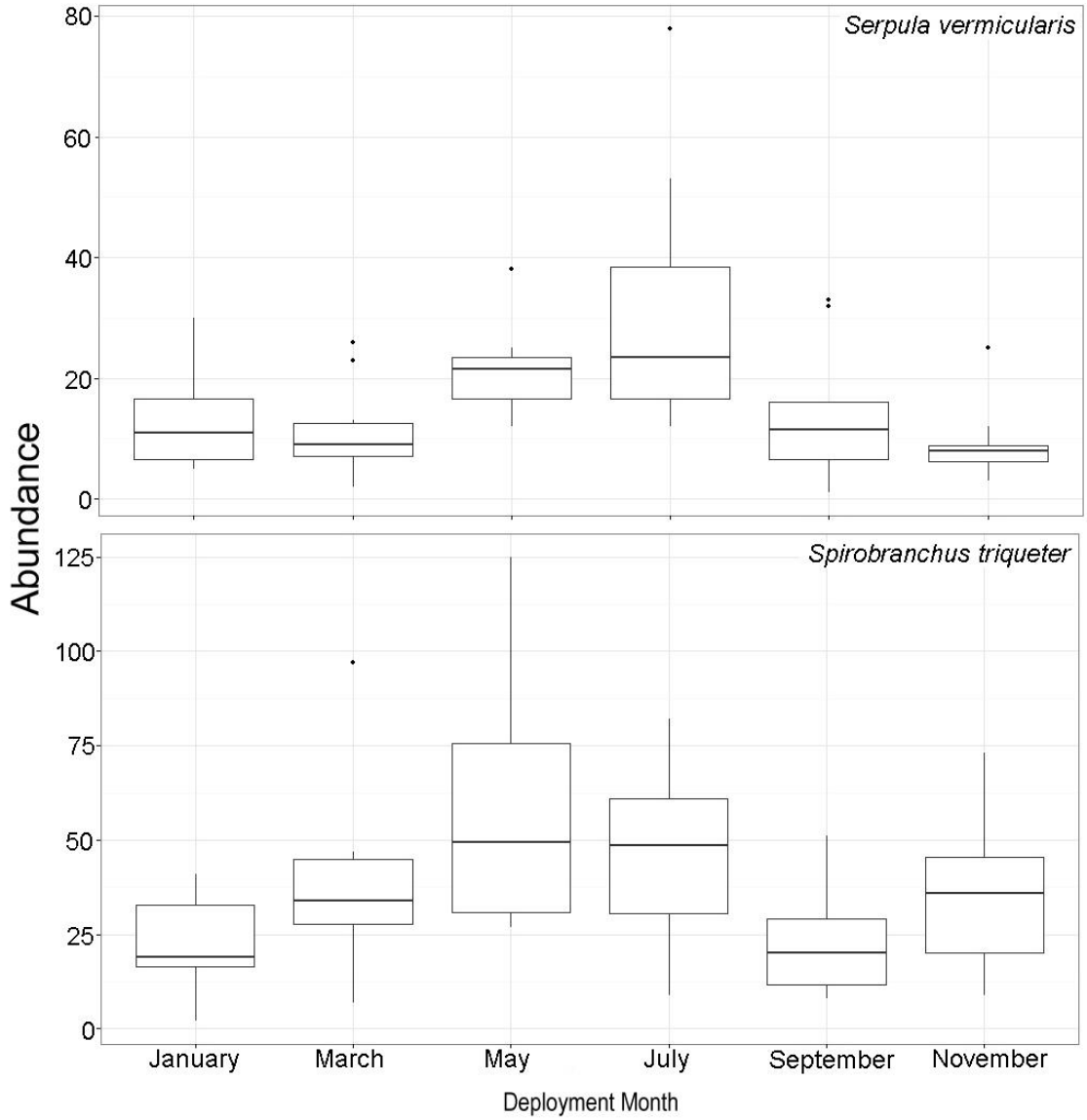

Figure 2.7. Abundance of *S. vermicularis* and *S. triqueter* per tile when deployed bimonthly during 2012. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range.

Photo monitoring

All the deployed settlement tiles at the Main Site were photographed every two months between May 2012 and September 2013. The tiles deployed after May were not photographed until they had been deployed for 2 months. Some tiles were not photographed on every monitoring visit due to reduced underwater visibility resulting in some tiles being missed. The photo monitoring of the tiles recorded 15 different taxa in total. *S. vermicularis* tubes were only accurately identifiable when they exceeded ~ 4 cm in length. Tubes shorter than this, in particular less than 1 cm were indistinguishable between serpulid species. As a result of the taxonomic uncertainty, counts for all serpulid species were pooled to give total serpulid abundance. The pooled data from all

photographs taken over the 22-month study revealed increased settlement of serpulid species on tiles deployed in May and July (Figure 2.8). This was statistically significant with Z always > 3 and P < 0.05, except for the increase in abundance between July and January/March. This closely resembles the results seen in Figure 2.7 which shows the abundances of *S. vermicularis* and *S. triqueter*. These two species were the most abundant serpulid species, and other Serpulidae only accounted for 3 % of the total abundance of all serpulid species, across all settlement tiles.

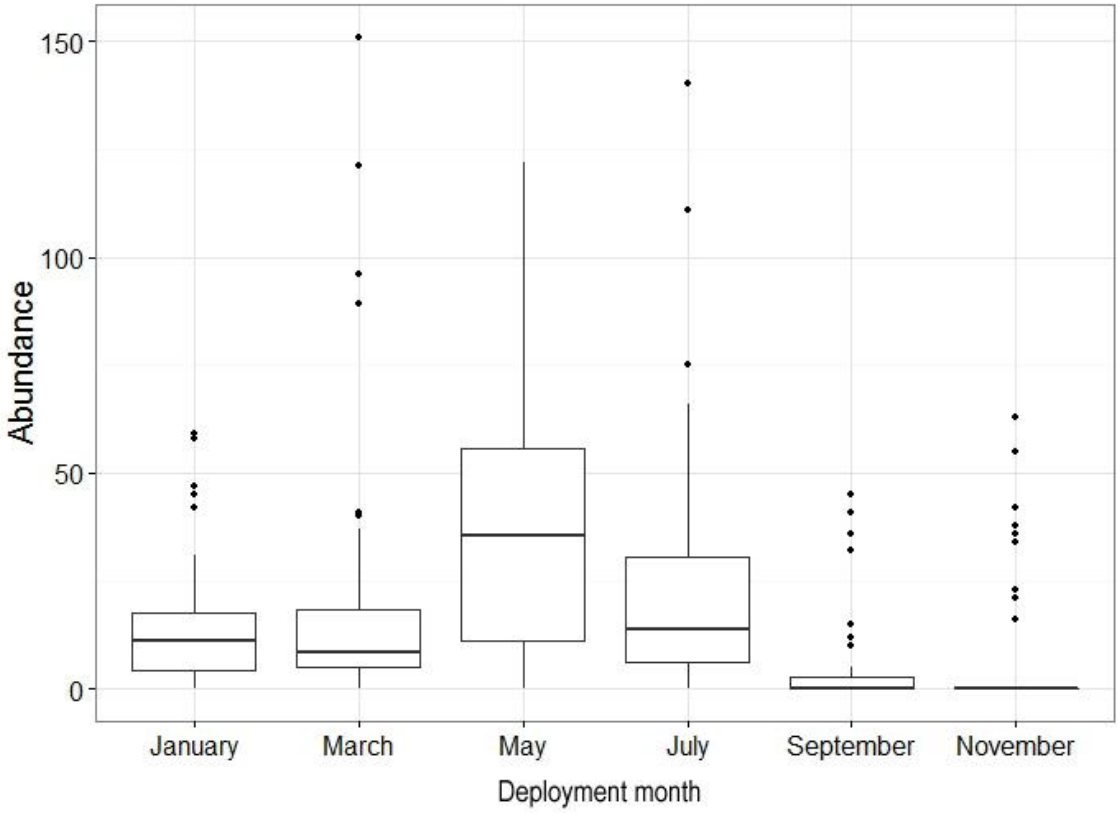

Figure 2.8. Abundance of serpulids per tile from the pooled photo monitoring data for tiles deployed bimonthly during 2012. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range.

When using the date the photographs were taken the photo monitoring data revealed that abundances peaked on all tiles in the autumn regardless of when they were deployed. The abundance of serpulids then declined over the winter, before increasing in abundance during the following summer (Figure 2.9). The photo monitoring did not record any significant colonisation by macro-organisms prior to serpulid settlement which may have competed for space or inhibited serpulid settlement. Figure 2.10 shows the minimal colonisation of a settlement tile 4 months after its deployment in March. This reduced settlement was typical of all tiles deployed before May.

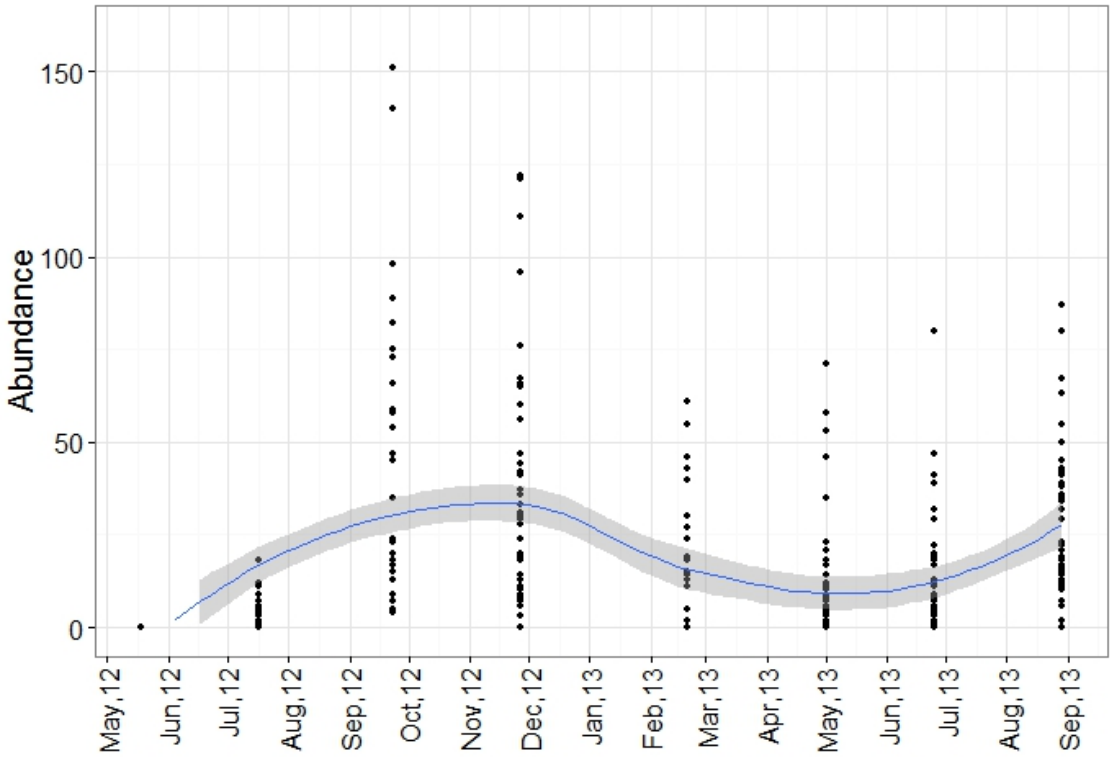

Figure 2.9. Abundance of serpulids recorded per settlement tile over the duration the tiles were deployed, from the photo monitoring data. Curve calculated using locally weighted scatterplot smoothing, with the shaded area the standard error.

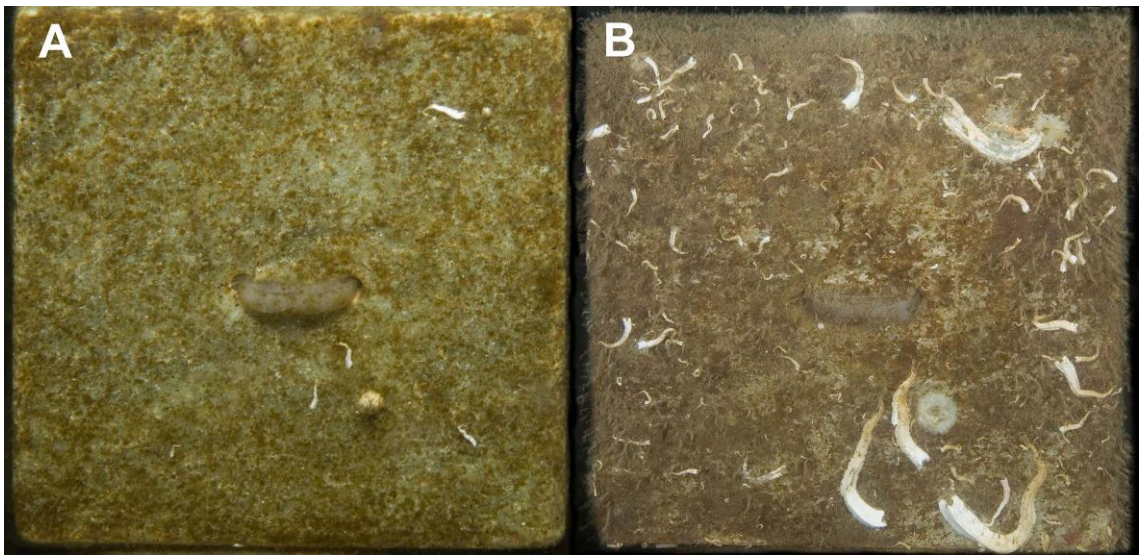

Figure 2.10. Photographs taken in-situ of the same tile deployed in March 2012 at the Main Site, Photo A shows the tile July 2012, Photo B shows the tile in November 2012.

Spatial effects

At the end of the study a total of 31 species were recorded from the 49 tiles recovered from the 5 sites around Loch Creran. Unfortunately a tile at the Rubha Mor was lost from

the 10 deployed there. There was a significant difference in the abundance of *S. vermicularis* due to location (Figure 2.11: F = 7.59, P <0.001). Pair-wise tests found significantly lower abundances at Rubha Mor compared to Mussel Farm, Kelco and Upper Basin (Z = 3.7-4.2, P always <0.002). The Main Site also had significantly fewer individuals compared to Kelco, Mussel Farm and Upper Basin (Z = 3.95-4.44, P always <0.001). Sites with existing reef areas (Main Site and Rubha Mor) had on average only a third of the *S. vermicularis* colonists that were recorded at the non-reef sites (Mussel Farm, Kelco and Upper Basin), with average abundances of 9.1 and 32.9 individuals per tile respectively. A GLM model fitted by maximum likelihood found the non-reef sites to have significantly more *S. vermicularis* than the reef sites, with site specified as a random factor to account for spatial pseudoreplication (Likelihood-Ratio Test (LRT) = 22.196 P >0.001).

Similarly to the tiles investigating temporal effects, the most abundant species recorded was *S. triqueter*. Its abundance across the 5 sites is given in Figure 2.11. A GLM revealed there was no significant interaction effect of *S. triqueter* on the abundance of *S. vermicularis* across the sites (F = 1.62, P = 0.342). A further GLM with *S. triqueter* abundance as the response variable, detected a significant difference in the abundance of *S. triqueter* due to location (F = 7.11, P <0.001). Pair wise tests found significantly more individuals at the Rubha Mor site compared to all other sites, with Z always >3.02 and P always <0.02.

Gregarious response

All 16 tiles that were deployed in the reef at the South Shian site were recovered in September 2014. Unfortunately, the tiles deployed near the reef acting as a location control became detached from their canes, so yielded no data. The abundance of *S. vermicularis* on the tiles in the reefs was much lower than at any other site with an average of only 0.5 individuals per tile (Figure 2.11). A GLM found the reduced settlement at the South Shian site to be significantly different to all other sites with Z always > 7.9 and P < 0.001. The abundance of *S. triqueter* was comparable to other sites, but substantially lower than the neighbouring site at Rubha Mor (Figure 2.11). A GLM confirmed this trend and found the South Shian site to only be significantly different from the Rubha Mor site (Z = 5.343, P <0.001). Unfortunately the loss of the South Shian control tiles resulted in the effect of location and treatment being confounded at this site. Therefore

these statistical tests should be treated with caution, additionally the deployment timing and duration of the tiles at the South Shian site were also different to the other sites.

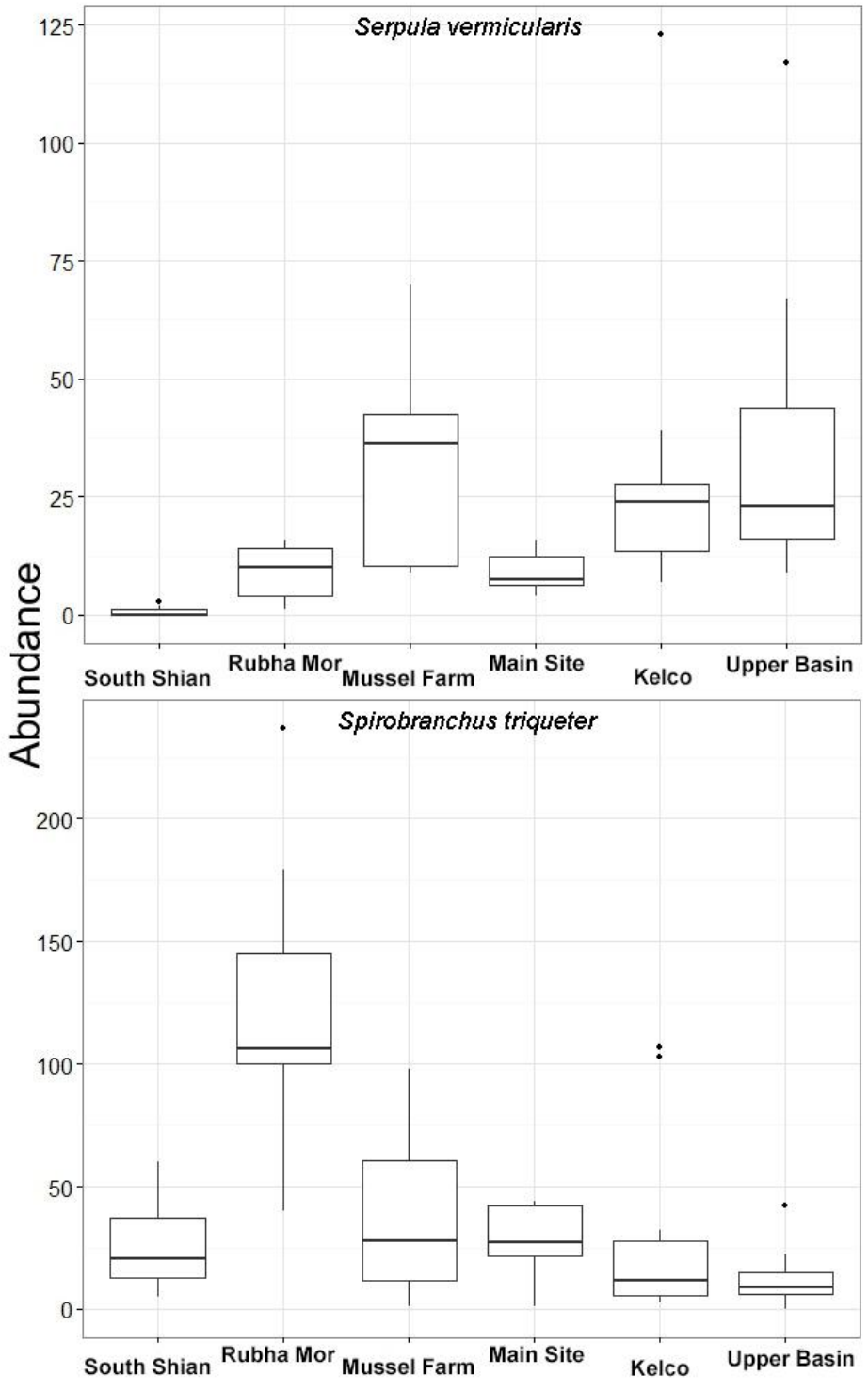

Figure 2.11. Abundance of *S. vermicularis* and *S. triqueter* per tile from tiles deployed in reefs at the South Shian site, in comparison to the original 5 sites in Loch Creran. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range.

The distance from all 60 tiles deployed at the Main Site, to the nearest live reef were measured to the nearest cm. All tiles were between 9 and 163 cm away from a live reef, with 90% of tiles between 10 and 70 cm away from live reefs. A GLMM tested the relationship between the abundance of *S. vermicularis* and the distance to the nearest live reef using data from all 60 tiles. Deployment month was set as a random variable to account for the pseudoreplication in the design. The model found there was no significant relationship between the abundance of *S. vermicularis* and distance to a live reef (Figure 2.12: T = -1.15, P = 0.25).

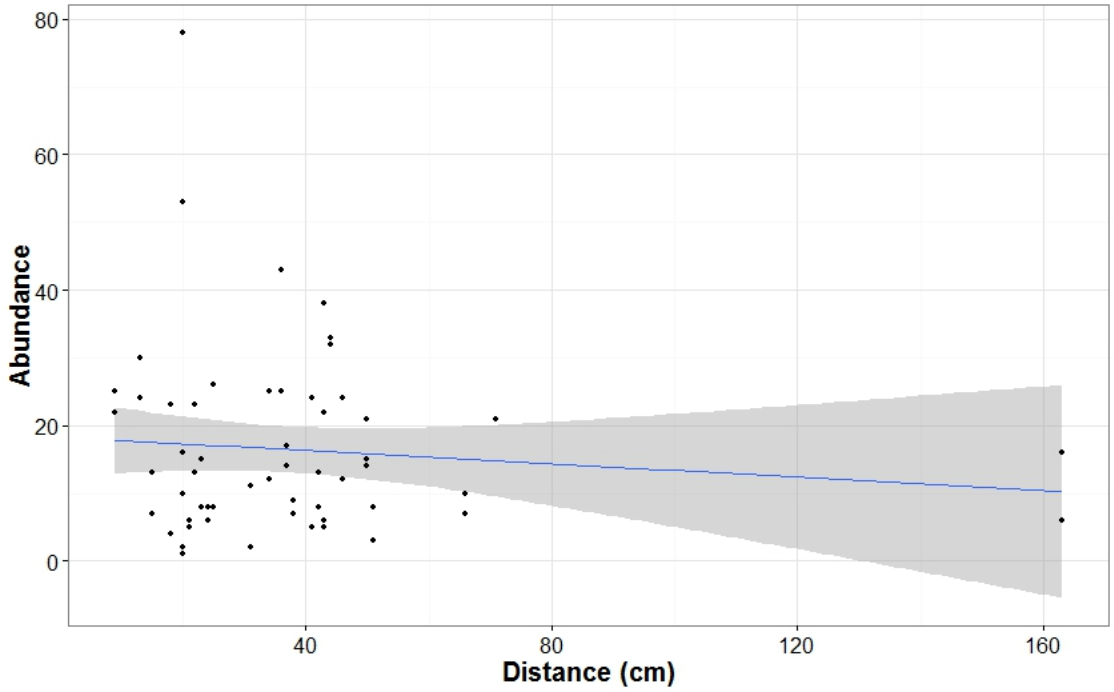

Figure 2.12. Abundance of *S. vermicularis* and distance in cm from the nearest live reef at the Main Site in Loch Creran. The line represents the fitted Generalised Linear Model, and the shaded area the standard error.

Environmental data

Temperature and salinity were recorded for 194 days at Kelco, 144 days at the Mussel Farm, 354 days at Rubha Mor, 302 at the Main Site and 357 at the Upper Basin. Data from 6 meters below chart datum were recorded from the CTD deployments throughout the year. These were then used to validate the data recorded by the sondes. The CTD measurement points were always within the inter-site variability of the sonde measurements, supporting their validity (Figure 2.14). Temperature did not vary by more than 1 $^{\circ}$C between the sites over the year, and follows an expected seasonal trend, with maximum seawater temperatures reached in September and minimum temperatures in

March. Salinity was much more variable throughout the year and followed no obvious seasonal trend. There were also greater variations in salinity between sites. A significant decline in salinity with a minimum drop of 3.5 ppt was recorded in January 2014 at all sites. This corresponded with an extreme rainfall event combined with significant snow melt (Hannaford *et al.*, 2014).

Due to logger failures at different periods of the year, statistical comparisons could only be made between Rubha Mor, Main Site and the Upper Basin over the same 284 days for temperature, and 227 days for salinity. There was no significant difference between the temperatures recoded at the 3 sites (Chi-Squared = 0.142, P = 0.931). There was however a significant difference in the salinity between the three sites (Chi-Squared = 95.59, P = <0.001). The Main Site had on average slightly higher salinities through the year than the other sites, and the Upper Basin site had lower salinities with a greater range (Figure 2.13). Pairwise tests found these differences in salinity to be significant between all three sites, with P always <0.01.

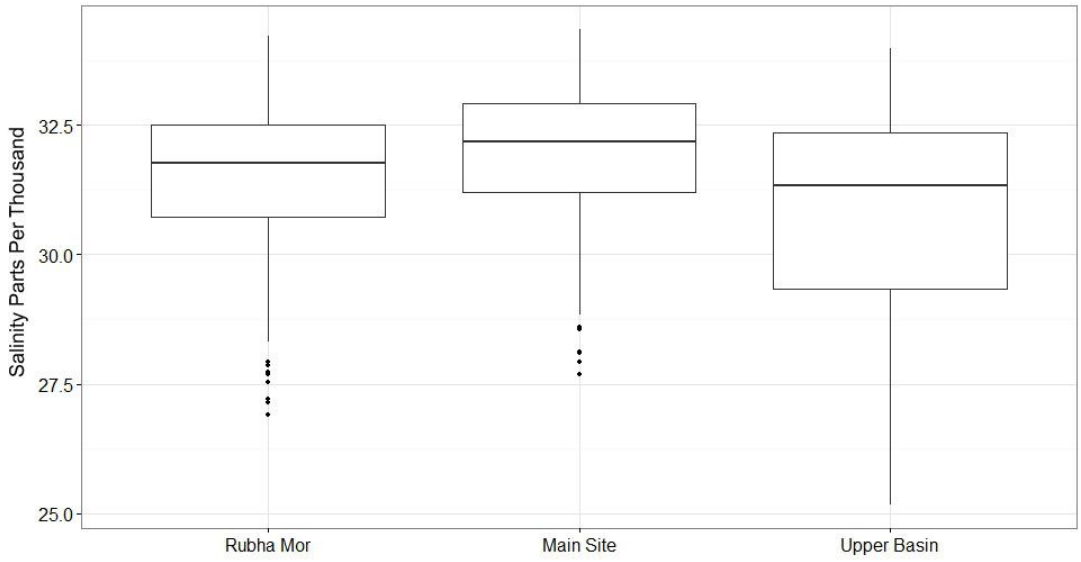

Figure 2.13. Salinity (PPT) at the 3 sites in Loch Creran. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range.

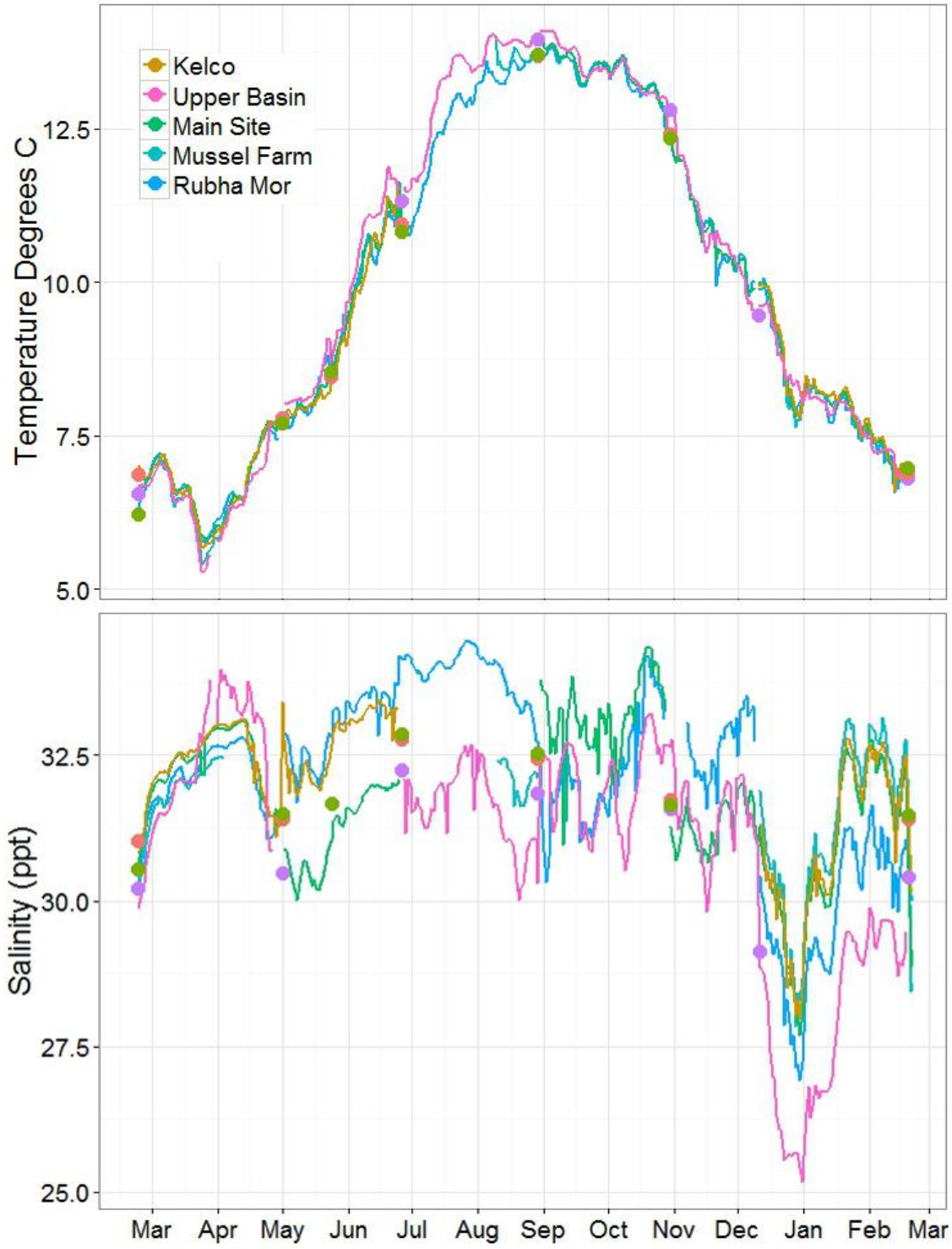

Figure 2.14. Daily averages for temperature (°c) and salinity (ppt) at the 5 study sites (Figure 2.1), from February 2013 to February 2014. Spot data points represent 6m CTD readings from the three locations in Loch Creran (Table 2.2).

The results of the PSA are shown in Table 2.3. The median grain size at each site varied from 0.077 mm at the Main Site to 0.616 mm at Kelco. These data were combined with temperature, salinity, and locational measures from each site, giving 14 environmental

variables in total. A BIO-ENV analysis within Primer v7 identified the environmental variables of maximum salinity and distance to the mouth of Loch Creran as best explaining the biological patterns (Figure 2.15: Rho = 0.311, P = 0.01). Forcing the BIO-ENV routine to only include the PSA data revealed no significant correlation with the biological data with Rho always < 0.11 and P > 0.9.

To validate this, a GLM of the 14 environmental variables as predictor variables for the abundance of *S. vermicularis* was created. The non-significant regression parameters were removed using the automatic selection process of the stepAIC() routine. This left only two significant environmental variables, maximum salinity and distance to the mouth of Loch Creran, with F = 12.97 + 11.14 respectively and P always <0.001. There was also no significant interaction between these two variables. This model was able to explain 32.7 % of the deviance in the abundance of *S. vermicularis* (Figure 2.16).

Table 2.3. PSA Results. Values expressed as % contribution.

Sieved Fraction (µm)	Wentworth Class	Kelco	Mussel Farm	Upper Basin	Rubha Mor	Main Site
>16000	Coarse Gravel	17.45	0.00	6.49	0.00	0.00
8000 - 16000	Medium Gravel	15.49	0.95	3.46	3.88	4.04
4000 - 8000	Fine gravel	8.80	2.18	3.94	6.34	2.87
2000 - 4000	Very fine gravel	3.94	2.83	7.98	6.12	4.50
1000 - 2000	Very coarse sand	2.44	3.70	10.76	7.96	4.01
500 - 1000	Coarse sand	1.61	5.01	12.28	8.89	4.20
250 - 500	Medium sand	1.99	10.70	19.51	10.90	4.61
125 - 250	Fine sand	2.66	14.84	21.20	17.41	4.26
63 - 125	Very fine sand	6.95	30.96	8.62	23.83	12.87
<63	Silt & Clay	38.67	28.83	5.76	14.67	58.63

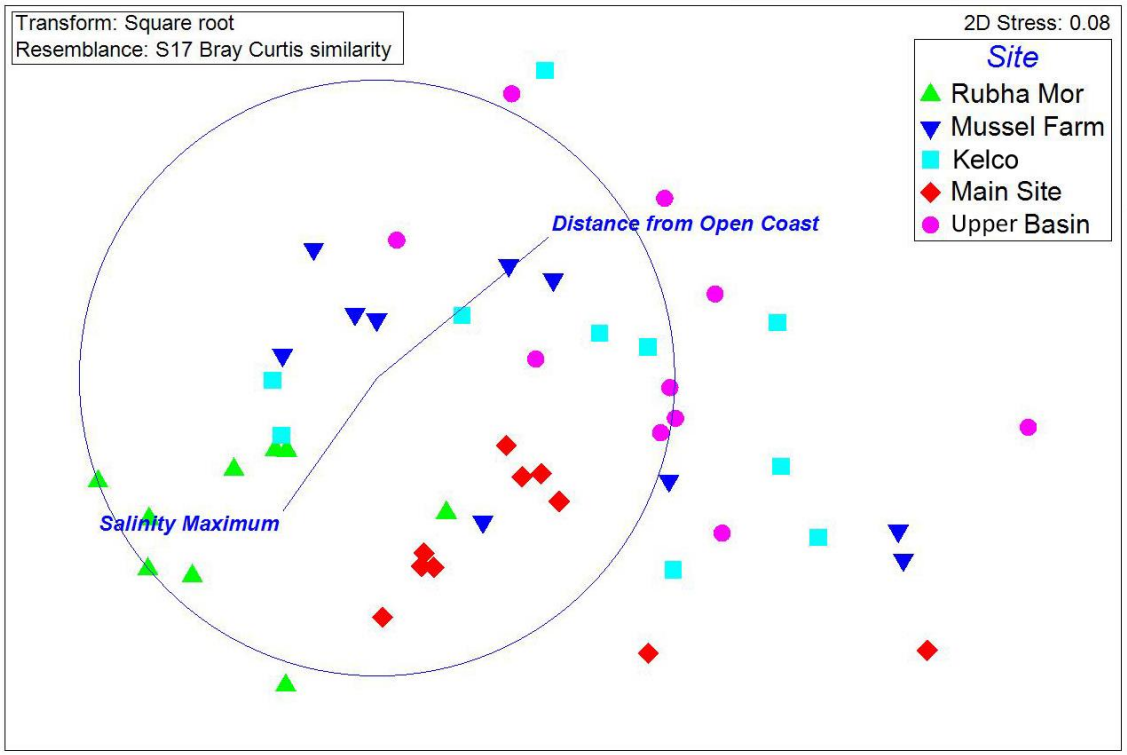

Figure 2.15. Multi-dimensional scaling plot, showing the similarity between samples created by the abundance of *S. vermicularis* and *S. triqueter*. The lines denote the two environmental variables that best correlate to the biological data, and their trajectory. Distance to mouth, refers to the distance to the mouth of Loch Creran.

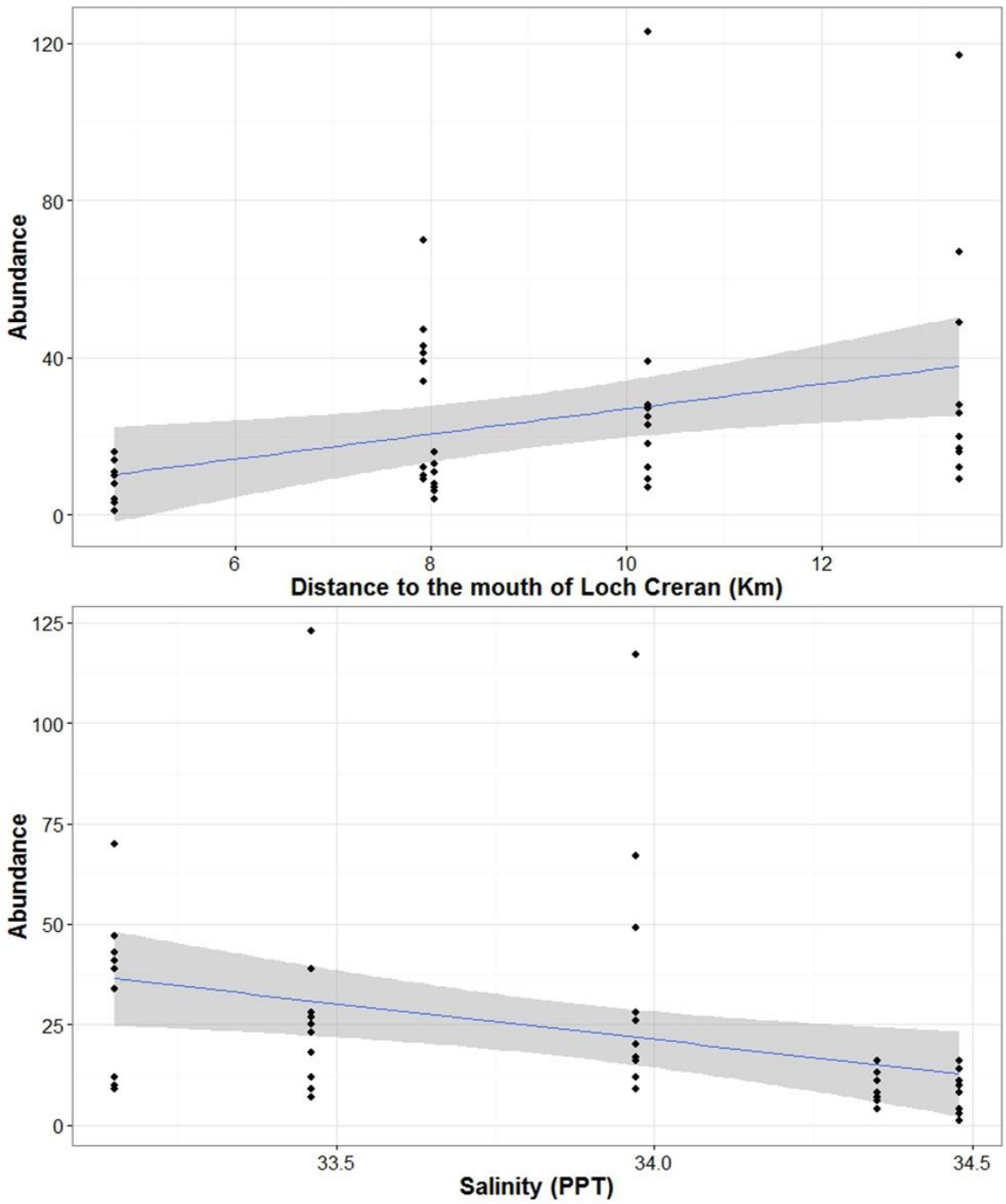

Figure 2.16. Abundance of *S. vermicularis* with distance from the mouth of Loch Creran, and the maximum salinity recorded. Lines represent the fitted generalised linear model, and the shaded area the standard error.

2.4 Discussion

Effects of deployment timing

Materials deployed in July had significantly more *Serpula vermicularis* colonists than tiles deployed at other times of the year (Figure 2.7), therefore allowing the initial alternative hypothesis to be accepted. The greatest difference was between tiles deployed in July, which had 61 % more colonists than tiles deployed in November. This work

confirms other studies, with the peak settlement for *S. vermicularis* occurring between July and September and materials deployed either side of this period having significantly fewer colonists (Cotter *et al.*, 2003; Chapman *et al.*, 2007). The results show the abundance of *S. vermicularis* on tiles was not correlated with the length of time they were submersed. Although the results did show that the month the tiles were deployed in had a significant effect on the abundance of *S. vermicularis* over a 22-month period (Figure 2.7).

The photo monitoring of the settlement tiles revealed that tiles deployed before July were not colonised by any other visible organisms, which may have outcompeted or inhibited *S. vermicularis* recruitment (Figure 2.10). The difference in the abundance of serpulids between deployment months may be caused by the establishment of biofilms on the tiles. The tiles deployed before May could have developed a biofilm before the peak *S. vermicularis* recruitment period in July - September (Chapman *et al.*, 2007). Bacterial biofilms have been shown to inhibit invertebrate larval settlement in several studies reviewed in Dobretsov *et al.* (2013). Most studies into larval inhibition by biofilms have been laboratory studies, and the conditions may not be representative of open marine systems (Holmström *et al.*, 2000). A field experiment found the bacterium *Pseudoalteromonas tunicata* inhibited larval settlement in Sydney harbour for 7 weeks (Dobretsov *et al.*, 2013). So it is plausible that a bacterial film may have developed on the tiles deployed before May, which inhibited *S. vermicularis* from settling.

Conversely other studies have shown that biofilmed surfaces are preferred by settling serpulid larvae (Chan and Walker, 1998; Hamer *et al.*, 2001). Chan and Walker, (1998) found *Spirobranchus lamarckii* preferentially settled on biofilmed surface that had been allowed to develop for 3 weeks. This study was corroborated by Hamer *et al.*, (2001) as they found larvae of *S. lamarckii* settled consistently on the oldest biofilmed surface. Both of these studies however were conducted under laboratory conditions and only studied the effects of biofilms up to 28 days old. Therefore biofilms may still be responsible for the inhibition of settling *S. vermicularis,* but this is likely to result from a change in the biofilm community after at least a month. Further work is needed to understand the development and succession of biofilm communities and their role in either inhibiting or attracting serpulid larvae after 1 month. Without this knowledge the role of biofilms still seems a possible cause of the temporal trends seen in this chapter, but their exact role remains unclear. It also remains unknown whether the decreased abundances observed

over the winter of 2012/2013 (Figure 2.9), were typical or the result of an extreme salinity event such as that recorded in January 2014 (Figure 2.14).

Spirobranchus triqueter showed increased recruitment on tiles deployed in May, compared to other deployment periods. This was two months earlier than the peak recorded for *S. vermicularis* (Figure 2.7). Chapman *et al*., (2007) also observed *S. triqueter* settlement to peak during May and June in Loch Creran. Cotter *et al*., (2003) similarly found *S. triqueter* recruitment to peak during June although this did vary between years and sites. Both studies also recorded other smaller peaks in recruitment from May to October (Cotter *et al.,* 2003; Chapman *et al.,* 2007). These results suggest that for the two dominant serpulid species in Loch Creran settlement is maximal on materials deployed into the Loch just before their annual recruitment peaks.

Effects of deployment location and environment

The results found significantly fewer individuals at Rubha Mor and Main Site compared to the other sites (Figure 2.11). This reduction in settlement can be linked to the presence of live reefs at these sites. The non-reef sites had on average 72 % more colonists than the reef sites. Care must be taken with the inferences from these results, as the experiment was not designed solely to test for differences between reef and non-reef areas.

These data support the assumption that larval supply is not the limiting factor in reef distribution within Loch Creran, as tiles were colonised regardless of location. The results from both temporal and spatial studies found no interaction between *S. vermicularis* and *S. triqueter* over any treatment, meaning that settlement of *S. vermicularis* is not influenced positively or negatively by *S. triqueter.* This supports the conclusion that although *S. triqueter* is numerically the most abundant member of an *S. vermicularis* reef matrix (Chapman *et al.*, 2011), its presence is not linked to *S. vermicularis* settlement and colonisation.

These data on *S. vermicualris* recruitment patterns should be treated with a degree of caution as they were only collected over a two-year period. Whereas *S. vermicularis* are estimated to reach at least 6 years old (Hughes *et al.*, 2008), and similar serpulid species can live for several decades (Kupriyanova *et al.*, 2001). So these results cannot estimate yearly variations in recruitment. There is also some discussion in the literature whether *S. vermicularis* reefs are a persistent feature within Loch Creran and it has been suggested they may be a transient feature within the Loch (Hughes, 2011), and may suffer mass mortality events similar to those observed in Linne Mhuirich (Moore *et al.*, 1998).

Environmental conditions such as salinity in sheltered systems such as Loch Creran, can also be susceptible to climatic events. These events may have significant effects on recruitment for several years. These types of events are clearly shown by the extreme rainfall event during January 2014, where salinity within the Loch at 6m dropped by a minimum of 3.5 ppt at all sites (Figure 2.14) (Hannaford *et al.*, 2014).

There was a significant negative relationship between maximum salinity recorded during the 12-month period at each site and the abundance of *S. vermicularis* on settlement tiles (Figure 2.16). Overall reef sites had a salinity maximum of 34.4 ppt and lower abundances. This contrasted to the non-reef sites which had a lower maximum salinity of 33.5 ppt, and much higher abundances of *S. vermicularis*. These values correspond to salinity maxima recorded previously in Loch Creran (Gage, 1972). Distance to the mouth of Loch Creran also correlated significantly with the abundance of *S. vermicularis* on settlement tiles, with abundances increasing further into the loch. There was also no evidence of autocorrelation between the salinity maximum recorded and distance from the mouth of the loch. The sites with the higher maximum salinities were located centrally in the loch, with lower maximum salinities recorded at the Upper Basin and Rubha Mor sites.

These results seem to contradict the general distribution of *S. vermicularis* reefs within Loch Creran. As the abundance of reefs generally declines further into the loch (Moore *et al.*, 2009). The increasing abundance of recruits on settlement tiles may therefore be correlated to the decreasing abundance of live reefs as the spatial results show that settlement on tiles increased away from live reef areas.

Studies on *S. vermicularis* and other serpulid species have also found them to be very tolerant of salinity fluctuations (Hill, 1967; Hartman-Schroder, 1971; Bianchi and Morri, 2001). Chapman (2004) using salinity data from Gage (1972) speculated that salinities of 33 ppt or above were favourable for *S. vermicularis* larvae. The author linked a salinity of 33 ppt or higher in the summer with gamete release. When salinity then declined in the autumn gamete release ceased. The data presented in Figure 2.14 does not support such a predictable relationship between season and salinity. As the variability in salinity between sites and weeks was equal to between season variations. Low salinities have been seen to reduce gamete production and increase mortality levels in certain serpulid species. These effects were most pronounced in salinities below 25 ppt, which were not recorded in this study (Qiu and Qian, 1997, 1998). Temperature would therefore seem a more likely

controlling factor on gamete release, as it follows a more predictable seasonal trend (Hill, 1967).

Gregarious settlement behaviour

Gregarious settlement has been suggested as a factor in the creation of *S. vermicularis* reefs (Ten Hove, 1979). This gregarious response has been demonstrated in 10 % of the species within the Serpulidae family (Ten Hove, 1979; Toonen and Pawlik, 1994; Chan and Walker, 1998; Kupriyanova *et al.*, 2001). Gregariousness has obvious advantages for settling larvae, as by settling near adults they are choosing a habitat likely to support post larval growth. Chemical cues associated with adults have often been cited as the likely cause of a gregarious response (Bryan *et al.*, 1997). However specific chemical cues have proven elusive and the chemical structure of such compounds have only been derived for 5 marine invertebrate species (Toonen and Pawlik, 1996). Ten Hove (1979) found that using empty tubes of *Filcopomatus uschakovi* encouraged the settlement of recruits. When the tubes contained living animals this further enhanced settlement. The author also reported that broken tubes seem to repel the larvae. This would indicate that chemical composition, shape and height of the tubes are less important for settlement. A gregarious settlement cue however might only exist if a critical mass of adults is reached, as a certain density may be required to exude a strong enough settlement cue to attract recruits. Devoid of this cue the larvae become less discriminating in their choice of substrate (Toonen and Pawlik, 2001a).

To date there has been no evidence to support gregarious settlement behaviour in *S. vermicularis.* Chapman *et al*. (2007) found no significant difference in the settlement of *S. vermicularis* on fragments of live reef, fragments of dead reef and horizontally orientated scallop shell. However the author does state that differences in the surface area calculations between treatments, and the live reef fragments already having a biofilm, when the other treatments did not; may have led to discrepancies in the results (Chapman, 2004). Therefore if a gregarious larval settlement response is exhibited by *S. vermicularis* it is less clear than in other serpulid species. Despite the majority of studies into gregarious serpulid settlement only being evident in aquaria (Marsden, 1991; Toonen and Pawlik, 1996; Bryan *et al.*, 1998), it is clear that some sort of aggregating behaviour is present; otherwise the dense aggregations forming reefs would not be present as they are currently.

The increased settlement observed at the non-reef sites compared to the reef sites at first appears contradictory, if *S. vermicularis* larvae do not respond to gregarious cues.

However these findings may be caused by larval behavioural dimorphism. Toonen and Pawlik, (1994) found that the larvae of *Hydroides dianthus* can be "aggregators" and settle in response to a gregarious cue, or "founders" where they settle in response to an unoccupied biofilmed substrate. Additionally another source of variation in the settlement of larvae is the "desperate larvae hypothesis". As the larvae age and their energetic reserves decline they became less discriminating in their choice of substrate (Toonen and Pawlik, 1994, 2001a, 2001b, 2001c; Marshall and Keough, 2003).

These factors may explain the decreased settlement on tiles in reef areas. At the reef sites the larvae that respond to a gregarious cue (the "aggregators") will preferentially settle on adult conspecifics. This results in reduced settlement on the tiles and other substrate in the area. Whereas at the non-reef sites the aggregators may switch to becoming founders, as energetic reserves decline in the absence of a gregarious cue. This would result in high abundances on the settlement tiles.

Testing for a correlation between settlement tile abundance and distance to the nearest live reef, did not reveal any trend (Figure 2.12). Although this suggests a lack of any gregarious settlement response, the results may be the result of scale. A soluble chemical gregarious cue is likely to dilute rapidly in turbulent flow (Toonen and Pawlik, 1996). Toonen and Pawlik, (1996) found that settlement of *Hydroides dianthus* increased over a scale of millimetres, with more than 75 % of settlement occurring on the anterior half of adult tubes. They attributed this to the expectation that the soluble cue is only likely to be detectable in the boundary layer flow around the substrate. The minimum distance from any tile at the Main Site to a live reef was 9 cm. The tiles placed in the reef were also ~ 1 cm from adult tubes. This will potentially have resulted in any gregarious cue being undetectable by larvae on any of the deployed settlement tiles. Additionally the closer the tiles were to adult *S. vermicularis* the greater the chance of them detecting any gregarious cue and settling onto the tubes of nearby adults. This may also explain the reduced settlement on the tiles deployed in the reef at South Shian compared to the other reef sites (Figure 2.11).

Such a gregarious settlement cue encouraging settlement onto the tubes of live adults, has been seen in other studies (Ten Hove, 1979; Toonen and Pawlik, 1996), although evidence for this settlement behaviour is divided for *S. vermicularis*. As stated earlier Chapman *et al.* (2007) found settlement on to live reef fragments to be indistinguishable from that on dead reef fragments and less than for scallop shell. The study was conducted at 1 site within an area of live reef from mid-August, with only 4 replicates for each treatment.

The results presented here suggest that having conducted the previous experiment within a reef area may have reduced settlement rates and could have confounded the results of the experiment. Bosence (1979) provides some evidence to support this proximal gregarious cue. Measuring the distance to the nearest neighbour from the tube opening of *S. vermicularis* in a reef aggregation they found a bimodal relationship, with most tubes being 5 mm from the nearest neighbour, with a second peak at 10-15 mm from the nearest neighbour. This corresponds with the increased settlement observed on the anterior half of *H. dianthus* tubes by Toonen and Pawlik (1996). Although Bosence, (1979) speculates this settlement pattern was a function of creating optimum strength in the reef structures and optimum spacing for suspension feeding. However neither of these studies provide enough evidence to either support or disprove the occurrence of a gregarious cue. Further work investigating this hypothesis would therefore be needed to strengthen the case for or against gregarious settlement behaviour in *S. vermicularis.* It is the author's opinion that *S. vermicularis* do exhibit a gregarious settlement response, but this response will only increase settlement onto the anterior third of adult conspecifics. As the chemical cue is likely to only be detectable within a few mm of adult conspecifics, but the effect reduces the density of larvae, in the plankton around *S. vermicularis* reefs. This creates an effect where settlement tiles deployed away from reefs display higher settlement rates than tiles within reef areas.

Conclusions

These data show that deployment timing could have significant implications for a potential restoration project. Restoration materials deployed in July will have significantly more *S. vermicularis* than materials deployed at other times of the year. This increased settlement was still significant after 2 years, and was up to 61% higher than materials deployed at other times of the year.

These data also show that differences in location even within a discrete enclosed system can have a significant impact on the colonisation of restoration materials. The exact mechanisms creating reduced settlement at sites within existing reef areas remain unclear, although the trend is quite pronounced. This information could allow nursery areas to be established in non-reef areas. After a year these colonised materials could be transported to damaged reef areas. This would be similar to "coral gardening" which has been successful in restoring small areas of coral reefs (Japp, 2000; Rinkevich, 2005), although the scale and cost of such techniques may prove prohibitive (Rinkevich, 2008).

Chapter 3. Developing successful techniques for the restoration of *Serpula vermicularis* reefs: effects of deploying different substrates

3.1 Introduction

Serpula vermicularis reefs are regarded as biogenic reefs in the UK, and are of conservation importance (Holt *et al.*, 1998; Moore *et al.*, 1998). The reefs within Loch Creran support high levels of biodiversity and abundance with 163 taxa and 12,756 individuals in a 0.1 m^2 reef area, compared to 17 - 67 taxa and 61 – 1,155 individuals from comparable sediment substrates (Chapman *et al.*, 2011). The increased biodiversity and abundances have been attributed to increased hard calcareous substrate and increased crevice habitats compared to the surrounding substrate (Chapman *et al.*, 2011). This increase in hard substrate and complex interstitial spaces has been cited as an important factor in the provision of ecological services of several biogenic reef-forming species (Nestlerode *et al.*, 2007; Rees *et al.*, 2008; Chapman *et al.*, 2011; Ragnarsson and Burgos, 2012).

This study builds on the knowledge of restoring *S. vermicularis* reefs gained in Chapter 2, along with the work of Chapman *et al.*, (2007) and Moore *et al.*, (1998, 2003, 2009). Chapter 2 found that restoration materials deployed in July had significantly more recruits than materials deployed at other times of the year. The results also found significantly more recruits on materials deployed away from extant reef areas within Loch Creran. The current chapter aims to identify whether some substrate types perform better than others in the restoration of *S. vermicularis* reefs.

The provision of hard substrate has often been used as a restoration technique for biogenic reefs (Nestlerode *et al.*, 2007; Miller *et al.*, 2009; Roberts *et al.*, 2011). In particular this technique has been used extensively in the restoration of oysters (*Crassostrea virginica*) on the east coast of the U.S.A. (Nestlerode *et al.*, 2007; Brumbaugh and Coen, 2009; Beck *et al.*, 2011). The most desirable material used in these oyster restoration projects is *C. virginica* shell either from local fisheries or dredged historical deposits. These piles of oyster shells provide settlement habitat, predation protection, reduced physical stresses and epifaunal competition, and have been proven to be successful restoration materials (Gutierrez *et al.*, 2003; Schulte *et al.*, 2009). The limited supply of oyster shell has prompted the examination of suitable alternative restoration materials (O'Beirn *et al.*, 2000). In the case of *S. vermicularis* and other rare biogenic reef-forming species this limitation of such material is even more critical, due to the lack of significant

accumulations of conspecific material. Any *S. vermicularis* restoration project of an ecologically significant scale would therefore need to consider alternative substrates to ensure an adequate supply of material.

The use of alternative substrate materials for restoration projects has only been studied in a limited number of cases (O'Beirn *et al.*, 2000; Mann and Powell, 2007; Nestlerode *et al.*, 2007; Fariñas Franco *et al.*, 2013). Substrate roughness, orientation, colour and chemical composition have all been cited as factors which may influence the settlement of *S. vermicularis* (Richmond and Seed, 1991; Brown, 2005; Chapman *et al.*, 2007). Chapman *et al.*, (2007) found *S. vermicularis* preferred to settle on the underside of scallop shells over other treatment options. This may be due to the phototactic behaviour of larvae, avoidance of siltation, or decreased predation. Moore *et al.*, (1998) also observed that extant reefs in Creran predominantly colonise bivalve shells. The effect of elevating substrate materials above the seabed has also been studied in several restoration projects. Oyster restoration projects have found that elevated reefs are more successful as they are less susceptible to siltation and anoxic bottom water (Lenihan and Peterson, 1998; Gregalis *et al.*, 2008). Similarly in an experimental *M. modiolus* restoration project, Fariñas-Franco and Roberts (2014) found the elevation of restoration materials offered significant advantages.

Aims and hypotheses

The main aim of this study was to test the effectiveness of different hard substrates as potential restoration materials. The null and alternative hypotheses being.

H_0: There would be no significant difference in the abundance of serpulids between any of the restoration treatments.

H_1: The scallop shell in large bag restoration treatment would support the highest abundance of serpulids compared to the other treatments.

The study also investigated the effect location of restoration materials within Loch Creran had on the abundance of serpulid recruits.

H_0: Restoration treatments at different locations within Loch Creran would have equal abundances of *S. vermicularis*.

H_2: Restoration materials deployed in areas without extant reefs would have higher abundances of *S. vermicularis*.

3.2 Methods

Study sites

The main study site (Main Site) was located off the southern shore of Loch Creran on the West coast of Scotland (Figure 3.1). An additional three sites were spread around the lower basin with one further site in the upper basin of the Loch (Figure 3.1). These sites were the same as those used in chapter 2 and the coordinates for the sites are given in Table 2.1. The sites were chosen to give an even geographic coverage around the loch and to include areas with (Main Site, Rubha Mor) and without (Mussel Farm, Upper Basin, Kelco) existing reefs (Moore *et al.*, 2006, 2009).

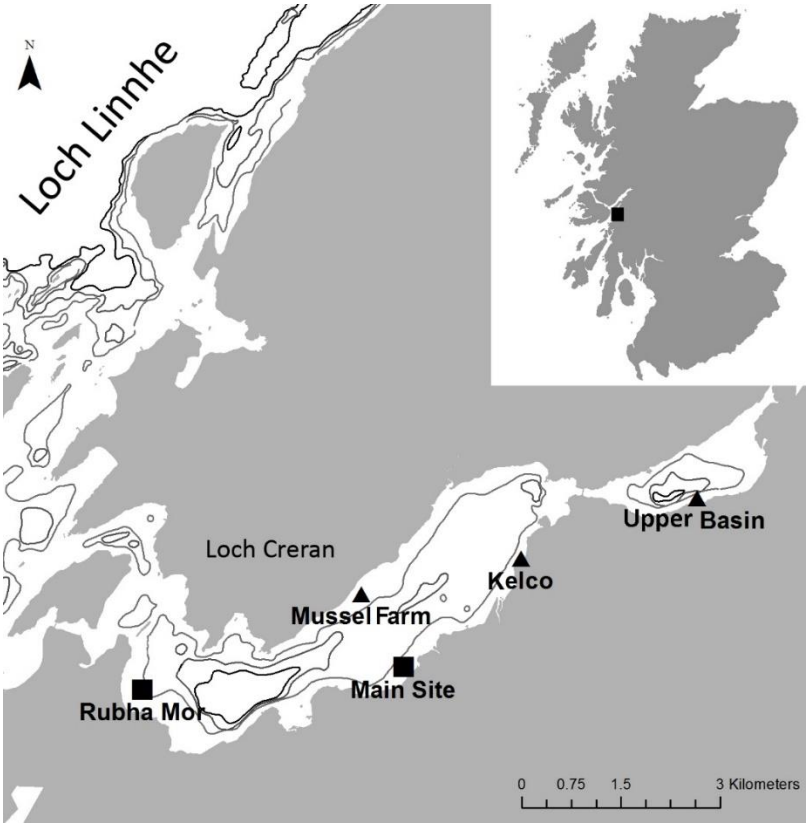

Figure 3.1. Loch Creran study site. Black squares indicate the location and names of the study sites with existing reefs, the triangles indicate the non-reef sites.

Restoration materials

Three different restoration materials were trialled at the Main Site. These materials were selected as they were all relatively cheap, easy to obtain and easy to deploy, therefore would be suitable for a large scale cost effective restoration project. Scallop shell was used in three different treatment options. Firstly, in loose piles to assess the effect of limited relief < 10 cm. Then secondly in small cylindrical mesh bags with a height off the seabed of 16 cm, a length of 25 cm and a mesh size of 2 cm and finally in large cylindrical

shaped mesh bags giving a height off the seabed of 32 cm, a length of 30 cm and a mesh size of 2 cm (Figure 3.2). In addition to the three scallop shell treatments, boulders (40-50 cm in diameter) from a local quarry were used as these would replicate similar rock types already found in the loch. For the final treatment cobbles measuring between 6.4 and 25.6 cm in diameter were held within the same size cylindrical mesh bags as scallop shell in large bags. The use of cobbles and boulders also allowed an assessment into whether the increased deployment cost of cobbles in large bags over using boulders outweighed any perceived restoration benefit. Cobbles in large bags would also be an easier substrate to acquire than scallop shell, so their comparison will allow an assessment of their benefits against their availability.

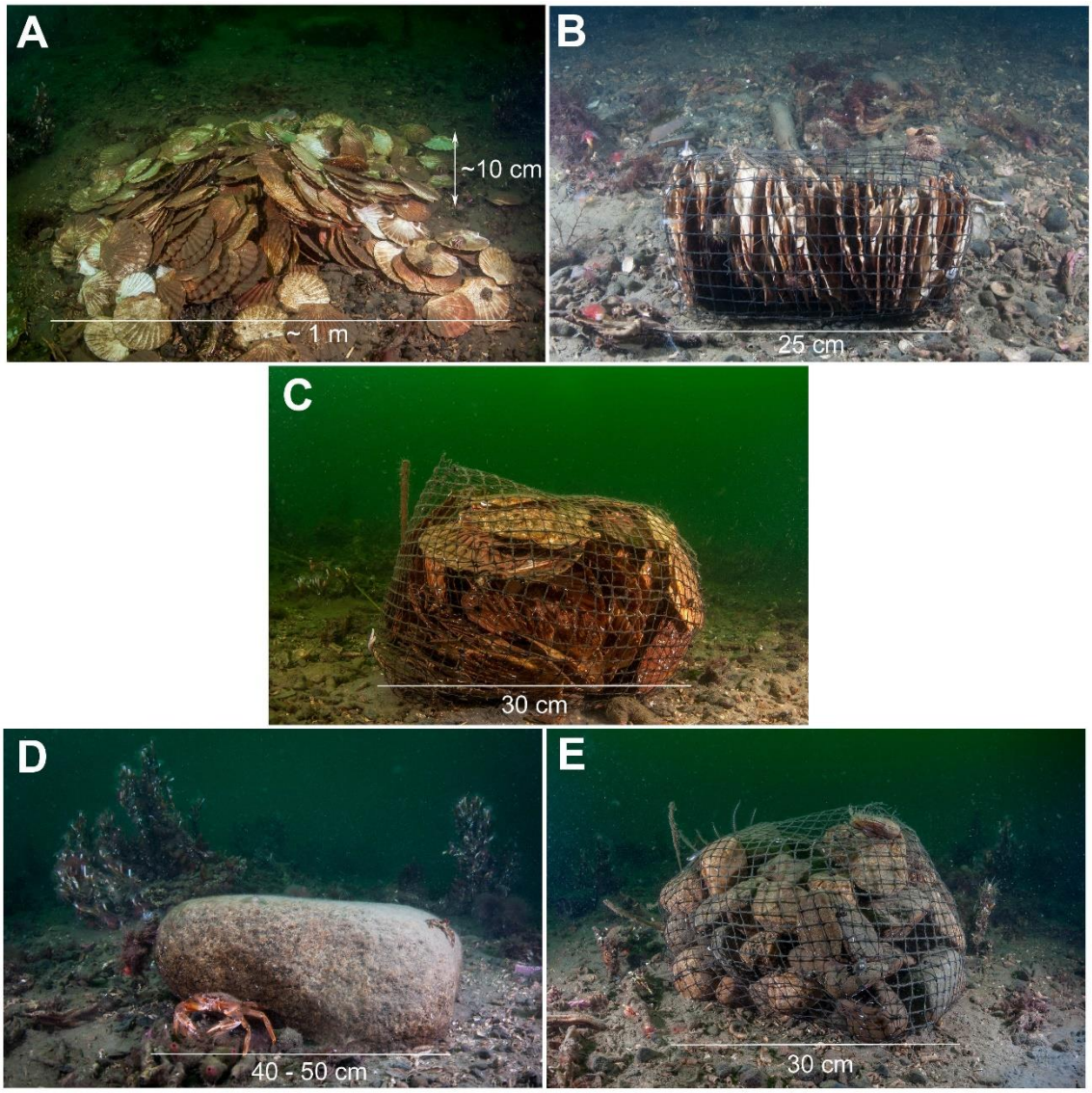

Figure 3.2. The five different restoration units deployed at the Main Site, A – pile of scallop shell, B – scallop shell in small bag, C – scallop shell in large bag, D – boulder, E – cobbles in large bag.

Six replicates of each restoration unit were deployed at the Main Site on the 27th March 2012. The units were set out in six discrete areas around the Main Site at a depth of 6 m below chart datum. Each area, labelled A-F contained a single restoration unit of each type, and had a 10 m separation to the next area (Figure 3.3). The restoration units were deployed by lowering them slowly to a position 2 m above the seabed. Divers then used lifting bags to carry the units to their allocated areas without damaging the surrounding *S. vermicularis* reefs.

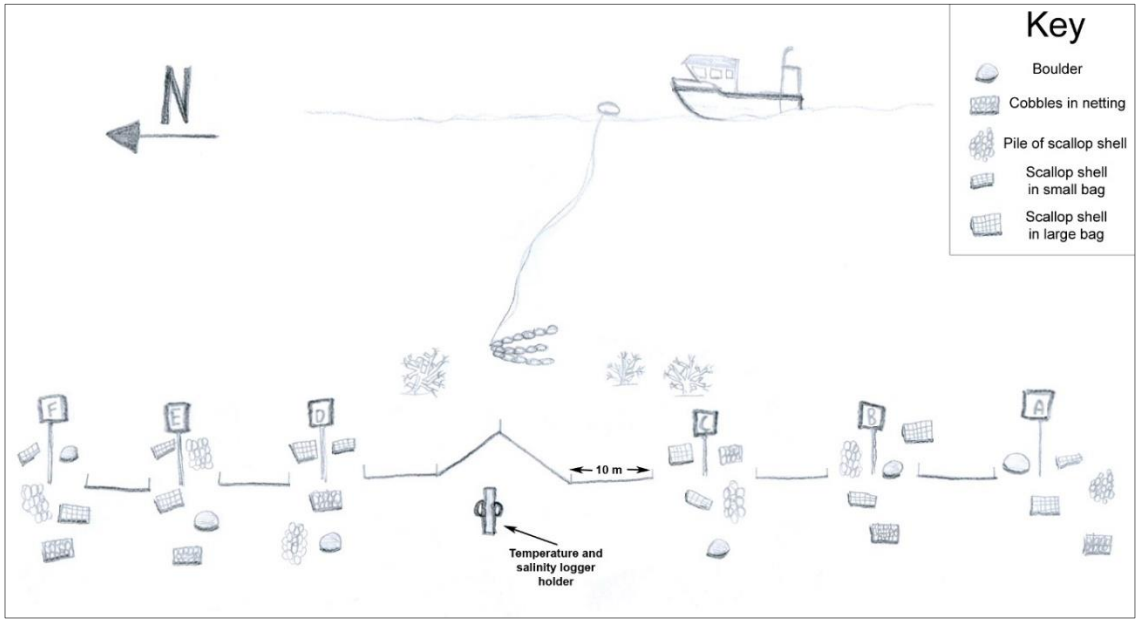

Figure 3.3. Diagram showing the general layout of restoration units at the Main Site. Diagram not to scale and all distance between pairs of pins is 10m. Diagram depicts the mooring line and large chain links that permanently mark the site. Each area A-F shows the approximate position of the 5 different restoration treatments seen in Figure 3.2.

Spatial effects

Five replicate units of scallop shell in large bags were deployed at each of the 5 sites around Loch Creran on the 27th of March 2012 (Figure 3.1). Due to logistics involved with deploying and monitoring the restoration units, only one treatment was deployed at all of the 5 sites. Scallop shell in large bags was chosen as it was expected to be the most effective restoration technique. At each site the restoration units had a minimum separation of 2 m and were positioned at a depth of 6 m below chart datum.

Monitoring

All sites were visited 5 times with a mean of 5 months between visits, the final monitoring visit being on the 30th August 2014. The individual restoration units at each site were

monitored on each visit using in-situ photography. Each restoration unit was photographed a minimum of 8 times, by a diver using a 101 mm by 76 mm quadrat frame (0.0077 m^2) to standardise the sampling area of each photograph. The photographs were taken from every side of each unit in a haphazard random manor to account for any differences in serpulid abundance created by orientation. The camera used was Nikon D70s with a 40 mm lens and a pair of Sea & Sea flash guns. Examples of the in-situ photographs taken are shown in Figure 3.4.

Figure 3.4. Photo quadrats (101 mm x 76 mm, 0.0077 m^2) taken of three different restoration units at the Main Site in August 2014. A is from cobbles in large bag, B is from a boulder, C is from scallop shell in large bag.

Analysis

The photographs collected over the 5 monitoring visits were sorted into appropriately labelled folders for each restoration unit, from each site, and at each time point. For further analysis, only 5 photographs were randomly selected from the 8 or more collected from each restoration unit. In total this gave 1250 quadrat photographs. The number of visible serpulid tubes were then counted in each photograph. Identification to species level was not possible, due to the need to view the operculum of each individual. Although from the work in Chapter 2 it was expected that 95 % of the observed serpulids would be either *S. vermicularis* or *Spirobranchus triqueter*. To standardise the counting between photographs only tubes with openings visible inside each quadrat were counted, and all photographs viewed and enumerated at 100 % (1:1 pixel size). Only counting tubes with openings inside quadrats will have reduced the density of serpulids observed. However, it was seen as the only practical option for assessing abundance, also the proportion of tubes with openings outside of quadrats compared to inside was observed to be similar across treatments. All graphical interpretations were conducted using the ggplot2 package within R (Wickham, 2009; R Core Team, 2015).

A two way crossed design with interactions was used to assess the effect of restoration treatment and location had on the abundance of serpulids across all treatments. This test was conducted using a permutational analysis of variance (PERMANOVA) routine in PRIMER v7 with the PERMANOVA package (Anderson *et al.*, 2008). PERMANOVA was chosen over standard univariate techniques to account for the highly skewed non-normal data and the temporal pseudoreplication and non-independence in the dataset (Anderson, 2001a). This technique also allowed the data to be analysed without transformation, which has been shown to perform poorly on count data and can obscure significant interaction terms (Anderson *et al.*, 2008; O'Hara and Kotze, 2010). Counts of serpulids were modelled as a function of site or restoration treatment as fixed categorical factors and monitoring date was set as a random factor. This accounted for the temporal pseudoreplication created by samples being collected at different times (Millar and Anderson, 2004). The test used a resemblance matrix calculated using Euclidean distance, without any data transformations. P values were calculated using Type III Sum of Squares and 9999 permutations of residuals under a reduced model. These options within PERMANOVA gave the greatest statistical power and have proved the most accurate in avoiding type I errors in multi factorial models (Anderson, 2001b; Anderson *et al.*, 2008). Pairwise tests were used to investigate any significant factors and interactions; this was done within the PERMANOVA routine on repeat routines (Anderson *et al.*, 2008). Significance was accepted at P-values of 0.05 or less.

3.3 Results

Restoration materials

In total 745 photo quadrats were taken of the 5 different restoration treatments at 5 different time points. On average across all monitoring time points scallop shell in large bags had more serpulid tubes present than any other treatment with 7151 per m^2. The next most successful treatment was cobbles in big bags with an average of 5747 per m^2. The piles of loose scallop shell proved the least effective treatment with only 627 per m^2. These results split into the 5 different monitoring time points are shown in Figure 3.5.

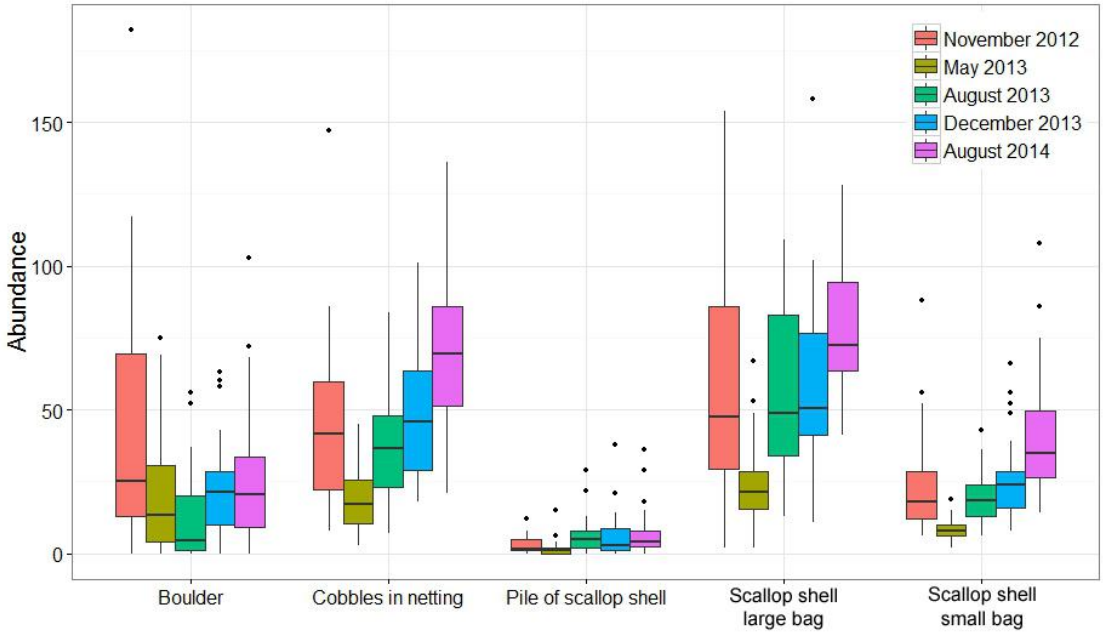

Figure 3.5. Abundance of Serpulidae per 0.0077 m^2 quadrat from the 5 restoration treatments separated into the 5 monitoring time points. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range.

The PERMANOVA routine found a significant difference in the abundance of serpulids due to restoration treatment (Table 3.1). The effect of monitoring date, which was set as a random factor can be seen in Figure 3.5. The model found that monitoring date explained 11 % of the variance within the model, whereas restoration treatment explained 35 % of the variance. Pairwise tests within PERMANOVA found significant differences between most treatment combinations with T always >3.23 and P always < 0.045. The exceptions being between Boulders and Cobbles (T = 2.15, P = 0.09) and Boulder and Scallop shell in small bags (T = 0.27, P = 0.79).

Table 3.1. Results from PERMANOVA, using Euclidean distance to test for treatment effects with monitoring date as random factor. The test statistic Pseudo-F and (P) are calculated using 9999 permutations with n=745.

Source	df	SS	MS	Pseudo-F	P
Treatment	4	223960	55990	18.352	0.0001
Date	4	73225	18306	40.848	0.0001
Treatment*Date	16	48843	3052.7	6.8117	0.0001
Residual	720	322670	448.15		
Total	744	669460			

Analysis of the data recorded from the final monitoring visit in August 2014 was conducted independently to the earlier monitoring data (Figure 3.6), the rational being the that final monitoring visit represents the most reliable time point at which to judge the restoration units, without the variability created by succession and seasonal changes. Scallop shell in large bags still had the greatest abundance of serpulids, however cobbles in large bags only had 9.6 % fewer serpulids on average (Figure 3.6). A PERMANVOA routine using only the August 2014 monitoring data found a significant difference in the abundance of serpulids due to treatment with Pseudo-F = 59.9 and P < 0.001. The effect of treatment now explained 66 % of the models variance. Pairwise tests found significant differences between all treatments, except between scallop shell in big bags and cobbles in netting (P = 0.237). These significant pairwise comparisons are shown in Figure 3.6.

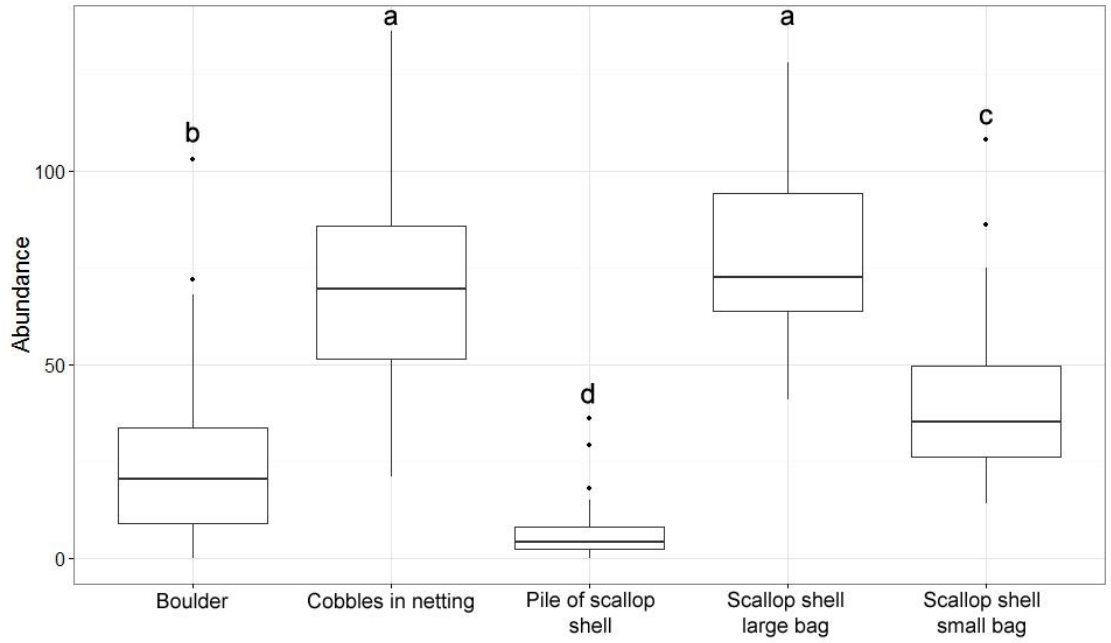

Figure 3.6. Abundance of Serpulidae per 0.0077 m^2 quadrat from the 5 restoration treatments in August 2014. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range. Plots not sharing a letter are significantly different at p <0.05.

Spatial effects

In total 650 quadrat photos were taken at the 5 sites in Loch Creran, over the 5 monitoring visits. These results show the Main Site on average having the lowest abundance of Serpulidae of the 5 sites at 7150 per m^2, compared to Kelco the most abundant site with 11995 per m^2 (Figure 3.7). The Mussel Farm, Kelco and Rubha Mor sites all displayed similar average abundances of serpulids with less than 1826 per m^2 separating the three sites.

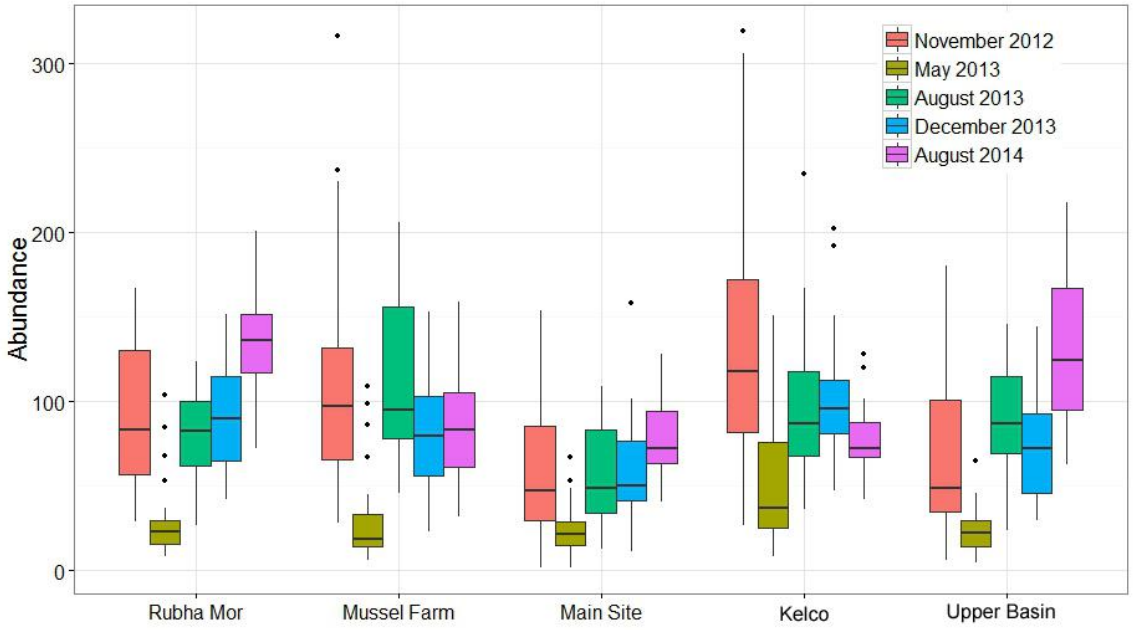

Figure 3.7. Abundance of Serpulidae per 0.0077 m^2 quadrat from the 5 sites separated into the 5 monitoring time points. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range.

Using PERMANOVA differences due to location were tested. A two-way crossed mixed model using monitoring date as a random factor and site as a fixed factor, found no significant difference in the abundance of serpulids due to location (Table 3.2). The variance in the dataset created by monitoring date is visible in Figure 3.7. The model found that monitoring date explained 28 % of the variance in the model, whereas site only explained 5 % of the variance. Testing for differences between the reef sites (Main Site and Rubha Mor) and the other non-reef sites, also found no significant difference in the abundance of serpulids (Pseudo-F = 3.886, P = 0.1248). The factor of reef or non-reef was only able to explain 3 % of the model variance.

Table 3.2. Results from PERMANOVA, using Euclidean distance to test for spatial effects with monitoring Date as random factor. The test statistic Pseudo-F value and (P) are calculated using 9999 permutation with n=650.

Source	df	SS	MS	Pseudo-F	P
Site	4	113430	28359	3.002	0.0539
Date	4	398280	99569	67.580	0.0001
Site*Date	16	152010	9500	6.448	0.0001
Residual	625	920850	1473		
Total	649	158560			

The data recorded from the final monitoring time point in August 2014 were analysed independently to the other monitoring data. In comparison to the combined dataset this highlighted an increased abundance of serpulids at the Rubha Mor and Upper Basin sites (Figure 3.8). A reduced PERMANVOA routine found a significant difference in the abundance of serpulids due to location with Pseudo-F = 19.801 and P = 0.001. The effect of location now explained 43 % of the model variance. Pairwise tests found that the Rubha Mor and Upper Basin sites were not significantly different to each other (P = 0.679), but they were significantly different from all other sites (P always <0.001). All other pairwise site combinations were not significantly different to each other. All non-significant pairwise comparisons are shown in Figure 3.8. The effect of reef and non-reef sites were then tested on the reduced dataset using PERMANOVA. This found no significant difference in the abundance of serpulids between reef sites (Rubha Mor and Main Site) and the remaining non-reef sites (Pseudo-F = 1.05, P = 0.316).

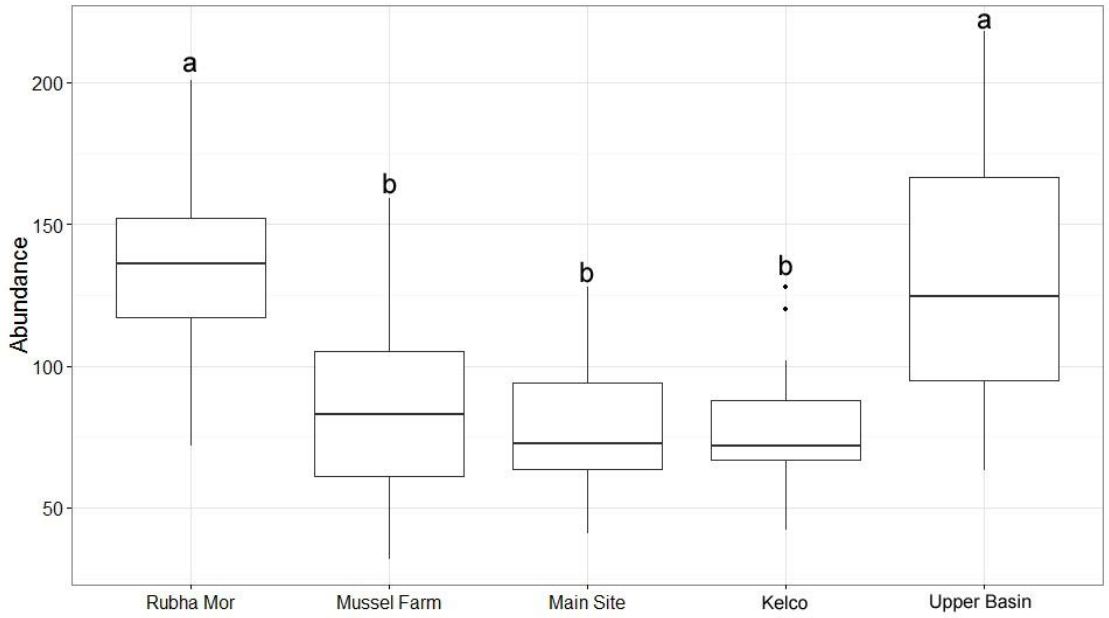

Figure 3.8. Abundance of Serpulidae per 0.0077 m^2 quadrat from the 5 sites from the monitoring conducted in August 2014. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range. Means with the same letter are not significantly different.

3.4 Discussion

Restoration materials

The aim of this study was to identify a potential substrate that could be used in the restoration of *S. vermicularis*. The original hypothesis was that scallop shell treatment in large bags would support the greatest abundance of serpulid recruits. The results tend to

support this hypothesis, with scallop shell in large bags having significantly higher abundances of serpulids overall than other treatments, when all monitoring data were included (Figure 3.5). When just analysing the final monitoring data, however scallop shell in large bags still had higher abundances of serpulids than other treatments, although these were not significantly different from cobbles in netting (Figure 3.6).

Scallop shell was thought to be an effective restoration treatment based on the extensive use of oyster and other bivalve shells in oyster restoration projects in the U.S.A (O'Beirn *et al.*, 2000; Nestlerode *et al.*, 2007), along with previous work on *S. vermicularis* recruitment (Chapman *et al.*, 2007). A possible explanation for advantage scallop shell has as a restoration treatment is the complex substrate it creates. A relationship between increased recruitment and substrate complexity and has been recorded in other restoration studies. O'Beirn *et al.*, (2000) tested the restoration potential of oyster shell, clam shell and coal ash pellets for *C. virginica* reefs. The study found that oyster shells had the greatest interstitial volume at 0.7 L per 1 L of substrate, compared to 0.58 L for clam shell and 0.45 L for coal ash. The reefs constructed from oyster shells also had significantly greater abundances of oysters two years after deployment. Across all reef designs and tidal elevations the reefs constructed from oyster shell had an average of 935 oysters per m^2, compared to 149 per m^2 for the clam reefs and 141 per m^2 for the ash reef. The better performance of the oyster shell as a restoration material was further emphasised by the other treatments being dominated by oysters <20 mm, whereas the oyster shell reefs had 22 % of their oyster population >60 mm. This latter represents a large proportion of oysters that can contribute more rapidly to future reproductive outputs and increases the sustainability of the restored reef (O'Beirn *et al.*, 2000; Lipcius *et al.*, 2008). O'Beirn *et al.*, (2000) related the increased abundance of oysters on the oyster shell reefs to several factors relating to increased interstitial space. Firstly the increased interstitial space provides more space for settling larvae compared to the more compacted restoration materials. Secondly the protection to juvenile oysters afforded by the complex interstitial spaces in the oyster shell reefs (O'Beirn *et al.*, 2000; Nestlerode *et al.*, 2007). Bartol and Mann, (1999) demonstrated that the complex interstitial spaces created in oyster reefs protect juvenile oysters from predation as well as buffering them from climatic extremes, namely storm damage. Finally O'Beirn *et al.*, (2000) suggested the increased settlement on oyster shell reefs in the first year created a positive density dependence; the increased abundance of living oysters after 12 months created further interstitial spaces, therefore more refuge for the next cohort of oyster larvae. Large numbers of oysters may also

enhance further settlement through gregarious chemical cues (Turner *et al.*, 1994). The possible gregarious settlement of *S. vermicularis* is discussed in Chapter 2. If a settlement cue is exhibited by *S. vermicularis* it is likely to further increase the abundance of serpulids on the scallop shell in large bags treatment, as the greater initial settlement of serpulids will create a stronger gregarious chemical cue during subsequent settlement periods. The significant difference between the scallop shell in large bags and boulder treatments, maybe a result of the complex substrate afforded by the former, although grazing pressure may also have had an effect. Grazing pressure would potentially have been higher on the boulder treatment due to lack of protective netting, unfortunately observations of potential grazers were not made during the study, which may have allowing a better insight into the difference between these treatments.

The lower relief of the scallop shell in piles treatment (<10 cm) compared to the other treatments is a possible cause of it having the lowest abundance of serpulids of any treatment (Figure 3.6). The significant difference between scallop shell in large and small bags may also be attributed to their difference in relief (Figure 3.2). The elevation from the seabed of artificially constructed oysters reefs has been seen to have a significant positive effect on recruitment successes (Lenihan and Peterson, 1998; Nestlerode *et al.*, 2007; Gregalis *et al.*, 2008). While Lenihan and Peterson, (1998) attributed the more dependable habitat created by elevated oyster reefs to an avoidance of hypoxic/anoxic bottom waters. There is no evidence to suggest the presence of hypoxic/anoxic conditions within Loch Creran (Gage, 1972; Almroth-Rosell and Tengberg, 2012). However Lenihan and Peterson, (1998) also suggest that decreased elevation would increase the relative sedimentation and burial rates compared to taller reefs. This increased sedimentation of the low lying treatments was visually apparent during the repeated monitoring visits, although was not formally assessed. Loch Creran receives significant inputs of terrigenous organic matter like many fjordic sea lochs, resulting in rapid sediment accumulation (Ansell, 1974a). The failure of two restored oyster reefs due to sediment burial was studied by Powers *et al.*, (2009). They attributed the loss of these reefs to the highly energetic environment found there. They reflected that a more informed site selection would have avoided this problem as the presence of coarse substrate at the sites indicated an energetic environment (Powers *et al.*, 2009). This level of sediment transport and resuspension of coarse sand however was not evident at any of the study sites. The avoidance of silt by settling serpulid larvae has been suggested as the reason behind the selection of the underside of surfaces in several studies (Bosence, 1979;

Young and Chia, 1982; Cotter *et al.*, 2003). Whilst the avoidance of siltation has not been proven for *S. vermicularis* larvae it is likely to have a negative effect on site selection by larvae and has been recorded in other invertebrates (Rodriguez *et al.*, 1993; Chapman *et al.*, 2007). The reduced elevation of the pile of scallop shell and scallop shell in small bag treatments would increases the relative sedimentation and burial rates compared to taller reefs, perhaps resulting in the lower settlement rates observed.

Spatial effects

It was hypothesised that the restoration units deployed at locations without extant *S. vermicularis* reefs would have higher abundances of serpulids, as previously observed in Chapter 2. However, reef presence was found to have no significant effect on the total abundance of serpulids. This discrepancy between these data and those of Chapter 2 can be explained by the photo monitoring methodology. Photo monitoring was the only practicable option for the monitoring of the restoration treatments, due to their size and quantity of substrate that composed some treatments. However identification of serpulids to species level requires a clear view of the operculum and this was not possible for every individual serpulid in each photograph (Hayward and Ryland, 2003; Ten Hove and Kupriyanova, 2009). Therefore, the identification of serpulids in the monitoring photographs to species level was not possible. The results of Chapter 2 found the species composition of serpulids on settlement tiles to be dominated by *S. triqueter* and *S. vermicularis*. These two species across all settlement tiles made up 95 % of the serpulid species recorded. Analysis of these data however showed there to be no correlation between the abundance of *S. triqueter* and *S. vermicularis* ($F=1.62$, $P=0.342$). The composition of these two dominant species varied across the 5 study sites, and were not correlated to any measured environmental factor (Figure 3.9 & Chapter 2).

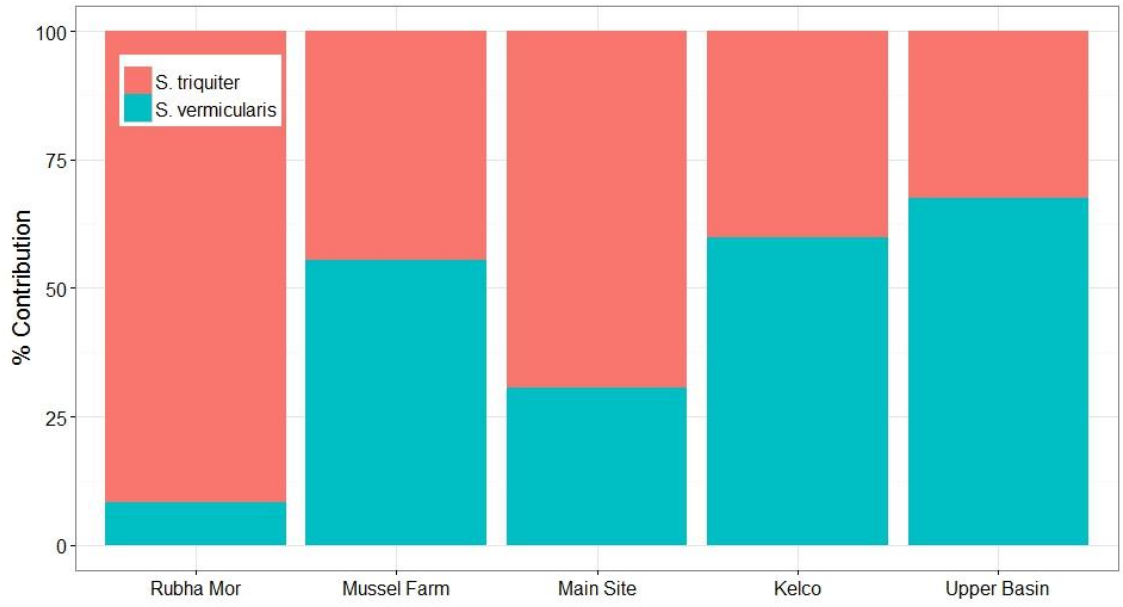

Figure 3.9. Percentage contribution of *S. triqueter* and *S. vermicularis* to the serpulid population at each site. Data from the settlement tiles used to assess the spatial differences in Chapter 2.

Due to the uncertainty in the relative abundance of these two species it is impossible to assess the effect proximity to *S. vermicularis* reefs had on the colonisation of restoration units. If the results obtained from the settlement tiles in Chapter 2 are comparable to the restoration units in this chapter (Figure 3.9), then a smaller proportion of the serpulids recorded at the Main Site and Rubha Mor would be *S. vermicularis*, compared to the other sites. The likely consequence of this would be a significant difference in the abundance of *S. vermicularis* due to the presence of extant reefs, therefore corroborating the results of Chapter 2.

A direct comparison of the data from this chapter was made with the photo monitoring data of Chapter 2 which also did not differentiate serpulid species. This comparison was made to help validate the settlement and mortality cycle observed in Figure 2.9, and increase the observation time period from 15 months to 27 months. This comparison is shown in Figure 3.10, with both datasets recording similar seasonal trends during the same period. This helps confirm that the abundance of newly settled serpulids in Loch Creran peaks around October, with April to May having the lowest abundances. The increased abundances of serpulids on the scallop shell restoration units (this chapter) compared to the settlement tiles (Chapter 2) seen in Figure 3.10, may be explained by the difference in substrate type (Chapman *et al.*, 2007; Nestlerode *et al.*, 2007).

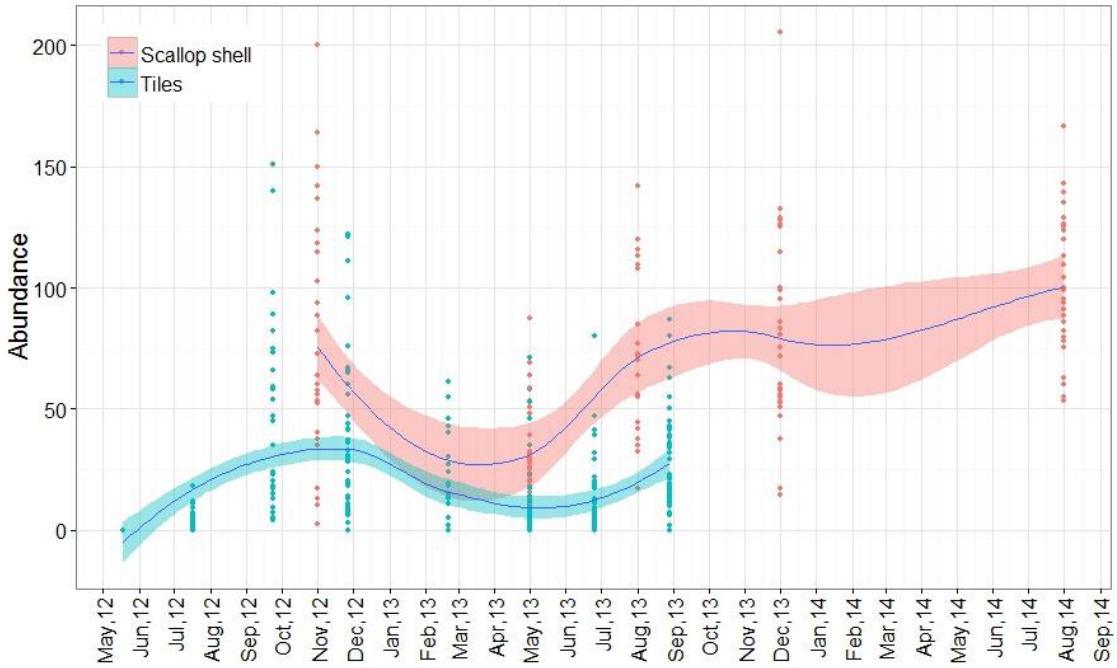

Figure 3.10. Abundance of serpulids recorded per 10 cm^2 from the Main Site, data from the settlement tiles from Chapter 2 (blue) and scallop shell in large bags restoration treatment (red). Curves calculated using locally weighted scatterplot smoothing.

Conclusions

These results build on the conclusions of Chapter 2 in helping to establish the techniques required to restore *Serpula vermicularis* reefs in Loch Creran. The results support the original hypothesis that scallop shell in large bags would be the most successful restoration technique. By the end of the study scallop shell in large bags had 9.6 % more serpulids than cobbles in large bags, although this difference was not significant. Significant differences in the abundance of serpulids between these two treatments and the other treatments may possibly be attributed to the greater substrate complexity afforded by scallop shell and cobbles in large bags. These results therefore imply that either cobbles or scallop shell in large bags could be successfully used in a future restoration project. Scallop shell may attract marginally more serpulids and be seen as the preferred material, however the cost and logistics of acquiring large volumes of scallop shell may outweigh its marginal gains.

The results also highlight the importance height of the restoration materials has. With loose piles of scallop shell having 91 % fewer serpulids than scallop shell in large bags. This reduced settlement might have resulted from increased sedimentation around the loose piles of scallop shells, although this was not measured (Rodriguez *et al.*, 1993; Chapman *et al.*, 2007). Therefore a future restoration project would be advised to ensure

sufficient height of any deployed restoration materials, despite the increased logistics and costs it would create.

The results also found no difference in the abundance of serpulids between the reef and non-reef sites which is in disagreement with the results of Chapter 2. This discrepancy, however, is the likely result of photo monitoring being unable to differentiate serpulid species. Future monitoring should therefore attempt to quantify the relative abundances of *S. vermicularis* and *S. triqueter* at the 5 sites, in order to fully understand the effect location has on the recruitment of *S. vermicularis* onto restoration materials within Loch Creran.

Chapter 4. Novel techniques for the restoration of *Limaria hians* reefs

4.1. Introduction

The known distribution of *Limaria hians* is from the Canary Islands and the Mediterranean to the Lofoten Islands, Norway. In the British Isles it is absent on the East Coast, and is most common on the Scottish west coast (Tebble, 1976; Seaward, 1990). Connor *et al.*, (2004) describes *L. hians* communities as being commonly encountered on shallow sub-littoral ground composed of mixed muddy gravel and sand, in weak to strong tidal currents (0.25 – 1.5 m / s). *Limaria hians* is unusual within the Bivalvia in that it cannot retract the soft parts of its body within its shell, as a result of which *L. hians* has developed defensive adaptations. Its tentacles, which extend from its mantle margin, can be autotomized as well as secreting acrid smelling mucus from its epidermal glands, making it distasteful to potential predators (Gilmour, 1967). *Limaria hians* also constructs protective nests by binding together material with their byssus threads (Gilmour, 1967; Hall-Spencer and Moore, 2000a).

In some locations the nests created by *L. hians* form areas of biogenic reef which can contain > 600 individuals per m^2 and can be 5 – 20 cm deep (Hall-Spencer and Moore, 2000a; Trigg *et al.*, 2011). These semi-infaunal bivalve reefs can form continuous cover for several hectares in the tidal narrows of sea lochs (Holt *et al.*, 1998; Hall-Spencer and Moore, 2000a; Moore *et al.*, 2013). The reefs can be difficult to distinguish from the surrounding seabed because the nest material incorporates algae, sand and gravel into the reef matrix and the surface is colonised by epibiota (Trigg *et al.*, 2011). The reefs support a very rich community, with many more species being present than would be found on the same substrate without *L. hians*. Trigg *et al.*, (2011) found 282 species from a 0.16 m^2 area from two *L. hians* reefs off Port Appin and in Loch Creran. This is supported by a qualitative study of a reef in Loch Fyne where 280 species were recorded from a 0.29 m^2 area (Hall-Spencer and Moore, 2000a). *Limaria hians* reefs were omitted from the UK biogenic reef classification, and are not included as an Annex I habitat in the Habitats Directive (Holt *et al.*, 1998). However following the work of several *L. hians* researchers, they are now considered a biogenic reef-forming species of conservation importance (Hall-Spencer and Moore, 2000a; Trigg *et al.*, 2011). Currently they are considered a priority habitat for conservation under the UK Biodiversity Action Plan and recently they have become protected features in 5 new Nature Conservation Marine Protected Areas in Scotland through the Marine (Scotland) Act 2010.

There is a relatively little literature on *L. hians* with only three peer reviewed publications within the last 20 years (Hall-Spencer and Moore, 2000a; Trigg and Moore, 2009; Trigg *et al.*, 2011). The reproduction of *L. hians* has been studied by Ansell (1974b) who suggested that *L. hians* spawns from July to September in the Clyde sea area. This was based on the observation that the weight of *L. hians* gonads increased from April to July, and then started to decline from July to March, which occurred rapidly at first due to spawning. Two studies into the reproductive cycle of *L. hians* found it easy to differentiate the sexes during spawning periods, with females having red gonads and males white (Hrs-Brenko, 1973; Ansell, 1974b). Work on other Limidae species have found them to be protandrous hermaphrodites, although this has not been studied in *L. hians* (Lodeiros and Himmelman, 1999; Järnegren *et al.*, 2007).

An early study by Lebour (1937), found Limidae veligers to be most abundant later in the year between October and November off Plymouth. However in the Adriatic, Hrs-Brenko, (1973) found that *L. hians* was reproductively active throughout the whole year. Plankton samples taken approximately every 10 days from 1967 - 1970 also recorded the presence of *L. hians* larvae throughout the year. The study found higher abundances of larvae in spring and summer, from which they inferred that the main spawning period for *L. hians* was during spring and summer (Hrs-Brenko, 1973). Trigg (2009), concurred with Ansell (1974b); and found peak settlement to occurr in July and August on his study sites in Scotland. The difference between Adriatic and Scottish populations is likely a function of latitude. A decrease in latitude sees a decrease in the seasonal water temperature fluctuations. This can lead to differences in the timing of gamete development and spawning as well as a more protracted spawning period (Sastry, 1966, 1970; Dukeman *et al.*, 2005).

It is thought that *L. hians* reefs were once more common. There are records of sites where abundant dead *L. hians* shells are present but living reefs are no longer present. Examples include, Orkney, the Scilly Isles and Cardigan Bay (Seaward, 1990). More recently, large declines have been seen in the Clyde which have been attributed to demersal fishing activities such as scallop dredging (Hall-Spencer and Moore, 2000a). Disturbance by bottom towed fishing gear is regarded as the major threat to *L. hians* reefs in the UK (Hall-Spencer and Moore, 2000a; Trigg and Moore, 2009). The recovery rate of a *L. hians* reef following experimental disturbance was tested by artificially clearing 0.25 m^2 plots and monitoring recovery off Port Appin on the west coast of Scotland (Trigg and Moore, 2009). The results found an average recovery of 24.2 % for the area in the cleared plots,

after 12 months. This recovered nest material was however thinner than the original material. They found that recovery started from the edges of the existing nest material or on pebbles at the margins of experimentally cleared plots. The authors speculated that this was a result of increased predation protection and physical stability gained from existing reef material and stable substrates. Using linear estimates, the authors calculated the rate of recovery at 3.2 cm per annum. A Newhaven scallop dredge has a 7.5 m wide swath. Extrapolating the calculated recovery rate, the disturbance created by a scallop dredge would take an estimated 117 years to recover (Trigg and Moore, 2009). This calculation however does not take into account several factors such as variable recruitment, the severity and scale of the destruction. The only other study on the recovery of a *L. hians* reef was by Minchin, (1995). Tributyltin (TBT) was used as antifouling on salmon farms in Mulroy Bay in Ireland from 1981-1985. During this period, settlement of *L. hians* declined and failed and the nest material was observed to thin and break up with sand patches appearing. On revisiting the site in 1994 the population was similar to a baseline recorded in 1980, with no extensive sand patches visible (Minchin, 1995). This recovery within 9 years contrasts to the lengthy recovery times reported by Trigg and Moore, (2009). However as Trigg and Moore (2009) concluded, recovery could depend on the amount of *L. hians* nest material remaining. The thinning of nest material leaving only small gaps may recover relatively quickly because the remaining nest material can grow and expand. However if all nest material is removed, as would occur with the passage of a dredge, then recovery might take significantly longer.

L. hians differs from *M. modiolus* and *S. vermicularis* in the form of the biogenic reefs they create. *L. hians* binds together the upper substrate surface and builds its nest structures over it, whereas *S. vermicularis* reefs build up in aggregations from a stone or shell leaving the majority of the substrate in an area unaltered. *M. modiolus* conversely are more infaunal than *L. hians* and reefs build up over tens of years from a substrate that is commonly composed of fine sediments and shell fragments (Gilmour, 1967; Lindenbaum *et al.*, 2008; Trigg and Moore, 2009). The construction of artificial reefs and the addition of "cultch" has been frequently used in the restoration of oyster reefs (Caddy and Defeo, 2003; Luckenbach *et al.*, 2005; Schulte *et al.*, 2009). This technique is thought to be successful as it creates a stable substrate for settling recruits, and increases complex interstitial spaces which affords increased predation protection and settlement opportunities (Cranfield *et al.*, 2004; Luckenbach *et al.*, 2005). The technique has been

proven effective in increasing settlement and survival of juvenile oysters (Bartol and Mann, 1997; Luckenbach *et al.*, 2005; Brumbaugh *et al.*, 2006).

Observations suggest there is little difference between the substrates found underneath existing *L. hians* reefs and those seen in areas where former *L. hian*s reefs have been lost or damaged (Cook, pers. obs.). Restoration efforts for *L. hians* should therefore not just focus on the provision of habitat through the addition of cultch, as a suitable substrate may already be present in many degraded areas (Trigg *et al.*, 2011; Moore *et al.*, 2012).

Aims and hypotheses

The scope of the project was twofold; the first aim was to investigate a reported decline in the *L. hians* reef off Port Appin. Comparing any change in reef extent since the initial decline reported in 2011 by Moore *et al.* (2012). The null and alternative hypothesis being.

- H_0: The extent of the *L. hains* reef off Port Appin would not have significantly changed since it was last estimated in 2011.
- H_1: The extent of the *L. hians* reef off Port Appin will have significantly declined since 2011.

The second part of the project was to investigate novel ways to restore damaged *L. hians* reefs. This investigation was split into three separate aims. Firstly, following the observations of Trigg and Moore, (2009) it was thought that artificially stabilising the substrate at restoration sites would increase *L. hians* recruitment and promote reef development. The null and alternative hypothesis being.

- H_0: Artificially stabilising the sediment in areas of damaged *L. hians* reef would not increase the abundance of *L. hians* compared to un-stabilised areas.
- H_1: Artificially stabilising the sediment in areas of damaged *L. hians* reef would significantly increase the abundance of *L. hians* compared to un-stabilised areas.

The second aim was to investigate stock enhancement as a restoration technique by seeding areas with juvenile *L. hians* collected on artificial spat collectors, as well as translocating small patches of *L. hians* reef. The null and alternative hypothesis being.

- H_0: Stock enhancement techniques would not significantly increase the abundance of *L. hians* in restoration plots compared to other techniques.
- H_1: Stock enhancement techniques would significantly increase the abundance of *L. hians* in restoration plots compared to other techniques.

The final aim was to investigate if the provision of substrate would increase *L. hians* recruitment and aid reef development. The null and alternative hypothesis being.

- H_0: Providing additional hard substrate would not significantly increase the abundance of *L. hians* in restoration plots compared to other techniques.
- H_1: Providing additional hard substrate would significantly increase the abundance of *L. hians* in restoration plots compared to other techniques.

4.2. Methods

Site information and decline of the Port Appin reef

Two study sites were located off Port Appin, and two study sites were located in the narrow entrance to Loch Creran (Figure 4.1). The two sites at each location were selected to give one site within an extant *L. hians* reef and another in close proximity to a reef. All sites were exposed to strong tidal flow in excess of 0.4 m/s and all sites were between 8 m and 11.5 m below Chart Datum.

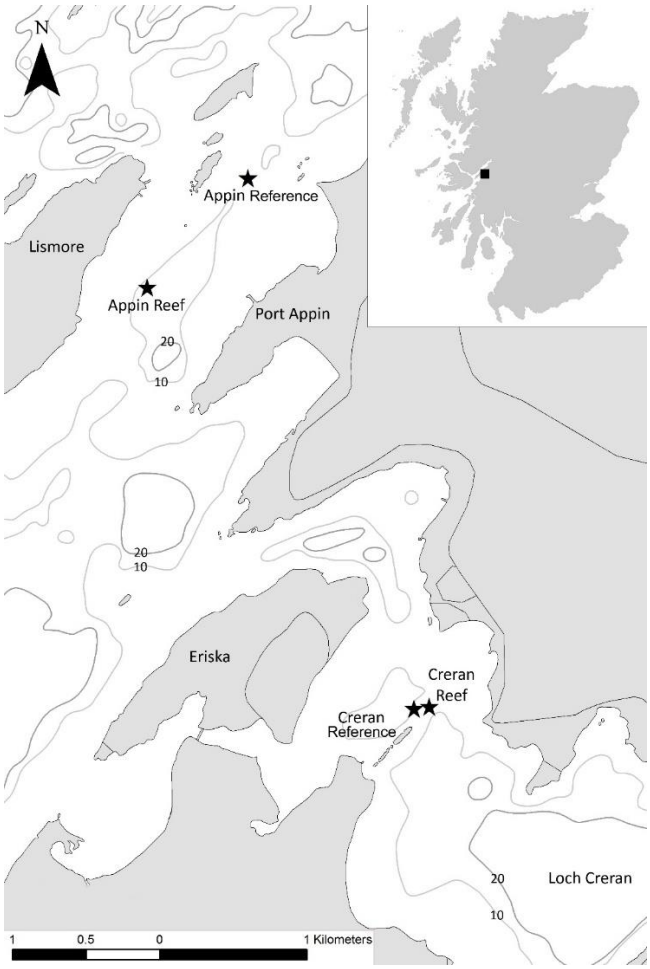

Figure 4.1. Map of the 4 study sites (stars) used in this study of *Limaria hians* restoration techniques.

The *L. hians* reef off Port Appin was first recorded by Connor, (1990). Although the precise position of this record is unknown, it is assumed to be from the southern end of the reef. In 2001, 3 diver transects revealed an extensive reef area with up to 100 % cover of nest material (Moore, unpublished). Subsequent studies between 2003 and 2006 revealed a reef of approximately 40.5 hectares (O'Malley, 2004; Forrest, 2005; Trigg and Moore, 2009; Trigg *et al.*, 2011) (Figure 4.2). This would have made it one of the largest known reefs in Scotland (Moore *et al.*, 2012, 2013). In 2011 the whole reef area was revisited utilising divers and drop down video to record its extent (Moore *et al.*, 2012). This survey revealed a large reduction in the extent of the reef. The remaining reef was fragmented into 3 smaller areas, giving a remaining total area of 4.47 hectares, which equates to an 89 % decline in the reef area (Figure 4.2).

The Appin Reef site was located in one of these patches, at the same location as the FS01 site surveyed by Moore *et al.*, (2012) (Figure 4.2) and had 80% cover of *L. hians.* The experimental area was located on a patch of pebbles and gravel within this reef area. The Appin Reference site was located in an historical area of *L. hians* which has had no records of living reef since 2011, and is currently 300 m from an area of extant reef. This site supported a forest of *Laminaria hyperborea* on a mixed substrate of cobbles, pebbles and gravel.

The *L. hians* reef in the entrance narrows to Loch Creran, has only recently been mapped revealing an extent of 18 ha (Moore *et al.*, 2013). The Creran Reef site was located in an area of 100 % cover of *L. hians* reef, which has been known since 2005. The site has been used in previous studies, notably Trigg and Moore (2009) and Trigg *et al.* (2011). The Creran Reference site was located 100m west of the Reef site. The site is protected from the ebb tide by a rocky barrier, creating a weaker tidal flow. The site had no records of *L. hians* presence, and was approximately 80m from the reef boundary as defined in Moore *et al.* (2013) (Figure 4.1).

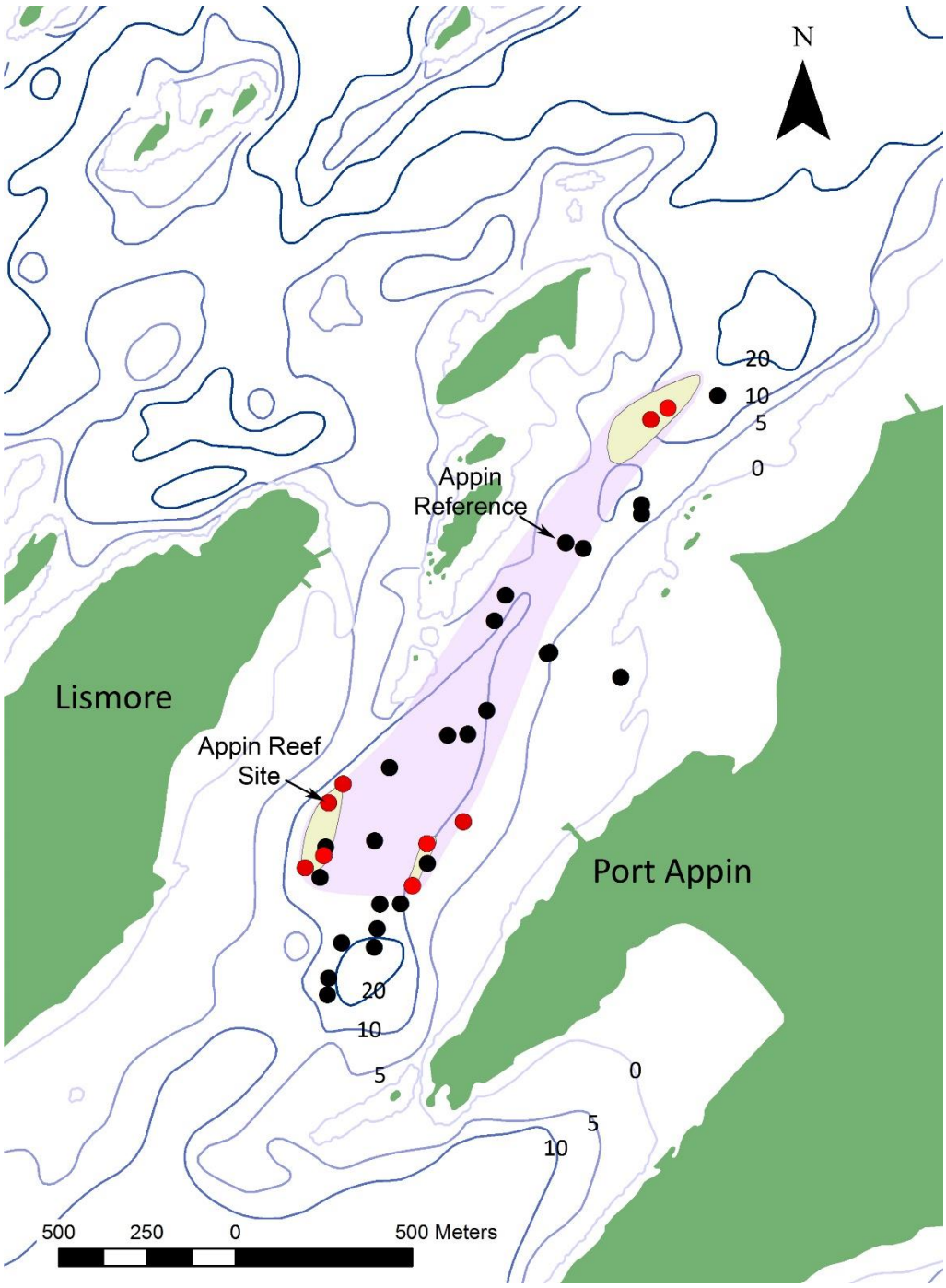

Figure 4.2. Historical extent of the *Limaria hians* reef at Port Appin shown in purple, and the extent in in 2011 in yellow. The 2011 survey records show the presence (red circles) and absence (black circles) of *L. hians*. Data from (Moore *et al.*, 2012).

Sediment stabilisation

The first restoration technique was based on the concept that sediment stabilisation would prove an effective restoration strategy. Netting was used to stabilise the sediment in areas of strong tidal flow improving anchorage for byssus threads and providing predation protection. The experiment used 0.25 m^2 plastic netting panels with 16 mm mesh to stabilise the substrate within the experimental plots. Five netting panels were deployed

by divers at each site in July 2013, with the corners of each panel being held in place with a metal peg. At the Appin and Creran Reef sites any existing *L. hians* reef was cleared, before the netting was pegged down. This allowed the recovery of these cleared reef areas to be assessed.

The netted plots were recovered by divers in August 2014. 5 litre buckets were used to collect the netting along with the first ~2cm of the sediment under the netting. Additionally at the Creran Reference site four randomly selected 0.25 m^2 plots were cleared using the same method. These were to act as reference plots for the experimental plots. The aim of these reference plots was to quantify the presence of any *L. hians* that were not visually apparent when initially establishing the site. All samples of netting and sediment were then carefully picked through for *L. hians* within 2 days of collection, with the aid of a Leica MZ75 dissection microscope.

Stock enhancement

Thirty spat collectors were deployed at each of the 4 sites in July 2012. Spat collectors were constructed from 50 cm x 50 cm squares of 16 mm plastic mesh. This mesh was then folded several times to create a complex 3D structure. This was of a similar design to the spat collector used previously on *L. hians* reefs (Trigg, 2009). These spat collectors were then arranged into bunches of three with a minimum 2 cm gap between them. These bunches were then attached to one-meter-long metal road pins, and 10 road pins were deployed at each site. The road pins were hammered into the seabed at each site by divers with the spat collectors attached 20 cm above the seabed.

To assess the effectiveness of the spat collectors, and any inter-annual recruitment variability, 4 spat collectors were randomly removed from the 30 deployed at each site after 1 and 2 years. These spat collectors were recovered by divers using snips to remove them from the road pins. The spat collectors were placed into sealable sample bags to avoid losing any spat during recovery to the surface. The spat collectors were then analysed within 2-3 days of collection using a dissection microscope. All bivalves found within the spat collectors were identified to species level using up to date taxonomic literature and enumerated.

The use of spat collectors as a restoration technique was assessed by transplanting spat collectors which had been collecting spat at each site for a year. At each site 5 spat collectors were removed from their road pins in July 2013 and relocated to the surrounding seabed. They were then covered and held in place with the same 0.25 m^2 of

netting and pegs as used for the other treatments. At the two reef sites any trace of *L. hians* reef was first removed from the experimental plots. These plots were then recovered by divers in August 2014. Five litre buckets were used to collect the netting and spat collectors along with the first ~2cm of the sediment under the netting. All samples of netting, spat collectors and sediment were then carefully picked through for *L. hians* within 2 days of collection, with the aid of a dissection microscope.

The final restoration technique involved transplanting five 0.031 m^2 sections of *L. hians* reef from the nearest reef to each of the reference sites. Each section was collected using a 5 litre bucket with diameter of 20 cm. The translocated *L. hians* and reef material were held in place with the same 0.25 m^2 of netting and pegs as used for the other treatments. These plots were deployed in July 2013 and then recovered by divers in August 2014. Empty five litre buckets were used to collect the netting and any remaining translocated material along with the first ~2cm of the sediment under the netting. These samples of netting, translocated material and sediment were then carefully picked through for *L. hians* within 2 days of collection, with the aid of a dissection microscope.

Provision of substrate

In July 2013 five restoration units were deployed at each of the 4 sites using a similar methodology to those trialled for *M. modiolus* restoration (Chapter 5). The units used 10 kg of crushed *Pecten maximus* shell with a size of ~2 cm^2 to fill mesh bags. These mesh bags measured 0.5 m in length by 0.5 m wide and 10 cm high and are commonly used in the cultivation of oysters. These units would create complex interstitial spaces as well as elevation from the seabed and substrate stability. These units were recovered in August 2014, giving a deployment duration of 13 months. The crushed shell bags were recovered by divers using 1 mm mesh bags to enclose the sample. Once enclosed they were lifted to the surface using a lifting bag and recovered on board using a winch. The samples were then picked through for *L. hians* within 2 days of collection with the aid of a dissection microscope. The lengths of all *L. hians* found were recorded using an electronic Vernier calliper with a precision of 0.01 mm.

Analysis

All graphical interpretations were conducted using the ggplot2 package within R (Wickham, 2009; R Core Team, 2015). The data from the restoration units were standardised to give values per 0.5 m^2 to account for the differences in sample size, before testing for differences between treatments.

Differences in the abundances of *L. hians* between restoration treatments were investigated using a Generalised Linear Mixed Models (GLMM) in R, using the lme4 package (Bates *et al.*, 2013; R Core Team, 2015). The model was fitted using a Poisson error structure, to account for the non-normal count data (Bolker *et al.*, 2009; O'Hara and Kotze, 2010). Site was specified as a random effect within the model, to account for the spatial pseudoreplication within the model. This allowed the effect of restoration treatment to tested across all sites, whilst accounting for the variability created by the different sites (Millar and Anderson, 2004). This technique allows the model to utilise data from all sites, whilst accommodating the spatial variability in the data. The null hypotheses of no treatment effect was tested with a Likelihood-ratio test (LRT) of deletion, by comparing the original model to a reduced model (Crawley, 2007). If the null hypothesis was rejected then pair wise tests between the different treatments could be conducted. This would be conducted using the general linear hypothesis routine within the multcomp package (Hothorn *et al.*, 2008).

A Generalised Linear Model (GLM) was used to test for differences between sites in the abundance of *L. hians* from the crushed shell restoration treatment. The GLM was fitted using the MASS package, within R (Venables and Ripley, 2002; R Core Team, 2015). The model was initially fitted using Poisson regression to account for the non-normal count data, (Bolker *et al.*, 2009; O'Hara and Kotze, 2010). If the fitted model exhibited over dispersion, it was refitted using negative binominal regression. This technique is commonly used when dealing with count data in ecology, which are often over dispersed (Ver Hoef and Boveng, 2007). The null hypotheses of no site effect was tested by comparing the original model to a reduced model using a Wald chi-squared test. If the effect of site proved significant, pair wise tests between the different sites were undertaken. This was conducted using the general linear hypothesis routine within the multcomp package (Hothorn *et al.*, 2008).

The length frequency of *L. hians* measured from the crushed shell samples were neither normally distributed nor did they conform to any common distribution without transformation. Differences in the length frequency of *L. hians* between sites were tested using a non-parametric Kruskal Wallis test within R. If significant differences between sites were detected pair wise comparisons were carried out using a Pair Wise Wilcoxon rank sum test with a Bonferroni correction (Crawley, 2007).

4.3. Results

Decline of the Port Appin reef

At the Port Appin Reef site in 2013 whilst retrieving the spat collectors placed in 2012 it was noticed that the reef surrounding the experimental area had disappeared. It was apparent on this visit and subsequent dives that the site had changed dramatically, and there was no visible reef material within 50 meters of the site. The site was visited again in 2014 to recover the remaining spat collectors and experimental units, and again no evidence of *L. hians* reef was recorded. In April 2015 a series of dives were conducted to confirm the presence of *L. hians* in the remaining reef patches identified in 2011 (Figure 4.2). These dives used the same "spot dive" methodology as outlined in Moore *et al.*, (2012). Two of these dives were conducted during a drift over the reef therefore giving several spot recordings per dive with more than 100m between recordings. These dives found the two southern patches had disappeared, and the northern patch had reduced in extent slightly. The dives also found no sign of *L. hians* reef material within the historical reef area, which would have indicated signs of recovery. These data are shown in Figure 4.3. The remaining reef patch has an extent of 2.73 hectares. This then equates to a 38 % loss in the extent of the Port Appin reef since 2011 and a 93 % loss within a decade (Figure 4.3).

Sediment stabilisation

Unfortunately a large number of experimental units were lost after their deployment. This was likely the result of the strong tidal currents, compounded by large kelp plants and creels being dragged over the site (Cook, pers. obs.). Additionally some of the labels differentiating treatments with and without translocated nest material were also missing, so these units could not be used without confounding the results. At the Creran Reef Site and the Appin Reference site the majority of the experimental plots were missing, and no more than three samples per treatment could be located. As a result these units were not recovered and the sites excluded from further analysis.

At the Appin Reef Site only a single Netting sample was recorded. The Creran Reference site yielded 6 samples. On recovery in 2014 these samples revealed an average of 66.3 ± 28.7 *L. hians* per m^2 from the experimental plots across both sites. The four cleared reference plots at the Creran Reference site only recorded one *L. hians*, therefore giving an abundance of 4 *L. hians* per m^2. A generalised linear model comparing all treatments found there were significantly more *L. hians* in the experimental plots opposed to the

control clearance plots (P = 0.02). These data along with the data from the stock enhancement experiments are shown in Figure 4.4.

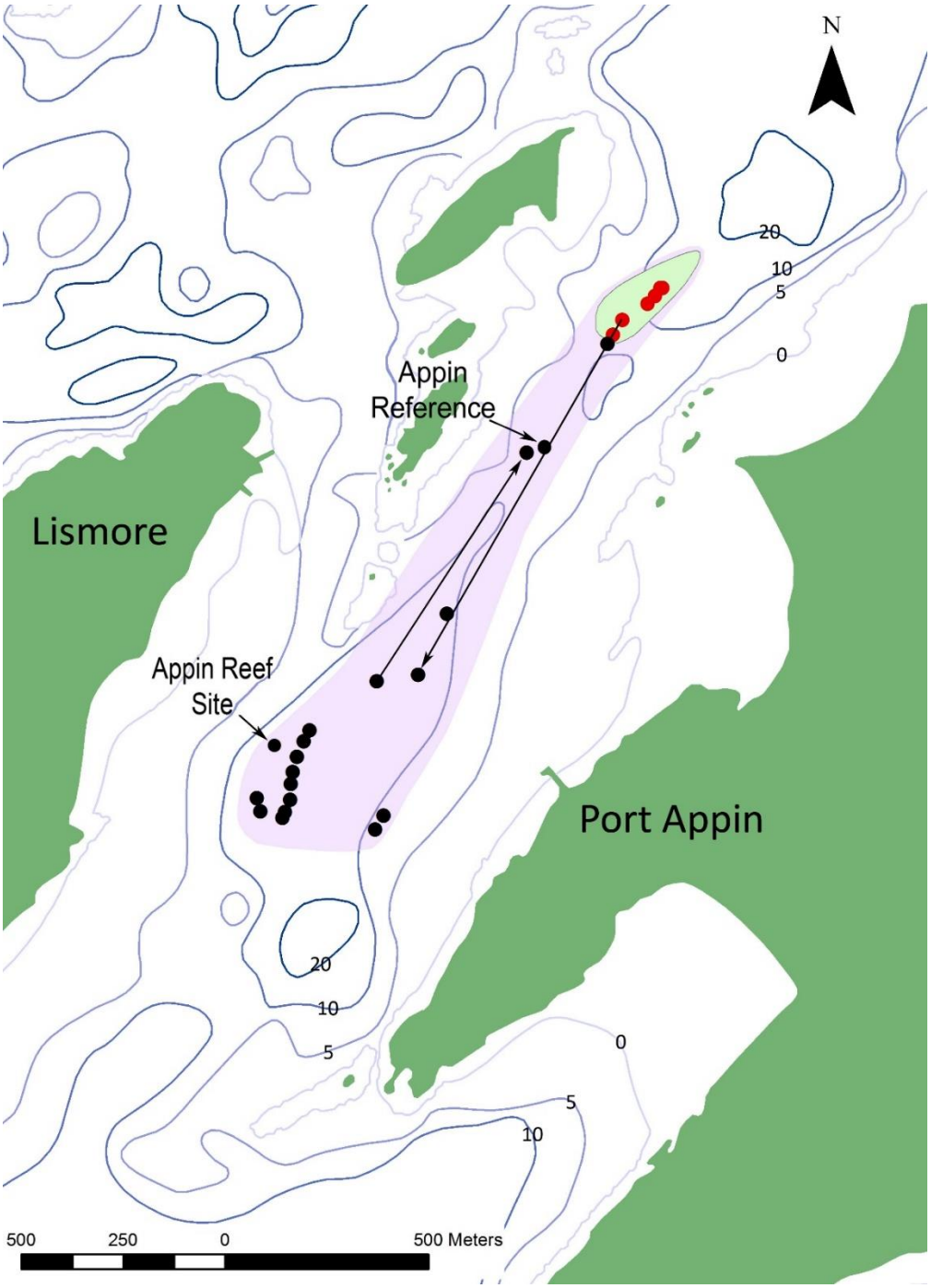

Figure 4.3. Historical extent of the *Limaria hians* reef at Port Appin in purple, and the extent following the 2015 survey. The 2015 records show the presence (red circles) and absence (black circles) of *L. hians*. The direction and path of the two drift dives are shown by black arrows.

Stock enhancement

Unfortunately the spat collectors at the Appin Reference site could not be located in 2013 and only a single individual *L. hians* was recorded from the 12 spat collectors across the other 3 sites. This single *L. hians* was recorded from the Creran Reference site. In 2014 after a 2-year deployment, a further 20 spat collectors were recovered from all 4 sites. From these only the 5 spat collectors from the Creran Reef Site contained any *L. hians* with an average of 12.4 individuals per collector.

Of the spat collectors redeployed in 2013 and covered with netting, only three were recovered from the Creran Reference site. On average these plots had 117.3 *L. hians* per m^2. Nest material and netting yielded more replicates with 5 recovered at the Creran Reference site and 5 from the Appin Reef site. These samples of fewer *L. hians* present than the spat collectors covered with netting with an average 81.6 *L. hians* per m^2. These data along with the other restoration techniques are displayed in Figure 4.4.

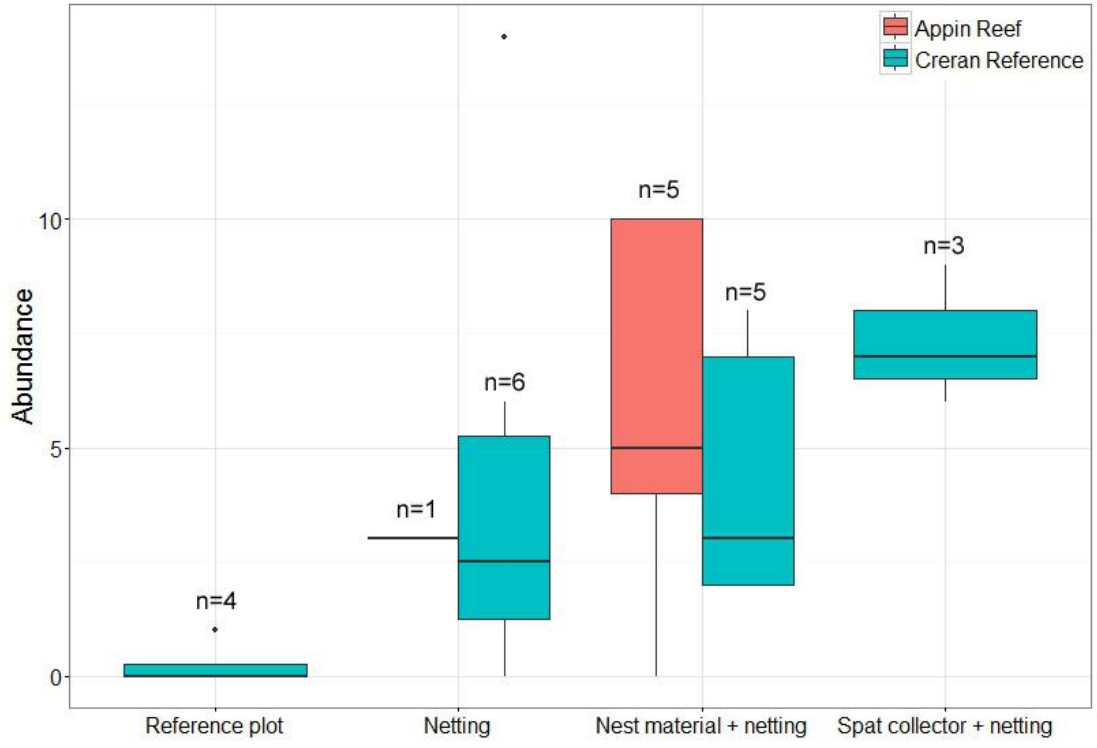

Figure 4.4. Abundance of *Limaria hians* from each treatment (0.25 m^2) from the 2 sites, in 2014. Box plots represent inter-quartile range, median, maximum and values or points representing outliers if greater than 1.5 * the inter quartile range. Number of replicates per treatment shown as n.

A Generalised Linear Mixed Model (GLMM), with site specified as a random term was used to test for differences between treatments. Site was specified as a random term to avoid spatial pseudoreplication, as the remaining experiment was unbalanced and

comparisons between sites would have been unreliable. The model used Poisson regression to control for the non-normal count data. The GLMM found a significant difference in the abundance of *L. hians* between treatments (Chi = 33.44; P < 0.001). Pairwise tests found significantly more *L. hians* in the three restoration treatments (Nest material + netting, spat collector + netting and netting) compared to the control clearance plots, with P always < 0.02. However the pairwise comparisons found no significant difference in the abundance of *L. hians* between the three restoration treatments, with P always > 0.1 (Figure 4.4).

Provision of substrate

The crushed shell bags were the only restoration treatment to be recovered from all 4 sites. The two reef sites had on average 157 ± 40 *L. hians* per sample, whereas the reference sites had 38 ± 10 *L. hians* per samples (Figure 4.5). A generalised linear model using negative binomial regression found significant differences in the abundance of *L. hians* due to site (F=22.58; P = 0.001; Figure 4.5). Pairwise tests found no significant difference in the abundance of *L. hians* between the two reef sites (Z=0.2; P = 0.99). However there were significant differences between all other pairs of sites with Z always > 3.95 and P < 0.001. Non-significant pairwise site comparisons are shown in Figure 4.5.

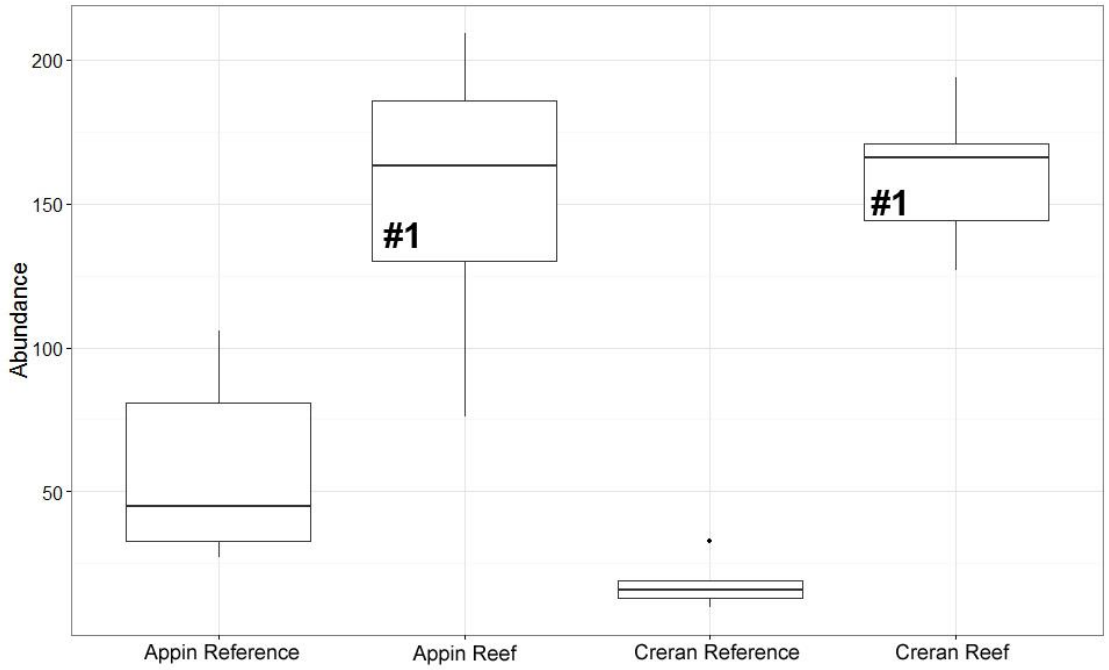

Figure 4.5. Average abundance of *L. hians* in each crushed shell sample (0.5 m^2) from the 4 sites in 2014 Box plots represent inter-quartile range, median, maximum and values or points representing outliers if greater than 1.5 * the inter quartile range. #1 represent non-significant treatment combination.

The lengths of all the *L. hians* recovered from the crushed shell samples were also recorded. These data revealed the two Appin sites had smaller *L. hians* than the Creran sites (Figure 4.6). They also show a bimodal distribution in the lengths of *L. hians* from the Creran Reference site. These length frequency data were not normally distributed, necessitating the use of non-parametric analysis. A Kruskal-Wallis test found significant differences between sites (Chi = 872: P < 0.001). Pairwise Wilcox rank sum tests using a Bonferroni correction found significant differences between all site combinations, with P always <0.014.

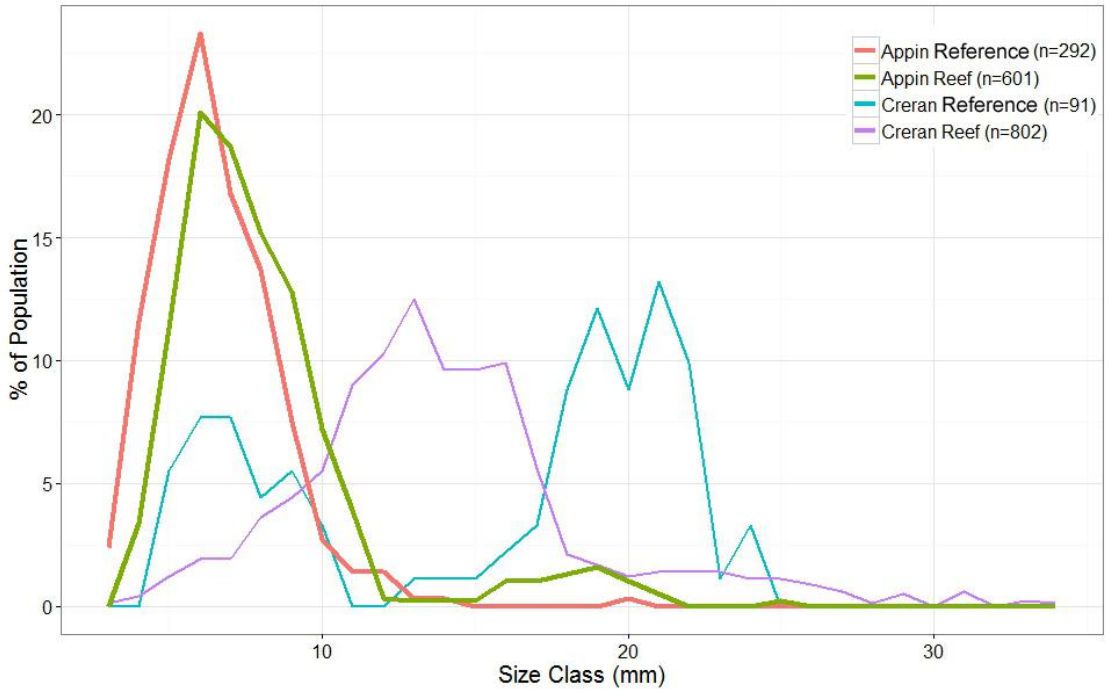

Figure 4.6. Size class data expressed in percentage of population to standardise for differences in abundance between sites. Thicker lines indicate the two Appin sites.

The data from all the netted plot experiments (spat collectors + netting, nest material + netting, netting) could be combined as there were no significant differences between the treatments (Figure 4.4). Following standardisation to give values per 0.5 m^2, these combined data could then be compared to the data from the crushed shell units. On average across all treatments and sites the netted plots had an average of 24 ± 3 *L. hians* per 0.5 m^2. Whereas the crushed shell samples on average had 95 ± 16 *L. hians* per 0.5 m^2 sample. This difference between netted and crushed shell restoration techniques was tested using a GLMM, with site specified as a random term. The model found the abundances of *L. hians* in the crushed shell samples were significantly higher than the netted samples when compared against a null model of no treatment effect (LRT = 8.49; P = 0.003).

4.4. Discussion

Decline of the Port Appin reef

These results highlight a dramatic decline of a biogenic reef of recognised conservation importance (Hall-Spencer and Moore, 2000a; Trigg *et al.*, 2011). This decline was first observed by Moore *et al.*, (2012), who recorded a 89 % reduction in the reef extent by 2011. These results show a further 38 % decline in reef extent since 2011, resulting in a remaining reef area of only 2.73 hectares. Moore *et al*., (2012) reported observing creel fishing taking place over the historical area of the reef during 2011 and subsequently. When the strings of creels were being recovered the rope between them was observed to be covered in kelp. These creel strings were being deployed across the current. On retrieval the boat drifted down current dragging the creel string along the sea bed along with numerous kelp plants attached to cobbles or small boulders. Observations at the Creran Reef Site in 2013 support this as a large "hedge" of kelp had built up against the metal road pins supporting the spat collectors. Observations by divers and remote video also support this explanation. Hedges of kelp plants were observed building up in longitudinal cross-tide lines, on the seabed. One of these kelp hedges was observed to have a lost string of creels entrained within it (Cook, pers. obs.). Movement of kelp, attached cobbles and small boulders across the seabed are likely to have disturbed the seabed potentially leading to the degradation and loss of the *L. hians* reef.

To our knowledge this is the first evidence that creel fishing has had a detrimental effect on sensitive habitats. Eno *et al.*, (2001) to date is the only other study to investigate the disturbance caused by creels. Their observations provide evidence of impacts on a single species (Ross coral (*Pentapora foliacea*)) and were conducted in areas of limited tidal flow where the effects of dragging creels were not studied. An additional possible cause for the decline in the reef is recruitment failure of *L. hians* over the last 10 years, although the limited data available do not support this hypothesis, as Macleod, (2012) found juvenile *L. hians* to be abundant in the remaining areas of reef sampled in 2011. The crushed shell restoration units also recorded abundant juvenile *L. hians* both at the reef and reference sites. The present results do not provide direct quantifiable evidence that creel fishing is the cause of the decline, but do provide support for it being a contributing factor. The loss of the *L. hians* reef surrounding the Appin Reef site has affected the inferences that can be drawn from experimental work at that site.

Sediment stabilisation

The aim of this experiment was to test the hypothesis that stabilising areas of the seabed with netting would improve the recruitment and reef formation of *L. hians.* Unfortunately most of the experimental plots were either lost or unidentifiable due to the strong tidal flow at the sites. It is also probable that the physical abrasion caused by the movement of kelp plants at the Appin Reef Site damaged some of the experimental plots. Despite this the results showed that netting areas of the seabed significantly increased the recruitment of *L. hians*, when compared to reference clearance plots. Substrate stabilisation is not commonly employed as a marine restoration technique. It has often been seen as having a favourable restoration outcome in oyster restoration studies, as it provides shoreline stabilisation and coastal defence (Beck *et al.*, 2011; La Peyre *et al.*, 2014). Substrate stabilisation using oyster shell cultch has also been conducted to reduce physical pressures such as increased sedimentation and erosion to aid marsh-land restoration projects (Meyer *et al.*, 1997). Substrate stabilisation has also been used as an effective technique on coral reef restoration projects (Rinkevich, 2005). Such techniques have been employed in areas where coral reefs have been degraded to rubble due to storm damage or anthropogenic impacts such as blast fishing. The remaining substrate is highly mobile due to tidal and wave driven currents. This movement increases mortality due to abrasion and the overturning of the remaining corals. Various methods have been used to stabilise these substrates, from laying artificial material over a reef, to tying corals to the seabed. These methods have been an important technique in the last 10 years of coral reef restoration, particularly for improving the success of coral transplantation experiments and facilitating natural recovery (Fox *et al.*, 2003; Rinkevich, 2005). Given the results of this project and examples from coral reef projects, substrate stabilisation in conjunction with other techniques seems promising as an appropriate restoration technique for *L. hians* reefs.

Stock enhancement

Spat collectors of various designs are commonly used to assess bivalve recruitment, and monitor restoration projects (Peterson *et al.*, 1996; Brumbaugh *et al.*, 2006; Roberts *et al.*, 2011). Unfortunately the spat collectors used in this study proved unreliable. During 2013 only a single *L. hians* was recorded from a total of 12 spat collectors distributed across 3 sites. In 2014 the only *L. hians* present were in the spat collectors from the Creran Reef Site. This contrasts with the results of the crushed shell bags, where *L. hians* were present at all sites (Figure 4.5). Larval supply of *L. hians* is confirmed by the settlement

in the bags of crushed scallop and the viability of the spat collectors is demonstrated by the settlement of other bivalve species in the spat collectors. An average of 30 ± 7.4 *Modiolarca tumida* and 31 ± 7.6 *Mytilus edulis* were found in each recovered spat collector. These data show that this design of spat collector is capable of facilitating settlement of some taxa, but not *L. hians*. Alternative spat collector designs, such as small crushed shell bags or monofilament based spat collectors as used by Trigg (2009) may prove more appropriate.

It was thought that the use of stock enhancement techniques would increase the recruitment of *L. hians* in restoration plots. However there was no significant difference between the two stock enhancement techniques and the netting only treatment. This may be accounted for by a number of factors. The rationale behind the use of spat collectors under netting was to provide appropriate substrate for *L. hians*, hopefully maximising recruitment in the first year. The initial elevation of the spat collectors from the seabed would reduce post settlement mortality, further enhancing recruitment. These spat collectors could then be used to seed areas of seabed. However the failure of the spat collectors to attract *L. hians* led to this treatment being indistinguishable from the other treatments. Using translocated *L. hians* and nest material also yielded no significant difference to netted only areas. The length of the *L. hians* recorded from these plots only found a maximum of 2 individuals from each plot that were over 2 years old (Trigg, 2009), whereas the original translocated reef material contained considerably more individuals aged 2 years and above. This suggests the survival of translocated individuals was low, and the use of translocated nest material would therefore not be an effective restoration technique.

Provision of substrate and comparison to other techniques

The crushed shell restoration units proved to be the most successful restoration technique trialled. This led to the rejection of the original null hypothesis that providing hard substrate would not significantly increase the abundance of *L. hians* compared to other techniques. The addition of crushed shell increased the abundance of *L. hians* by 79 % at the Appin reef site compared to the netted treatments, and by 339 % at the Creran Reference site. Additionally, the ability of the restoration units to remain stable in the strong tidal flows made them a much more effective restoration technique. Of the 20 crushed shell bags deployed only one was lost. This can be attributed to their weight of approximately 20 kg which stopped them being moved in the strong tidal currents.

The crushed shell units had high numbers of juvenile *L. hians* at all sites, in contrast to other treatments. The two reef sites showed the highest abundances of *L. hians* with no significant difference between the two sites. Given the loss of the reef surrounding the Appin Reef Site this result was not expected as the Appin Reef site was now the furthest away from a known *L. hians* reef (Figure 4.7). Larval density is expected to be a function of distance from source as recorded in other bivalve species (Elsäßer *et al.*, 2013), this possibly indicates an undetected larval source close to the Appin Reef site. The lower abundances of *L. hians* at the Creran Reference site, are possibly attributable to the reduced tidal flow at that location. This may have reduced the larval connectivity with the neighbouring reef, or reduced the seston available in the benthic boundary layer enough to no longer support a *L. hians* reef. The exact relationship between distance from a restoration site to an extant reef and larval supply is unclear from these data. Generally however the closer restoration materials are deployed to an extant biogenic reef with adequate natural larval supply the greater the likelihood of achieving sustainable recruitment at the restoration site is without stock enhancement (Lipcius *et al.*, 2008; Elsäßer *et al.*, 2013).

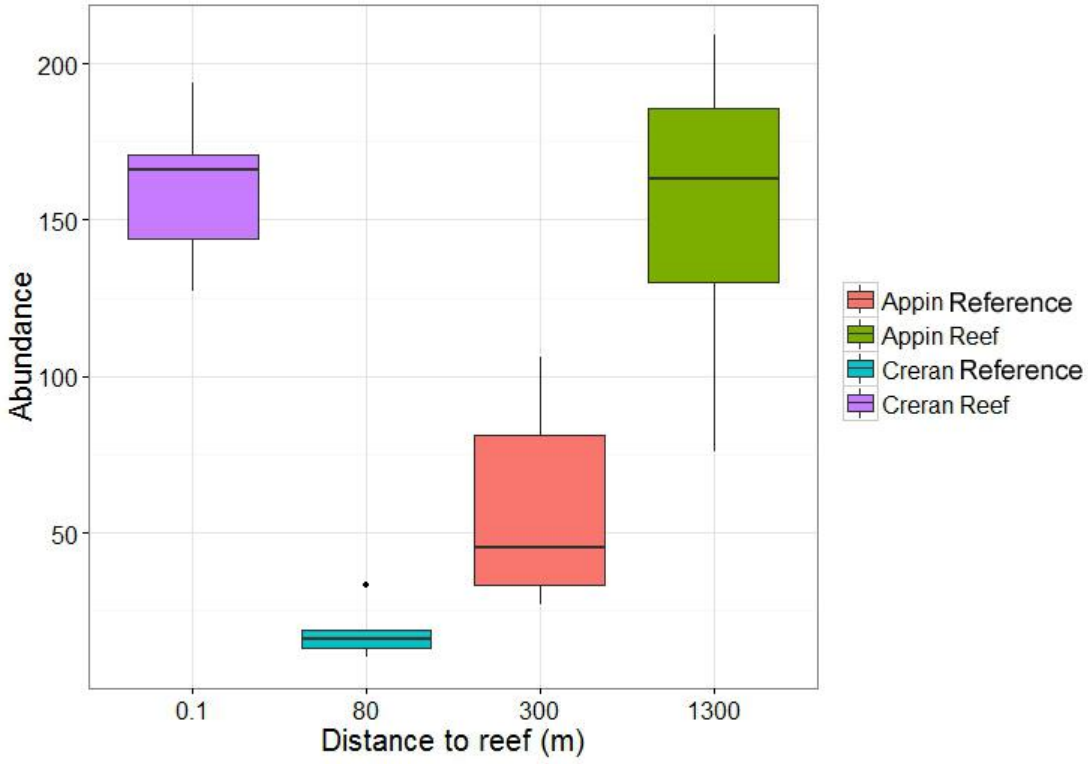

Figure 4.7. Average abundance of *L. hians* per crushed shell restoration unit from each site, plotted against that sites distance to the nearest known *L. hians* reef in meters. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range.

These results show that despite the loss of 93 % of the reef off Port Appin larval supply within the historical reef area was not limited. The recognition that the remaining reef patch off Port Appin is a source habitat is an important contribution to any future restoration attempts. A source habitat is defined as self-sustaining and able to act as a larval source for neighbouring areas (Caddy and Defeo, 2003; Lipcius *et al.*, 2008). Source habitats are commonly referred to as substrate limited, and restoration efforts should focus on strategies to increase the habitat availability for juveniles and adults (Brumbaugh *et al.*, 2006). The techniques trialled in this study are more likely to succeed than those in recruitment limited habitats, which require stock enhancement techniques (Mann and Powell, 2007; Geraldi *et al.*, 2013). The factors governing larval connectivity between different *L. hians* reefs are unknown, so inferences about whether the remaining reef patch is isolated or connected to other larval sources cannot be made. There is a very little literature on *L. hians* larvae and the length of time the larvae spend in the water column is unknown. Lebour (1937), recorded the veligers "grow well and soon lost the velum" and "at this size loses the velum and goes down to the bottom". Unfortunately no specific time is given for this process, which makes it impossible to estimate the distance larvae may travel in the water column. However given the short distance between the Appin and Creran reefs, it would plausible to assume they are part of the same metapopulation (Lipcius *et al.*, 2008). Despite the high larval supply within the historical reef area, any future restoration project must also remove the physical pressures from the area if they are to be successful (Brumbaugh *et al.*, 2006; Powers *et al.*, 2009; Elsäßer *et al.*, 2013).

Length frequency data highlighted a bimodal distribution for lengths of *L. hians* recorded at the Creran Reference site, whereas the two Appin sites only contained smaller individuals (Figure 4.6). Trigg (2009) used acetate peels to accurately age individual *L. hians* using the methods outlined in Anwar *et al.* (1990). From age at length measurements von Bertalanffy growth curves could be calculated. These growth curves estimated 2 year old *L. hians* to be between 14.5 and 21 mm in length. These length estimates correspond with the second peak in the length frequency distribution at the Creran Reference site (Figure 4.6). As the experiments were only deployed for 12 months, *L. hians* must be moving into the crushed shell bags from the surrounding seabed. *L. hians* are able to swim, although this is considered a defensive strategy and generally use their foot for locomotion (Gilmour, 1967; Trigg, 2009). This migration of individuals has not been recorded before. Although only 10 *L. hians* were estimated to have migrated during

this study. This migration effect may prove significant as reef expansion and natural recovery of damaged areas was previously recorded as only accreting from the margins of the existing reef (Trigg and Moore, 2009). This ability of juvenile *L. hians* to migrate to nearby suitable substrates, may allow faster recovery and reef expansion than previously calculated by Trigg and Moore, (2009), and consistent with the observations of Minchin (1995).

Conclusions

The results presented here highlight a number of issues relevant to restoring this relatively unstudied biogenic reef forming species. Despite the limitations of the high current flow environment, deployment of crushed shell units proved an effective restoration technique. These units enhanced the recruitment of *L. hians* at sites both within and outside existing reef areas. Substrate stabilisation using netting also proved an effective restoration technique, and enhanced recruitment compared to background areas, although the netted restoration plots attracted fewer recruits and were less likely to remain in place due to the high current flow than the crushed shell units. The success of the crushed shell units suggests that predation protection provided by the complex substrate and netting was a significant factor in their success. The results also showed that stock enhancement techniques were ineffective in increasing the recruitment of *L. hians*. The failure of the spat collectors demonstrates that they may be unreliable as a restoration technique at least in their current design for *L. hians*. It was also evident that the translocation of adult *L. hians* would probably not prove a successful technique due to the high mortality rates of the translocated individuals.

The work also further highlighted the vulnerability of these reefs to damage, with the Port Appin reef losing 93% of its extent within 10 years. A greater understanding of the pressures on *L. hians* and how to reduce these needs to be developed, before any successful restoration project can be undertaken.

Chapter 5. Developing techniques for the restoration of *Modiolus modiolus* reefs

5.1. Introduction

The horse mussel *Modiolus modiolus* (Linnaeus, 1758) is an Arctic - Boreal species, found in both the Atlantic and Pacific oceans. In the North East Atlantic they occur from the Bay of Biscay in the south, to northern Norway including the White Sea and Iceland (Schweinitz and Lutz, 1976; Brown, 1984; OSPAR Commission, 2009b). Individually *M. modiolus* are a widely distributed and common species. Aggregations of *M. modiolus* can build up to form reefs with densities ranging from between 10 and 619 mussels per m^2 at depths of up to 70m (Rees *et al.*, 2008). These reefs have a more limited distribution and are absent or scarce towards the geographic limits of their range (OSPAR Commission, 2009b; Gormley *et al.*, 2013). Reefs occur on different substrates from cobbles to muddy gravels and sand, and in fully saline waters that are usually moderately tide swept (Lindenbaum *et al.*, 2008; OSPAR Commission, 2009b). On the east coast of the UK they are not found south of the Humber Estuary with only infrequent reefs present northwards until Noss Head near Wick. Reefs are more abundant on the west coast with several reefs in the Irish Sea north of the Lleyn Peninsula and scattered records throughout the west coast of Scotland up to Orkney and Shetland (Holt *et al.*, 1998; OSPAR Commission, 2009b; Gormley *et al.*, 2013).

M. modiolus reefs are considered to be of conservation importance, due in part to the diverse nature of the flora and fauna associated with the biogenic reefs, as well as through seabed stabilisation and enhanced benthic productivity (Navarro and Thompson, 1997; Lindenbaum *et al.*, 2008; Sanderson *et al.*, 2008; Ragnarsson and Burgos, 2012). Studies on the large reef off the Lleyn Peninsula in Wales found very rich epifaunal and infaunal communities associated with the reef, with 213 different taxa recorded from infaunal cores and 64 taxa from *in-situ* quadrat counts (Rees *et al.*, 2008; Sanderson *et al.*, 2008). *M. modiolus* reefs have been identified as biogenic reefs and are key features in several Special Areas of Conservation (SAC) (1992 EC Habitats and Species Directive: Council Directive 92/43 EEC), and more recently as Protected Marine Features through the Marine (Scotland) Act 2010. *M. modiolus* reefs have also been identified by OSPAR as a priority marine habitat and are listed in the UK Biodiversity Action Plan. The high levels of biodiversity seen on *M. modiolus* reefs are a result of their habitat complexity and the trophic richness resulting from the high levels of biodeposition by the mussels (Navarro

and Thompson, 1997; Rees *et al.*, 2008). The structural complexity of a *M. modiolus* reef has three primary components. Firstly, a very dense layer of living and dead mussels which creates a framework in either single or multiple layers. Then there is a diverse community of free living and sessile epifauna and finally there is a very diverse community of crevice infauna, which live in-between the *M. modiolus* shells in the rich faecal deposits (Rees *et al.*, 2008; Ragnarsson and Burgos, 2012).

M. modiolus reefs have proven to be very stable and persistent features, the reef off the Lleyn Peninsula having been present for over 160 years with very little change in extent recorded over recent years (Lindenbaum *et al.*, 2008). Many *M. modiolus* reefs are seen to be under threat and in many cases are declining. They are particularly vulnerable to physical disturbance from mobile fishing gear (Magorrian and Service, 1998; Roberts *et al.*, 2011; Strain *et al.*, 2012; Cook *et al.*, 2013). Declines due to natural factors and climate change have also been suggested (Holt *et al.*, 1998; Mair *et al.*, 2000; Gormley *et al.*, 2013). Large scale loss of the *M. modiolus* reefs in Strangford Lough, has been directly attributed to the indirect and direct effects of fishing activity (Service and Magorrian, 1997; Roberts *et al.*, 2011; Strain *et al.*, 2012). Additionally the large scale reduction of the *M. modiolus* reef south of the Isle of Man has been reported since the original survey of the area, which has been attributed to impacts from bottom towed fishing gear (Jones, 1951; Holt *et al.*, 1998). A further study found that 90% of all epifaunal organisms were removed following the single pass of a trawl, on a reef north of the Isle of Man (Cook *et al.*, 2013). Natural recovery of these reefs has not been observed, and is unlikely to occur without intervention (Roberts *et al.*, 2011; Cook *et al.*, 2013; Fariñas Franco *et al.*, 2013). *M. modiolus* are long lived organisms commonly reaching more than 40 years of age (Anwar *et al.*, 1990). The re-establishment of a reef and its associated community to a pre-impacted state is likely to take decades (Holt *et al.*, 1998; OSPAR Commission, 2009b; Fariñas Franco *et al.*, 2013).

The global loss of shellfish reefs has only recently been fully realised, and the restoration of their ecosystem services has lagged behind terrestrial projects (Elliott *et al.*, 2007; Beck *et al.*, 2011). This awakening has led to an emergence of marine restoration projects with a particular focus on oyster restoration on the American east coast (Coen and Luckenbach, 2000; Schulte *et al.*, 2009; Kennedy *et al.*, 2011). The construction of artificial reefs and the addition of "cultch" has been the most widely used shellfish restoration technique (Caddy and Defeo, 2003; Luckenbach *et al.*, 2005; Schulte *et al.*, 2009). The addition of cultch has proved successful, as it helps replace the structural complexity of a healthy

reef. This complex structure increases sediment stabilisation and settlement opportunities, by providing suitable settlement substrates and enhanced refuge from predation (Bartol and Mann, 1997; Cranfield *et al.*, 2004; Luckenbach *et al.*, 2005; Brumbaugh *et al.*, 2006; Fariñas Franco and Roberts, 2014). In addition to the construction of artificial reefs, stock enhancement is often used to help restore or rehabilitate shellfish reefs. This stock enhancement takes two forms, either the translocation of adults from neighbouring areas, which maybe threatened or have successful sustainable settlement (Peterson *et al.*, 1996; Fariñas Franco and Roberts, 2014), or through the release of hatchery reared juveniles (Coen and Luckenbach, 2000; Thayer *et al.*, 2005). It is speculated that adult *M. modiolus* may attract *M. modiolus* larvae through a gregarious response, therefore suggesting the possibility of boosting the performance of materials with translocated mussels. This gregarious response has only been speculated for *M. modiolus* but has been observed in other reef-forming bivalves (Bayne, 1969; McGrath *et al.*, 1988; Zimmer-Faust and Tamburri, 1994). The success and comparison of restoration projects is often hard to judge, either due to differing restoration targets (Coen and Luckenbach, 2000), or the inadequate monitoring of comparable ecosystem services on relevant spatial and temporal scales (Kennedy *et al.*, 2011; La Peyre *et al.*, 2014). A comparison of different restoration materials, and the effect of varying stocking densities for translocated individuals has received very little attention in the literature (Mann and Powell, 2007; Nestlerode *et al.*, 2007; Fariñas Franco *et al.*, 2013).

Aims and hypotheses

The aim of this study was to investigate whether habitat provision would be a successful technique for the restoration of a damaged *M. modiolus* reef, and to investigate the restoration potential of different materials. The null and alternate hypothesis being.

H_0: There would be no significant difference in abundance of juvenile *M. modiolus* between the different restoration treatments and a control treatment.

H_1: One of the restoration treatments tested would have significantly higher *M. modiolus* recruitment compared to the control treatment.

The secondary aim of this study was to test the effect of translocating adult mussels from a healthy area of reef to a damaged area. The null and alternative hypothesis being.

H_0: The use of translocated adult *M. modiolus* in restoration units would not significantly increase the abundance of juveniles within them.

H_1: The use of translocated adult *M. modiolus* in restoration units would significantly increase the abundance of juveniles within the units.

5.2. Methods

Study sites

The three experimental sites were located in three separate *M. modiolus* reefs (Figure 5.1; Table 5.1). The reefs were selected so that they each had different characteristics, allowing the effect of trialled restoration techniques to be judged against a range of reef types. The first site was located in the large reef (~349 ha) North of the Lleyn Peninsula in Wales (Lindenbaum *et al.*, 2008). The reef lies within and forms a feature of the Pen Llŷn a'r Sarnau SAC. The reef is very rich, Rees *et al.* (2008) finding 213 infaunal taxa. *In-situ* quadrat records estimated *M. modiolus* density to be 100 individuals per m^2. The actual density of *M. modiolus* from infaunal samples is much higher at ~736 individuals per m^2 (Rees *et al.*, 2008), however infaunal density estimates were not comparable between the sites used in this thesis, due to the different and often semi-quantitative sampling methodologies employed. The site is exposed to strong tidal currents, modelled at 1.11 m/s (BERR, 2008), moderate wave action with a fetch >100km with a prevailing south westerly wind, and is 29m below Chart Datum.

The second site was located within a *M. modiolus* reef in Scapa Flow, Orkney. The experimental site was located near the wreck of First World War German light cruiser the SMS Karlsruhe. The site is referred to as the "Karlsruhe site" throughout this chapter. A recent study found the reef to support moderate levels of diversity with 63 taxa recorded from clump samples (Sanderson *et al.*, 2014). The reef is less dense than the Lleyn Peninsula reef with a density of ~80 individuals per m^2 from *in-situ* counts (Grieve 2015, pers. comm.). The site is also exposed to less tidal flow, 0.7 m/s (BERR, 2008), and less wave energy, with a 2km fetch from the prevailing south westerly wind, and a maximum 14km fetch from any wind direction. The site is 24m below Chart Datum.

The third site was located on the *M. modiolus* reef in the upper basin of Loch Creran. This reef is a key feature of the Loch Creran SAC, and as such is afforded protection from bottom towed fishing gear (Moore *et al.*, 2006). The Lleyn Peninsula reef is also protected from bottom towed fishing gear through its SAC designation (Cook *et al.*, 2013) and the Scapa Flow reefs is protected, by virtue of its position next to the large ship wreck. Mair *et al.*, (2000) found the Loch Creran reef to have a patchy density with up to 28 individuals per m^2 from transect estimates. The reef had higher levels of diversity than the Scapa

Flow however, with 158 taxa recorded from clump samples (Mair *et al.*, 2000). The reef is also very sheltered from tidal flow and wave action with a maximum fetch in any direction of 2.3 km. The site is 13m below Chart Datum.

Figure 5.1. Locations of three *M. modiolus* study sites across the UK (stars).

Table 5.1. Coordinates of the three *M. modiolus* study sites, all positions derived using the datum WGS1984.

Site	North	West
Lleyn Peninsula	52° 32.212	04° 39.029
Loch Creran	56° 32.745	05° 16.180
Karlsruhe	58° 53.377	03° 11.402

Experimental design

To assess the effects of using different materials to enhance the settlement and development of *M. modiolus*, 5 different restoration treatments were used. Whole scallop shell (*Pecten maximus*) was considered as the mostly likely successful substrate and has been used in other *M. modiolus* restoration trials (Roberts *et al.*, 2011; Fariñas Franco and Roberts, 2014). In addition to whole scallop shells alone two additional treatments were seeded with adult *M. modiolus*; "High" seeded contained 11 adult mussels and "Low" seeded contained 4 adult mussels. The numbers of mussels chosen to seed the samples was based on the total number available at the first site and then standardise at these numbers for the other two sites. The original aim was for the low treatment to have half the density of the high treatment. The mussels used to seed the samples were collected by divers from the study sites between 1 and 7 days before the deployment of the different restoration materials. The mussels were cleaned of epifauna and attached material before being placed in the High and Low samples. A further two treatments of crushed scallop, which had a uniformed size of ~2cm^2, and concrete building rubble with each sample containing assorted sizes of rubble weighing between 0.5 and 1 kg each. Each sample comprised 10kg of material sealed inside a sturdy plastic mesh bag measuring 0.5m by 1m. These bags are primarily used in the cultivation of oysters on the intertidal (Figure 5.2). Empty mesh bags containing a 0.5kg lead weight were used as a control treatment.

Figure 5.2. An experimental sample unit, an oyster bag filled with scallop shell at the Karlsruhe site. Image courtesy of Paul Kay.

The five restoration treatments were deployed at each of the three study sites, each treatment was comprised of 15 replicate units. This allowed for the recovery of five units of each treatment after one and two years, leaving five samples of each treatment *in-situ* for a future study. In total this gave 90 samples per location and 30 per time point resulting in 270 samples in total. The sampling design is shown in Figure 5.3. The initial deployment at each site was done by loading the sample bags haphazardly into 5 one tonne builders' sacks before loading the builders' sacks onto a boat. The boat was positioned over the site using GPS coordinates at slack water and the builders' sacks were then deployed overboard. Over the course of the next 2-5 days divers then relocated the builders' sacks on the seafloor and unpacked them on the surrounding seafloor. Each sample bag was positioned randomly a minimum of 2m away from an adjacent bag. The bags were positioned so as to not cover any live *M. modiolus* already present at that site (Figure 5.2).

Anwar *et al.*, (1990) showed that *M. modiolus* < 30mm in length are up to 4 years old, therefore the *in-situ* identification and quantification of juvenile mussels was not realistically possible, especially given the short working times for divers due to the depths and slack water times of the study sites. To recover the sample bags divers used custom made 1mm plastic mesh bags to first enclose the samples. This ensured retention of any juvenile mussels that may have been dislodged during the recovery process. The samples were then lifted to the surface using lifting bags, and back on-board the vessel either using a winch or by hand. Plastic tags on the samples bags allowed identification of the various treatments, and sample bags were chosen haphazardly from across the site. After recovery the restoration material and sample bag from each sample was carefully washed and picked through. The resulting biological sample was then fixed in 10 % buffered formaldehyde solution. These samples were then picked through carefully for juvenile *M. modiolus* with the aid of a dissection microscope where necessary. Juvenile *M. modiolus* were initially identified and separated from other Mytilidae species using external shell characteristics (Hayward and Ryland, 2003; Oliver *et al.*, 2010). This was due to the large number of juvenile mussels recovered and the time consuming nature of identification using hinge line characteristics. Along with the abundance of mussels per sample the length of all mussels was measured to 0.01 mm with digital Vernier callipers. Mussels less than 3mm were recorded as being 2.99mm in length, as accurate measures in this sixe class were not practically possible for such large numbers of mussels.

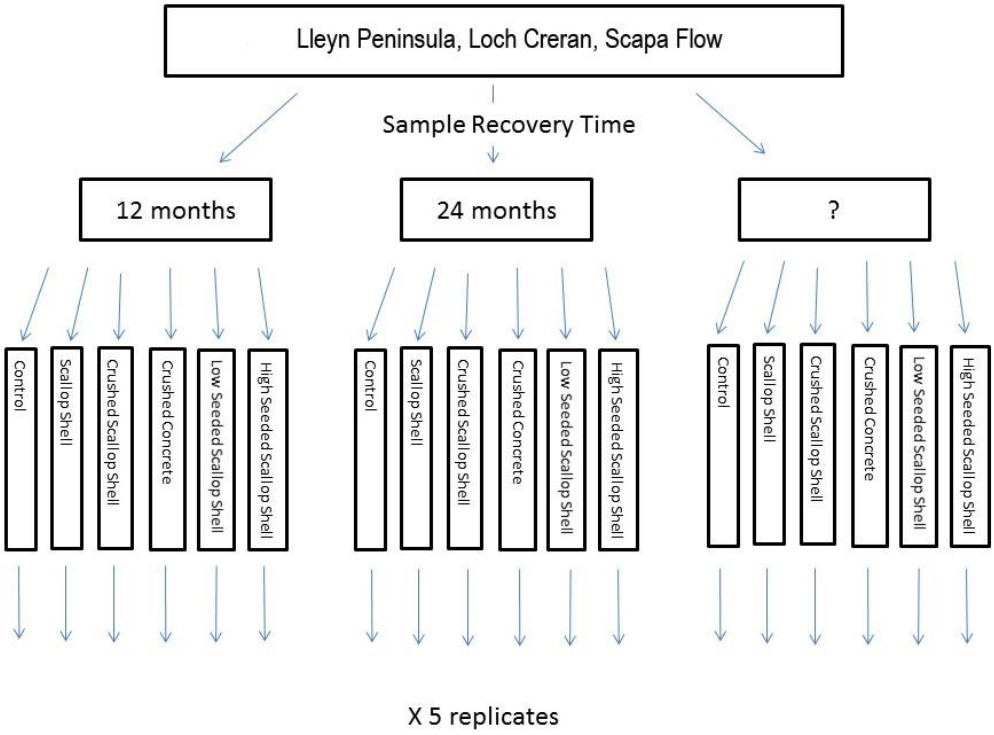

Figure 5.3. Experimental design for *Modiolus modiolus* recruitment experiment. The recovery time for the third set of samples is unknown so is shown as (?)

Identification of juvenile M. modiolus

Chapter 6 describes the use of DNA barcoding techniques to assess the reliability of identifying juvenile *M. modiolus* using external shell characteristics. The results show that juvenile Mytilidae identified using external shell characteristics belonged to at least three separate species. The results did however conclude that *M. modiolus* could be distinguished from the two other species due to a lack of crenulations on the internal hinge line of the shells (Oliver *et al.*, 2010). The mussels enumerated from the restoration samples were initially identified as *M. modiolus* using external shell characteristics. Following the findings of Chapter 6, a subset of 40 – 50 mussels from each of the three sites were randomly selected for more detailed identification using hinge line characteristics, following the methodology outlined in Chapter 6. The aim was to assess the proportion of juvenile mussels recorded at each site that were *M. modiolus* rather than morphologically similar juvenile Mytilidae species. These results were then used to adjust the total abundances of *M. modiolus* from the restoration samples.

Spat collectors

Spat collectors had historically been used on the Lleyn Peninsula reef to monitor annual settlement of *M. modiolus*. They were deployed and collected annually from 2005 to 2009. On collection the spat collectors had been fixed in ~5% formaldehyde solution but the abundance of *M. modiolus* juveniles had never been enumerated. The spat collectors were constructed of three layers of green pan scourer, which varied slightly in size from 10.1 – 11 cm long by 7.4 - 8 cm wide. The three layers of pan scourers were held together with a cable tie, and attached to a metal pyramid on the Lleyn Peninsula reef at 52^{o} 56'.516 North, 04^{o} 38'.070 West. The number of replicate spat collectors varied each year from 3 to 6. As part of this current study, three replicate spat collectors using the same construction were deployed at the Loch Creran site in 2012 and recovered 12 months later. All spat collectors were rinsed with fresh seawater and split apart over a 0.5 mm sieve, the individual layers of spat collector and the washings were then examined under a dissection microscope and the abundance of *M. modiolus* recorded.

Environmental data

Current speed estimates were only available for Lleyn Peninsula site and the Karlsruhe site. These estimates are based on modelled tidal flow data for surface currents. Differences in benthic boundary layer flow are more ecologically relevant than these data (Wildish *et al.*, 2008; Dame, 2012). Therefore a MIDAS Electromagnetic current meter made by Valeport was deployed at each of the sites. The current meter was fitted inside a multi-core frame for protection and lowered to the seabed at each site. The current meter's sensor was positioned approximately 20 cm above the seafloor. Boat traffic, strong tidal currents and security considerations, meant the current meter was left unmarked and recovery was conducted using divers to attach a lifting line and lift bag. The instrument recorded pressure (decibar), salinity (PSU), current speed (m/s) and current flow direction in magnetic degrees. Data were recorded 3 times 10 minutes and these three recordings were averaged within the instrument to give 1 reading ± SE, every 10 minutes. The instrument was in place for 41 days in Loch Creran, for 5 days at the Karlsruhe site and 6 days at the Lleyn Peninsula site.

Data analysis

All graphical interpretations were conducted using the ggplot2 package within R (Wickham, 2009; R Core Team, 2015). A two way crossed design with interactions was used to assess the effect site and year had on the abundance of *M. modiolus* recorded across all treatments. This test was conducted using a permutational analysis of variance

(PERMANOVA) routine in PRIMER v7 with the PERMANOVA package (Anderson *et al.*, 2008). PERMANOVA was chosen over standard univariate techniques to account for the presences of multiple zero counts and the highly skewed non-normal data (Anderson, 2001a). This technique also allowed the data to be analysed without a transformation, which have been seen to perform poorly on count data and may have hidden a significant interaction term (Anderson *et al.*, 2008; O'Hara and Kotze, 2010). Counts of *M. modiolus* were modelled as a function of site and year with an interaction term, with both site and year treated as fixed categorical factors. The test used a resemblance matrix calculated using Euclidean distance, without any data transformations. P values were calculated using Type III Sum of Squares and 9999 permutations of residuals under a reduced model, as this gives the greatest power and most accurate type I error for multi factorial models (Anderson, 2001b; Anderson *et al.*, 2008). Pairwise tests were used to investigate any significant factors and interactions; this was done within the PERMANOVA routine on repeat routines (Anderson *et al.*, 2008).

The effect of restoration treatment was investigated using Generalised Linear Mixed Models (GLMM) in R, (R Core Team, 2015). The effect of treatment was investigated at each site separately to avoid data transformations given the larger than expected variation in *M. modiolus* abundance between sites (O'Hara and Kotze, 2010). GLMMs were used rather than a PERMANOVA routine to test for differences due to treatment as they allowed for more accurate modelling of the data on a site by site basis. Initially a zero inflated Generalised Linear Model (GLM) using the pscl package (Zeileis *et al.*, 2008), and a standard GLM using the MASS package (Venables and Ripley, 2002), were fitted to the count data at each site. Negative binomial regression were used in both models, with treatment as a fixed factor. The two models from each site were compared using Akaike's Information Criterion (AIC) and a Likelihood Ratio test (LRT), to assess the effective of accounting for the presence of zero counts in the model. Following this a GLMM using the MASS package (Venables and Ripley, 2002), was used to model the effect of treatment on the abundance of *M. modiolus.* The model was initially fitted using Poisson regression, to account for the non-normal count data (Bolker *et al.*, 2009; O'Hara and Kotze, 2010). To account for temporal pseudoreplication created by samples being collected in different years, year was used as a random error term in the model (Millar and Anderson, 2004). If the fitted model exhibited overdispersion it was refitted using negative binominal regression with the glmmADMB package (Fournier *et al.*, 2012). The null hypotheses of no treatment effect, was tested by comparing the original model

to a reduced model, with a Wald chi-squared test (Crawley, 2007). If the effect of treatment proved significant, pair wise analysis of the different treatment types were conducted using the general linear hypothesis routine within the multcomp package (Hothorn *et al.*, 2008).

5.3. Results

Count data

All 30 experimental units were recovered from Loch Creran in 2013 and 2014 and all 30 units were recovered from the Karlsruhe site in 2013, but 1 sample was lost in 2014. The Lleyn Peninsula site proved more challenging due to the exposed location and strong tidal flow. As a result, 23 samples were recovered in 2013 and 28 samples in 2014.

The examination of the shell hinge line characteristics from the 40-50 random individuals at each site firstly confirmed the identity of all the mussels from the Lleyn Peninsula as *M. modiolus*. However only 20 % of the mussels from the Karlsruhe site, and 10 % of the mussels from the Loch Creran site were identified as *M. modiolus*. The percentages of *M. modiolus* from these two sites were then used to convert the original counts of suspected *M. modiolus* (identified using external characteristics) to actual numbers of *M. modiolus* per experimental unit.

There was a marked difference of nearly an order of magnitude between the average numbers of juvenile *M. modiolus* found in samples between sites. The Karlsruhe site had the lowest average abundance of 1.03 juvenile *M. modiolus* per sample whereas Loch Creran had an average of 1.15 and the Lleyn Peninsula site had an average of 70. Both the Creran and Karlsruhe sites had more juvenile *M. modiolus* in 2014, whereas the Lleyn Peninsula site had fewer *M. modiolus* in 2014 (Figure 5.4).

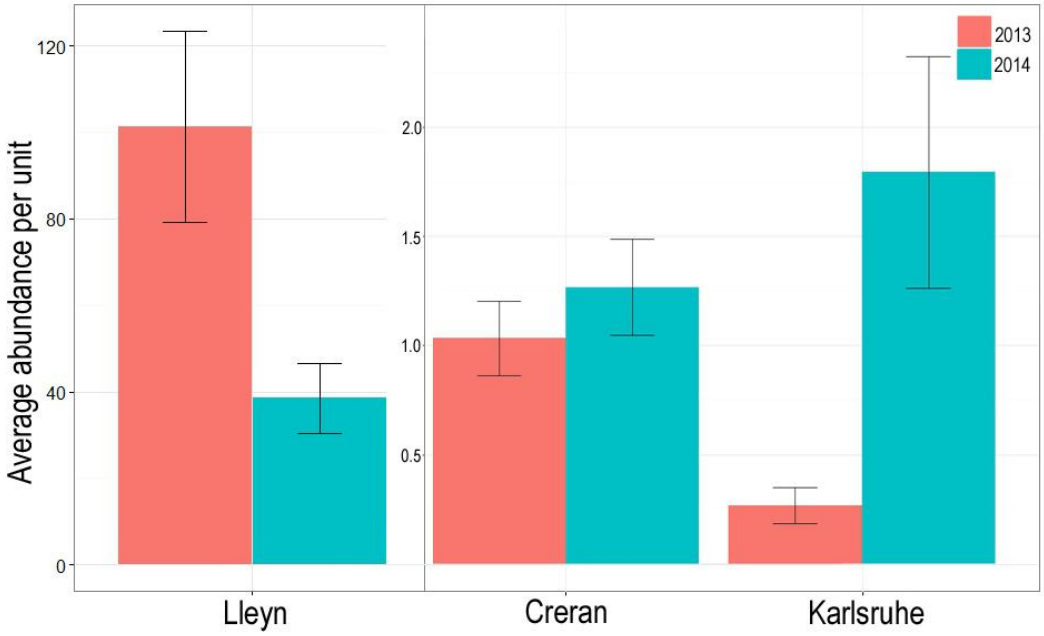

Figure 5.4. Average abundance of *M. modiolus* using the corrected counts per sample from the 3 sites, in 2013 and 2014. Error bars indicate standard error around the mean.

Using PERMANOVA a two-way crossed model found that both Year and Site along with the interaction between Year and Site were significant (Table 5.2). Pairwise tests within PERMANOVA found significant differences between the Lleyn Peninsula site and the Karlsruhe site (T = 6.81, P = 0.0001), and between the Lleyn Peninsula site and the Loch Creran site (T = 6.83, P = 0.0001). However there was no significant difference between the Loch Creran site and the Karlsruhe site (T = 1.95, P = 0.052). The interaction term between site and year found the effect of year was significant at the Lleyn Peninsula site (T = 2.86; P = 0.003), and the Karlsruhe site (T = 3.72; P = 0.004), but was not at the Loch Creran site (T = 0.84; P = 0.478).

Table 5.2. Results from PERMANOVA, using Euclidean distance to test for Site and Year effects and the interaction between the two. The test statistic pseudo F value and P are calculated using 9999 permutations with n=170.

Source	df	SS	MS	Pseudo-F	P
Site	2	168810	84403	46.48	0.0001
Year	1	17720	17720	9.7592	0.0007
Site*Year	2	35629	17815	9.811	0.0001
Residual	164	297790	1815.8		
Total	169	502720			

The decrease in the abundance of *M. modiolus* at the Lleyn Peninsula site in 2014 was not equal across all treatments (Figure 5.5). Reductions in shell and crushed treatments were greatest in 2014, with crushed shell treatments averaging 300.5 *M. modiolus* in 2013, but only 79.6 in 2014. These declines are likely to be caused by increased siltation in 2014, and are discussed later. The outlying crushed shell sample in 2014 (Figure 5.5), shows that this decline in *M. modiolus* was not seen in all samples.

As a result of these data the effect of restoration treatment was tested on individual site datasets. This allowed each dataset to be analysed without applying a transformation, and fitting individual models allowed over dispersion and the effect of zero counts to be assessed on a site by site basis.

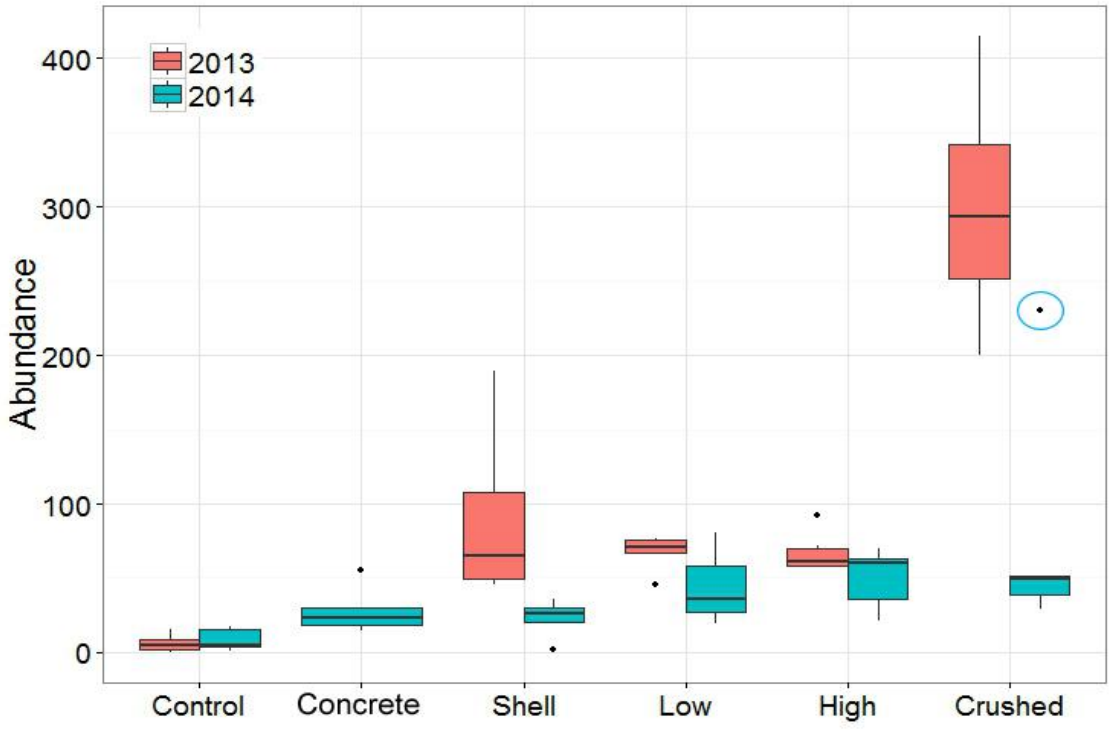

Figure 5.5. Abundance of *M. modiolus* from the Lleyn Peninsula site in 2013 and 2014. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range. The blue circle highlights the significant outlying crushed shell sample in 2014.

At the Lleyn Peninsula site there were no zero count samples, so a zero inflated model was not considered. A Generalised Linear Mixed Model (GLMM) with year as a categorical random value, using negative binomial regression to account for over dispersion found a significant difference due to restoration treatment (LRT = 49.17: P = <0.001: Figure 5.6). Pairwise tests found significantly fewer *M. modiolus* in the Control and Concrete samples and significantly more *M. modiolus* in the Crushed shell samples, compared to the other treatments. There was also no significant difference between the 3 whole scallop shell treatments (Table 5.3).

At the Loch Creran site there were 21 samples with zero counts, however a zero inflated model had a higher AIC score and was not significantly different from a standard model (ChiSq = 0.326; P = 0.99), so was not used. A GLMM with year as a categorical random value, using Poisson regression found significant differences due to restoration treatment (LRT=60.41: P = <0.001: Figure 5.6). Pairwise tests mirrored those seen at the Lleyn Peninsula site, except there was no significant difference between Control and Concrete samples (Table 5.3).

At the Karlsruhe site there were 33 samples with zero counts, however a zero inflated model had a higher AIC score and was not significantly different from a standard model (ChiSq=0.1322; P=1) so was not used. A GLMM using Poisson regression, with year as categorical random factor found significant differences due to restoration treatment (LRT= 43.786: P = <0.001: Figure 5.6). Pairwise tests found the Crushed shell samples had significantly more *M. modiolus* than Concrete, Shell or Low seeded samples. All other samples were not significant difference to each other (Table 5.3).

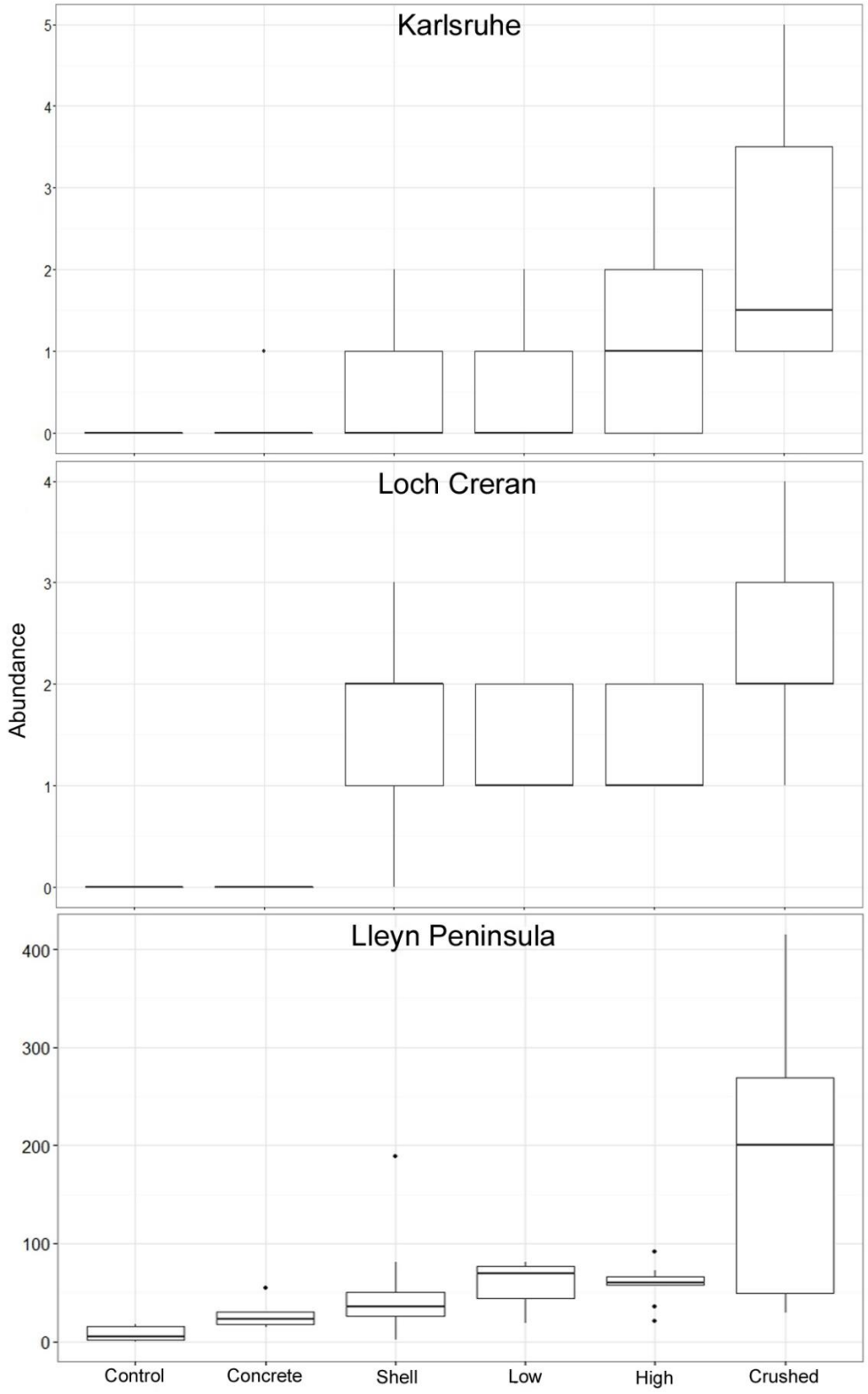

Figure 5.6. Abundance of *M. modiolus* per treatment from each of the 3 sites, from samples collected in 2013 and 2014. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range.

Table 5.3. Pairwise comparison matrix following general linear mixed models, for all three sites using data from both years. Bold values highlight significant results when P <0.05. Number of samples (n) is given in the left hand column.

Lleyn Peninsula						
	Control	Concrete	Shell	Low	High	Crushed
Control (n=9)	0					
Concrete (n=5)	**0.001**	0				
Shell (n=9)	**0.001**	**0.001**	0			
Low (n=8)	**0.001**	**0.001**	0.999	0		
High (n=11)	**0.001**	**0.001**	0.993	1.000	0	
Crushed (n=9)	**0.001**	**0.001**	**0.001**	**0.006**	**0.018**	0

Loch Creran						
	Control	Concrete	Shell	Low	High	Crushed
Control (n=10)	0					
Concrete (n=10)	1	0				
Shell (n=10)	**0.001**	**0.001**	0			
Low (n=10)	**0.001**	**0.001**	0.977	0		
High (n=10)	**0.001**	**0.001**	0.977	1	0	
Crushed (n=10)	**0.001**	**0.001**	**0.011**	**0.001**	**0.001**	0

Karlsruhe						
	Control	Concrete	Shell	Low	High	Crushed
Control (n=10)	0					
Concrete (n=10)	1	0				
Shell (n=10)	1	0.847	0			
Low (n=10)	1	0.847	1	0		
High (n=9)	1	0.102	0.400	0.400	0	
Crushed (n=10)	1	**0.009**	**0.022**	**0.022**	0.663	0

Length frequency data

Due to the very low abundances of juvenile *M. modiolus* recorded from the Karlsruhe and Loch Creran sites, only data from Lleyn Peninsula site were analysed. The length frequency of *M. modiolus* from the Lleyn Peninsula site are displayed by year in Figure 5.7. Of the 3499 mussels recorded, 57 % were less than 3mm in length.

Growth curves for the *M. modiolus* population at the Lleyn Peninsula reef had been calculated using acetate peels of sectioned shells and fitting of a Von Bertalanffy growth curve (Brash, 2014), using the methods outlined in Anwar *et al.*, (1990). Using these values, it was possible to estimate that *M. modiolus* would on average reach 3.88 mm in length after 1 year and 10.0 mm after 2 years (Figure 5.7). After 1 year 19 % of the mussels recorded were longer than 3.88 mm, with 3 mussels over 20 mm in length. After

2 years 11% of the recorded mussels were larger than 10.0mm, with 6 mussels greater than 20mm (Figure 5.7).

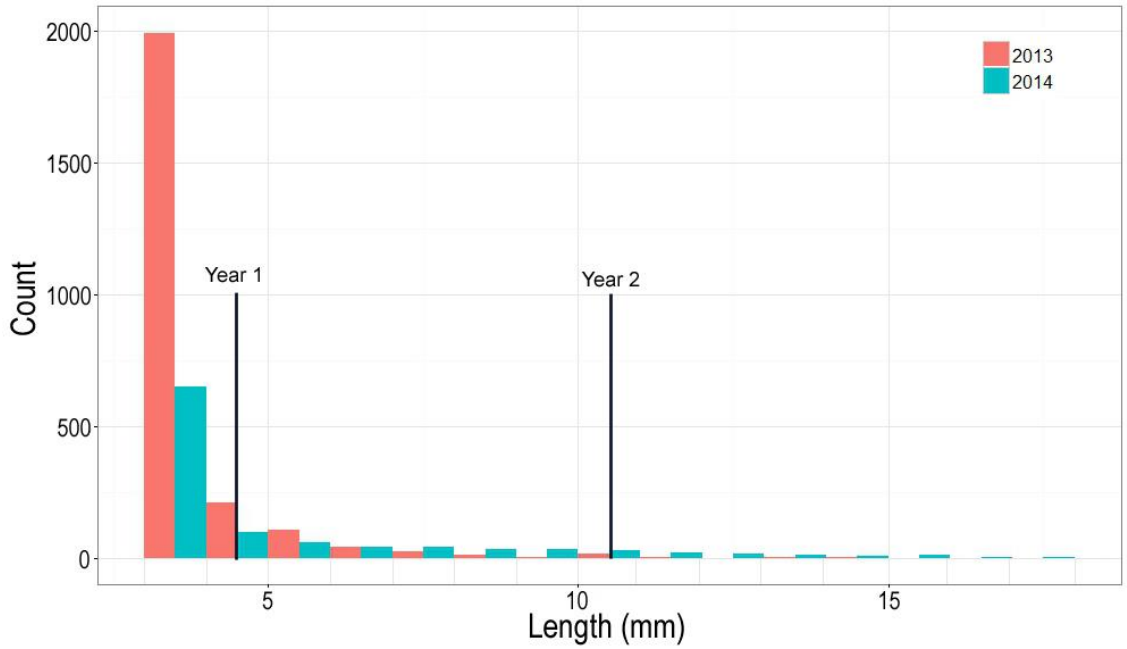

Figure 5.7. Length frequency of *M. modiolus* recorded from the Lleyn Peninsula site, expected length for mussels from one-year-old and two years old are shown using data from (Brash, 2014).

Spat collector data

The data collected from the historical spat collectors from the Lleyn Peninsula reef were of variable quality. All years had 6 replicate spat collectors with the exception of 2009 which only had 3. The condition of preserved spat collectors varied greatly between years and replicates. Some were fairly clean and intact, whereas others were damaged and clogged with sediment. The results obtained from these samples along with the additional 3 spat collectors recovered from Loch Creran in 2013 are shown in Figure 5.8. The results show a peak in recruitment in 2007 at Lleyn Peninsula reef, as well as the Loch Creran reef in 2014 had fewer *M. modiolus* than any of the 5 years of data from the Lleyn Peninsula reef.

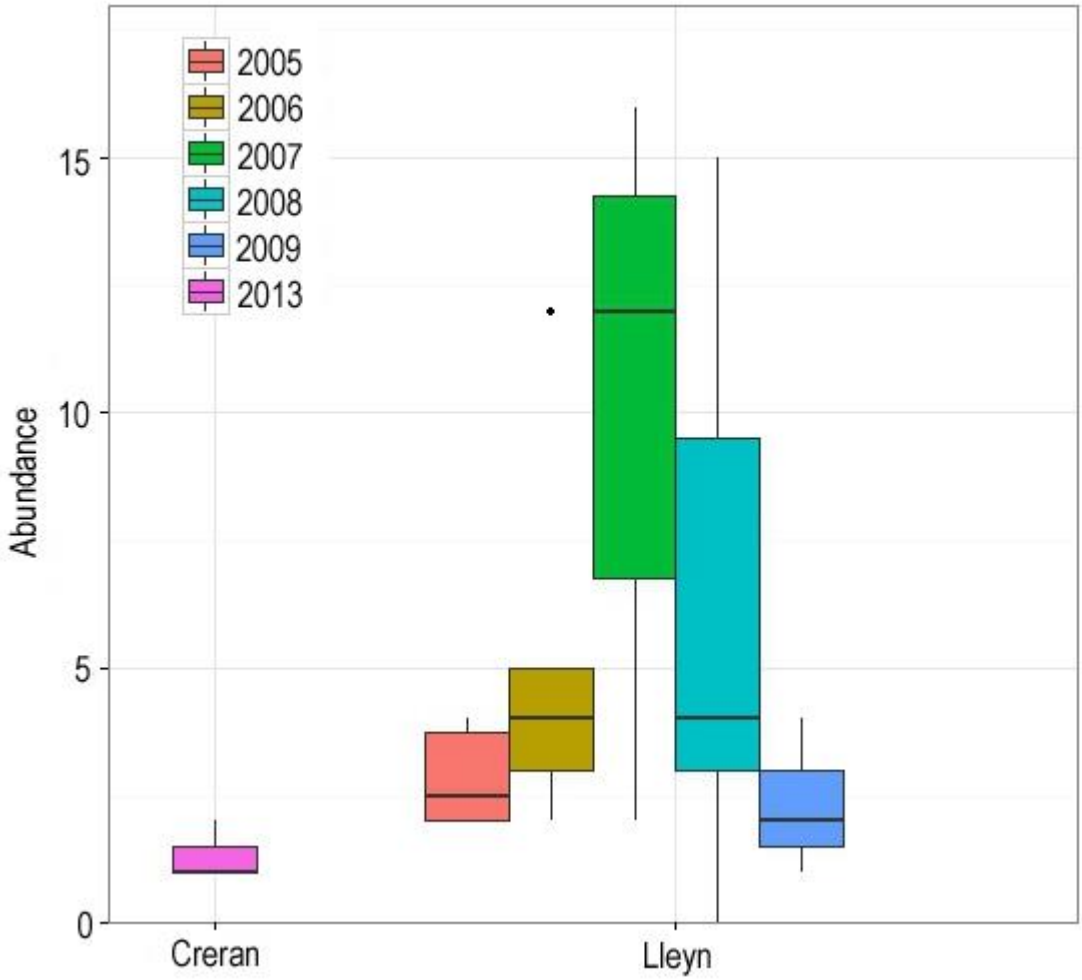

Figure 5.8. Abundance of juvenile *M. modiolus* from spat collectors at the Lleyn peninsula and Loch Creran reefs. Box plots represent inter-quartile range, median, maximum and minimum values or points representing outliers if greater than 1.5 * the inter quartile range.

Environmental data

The current meter was successfully deployed and recovered at the 3 study sites during 2013 and 2014. The maximum current speed at the Lleyn Peninsula site was 7.5 times that recoded at the Karlsruhe site. The difference between the maximum current speed at Karlsruhe site and the Loch Creran site was less than expected at only 0.05 m/s (Table 5.4). To standardise between spring and neap tidal variations, current speed data from the largest tidal range recorded at each site were plotted against time before and after high water at each site to visualise the difference in tidal flow between the three sites (Figure 5.9).

Table 5.4. Summary parameters recorded by the MIDAS ECM current meter deployed at the three study sites.

Site	Sampling dates	Maximum tidal range (m)	Current speed (m/s)		
			Max	Min	Mean
Karlsruhe	04.05.14 - 09.05.14	1.88	0.134	0.002	0.029
Loch Creran	10.12.14 - 31.12.13	3.48	0.083	0.000	0.013
Lleyn Peninsula	30.06.14 - 05.07.14	3.89	0.632	0.003	0.244

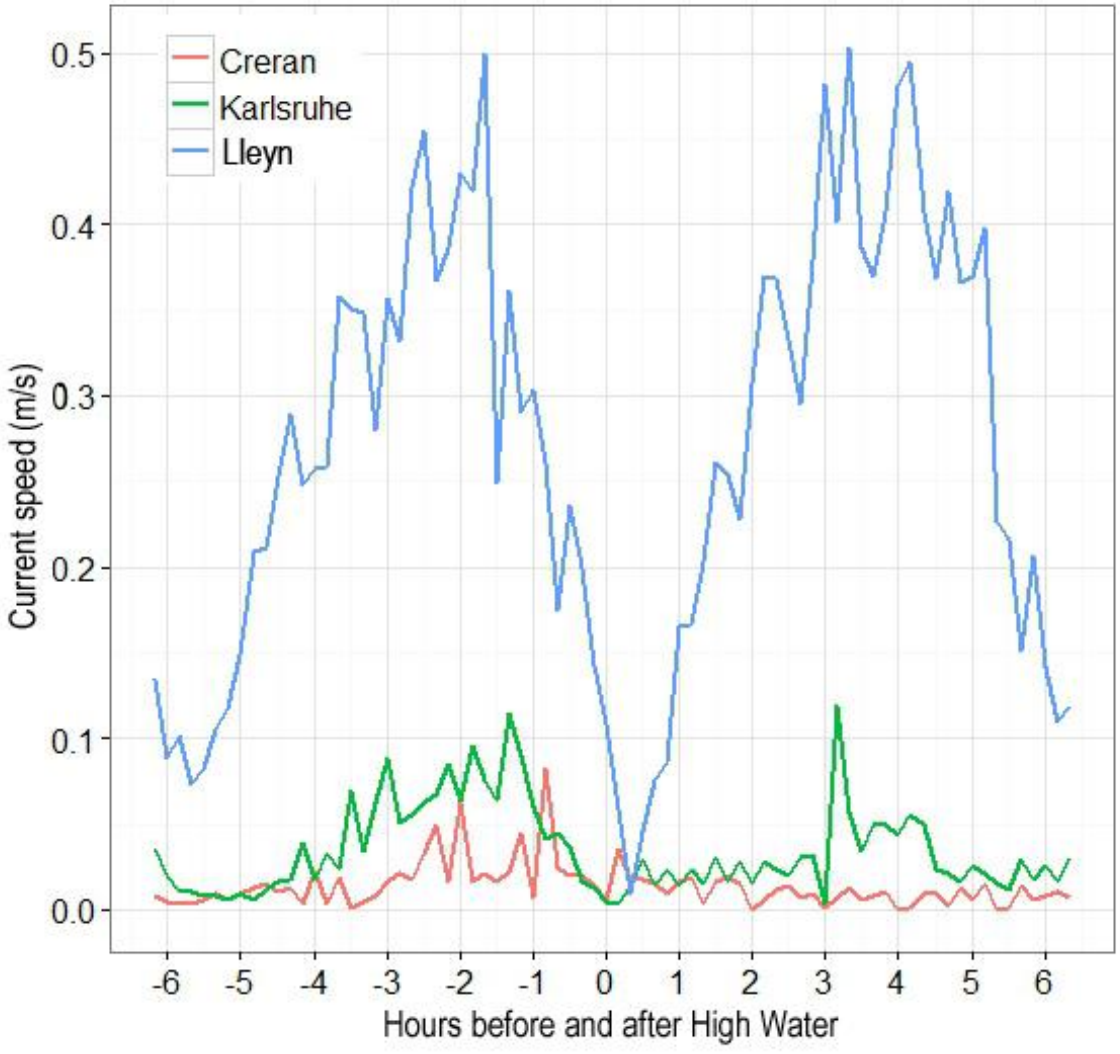

Figure 5.9. Tidal flow in meters per second, before and after high water at the three study sites. Data taken from the tidal cycle with the greatest range recorded during the deployment of the current meter at each site.

5.4. Discussion

Substrate preference

The presented results (Figure 5.6, Table 5.3), support the initial alternate hypothesis that one of the tested treatments would promote *M. modiolus* recruitment. The restoration of *M. modiolus* reefs through the provision of habitat, aims to increase natural recruitment and increase post settlement survival, and has been cited as the most likely restoration technique to succeed (Roberts *et al.*, 2011). The majority of studies utilising habitat provision to restore shellfish reefs have however only used a single substrate type (Brumbaugh and Coen, 2009; Schulte *et al.*, 2009).

Scallop shell was assumed would be the most successful restoration material, since it has been successfully used in other shellfish restoration projects (Luckenbach *et al.*, 2005; Roberts *et al.*, 2011). The use of *M. modiolus* shells might have been preferable, as the structures created would have had a greater resemblance to a natural reef. This then could be seen as true restoration as opposed to rehabilitation as defined by Hawkins *et al.* (2002). *M. modiolus* however are not commercially harvested or cultured in the UK, so large quantities of *M. modiolus* shell are not readily available from shellfish processing. It was for this reason they were not considered as a restoration material. Crushed concrete was chosen as it was readily available and would provide a textured surface, providing an increased surface area for settling larvae compared to a smooth substrate. Crushed concrete is also very cheap (<£5 per tonne) and very easy to source making it practical for restoration projects. Concrete has been used in shellfish restoration projects previously, but is usually deemed undesirable due to its appearance and permanence in the environment (Mann and Powell, 2007). These data show that crushed concrete was the poorest performing restoration treatment, and levels of recruitment were only detectable above the control treatment at the Lleyn Peninsula site (Figure 5.6; Table 5.3).

The crushed shell treatment had significantly more *M. modiolus* than any other treatment across all sites, with the exception of the High treatment at the Karlsruhe site (Figure 5.6; Table 5.3). The success of crushed shell can possibly be attributed to the complex nature of the substrate and the increased settlement surfaces it affords. Increased substrate complexity also has been seen to increase the settlement and survival of juvenile oysters, in addition to providing refuge for other species that inhabit the reef (Bartol and Mann, 1997; Cranfield *et al.*, 2004; Luckenbach *et al.*, 2005; Brumbaugh *et al.*, 2006; Fariñas Franco and Roberts, 2014). One of the few studies to test the effect of two different shell types on the restoration of *Crassostrea virginica*, supports these findings. Nestlerode *et*

al., (2007) recorded an oyster shell substrate as having more juvenile oysters than a surf clam shell substrate, and attributed this to a more complex structure and increased interstitial space. The study also highlights that differing substrates may create differing predation pressures. Smaller interstitial spaces such as those the rushed shell treatment creates may exclude larger predators, such as fish and large decapods, but conversely may create refuge for small predators such as portunid crabs. Several studies have stated that the rates of predation on young *M. modiolus* are high, although no attempts have been made to quantify this (Anwar *et al.*, 1990; Holt *et al.*, 1998; Mair *et al.*, 2000). This predation is thought to be primarily due to crabs and starfish, which are also seen as major predators of *Mytilus edulis* (Holt *et al.*, 1998).

Stock enhancement

The results of this study support the initial null hypothesis that the use of translocated adult mussels to enhance the recruitment of *M. modiolus* would be ineffective. Across all sites there was no significant difference between the abundances of *M. modiolus* in the seeded or un-seeded scallop shell treatments (Figure 5.6; Table 5.3).

These results however contradict the findings of the *M. modiolus* restoration trials in Strangford Lough (Roberts *et al.*, 2011; Fariñas Franco and Roberts, 2014). Roberts *et al.,* (2011) deployed clumps of adult mussels along with dead *M. modiolus* shell and scallop shell in the north and south basin of Strangford Lough. The study found spat only settled onto clumps of relocated adult mussels at the more intact reef in the south basin (Roberts *et al.*, 2011). This suggests a gregarious nature to *M. modiolus* larvae settlement. Gregarious settlement behaviour of reef-forming bivalves has been documented in several oyster species and *Mytilus edulis* (Bayne, 1969; McGrath *et al.*, 1988; Zimmer-Faust and Tamburri, 1994). Juvenile *M. modiolus* are commonly associated with byssus threads of adult mussels and are commonly referred to as gregarious (Wilson, 1977; Rees *et al.*, 2008; Roberts *et al.*, 2011). However, this association between juveniles and adults has not been linked to a gregarious settlement cue. It has also been suggested that juvenile *M. modiolus* living within the byssus threads of larger adults have a much greater chance of survival, as they are shielded from predation (Holt *et al.*, 1998). It is feasible, therefore, that protection from predation rather than a gregarious cue is creating increased recruitment in the byssus threads of adults (Nestlerode *et al.*, 2007).

A recent study by Carroll *et al.* (2015) however supports the findings of this study. Carroll *et al.* (2015) tested the recruitment of *Crassostrea virginica* in response to settlement cues and predation. They found that neither live adults nor chemical cues

enhanced settlement, which was contrary to their own expectation and previous studies (Zimmer-Faust and Tamburri, 1994; Smee *et al.*, 2013). They attributed these results to post settlement predation, as recruitment rates were 50% higher in plots protected from predation compared to open plots. Even within caged plots chemical cues did not enhance settlement. They suggest that larval supply and subsequent settlement are probably not limiting recruitment within their study area, and that post settlement mortality is the main effect controlling recruitment (Carroll *et al.*, 2015).

The three sites used in this study were not expected to have a limited larval supply, due to the relatively long planktonic larval stage of *M. modiolus*, with laboratory based studies indicate settlement taking between 19 and 38 days after fertilization (Ockelmann, 1965; Schweinitz and Lutz, 1976; Roberts *et al.*, 2011). All three study sites were located within existing reef areas, and a recent model of larval dispersion in Strangford Lough predicted the highest densities of larvae would be recorded within 500m of their parent reef (Elsäßer *et al.*, 2013). Therefore, post settlement predation may have had a stronger pressure on the abundance of *M. modiolus* recorded in this study, than a gregarious cue created by the translocated adults.

A study into the restoration of *M. modiolus* in Strangford Lough tested the use of translocated adults to seed artifical reefs constructed out of scallop shell (Fariñas Franco *et al.*, 2013; Fariñas Franco and Roberts, 2014). The authors found that after 1 year, artificial reefs with translocated adults had significantly richer communities and enhanced abundances of juvenile *M. modiolus* in comparison to unseeded reefs. Roberts *et al.*, (2011) also reported that spat collectors deployed in Strangford Lough away from clumps of adult *M. modiolus*, recorded negligible recruitment. This may indicate that larval supply is limited within Strangford Lough, with the few *M. modiolus* larvae produced settling within the byssus threads close to their release. Although judging larval supply in Strangford Lough is hard to judge with any confidence as recruitment is highly variable between locations and years (Roberts *et al.*, 2004, 2011; Fariñas Franco *et al.*, 2013). A recent study by Gormley *et al.* (2015) also found genetic evidence for the limited exchange of larvae between Strangford Lough and other *M. modiolus* reefs in the Irish Sea. Therefore, the juvenile *M. modiolus* recorded on the seeded reefs in the study by Fariñas Franco *et al.*, (2013) are likely the progeny of the translocated mussels. The fragmentation of the reef within Strangford Lough due to anthropogenic impacts, has now split the original metapopulation into isolated subpopulations, which may or may not be self-sustaining (Strain *et al.*, 2012; Elsäßer *et al.*, 2013).

The assessment of whether restoration sites are potential "sink" or "source" habitats, and the implications for restoration have become increasingly well documented (Caddy and Defeo, 2003; Mann and Powell, 2007; Lipcius *et al.*, 2008; Elsäßer *et al.*, 2013). Sinks are defined as habitats where the remaining spawning stock reproduction is unable to match or exceed post settlement mortality. A "source" habitat is defined as self-sustaining and able to become a larval source for nearby reefs (Lipcius *et al.*, 2008). The restoration of shellfish reefs in areas which act as sinks, commonly referred to as having a recruitment bottleneck (Caddy and Defeo, 2003). Stock enhancement through translocation or hatchery schemes, has been the primary method of overcoming this (Caddy and Defeo, 2003; Brumbaugh *et al.*, 2006). However, stock enhancement may not be able to achieve restoration objectives such as a self-sustaining population and has a poor track record. Therefore restoration goals are probably more attainable in locations with a natural larval supply and connectivity to other populations (Wallace *et al.*, 2002; Mann and Powell, 2007; Geraldi *et al.*, 2013).

Roberts *et al.*, (2011) have shown hatchery based stock enhancement for *M. modiolus* to currently not be appropriate. Brood stock from within Strangford Lough was used to cultivate spat in an aquaculture facility, and although the group managed to successfully produce larvae, only 4 then developed into the settled pediveliger stage. These pediveligers were only observed in the matrix created by brood stock mussels. It was estimated that to produce spat at 10mm length would take between 1 and 2 years. It has been reported that mortality of *M. modiolus* due to predation substantially declines once they reach 50mm in length (Seed and Brown, 1978; Anwar *et al.*, 1990). Given that post settlement mortality is so high in juvenile *M. modiolus*, it would be counterproductive to deploy mussels smaller than this. Using growth rates from Anwar *et al.* (1990) and Brash (2014), it would take between 6 and 11 years to achieve a length of 50mm (Figure 5.10). There might however be substantial variations in the relationship between predation rates and mussel length, at different locations. For example in areas of high tidal flow such as at Creagan in Scotland, predator size is restricted to those small enough to find shelter during the stronger periods of the tidal cycle (Comely, 1978). Roberts *et al.*, (2011) calculated the cost of running a *M. modiolus* hatchery at £6,500 per month, which when combined with poor survival rates, specific settlement requirements and a lengthy grow-out phase makes this method currently uneconomical.

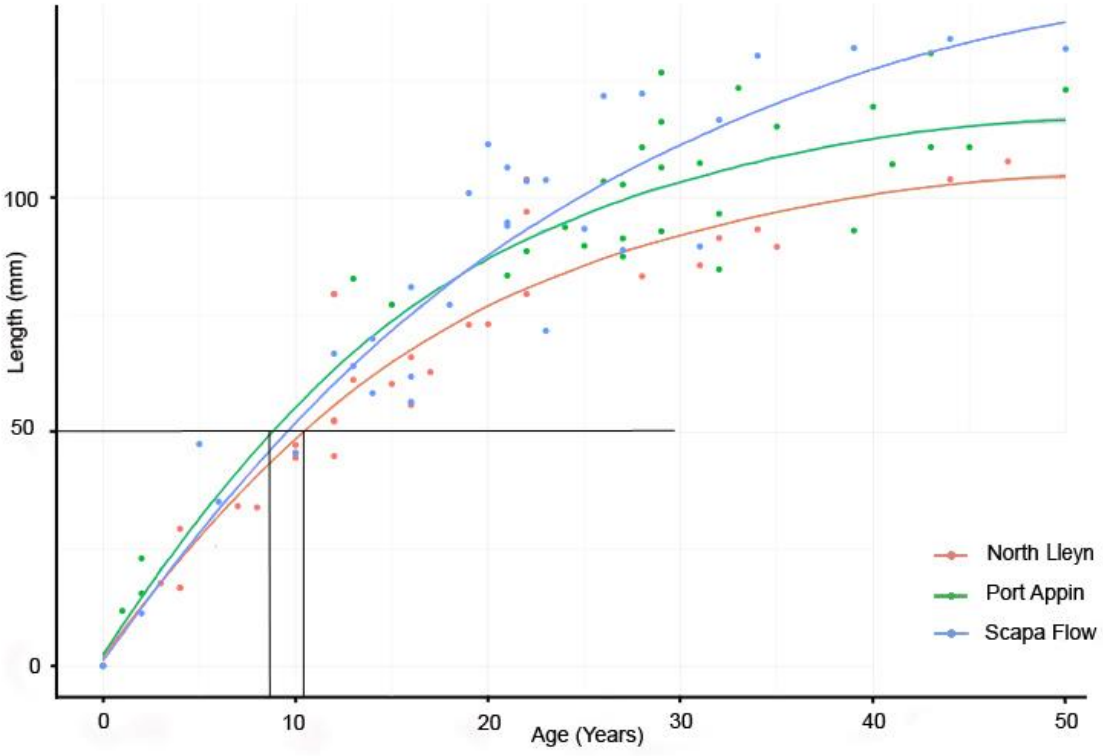

Figure 5.10. Taken from (Brash, 2014) von Bertalanffy growth curves (coloured lines) for three *M. modiolus* populations: Scapa Flow (Karlsruhe), Port Appin (Scotland) and Lleyn Peninsula reef. Point data represent the age and length of the mussels used to calculate the growth curves. Bold black lines identify the age range of mussels at 50mm in length.

While the use of large numbers of translocated adult mussels is likely to be impractical (McCay *et al.*, 2003), it has been suggested in the UK Biodiversity Action Plan that the replanting of *M. modiolus* from healthy areas to damaged areas may help the recovery of a reef. A feasibility study for the restoration of marine bivalve communities was conducted by McCay *et al.*, (2003). The study found that there is a great deal of uncertainty with a project of this kind. In particular the quantity of organisms needed to restore an area, when taking into account losses caused by environmental variables and the translocation process.

The results of this study found *M. modiolus* to be resilient to the translocation process. The mussels used to seed the High and Low treatments were necessarily treated roughly. After collection they were cleaned of epifauna and stored in mesh sacks suspended off piers and pontoons for several days before being placed into restoration units. They were also aerially exposed for several hours, before deployment. This allowed realistic mortality rates to be determined for translocated mussels. On larger restoration projects, keeping large numbers of mussels permanently submerged during transit would not be realistic. *M. modiolus* are found on intertidal shores (Wilson, 1977), however they are not

able to retain water in their mantle cavity. This implies they are not as well adapted to aerial exposure, as littoral species such as *Mytilus edulis* (Coleman and Trueman, 1971; Wilson, 1977). Mortality rates for the translocated *M. modiolus* were calculated during the processing of restoration units by counting the proportion of live and dead adult *M. modiolus* encountered. These results showed that on average across the three sites 90% of the translocated mussels survived the two-year deployment. Low mortality rates were also reported for translocated mussels used in the construction of the artificial reef in Strangford Lough (Fariñas Franco *et al.*, 2013). However the effects of aerial exposure on the reproductive outputs of *M. modiolus* have not been studied (Griffiths, 1981).

For a restoration project using translocated *M. modiolus* to be successful over a large scale, a self-sustaining source of adult *M. modiolus* would need to be found outside of existing protected areas (OSPAR Commission, 2009b; Pérez *et al.*, 2012). This could either be from other fragmented *M. modiolus* reefs in the surrounding area, or from further afield. Both of these options have significant obstacles. The recovery of damaged reefs is not fully understood and moving large numbers of individual mussels from scattered reefs to a single area rather than allowing for natural recovery may not be the most pragmatic approach (McCay *et al.*, 2003). Translocation of *M. modiolus* from other reefs, possibly from reefs threatened by anthropogenic activities areas may prove to be a feasible option. However the risks from introducing associated non-native species and genetic pollution, needs to be fully understood and possibly mitigated (Manchester and Bullock, 2000; McKay *et al.*, 2005). The morphological and physiological suitability of the translocated stock to the environmental niche of the restoration site would also need to be considered before such a project was undertaken (Pérez *et al.*, 2012; Fariñas Franco *et al.*, 2014).

Site differences

These results showed similar substrate preferences for *M. modiolus* recruitment across the three sites (Figure 5.6). However, the data from the Karlsruhe and Loch Creran sites were dominated by zero counts and extremely low abundances (Figure 5.4). There are substantial differences between *M. modiolus* reefs in the UK ranging from low density low energy, sheltered reefs to open coast, high energy, high density reefs (Holt *et al.*, 1998). The Lleyn Peninsula reef was predicted to have the highest level of recruitment, due to it having higher mussel densities and tidal flow than the other sites. This proved to be true. The Karlsruhe site was also predicted to have higher abundance of recruits than the Loch Creran site for the same reasons, which was not the case. The growth rates and production of bivalves has been seen to increase at higher tidal flows (Wildish and Peer,

1983). Production in high density bivalve reefs is often limited by depletion of food in the boundary layer (Wildish and Kristmanson, 1985). The relationship between increasing current flow and bivalve growth however is complicated by vertical mixing. Increasing current strength and bed rugosity leads to increased turbulent flow alleviating boundary layer depletion. The role of increasing current flow on larvae is not fully understood. Increasing flow aids in the delivery of plankton to surfaces but can also dislodge them (Koehl, 2007). The tidal flow at the Karlsruhe site was lower than modelled tidal flow data had predicted (BERR, 2008), making it more similar to the Loch Creran Site.

There were reduced abundances of *M. modiolus* at the Lleyn Peninsula site in 2014, particularly for crushed shell samples (Figure 5.5). Yearly variations in the recruitment of *M. modiolus* subpopulations have been recorded at this site previously (Figure 5.8: Holt *et al.*, 1998). The 2014 samples should contain the cumulative result of two years of recruitment, so this reduction equates to net loss of *M. modiolus* from 2013 to 2014, rather than just reduced recruitment between 2013 and 2014. The outlying sample for the crushed treatment in Figure 5.5, shows that the reduced abundances in 2014 were not observed for all samples. This makes failed recruitment and increased post settlement predation less likely, as it would have affected all samples. During sample retrieval in 2014 divers observed many of the bags were partially buried in the reef. Additionally during processing the majority of the samples were heavily laden with sediment. The Lleyn Peninsula reef has a complex topography, with ridges running perpendicular to the current comprised of faecal deposits and shell material. These ridges can have an amplitude of 1.2m, and a wavelength of up to 18m (Lindenbaum *et al.*, 2008; Rees *et al.*, 2008). Acoustic sub-bottom profiling revealed that a layer of shell and faecal material extended to over a meter below the surface of the reef (Lindenbaum *et al.*, 2008). Navarro and Thompson, (1997) recorded that an individual *M. modiolus* could produce 40.9 mg dry weight pseudofaeces a day during a spring phytoplankton bloom. Given these high rates of biodeposition, it is plausible that the interstitial spaces within the deployed substrates were beginning to fill with sediment. This could have led to the smothering of recruits which adult *M. modiolus* are known not to tolerate smoothing (Hutchison *et al.*, 2016). This brings into question the use of additional hard substrate at this site, as within a few years anything deployed may be totally buried. Although the adult *M. modiolus* currently inhabiting the Lleyn Peninsula reef, must be adapted to coping with these high rates of biodeposition, given the known persistence of the reef (Lindenbaum *et al.*, 2008).

Juvenile M. modiolus movement

The data also provided evidence for the movement of juvenile *M. modiolus*. The data showed that after 2 years approximately 11% of all mussels recorded were larger than could be expected given the length of substrate deployment. Some of these "oversized mussels" might be attributed to errors and estimations in the growth curves used to calculate length at age (Anwar *et al.*, 1990; Brash, 2014). Some of the oversized mussels however cannot be accounted for in this way, as their length at a given age falls far outside the expected range of values predicted by the growth curves. This means that some juvenile *M. modiolus* may have migrated into the samples from the surrounding seabed. Such movement of juvenile *M. modiolus* does have a precedent, having also been observed in another study (Flyachinskaya and Naumov, 2003). The numbers of *M. modiolus* apparently making such movements were small enough not to influence any restoration project. However, it does provide an insight into how juvenile mussels may move around within a clump of adult mussels to maximise protection and food supply as they grow.

Spat collectors

Spat collectors of various designs are commonly used to assess bivalve recruitment and monitor restoration projects (Peterson *et al.*, 1996; Brumbaugh *et al.*, 2006; Roberts *et al.*, 2011). The results from the spat collector data analysed during this study should be treated with caution. Unfortunately the pan scourer material used was not robust enough to cope with the tidal flow on the Lleyn Peninsula reef (Figure 5.9). As a result the amount of material per spat collector was not identical between replicates or years, so the data should be treated as semi quantitative. Additionally the spat collectors had varying amounts of sediment entrained within them, which resulted in lower abundances in the highly sediment-loaded samples. Generally the spat collectors showed high rates of recruitment on the Lleyn Peninsula reef. This varied over the 5-year deployment period with a possible peak in 2007. It is widely quoted that "recruitment of juveniles is very variable not only seasonally but between years" (Holt *et al.*, 1998). It has also been noted that on some reefs spawning is highly sporadic and may not occur for several years (Holt *et al.*, 1998; OSPAR Commission, 2009b; Halanych *et al.*, 2013). Data on the recruitment of *M. modiolus* from various reefs has traditionally been assessed using size frequency distribution (Comely, 1978; Seed and Brown, 1978; Anwar *et al.*, 1990). However this method fails to separate yearly fluctuation in settlement given the relatively slow growth of the species and requires destructive sampling (Holt *et al.*, 1998). Further work utilising

similar spat collectors outlined by Roberts *et al.* (2011), should be pursued particularly for pre-restoration monitoring. Such monitoring would allow a better identification of source and sink habitats, therefore allowing appropriate restoration objectives to be developed (Brumbaugh *et al.*, 2006; Lipcius *et al.*, 2008).

Conclusions

This work builds on the *M. modiolus* restoration work conducted in Strangford Lough (Roberts *et al.*, 2011; Elsäßer *et al.*, 2013; Fariñas Franco *et al.*, 2013; Fariñas Franco and Roberts, 2014). The results show that within areas of damaged reef where larval supply is not limited, the addition of crushed shell supported the greatest number of *M. modiolus* of any tested substrate after one year. The results also found significant differences in the abundance of *M. modiolus* recruits between the three sites. This suggests that restoration efforts at the Lleyn Peninsula site are far more likely to succeed than at either the Loch Creran or Karlsruhe sites. Whilst the data show that crushed scallop shell supported the greatest number of *M. modiolus* recruits; at restoration sites with high rates of deposition the substrates may be smothered before a *M. modiolus* community is established. Future monitoring of the remaining crushed shell samples at the Lleyn Peninsula site will hopefully increase our understanding in this area.

The results also showed that for the restoration of *M. modiolus* at locations where larval supply is not limited, the use of translocated mussels did not increase recruitment on deployed substrates. It should therefore be avoided as a restoration technique in order to preserve the donor population. In a sink habitat where natural recruitment does not equal or surpass settlement mortality, then translocation of adult mussels may be the only feasible technique in attempting to restore the lost shellfish reef. However this should be seen as a last resort and success is far from assured (Mann and Powell, 2007; Lipcius *et al.*, 2008).

Chapter 6. Identification of juvenile Mytilids from restoration samples using DNA barcoding and shell characteristics

6.1 Introduction

DNA barcoding has become a standard and broadly used genetic technique in the identification of known species and the discovery of undescribed species (Hebert, 2003). The technique uses a 648 base pair region of the mitochondrial cytochrome c oxidase subunit I gene (COI). Previous studies have shown this sequence diverges much more between species than within species (Hebert, 2003). This has led to its adoption as a global bio-identification system, allowing sequences from unknown species to be compared against a database of taxonomically identified specimens (Ratnasingham and Hebert, 2007).

Although there have been many genetic studies on marine molluscs including, biodiversity assessments (Puillandre *et al.*, 2012), species connectivity (Gormley *et al.*, 2015) and species identification (Barco *et al.*, 2016), there have been relatively few DNA barcoding studies and the identification of marine bivalves remains problematic (Layton *et al.*, 2014). Some of these taxonomic problems are linked to molluscs being one of the most diverse marine phyla with more than 50,000 described species (Appeltans *et al.*, 2012), but with only barcodes for 10,950 species as of February 2016 (Ratnasingham and Hebert, 2007). Bivalves can often have complex life cycles with major morphological differences from larval to adult stages in addition to significant phenotypic plasticity (Drent *et al.*, 2004). The majority of the identification literature is based on morphological characteristics of adult specimens, which can therefore make the identification of earlier life stages problematic (Schweinitz and Lutz, 1976; Drent *et al.*, 2004; Marko and Moran, 2009).

Bivalvia represent one of better studied classes within the Phylum Mollusca, with 64 % of the total estimated number of species being described. This compares to just 28-36 % for all classes with the Phylum Mollusca (Appeltans *et al.*, 2012). Whilst adult bivalve specimens are often identifiable using traditional morphological characteristics (Tebble, 1976; Hayward and Ryland, 2003), the identification of juvenile and larval specimens is often problematic (Hare *et al.*, 2000; Liu *et al.*, 2011). In Chapter 5 the focus was to assess the abundances of juvenile *M. modiolus* within different substrates which could be used in a restoration project. The analysis of these samples raised the possibility of the misidentification of juvenile *M. modiolus.* For example *Modiolula phaseolina* and

juvenile *Modiolus modiolus* are indistinguishable from external features and only the internal features allow the separation of these species (Hayward and Ryland, 2003) (Figure 6.1). The prevalence of *M. phaseolina* in *M. modiolus* reefs is also not known, leading to the possibility that misidentification could have a significant effect on the ecological studies of *M. modiolus* reefs due to the over estimation of juvenile abundances. The dissection and cleaning of the shells for accurate identification using internal features is extremely laborious when dealing with hundreds of individuals. Therefore, the ability to identify these juveniles quickly and with a high degree of accuracy is imperative for the ongoing ecological research of *M. modiolus* reefs.

Figure 6.1. Image of *Modiolula phaseolina* (left) and a juvenile *Modiolus modiolus* (right). Mussels approximately 5 mm in length.

Project aims

The aim of the project was to use DNA barcoding to assess the reliability of using external shell characteristics for the identification of juvenile bivalve molluscs from the restoration units in Chapter 5. The secondary aim of the project was to assess the internal hinge line characteristics of these juvenile bivalve molluscs, encase the external characteristics were not robust enough to accurately distinguish externally similar juvenile Mytilidae species, such as *Modiolus modiolus, Mytilus edulis* and *Modiolua phaseolina*.

6.2 Methods

Sample collection

During the collection of restoration units used to assess substrate preferences of *M. modiolus* in Chapter 5, a selection of possible juvenile *M. modiolus* were stored for genetic and morphometric analysis. These mussels were separated during the initial sorting of the substrates from the restoration units following their recovery from the seabed. During this initial sorting the first 20 mussels <1 cm in length which displayed the external shell characteristics of *M. modiolus* were retained. These were then labelled and fixed individually with 100 % ethanol in 2 ml Eppendorf tubes. Twenty mussels were collected from each of the three sites visited in Chapter 5, north of Lleyn Peninsula in Wales, the upper basin of Loch Creran and near the WW1 wreck of the SMS Karlsruhe in Scapa Flow in Scotland (Figure 6.2). All juvenile mussels were collected during 2014. On returning from fieldwork the tissue of each mussel was separated from their shell and re-fixed using fresh 100 % ethanol. This helped mitigate tissue degradation caused by sample dilution from the seawater within the mussel shells.

Figure 6.2. Location of the sites from which juvenile mussels were collected.

DNA extraction, amplification and sequencing

DNA was extracted from small (~25 mg) pieces of the adductor muscle from each of the 60 specimens collected across the three sites. For very small mussels <4 mm in length all available tissue was used for DNA extraction. DNA was extracted using the QIAGEN DNeasy Blood and Tissue kit. Each tissue sample was incubated with 20 µl of proteinase K and 180 µl of ATL buffer at 56°C for 3 hours. After 3 hours the samples were vortexed for 15 seconds and a pre mixed solution of 200 µl of AL buffer and 200 µl of 100 % ethanol was added before being vortexed again. The samples were then pipetted into a DNeasy mini spin column and centrifuged at 6000 x g for 1 min. The flow through was discarded and 200 µl of AW1 buffer was added to the mini spin columns before being centrifuged again at 6000 x g for 1 minute. The flow through again was discarded and the step repeated but using AW2 buffer and centrifuged at 20,000 x g for 3 minutes. Finally 50 µl of elution buffer was added to the mini spin columns and left to incubate at room temperature for 1 minute before being placed in the centrifuge for 1 minute at 6000 x g. An electrophoresis gel was then used to check the quality of the extracted DNA. Following the successful extraction of DNA from the 60 samples, the quantity of DNA in each sample was calculated using a photometer. The samples were then accordingly diluted to give a DNA concentration of 50 ng/µl.

Polymerase Chain Reaction (PCR) was employed using the mitochondrial COI universal primers LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) to generate an amplicon for each specimen. Each reaction used 1 µl of the extracted DNA at 50 ng/µl, with 2 µl of the LCO and HCO primers and 20 µl of High Performance Liquid Chromatography grade water (HPLC) to give a total reaction volume of 25 µl. All reactions used illustra™ PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare UK). The PCR thermal regime consisted of 3 minutes at 95°C, followed by 40 cycles of denaturation at 94°C for 30 seconds, then annealing at 45°C for 30 seconds and extension at 72°C for 1 minute. The reaction was ended with a final extension step at 72°C for 10 minutes.

Following PCR the samples were purified using the PureLink PCR purification kit from Invitrogen. Purification used 50 µl of each PCR product added to 200 µl of binding buffer B3 and pipetted into a PureLink spin column. These were then centrifuged at 10,000 x g for 1 minute, and the flow through discarded. The cleaned PCR product was then eluted from the column by applying 50 µl of elution buffer to the filter in the column, incubation at room temperature for 1 minute and then centrifuging for 2 minutes at 10,000 x g. The

purified product was then sent to Edinburgh Genomics for Sanger sequencing. Bidirectional sequencing was carried out following standard PCR protocol using the BigDye v3.1 Terminator Cycle Sequencing Kit (Thermofisher, UK) on an ABI 3730XL capillary sequencing instrument.

DNA barcoding analysis

The chromatograms of all the returned sequences were checked by eye, with poor quality sequence reads being discarded from further analysis. The remaining sequences were trimmed at each end by eye using Geospiza's Finch TV™. Samples with a good quality forward and reverse sequence were imported and aligned using MEGA v7 (Tamura *et al.*, 2013). This alignment was done by creating a reverse complement of the reverse sequence and aligning it with the forward sequence using ClustalW (Thompson *et al.*, 1994). Where any ambiguities were encountered between the forward and reverse sequence the original chromatograms were studied to locate the source of the ambiguity and correct it. The aligned sequences were then exported as a single contiguous sequence for further analysis.

The trimmed and aligned sequences were then imported into MEGA v7 along with sequences of 5 Mytilidae species from NCBI GenBank. These species were chosen as they represented the species most likely to be encountered in the restoration samples. A sequence of *Arctica islandica* was also included as a species outside the Mytilidae family (Table 6.1). All sequences were then aligned using ClustalW to give a consensus sequence of approximately 450 base pairs. MEGA's model selector was used to find the most appropriate nucleotide substitution model based on Akaike Information Criterion (AIC) values. A maximum likelihood phylogenetic tree was then constructed using the most appropriate model, with bootstrap support (500 replicates) (Tamura *et al.*, 2013).

Table 6.1. Sequences of Mytilidae species from GenBank used as reference samples in the construction of the phylogenetic tree. A sequence of *Arctica islandica* was included to act as an outgroup.

Sample Name	Gen Bank accession number	Sample Location	Reference
Mytilus trossulus	KF644032	Canada	(Layton *et al.*, 2014)
Mytilus galloprovincialis	KC789273	Turkey	(Keskin, 2013)
Mytilus edulis (A)	KR084882	North Sea	(Barco *et al.*, 2016)
Mytilus edulis (B)	KR084911	North Sea	(Barco *et al.*, 2016)
Modiolus barbatus (A)	KR084927	North Sea	(Barco *et al.*, 2016)
Modiolus barbatus (B)	KR084891	North Sea	(Barco *et al.*, 2016)
Modiolus modiolus (A)	KC119339	Iceland	(Halanych *et al.*, 2013)
Modiolus modiolus (B)	KR084900	North Sea	(Barco *et al.*, 2016)
Modiolus modiolus (C)	HM884246	Canada	(Layton *et al.*, 2014)
Artica islandica	KR084887	North Sea	(Barco *et al.*, 2016)

Mussel shell characteristics

The features used to differentiate between different Mytilidae species were examined with the 60 juvenile mussels collected from the restoration experiments. Firstly external features along with the total length of each mussel were recorded. The external features assessed were the position of the umbone being either terminal, as expected for *M. edulis* or subterminal as expected for *M. modiolus* or *M. barbatus* (Figure 6.3; Hayward and Ryland, 2003; Oliver *et al.*, 2010). The presence and shape of any periostracum spines were also noted, as serrated spines should allow differentiation between *M. barbatus* from *M. modiolus.* Additionally the identification literature does not mention the presence of spines on *M. edulis* (Tebble, 1976; Hayward and Ryland, 2003).

Following the external examination the mussels were carefully opened down the ventral margins, before being placed into individual Eppendorf tubes with 20 µl of proteinase K, diluted with HPLC water to ensure complete shell coverage. The samples were then incubated at 56 ^{0}C for 30 minutes, vortexed for 15 seconds and placed back in the incubator for a further 30 minutes. This procedure removed the periostracum and ligament and allowed a clearer identification of the hinge line characteristics. Following incubation the shells were rinsed with 100 % ethanol and air dried, before being examined with a Leica MZ75 dissection microscope. The hinge line characteristics were noted and then photographed using a Leica DC300 camera attached to the microscope.

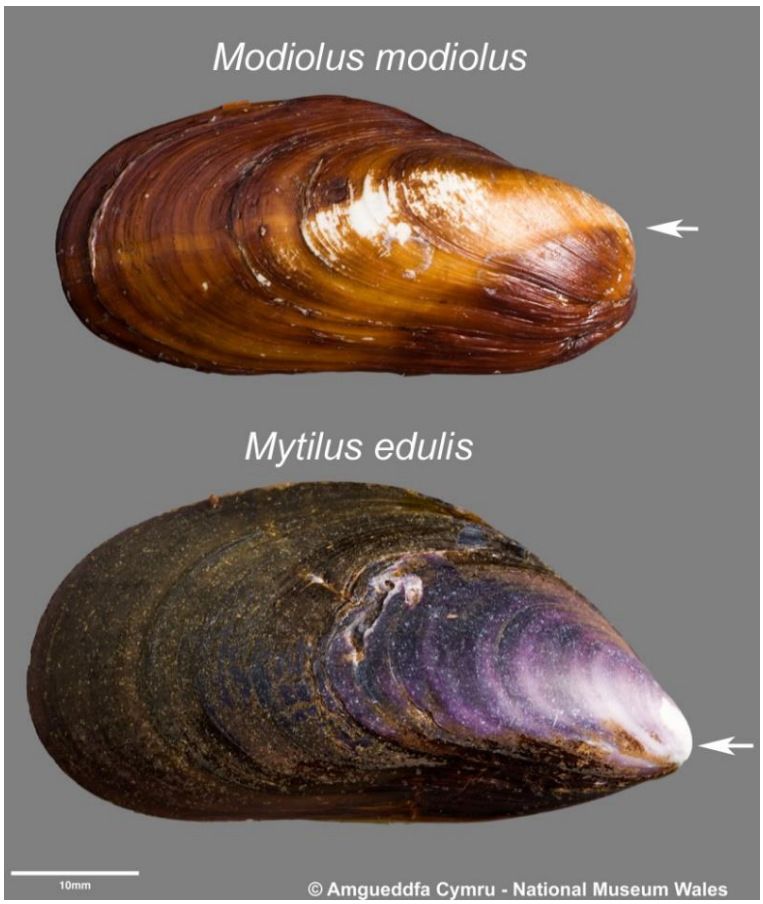

Figure 6.3. External shell characteristics of *M. modiolus* and *M. edulis*. Arrows indicate the position of the umbone being terminal for *M. edulis* and sub-terminal for *M. modiolus*, image courtesy of the National Museum Wales.

6.3 Results

DNA barcoding

The initial review of the returned chromatograms revealed 10 samples with good quality forward and reverse sequences, and a further 2 samples with good quality forward only sequences. The 10 samples with forward and reverse sequences were aligned to give a single contiguous sequence for that sample. These 10 sequences were then aligned with the 2 samples with forward only sequences and the 10 sequences from Genbank (Table 6.1). The evolutionary model selector in MEGA found the Tamura-Nei substitution model including invariant positions to be the best fit for these data (Tamura and Nei, 1993). The constructed maximum likelihood phylogenetic tree using the Tamura-Nei model is shown in Figure 6.4. The tree was rooted onto the *Arctica islandica* sequence as its known to be outside of the Mytilidae family. The phylogenetic tree clearly shows the separation of three clades with 100 % bootstrap support (Figure 6.4). The upper clade contains the 7 samples from Loch Creran, along with the references sequences of *M.*

trossulus, *M. galloprovincialis* and *M. edulis*. The lower two clades diverge after splitting from the upper clade. The first of these clades contains the sample Karlsruhe 20, along with the two reference sequences for *M. barbatus*. Finally, the lower clade contained the remaining 4 samples from the Karlsruhe site and the Lleyn site, along with the *M. modiolus* reference samples. These three clades therefore show that the juvenile mussels recovered from the restoration experiments are likely to represent three distinct species, potential more particular within upper Loch Creran clade. The 12 sequences were also entered into the online BOLD database, and compared against all the sequences with the database (Ratnasingham and Hebert, 2007). This collaborated the results of the phylogenetic tree and returned a minimum agreement of 99 % in the identification of the 7 Creran samples being *M. trossulus*, Karlsruhe 20 being *M. barbatus* and the remaining samples being *M. modiolus* (Table 6.2).

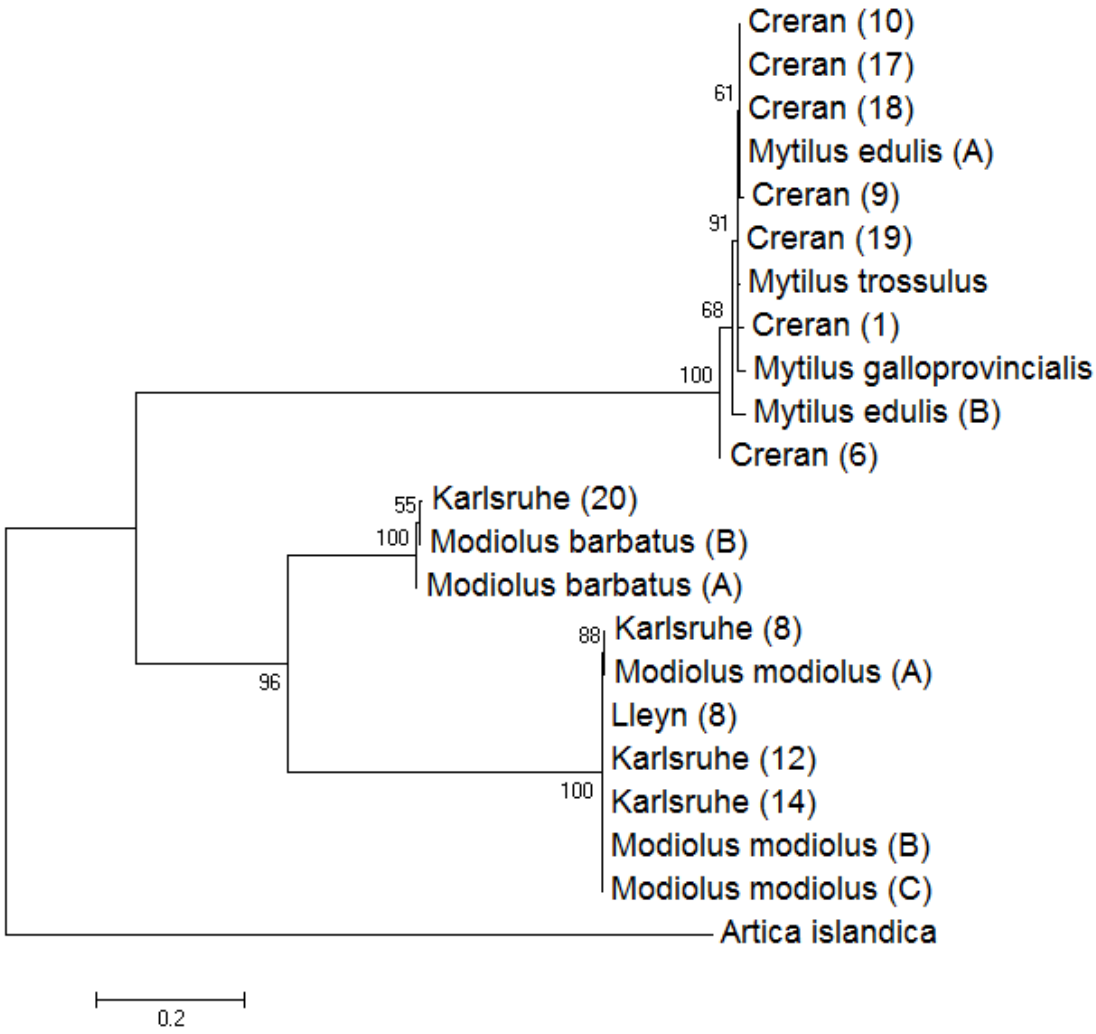

Figure 6.4. Phylogenetic tree constructed using the Maximum Likelihood method based on the Tamura-Nei model. The percentage support for each clade is shown next to each branch. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Table 6.2. Samples used for genetic barcoding along with their shell characteristics and identification using the BOLD database.

Sample	Umbone	Hinge margin	Posterior dorsal crenulations	Anterior ventral crenulations	Length (mm)	BOLD Identification
Creran 1	Sub terminal	Smooth	No	Yes	6.30	*M. trossulus*
Creran 6	Sub terminal	Small groove	No	Yes	8.28	*M. trossulus*
Creran 9	Sub terminal	Small groove	No	Yes	4.87	*M. trossulus*
Creran 10	Sub terminal	Smooth	No	Yes	4.38	*M. trossulus*
Creran 17	Sub terminal	Smooth	No	Yes	5.03	*M. trossulus*
Creran 18	Sub terminal	Smooth	No	Yes	4.25	*M. trossulus*
Creran 19	Sub terminal	Small groove	No	Yes	3.40	*M. trossulus*
Karlsruhe 8	Sub terminal	Groove	No	No	4.93	*M. modiolus*
Karlsruhe 12	Sub terminal	Groove	No	No	7.72	*M. modiolus*
Karlsruhe 14	Sub terminal	Large groove	No	No	6.90	*M. modiolus*
Karlsruhe 20	Sub terminal	Crenulations	Yes	Yes	7.83	*M. barbatus*
Lleyn 8	Sub terminal	Groove	No	No	7.43	*M. modiolus*

Mussel shell characteristics

The positions of the umbones on the 12 juvenile mussels that were successfully barcoded were judged to be all subterminal (Table 6.2). The 12 mussels also all had varying amounts of periostracum spines, although none of the spines were serrated. Therefore from the external shell characteristics all 12 individuals appeared to be the same species, contrary to the barcoding results. The internal shell characteristics were however able to distinguish the 12 juvenile mussels into three distinct clades supporting the DNA barcoding results. The analysis focused on the presence or absence of crenulations on the anterior ventral hinge line adjacent to the umbone and on the posterior dorsal hinge line (Table 6.2).

The 7 Loch Creran mussels all had a series of broad anterior ventral crenulations slightly offset from beneath the umbone which is indicative of *Mytilus* species (Figure 6.5, Image A). Sample Karlsruhe 20 displayed fine posterior dorsal crenulations running approximately a third of the way along the dorsal hinge line, in addition to a small batch of dense anterior ventral crenulations directly beneath the umbone (Figure 6.5, Image B). These features however were not indicative of *M. barbatus* which has no crenulations

(Oliver *et al.*, 2010), although the DNA barcoding identified it as being *M. barbatus.* The presence of these crenulations both beneath the umbone and on posterior dorsal margin are identifying features for *Modiolula phaseolina* and are shown in the identification literature provided by Oliver *et al.* (2010) (Figure 6.5, Image D). The remaining 3 mussels from the Karlsruhe site and the single mussel from the Lleyn site featured no crenulations along the hinge line. This lack of any hinge line crenulations is indicative of either *M. modiolus* or *M. barbatus* (Oliver *et al.*, 2010) (Figure 6.5, Image C).

Studying all 60 juvenile mussels recovered from the three sites, rather than just the samples used in the genetic analysis, allowed an estimation of the abundance of each clade at each of the sites. At the Loch Creran site only 3 individuals displayed *M. modiolus* like features, with 17 displaying *M. trossulus* like features. At the Karlsruhe site 4 samples displayed *M. modiolus* like features with the remaining 16 individuals displaying *M. phaseolina* like features. At the Lleyn peninsula site all 20 individuals displayed features characteristic of *M. modiolus*.

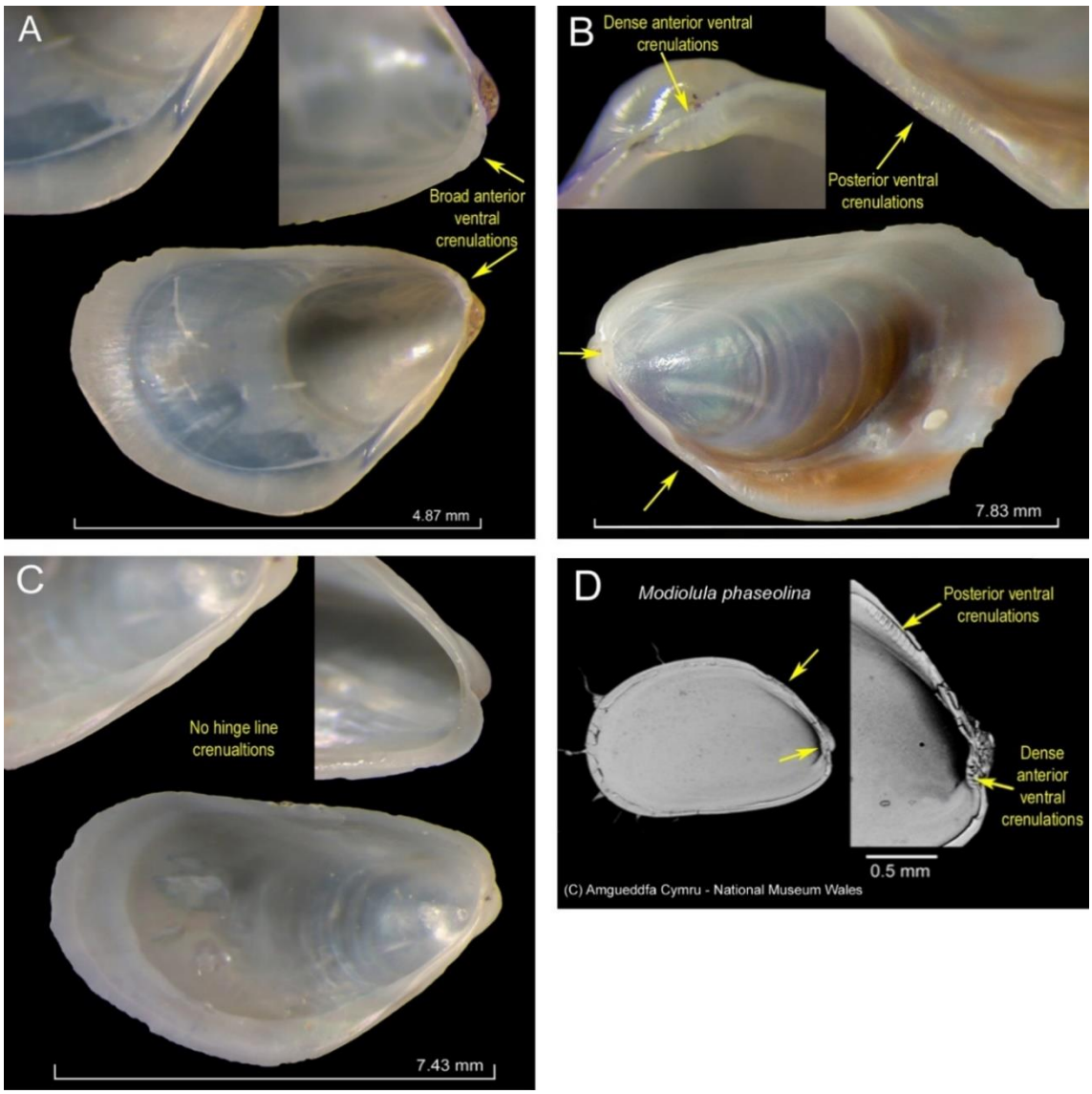

Figure 6.5. Images of the shell characteristics that define the three defined clades from the genetic analysis. Image A is sample Creran 9, *Mytilus trossulus*. Image B is sample Karlsruhe 20, *M. barbatus*. Image C is sample Lleyn 8, *M. modiolus*, Image D is *Modiolula phaseolina* taken from Oliver *et al.* (2010).

6.4 Discussion

Species identification

The project found that external shell characteristics could not be used to reliably differentiate juvenile *M. modiolus* from other Mytilidae species. The DNA barcoding results found the 12 juvenile mussels originally identified as *M. modiolus* using external characteristics, were actually from three separate clades (Figure 6.4). The project did however, show that juvenile *M. modiolus* can be reliably differentiated from the other Mytilidae species recorded in this study by the lack of any crenulations on their hinge line (Figure 6.5). These results allowed the proportion of the *M. modiolus* recovered during Chapter 5 to be identified using hinge line characteristics, so that the results of Chapter 5 could reliably express the abundances of juvenile *M. modiolus* recorded at each of the restoration sites.

The identification of the 7 mussels from Loch Creran as *Mytilus trossulus* was unexpected. The literature clearly separates *Mytilus* species from *Modiolus* species due to presence of terminal umbones (Figure 6.3; Tebble, 1976; Hayward and Ryland, 2003; Oliver *et al.*, 2010). However the umbone positions in all the juvenile mussels in this study were indistinct between species, leading to the conclusion that umbone position does not represent a reliable diagnostic characteristic in juvenile mussels (Table 6.2; Figure 6.5).

The presence and characteristics of periostracum spines also proved an unreliable identification characteristic. The identification literature either makes no mention of periostracum spines on *Mytilus* species (Tebble, 1976; Hayward and Ryland, 2003), or states the absence of spines (Oliver *et al.*, 2010). Despite this all juvenile mussels from Loch Creran displayed periostracum spines before they were removed using proteinase to allow for identification of the hinge line characteristics.

M. barbatus is recorded as being easily separated from *M. modiolus* by the presence of serrated periostracum spines (Hayward and Ryland, 2003; Oliver *et al.*, 2010). However the juvenile mussel Karlsruhe 20 which was identified as *M. barbatus* through DNA barcoding, did not display serrated periostracum spines (Figure 6.6). Inferences about the absence of serrated spines on this single mussel must be viewed with caution, and the lack of serrations may be an environmental response (Drent *et al.*, 2004). Tebble, (1976) also noted that specimens of *M. barbatus* from the lower shore of the English Channel had indistinct serrations and may represent a sub-species.

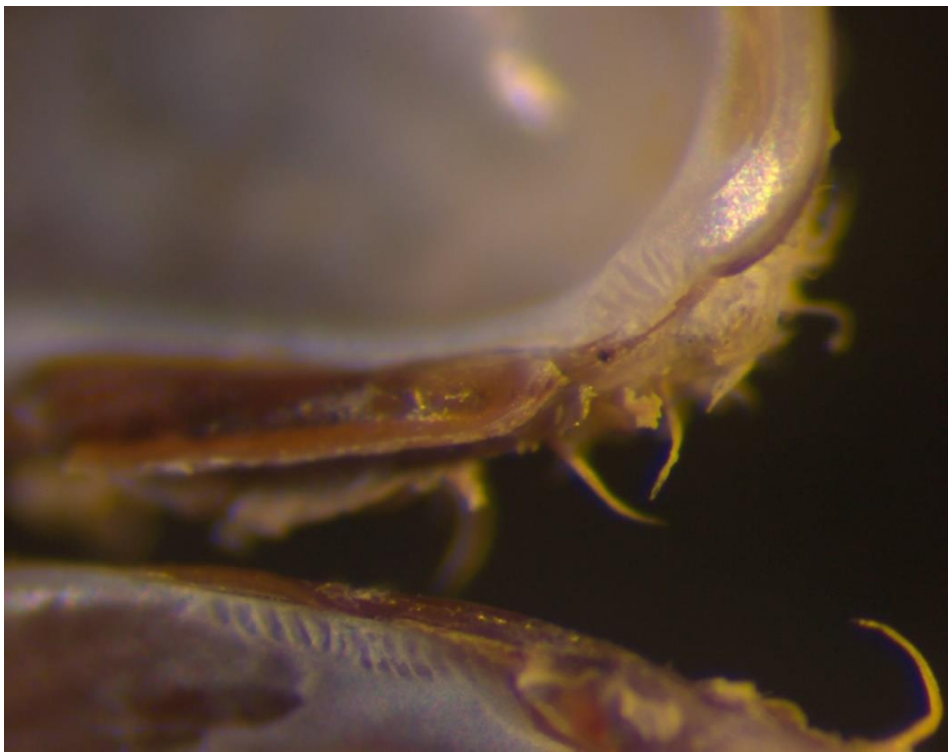

Figure 6.6. Juvenile mussel Karlsruhe 20 identified as *Modiolus barbatus* by DNA barcoding, but displaying unserrated periostracum spines and crenulations beneath the umbone and on posterior ventral margin, features associated with *Modiolula phaseolina*.

One of the principle objectives of this study was to differentiate *M. modiolus* and *Modiolula phaseolina,* which from external characteristics appear indistinguishable (Tebble, 1976; Hayward and Ryland, 2003; Oliver *et al.*, 2010). The mussel Karlsruhe 20 had crenulations beneath the umbone and on the posterior ventral margin (Figure 6.6), which would lead to its identification as *M. phaseolina* using the current literature (Tebble, 1976; Hayward and Ryland, 2003; Oliver *et al.*, 2010). However the DNA barcoding results for this specimen identify it as *M. barbatus* with 99 % certainty. The current literature states however that *M. barbatus* lacks any hinge line crenulations (Tebble, 1976; Hayward and Ryland, 2003; Oliver *et al.*, 2010). This discrepancy is not easily resolved, and would require further work. Unfortunately there are no COI reference sequences for *M. phaseolina* currently available which may help separate the two species. Ideally a future project studying the variability in COI sequences from specimens displaying serrated periostracum spines and those displaying hinge line crenulations would be able to resolve this issue. A potential hypothesis arising from this study would be that specimens with hinge line crenulations are *M. phaseolina* and that *M. barbatus* is a variant of *M. modiolus.* The presence or absence of serrated periostracum spines may be due to phenotypic plasticity related to local environmental conditions (Seed, 1968).

The DNA barcoding results were unable to accurately identify the juvenile mussels from Loch Creran to a single species. The phylogenetic tree of Figure 6.4 shows these mussels pooled within the same clade as reference samples for *M. edulis*, *M. trossulus* and *M. galloprovincialis.* This is may be explained by the hybridisation of these three species which has been observed in Loch Etive, 10 miles south of Loch Creran (Beaumont *et al.*, 2008).

It is thought that *M. trossulus* originated in the Pacific and colonised the North Atlantic through the Bering Strait 3.5 million years ago (Riginos and Cunningham, 2005). *M. edulis* is then thought to have arisen in the Atlantic due to allopatric speciation, and *M. galloprovincialis* separated when connectivity between the Mediterranean and Atlantic was restricted (Riginos and Cunningham, 2005; Beaumont *et al.*, 2008). A second influx of Pacific mussels into the Atlantic is then thought to have occurred between 3.5 million and 12,000 years ago. *M. edulis* then gradually outcompeted *M. trossulus* in Europe waters, with *M. trossulus* only surviving in the Baltic Sea due to their greater tolerance of low salinities. Since their separation significant hybridisation has occurred between these two species within the Baltic (Riginos and Cunningham, 2005). *M. edulis* is currently believed to be the most abundant mussel in European waters, but with *M. galloprovincialis* expanding out of the Mediterranean both as a pure species and as hybrids along the west coast of Europe to Scotland (Gosling, 1992). Recent studies have also found *M. trossulus* are not just restricted to the Baltic, with the species also being found in Norway, Netherlands and the White Sea (Vainola and Strelkov, 2011).

Of greatest relevance to this study are the findings of Beaumont *et al.*, (2008). They found mussels in Loch Etive which had a fragile shell and a different shell shape to *M. edulis*, were more closely related to *M. trossulus*. Allozyme analysis using the Me 15/16 loci found both *M. trossulus*, *M. edulis* and their hybrids were present within the Loch. Additionally, the study found *M. galloprovincialis* and *M. edulis* hybrids were present in significant numbers in Loch Ewe, approximately 80 miles to the north. The source of the *M. trossulus* population in Loch Etive is currently unclear. Beaumont *et al.*, (2008) suggests that the population is a post glacial relict, having survived in the low salinity upper reaches of the Loch much like the Baltic populations. The alternative explanation is the accidental introduction of *M. trossulus* through ballast water from vessels coming from the Baltic or Canada. This however seems unlikely due the minimal shipping activity in Loch Etive (Beaumont *et al.*, 2008).

The probable identification of the juvenile mussels in this study from Loch Creran as *M. trossulus*, supports the theory of there being relict populations of *M. trossulus* in the sea lochs of the Scottish west coast, as the accidental introduction of *M. trossulus* into the upper basin of Loch Creran is less likely than their introduction into Loch Etive. This is due to the presence of a shallow sill separating the upper and lower basins of the Loch. This severely limits vessel access making accidental introductions of *M. trossulus* using vessels as vectors highly unlikely (Tett and Wallis, 1978). Beaumont *et al.*, (2008) also note that unpublished data suggest Loch Etive is not the only location on the Scottish West coast with *M. trossulus* occurrence.

Further work on genetic analysis would be needed to accurately identify the juvenile mussels in Loch Creran to species level. Whilst the use of the mtDNA gene (COI) has proven very accurate and reliable in identifying species, it has limitations (Hebert, 2003; Moritz and Cicero, 2004; Paine *et al.*, 2008). One of these is the identification of species where the species boundaries are blurred by hybridisation or introgression (Hebert, 2003; Moritz and Cicero, 2004), as here with the likelihood of hybridisation between *M. edulis* and *M. trossulus* within Loch Creran and the possible presence of *M. galloprovincialis* and its hybrids (Beaumont *et al.*, 2008). The use of one or more nuclear DNA markers such as the Me 15/16 locus along with allozyme analysis should be used to resolve these three species. This type of analysis has proven effective in the separation of these species in other studies (Beaumont *et al.*, 2008; Vainola and Strelkov, 2011).

Implications for monitoring work

Whilst this study shows that external shell characteristics are unreliable in the identification of juvenile mussels (Figure 6.5), the lack of crenulations on the hinge line of juvenile *M. modiolus* allows for their accurate identification without resorting to genetic techniques.

The accurate identification and subsequent quantification of juvenile *M. modiolus* has been critical in a number of previous studies. These include: assessing the geographic variability in the reproduction and growth of *M. modiolus* populations (Seed and Brown, 1978; Brown, 1984; Jasim and Brand, 1989), the monitoring of protected *M. modiolus* reefs for conservation management (Mair *et al.*, 2000, 2009, Moore *et al.*, 2006, 2012), and the quantification of physical impacts to *M. modiolus* reefs (Cook *et al.*, 2013). There is no evidence to suspect that the misidentification of juvenile *M. modiolus* has occurred in any of these studies. However given the species variability in juvenile mussels from

the *M. modiolus* reefs observed in this study, and the quantity of juvenile mussels enumerated in the studies listed above, the possibility of misidentification does exist.

Due to the sometimes indistinct nature of the hinge line crenulations on Mytilidae species, the only way to accurately identify the majority of *M. modiolus* was through the use of proteinase to dissolve the ligament and periostracum. As these often obscured the hinge line crenulations, and the absence of crenulations is harder to judge than their presence. In some individuals this technique was not required as seen in Figure 6.6 and further work may improve the reliability of assessing these features without digesting the ligament and periostracum. Although the identification of mytilids using hinge line crenulations is a laborious process it seems prudent that it should be incorporated into future studies that rely on the accurate identification of juvenile *M. modiolus.* Whilst it may not be realistic for every individual mussel in a study (for example chapter 5 recorded over 4300 juvenile *M. modiolus*) a subset of juveniles should be examined for hinge line crenulations.

The results of this chapter were used in Chapter 5 to identify *M. modiolus* using hinge line characteristics in a subset of 40-50 mussels, originally identified as *M. modiolus* using external features from each of the 3 restoration sites. Whilst re-examination of all 4300 mussels was not a feasible option, this further detailed analysis of this subset of individuals allowed abundance corrections to be made to the data. This seems a prudent approach for ecological studies dealing with high abundances of hard to distinguish species, where accurate identification is critical to the conclusions of that study.

Conclusions

This study has shown that the identification of juvenile Mytilidae species is not possible using external shell morphological characteristics. However, the accurate identification of juvenile *M. modiolus* can be made using internal hinge line characteristics.

The DNA barcoding results have highlighted the potential presence of *M. trossulus* in Loch Creran, which would represent one of only a handful of recordings in UK waters and may be part of a relict population (Beaumont *et al.*, 2008). However further analysis using different genetic techniques would be needed to confirm this.

Identification of a specimen as *M. barbatus* using DNA barcoding which had the hinge line characteristics of *M. phaseolina*, raised the possibility that these two genera may not be distinct. This would require a further specific study to clarify, and distinguish the separation between these two genera.

Chapter 7. General discussion and conclusions

The overarching aim of the project was to develop techniques for the restoration of biogenic reefs created by *Serpula vermicularis*, *Limaria hians* and *Modiolus modiolus*. These reef-forming species are of conservation value in the UK and are protected features under the 1992 EC Habitats Directive, Marine (Scotland) Act 2010 and the Marine Act (Northern Ireland) 2013, among other designations. These reefs are considered to be of conservation value primarily due to high levels of biodiversity they support (Holt *et al.*, 1998; Hall-Spencer and Moore, 2000a; OSPAR, 2005). In comparison to many other marine restoration studies the objective of this study was not habit rehabilitation to restore a lost ecosystem service, or to restore biogenic reefs to areas where they are currently extinct. Rather the objective was to develop techniques that will aid the natural recovery of these reefs after physical disturbance, thereby restoring the high levels of biodiversity they support. This use of restoration ecology for biodiversity conservation is increasingly being seen as a method for achieving global biodiversity targets (Young, 2000; Egoh *et al.*, 2014). For any restoration project it is important to clearly define its scope and aim. Restoration ecologists are becoming increasingly aware that the full restoration of an ecosystem to a perceived "pre-human" reference point is an unrealistic and unachievable goal, particularly in the marine environment (Hawkins *et al.*, 2002; Hobbs, 2007). Therefore the setting of realistic goals based on the ecological realities of today are needed not only to accurately judge restoration efforts, but to avoid a loss of confidence that restoration can deliver useful outcomes (Elliott *et al.*, 2007; Hobbs, 2007; Suding, 2011).

The aim of restoring reef areas subjected to spatially limited yet severe physical disturbance is not only an important consideration in setting realistic objectives, but is of particular relevance to the reefs created by the three study species. The majority of the known UK reefs created by the three study species are located within Marine Protected Areas. Despite this protection the impacts created by bottom towed fishing gear represent their greatest threat (Hall-Spencer and Moore, 2000a; OSPAR, 2005; Moore *et al.*, 2009; Strain *et al.*, 2012; Cook *et al.*, 2013). Therefore the need to develop restoration techniques specific to this type of impact should form the highest research priority, and could be viewed as a risk based approach to restoration research. This differs from the majority of reactive temperate marine restoration research, where reef rehabilitation to

restore a fishery or a lost ecosystem service is the primary objective (Elliott *et al.*, 2007; Beck *et al.*, 2011).

A risk based approach to restoration research should be expanded to include a pragmatic element in guiding restoration policy for habitats of conservation importance. Generally the ecosystem services provided by these rarer habitats are not fully understood or are limited in scale and don't support a substantial fishery (Frid and Clark, 1999). To restore an ecosystem, an understanding of how it worked before it was impacted is required. The greater the knowledge of an ecosystem the greater the chance of its successful restoration (Hobbs, 2007). There is an increasing awareness that ecosystem dynamics are complex and often unpredictable and certain ecosystems may exist in multiple stable states (Loreau *et al.*, 2001; Knowlton, 2004). The temporal persistence of many marine habitats also remains uncertain. The North Lleyn *M. modiolus* reef has proven to be a stable and persistent feature for the last 160 years, but historical data for many habitats are not available (Lindenbaum *et al.*, 2008). The recent discovery of the rapid expansion of the *L. hians* reef in Loch Alsh correlates with the decline of the *M. modiolus* reef in the same area (Moore *et al.*, 2013). This raises the possibility that *L. hians* reefs are transient features and capable of faster reef development than previously thought (Trigg and Moore, 2009). There is also evidence for *S. vermicularis* reefs being transient habitats with the recent loss of the reefs in Linne Mhuirich without obvious cause, in addition to the lack of significant deposits of reef debris in Loch Creran despite the reefs first being recorded in 1882 (Moore *et al.*, 1998; Hughes *et al.*, 2008; Hughes, 2011). Therefore the restoration of these rarer less well understood habitats is unlikely to be successful, unless pragmatic and achievable objectives are defined based on the current ecological understanding of that habitat.

To be successful, all restoration programs must enhance the recruitment of the species they wish to restore (Mann and Powell, 2007). The provision of additional substrate to improve recruitment is a well established worldwide practice dating back at least 2000 years. This first record dates back to the writings of Pliny the Elder, where he describes the spreading of brush oak in Lake Avermis to encourage the settlement of *Ostrea edulis* (Mann and Powell, 2007). The results of this project for all three species show that increasing habitat provision through the addition of hard substrate enhanced natural recruitment, implying that over time it would become an effective restoration technique, as observed in other restoration projects (O'Beirn *et al.*, 2000; Luckenbach *et al.*, 2005; Schulte *et al.*, 2009). The provision of scallop shell either crushed or whole provided the

optimum substrate for the recruitment of all three study species. The success of the scallop shell substrates may be related attributed to their structural complexity and availability of settlement surfaces. This relationship of increased substrate complexity supporting higher abundances of recruits has been observed in other studies (Bartol and Mann, 1997; O'Beirn *et al.*, 2000; Cranfield *et al.*, 2004). There are two primary factors which may contribute to this trend. Firstly increased substrate complexity provides more space for settling larvae, and secondly provides increased predation protection (O'Beirn *et al.*, 2000; Nestlerode *et al.*, 2007).

The use of substrates that do not naturally form the foundation of biogenic reefs to enhance recruitment requires careful consideration and is discussed in Mann and Powell (2007). They conclude that whilst settlement occurs on many different materials including tyres, fly ash and concrete, none of them offer a practical alternative to oyster shell in large applications. Each of the substrates had at least one negative attribute, from stability in strong tidal currents, compaction and loss of interstitial space, fabrication costs and undesirable permanence after deployment. Compared to oyster shell which has a 60 million year proven track record of providing the optimum substrate for oyster recruitment (Mann and Powell, 2007). For rare biogenic reef-forming species however sources of conspecific calcareous material are usually not available. This is primarily due to the rarity of these habitats but also due to losses of conspecific material over time through erosion and bio-erosion (Holt *et al.*, 1998; Hughes, 2011). Therefore the search for substrates which closely replicate the original habitats is of vital importance. Even for habitats where conspecific calcareous material is available such as oyster reefs, restoration projects are being forced to consider alternative substrates. The availability of cheaper substrates such as clam shell from the offshore fishery coupled with dwindling supply of oyster shell has necessitated this change in focus (Powell *et al.*, 2006; Mann and Powell, 2007; Nestlerode *et al.*, 2007).

For the habitats considered in this study, maximising substrate complexity of deployed substrates is a robust and achievable approach to increasing recruitment. However as noted in the study by Mann and Powell (2007) artificial substrates may not increase post settlement survival or reef development. Chapter 5 hints that this was perhaps starting to occur, when crushed scallop shell was observed to support the greatest abundance of *M. modiolus* recruits compared to other treatments. However abundances of juvenile *M. modiolus* declined in the second year. This was associated with an observed increase in sediment accumulation within the restoration units which may have smothered juvenile

M. modiolus (Hutchison *et al.*, 2016). Therefore to be an effective restoration technique factors affecting the provision of complex substrates, such as sedimentation rates which affect the longer term post settlement survival and later reef development need to be evaluated. Unfortunately due to the time scale of this project, factors such as sedimentation cannot be accurately judged. The decline in juvenile *M. modiolus* at the North Lleyn site occurred after 2 years and may represent the limit of the sustainable abundances able to survive and develop into part of the reef. Conversely the abundances of *M. modiolus* may continue to decline as the interstitial spaces in the substrate fill with sediment. The restoration units left in place for both *S. vermicularis* and *M. modiolus* will hopefully continue to be monitored in the future, allowing the long term effectiveness of these restoration techniques to be judged.

Bivalves and serpulids are broadcast spawners and recruitment is most successful when they occur in dense aggregations (Kupriyanova *et al.*, 2001; Brumbaugh and Coen, 2009; Gormley *et al.*, 2015). Increasing the density of bivalves or serpulids within a given area is therefore likely to improve fertilization rates and larval production, potentially improving recruitment (Caddy and Defeo, 2003; Brumbaugh and Coen, 2009). This rationale has formed the foundation of using stock enhancement in overcoming recruitment bottlenecks often in conjunction with habitat provision to increase recruitment at restoration sites (Caddy and Defeo, 2003; Brumbaugh and Coen, 2009).

A study into the restoration of *M. modiolus* in Strangford Lough tested the use of translocated adults to seed artifical reefs constructed out of scallop shell (Fariñas Franco *et al.*, 2013; Fariñas Franco and Roberts, 2014). The authors found that after 1 year, artifical reefs with translocated adults had significantly richer communities and enhanced abundances of juvenile *M. modiolus* in comparison to unseeded reefs. Other studies, including the translocation of bay scallops (*Argopecten irradians concentricus*) have also recorded significant recruitment increases in seeded areas (Peterson *et al.*, 1996). Contrary to the studies by Peterson *et al.* (1996), Fariñas Franco *et al.* (2013) and Fariñas Franco and Roberts, (2014), Chapters 4 and 5 found translocated adult *L. hians* and *M. modiolus* had no effect on recruitment. This discrepancy between studies may be related to differences in the natural larval supply at the restoration sites.

The restoration sites selected by Peterson *et al.*, (1996) and Fariñas Franco *et al.*, (2014) could be described as having a limited natural larval supply. The study by Peterson *et al.*, (1996) aimed to a restore a scallop population to an estuarine basin where they had been virtually eliminated by a red tide outbreak. Similarly the study by Fariñas Franco and

Roberts, (2014), aimed to restore a *M. modiolus* reef to an area of historical *M. modiolus* reefs. Whilst *M. modiolus* reefs do remain in Strangford Lough they are greatly reduced and fragmented (Strong *et al.*, 2016). A modelling study has shown that within Strangford Lough *M. modiolus* larval supply declines rapidly at distances greater than 500 m from four remaining reef sites (Elsäßer *et al.*, 2013). A recent genetic study has also shown that larval supply into Strangford Lough from other reefs in the Irish Sea is limited (Gormley *et al.*, 2015). Therefore larval supply and recruitment at the restoration sites selected by Peterson *et al.*, (1996) and Fariñas Franco *et al.*, (2014), would be reliant on the translocated individuals. In contrast the study sites used throughout this thesis were located within reef areas so larval supply from the translocated individuals would have been insignificant compared to larval supply from the surrounding reef. This hypothesis is supported by a study by Geraldi *et al.*, (2013), who found seeding artificial reefs did not enhance oyster reef development. They found that natural oyster recruitment overwhelmed any benefit of seeding, and the seed oysters were not producing a strong enough chemical cue to attract larvae. In conclusion the results of Chapter 4 and 5 show that the translocation of adults is not an effective technique for restoring damaged areas of biogenic reefs, as larval supply is not limited to that provided by the translocated individuals. Even for the restoration of sites with limited natural larval supply, stock enhancement through translocation is unlikely to be an effective approach for threatened habitats of limited extent, as the physical disturbance and loss of biodiversity created by the removal of individuals from one reef, are unlikely to outweigh the benefits of restoring another. This is particularly relevant when the outcome of such restoration projects would be uncertain due to our lack of ecological knowledge of that ecosystem (Hobbs, 2007).

The use of hatchery reared stocks to overcome recruitment limitations as opposed to translocation has been applied extensively in oyster restoration projects (see Caddy and Defeo, 2003; Brumbaugh and Coen, 2009 for reviews). The use of hatchery reared stock was not directly considered for this project due to the time and resources required, as well as a lack of expertise and knowledge required for the aquaculture of these non-commercial species. Given the original aim of aiding natural recovery in damaged areas of existing biogenic reefs and the results of the translocation experiments, a hatchery based restoration project would have given no significant restoration advantage over habitat provision.

If the restoration of a biogenic reef were to be attempted in a recruitment limited location, Figure 7.1 could be used to initially evaluate the likelihood of a hatchery based program

being successful. Growth curves of *C. virginica* are presented along with growth curves for *M. edulis, S. vermicularis, M. modiolus and L. hians*. Data for *M. modiolus* are from Seed and Brown (1978) and Anwar *et al.* (1990). *Limaria hians* data are from Trigg (2009), and *S. vermicularis* from Chapman (2004) and Orton (1914). *M. edulis* data are from Bayne and Worrall (1980) and Seed (1968). *Crassostrea virginica* growth data are from the disease resistant strain Delaware Bay (DERBY) (Harding, 2007). This strain of *C. virginica* was selectively bred in the 1960s to produce a disease resistant strain and has been used extensively in restoration and rehabilitation efforts in the Chesapeake Bay (Haskin and Ford, 1979; Harding, 2007). Rates of maturation for *C. virginica* varied between studies, ranging from 4 months to 2 years (Galtsoff, 1964; Buroker, 1983). This is likely the result of environmental differences between populations; so an average maturation age of 1 year was used.

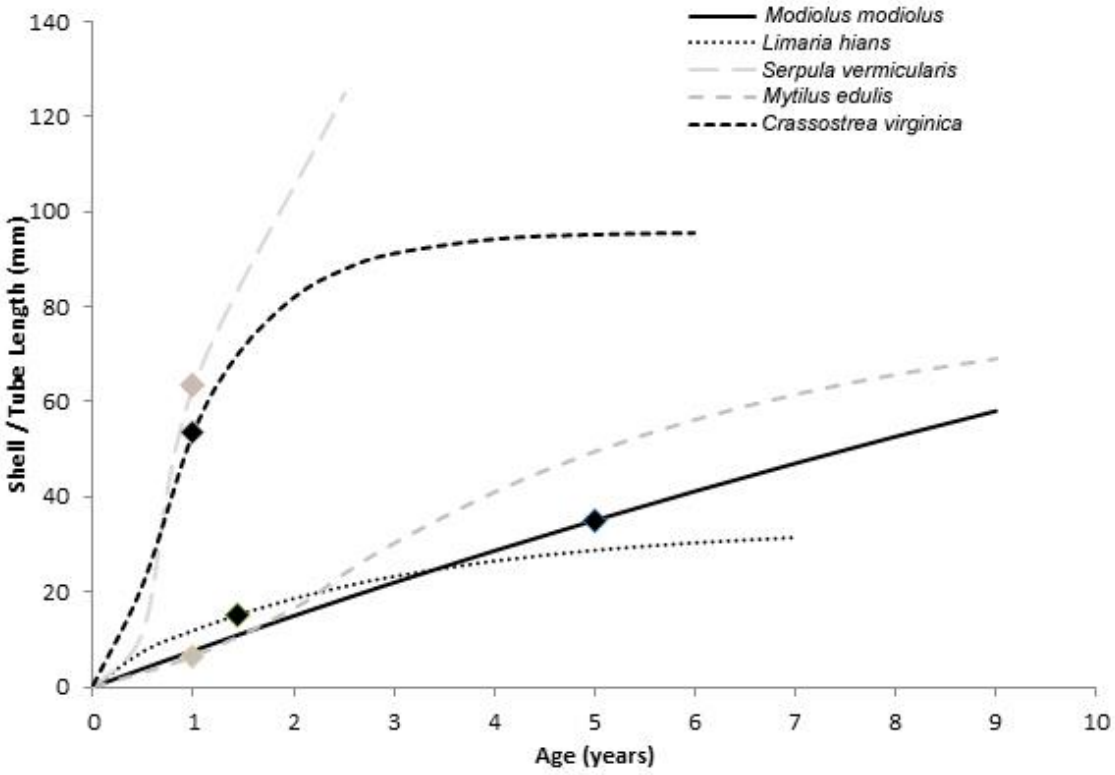

Figure 7.1. Growth curves from several species of restoration importance. Diamonds show the age of sexual maturation of each species.

Slower growth and the increased age at maturation increases the costs of aquaculture, which would make *M. modiolus* the least suitable of the presented species for a hatchery based program (Figure 7.1). The use of juvenile hatchery reared *M. modiolus* for a restoration project was trialled by Roberts *et al.* (2011). However they concluded that poor seed yield coupled with high running costs currently makes this an unviable

restoration technique. Rapid growth and early maturation makes a species more likely to be cost effective in a hatchery based stock enhancement program. This is corroborated by the various successful *C. virginica* hatchery programs (Caddy and Defeo, 2003; Brumbaugh and Coen, 2009). Whilst *L. hians* and *M. edulis* reach maturity at an early age, they remain relatively small, making them more susceptible to significant predation pressure when released (Wallace *et al.*, 2002; Carroll *et al.*, 2015). Greater knowledge of the survival rates of these juveniles, at a range of sizes, at the potential release sites would therefore be required before an informed decision about their suitability for a hatchery based stock enhancement project could be made.

The growth and maturation of *S. vermicularis* closely resembles that of *C. virginica* (Figure 7.1), therefore out of the three study species *S. vermicularis* represents the greatest potential for a hatchery based stock enhancement program. However unlike the release of hatchery reared shellfish *S. vermicularis* reef fragments would have to be deployed carefully to avoid damage, which would substantially increase the cost of any restoration attempt. Further research into the aquaculture of *S. vermicularis* may prove prudent however, and deployment methods could be developed from those already in practice for coral reef restoration (Rinkevich, 2005; Forrester *et al.*, 2014). Of the three study species it has the smallest geographical range as biogenic reef former and recent losses in Linne Mhuirich (Moore *et al.*, 1998) and declines in Loch Teacuis (SNH, 2015) make it the most vulnerable. Therefore any future restoration attempts are more likely to be faced with a limited natural larval supply scenario necessitating the need for stock enhancement (Caddy and Defeo, 2003; Brumbaugh and Coen, 2009).

The restoration experiments on *S. vermicularis* and *M. modiolus* also highlighted significant spatial and temporal variations in recruitment. Chapter 2 investigated the effect deployment timing had on the abundance of *S. vermicularis* recruits. The study found that deploying restoration substrates into Loch Creran in July yielded significantly higher abundances of recruits than at other times of the year. This increased abundance on substrates deployed in July was still significant 2 years later. Whilst this supports the earlier work into *S. vermicularis* recruitment patterns by Chapman *et al.*, (2007), it also shows that the timing of a restoration project can have a significant effect on its outcome. Chapters 2, 3 and 5 highlight differences in the recruitment to restoration materials related to spatial variances. Chapter 5 shows that the recruitment of juvenile *M. modiolus* varies greatly between reef locations and reef types, with only the North Lleyn reef exhibiting significant recruitment. The inter annual recruitment of *M. modiolus* is known to be

variable and poorly understood with irregular recruitment and gaps of several years reported for several reefs (Seed and Brown, 1977; Comely, 1978; Jasim and Brand, 1989; Holt *et al.*, 1998). Based on these findings only the restoration of damaged areas of the North Llyn reef would present a feasible objective. A greater understanding of the recruitment processes at the two other reefs may allow a pragmatic approach to their potential restoration in the future, either through habitat provision or stock enhancement. However without this ecological understanding any restoration goals would be hard if not impossible to achieve (Hobbs, 2007).

Chapters 2 and 3 highlight spatial variations in the recruitment of *S. vermicularis* across smaller scales within Loch Creran. The differences between sites are related to the presence of extant reefs, with sites away from extant reefs having higher abundances of *S. vermicularis*. Whilst the exact mechanisms for this trend remain unclear, the role of gregarious settlement cues as observed in several other Serpulidae species are likely to be crucial (Toonen and Pawlik, 1994). Unfortunately the timescale of the project only allowed the initial colonisation of the restoration units in Loch Creran to be assessed. As highlighted by Mann and Powell (2007) enhanced recruitment does not guarantee the achievement of restoration objectives, and only provides the first step in the restoration of biogenic reefs. It is hoped that future monitoring of the restoration units in Loch Creran will help answer whether the site differences in Loch Creran remain significant, as the *S. vermicularis* recruits continue to grow and form reefs. Recent visual observations made during recent brief dives at some of the study sites already suggest that reef development at sites in the presence of extant reefs is enhanced compared to sites away from existing reefs, therefore reversing the trends of the initial colonisation (Hermitage, 2016).

As well as developing and testing restoration techniques the project encountered problems that affected the ability to judge the success of the restoration efforts. This has often been a major criticism of many marine restoration projects (Underwood, 1996; Mann and Powell, 2007). A major obstacle that arose during the project was the uncertain identification of juvenile *M. modiolus* using external shell characteristics. Chapter 6 used DNA barcoding to validate the identification of juvenile *M. modiolus* from Chapter 5 using internal hinge line features. This allowed more accurate abundances of *M. modiolus* in the restoration units to be determined, without this knowledge abundances of *M. modiolus* may have been over estimated by as much as 90%. Taxonomic issues such as this have been encountered in various fields of ecology and emphasise the need for

continued research, as accurate identification underpins the results of many ecological studies (Tyler *et al.*, 2012).

The basic principles for the restoration of biogenic reefs are simple, and have been outlined in a number of reviews (Hawkins *et al.*, 2002; Brumbaugh *et al.*, 2006; Elliott *et al.*, 2007; Hobbs, 2007). The first and most important step in any restoration project is the removal or reduction of stressors on an ecosystem, therefore allowing natural recovery to occur if possible. The loss of 93 % of the Port Appin *L. hians* reef within a decade demonstrates the consequences of failing to meet this first principle. This first principle should also be seen as the most cost effective restoration technique, and further direct intervention would be substantially more expensive. If however natural recovery is unable to restore the ecosystem, perhaps due to shift to an undesirable stable state (Elliott *et al.*, 2007), then the second step is to enhance natural recruitment. This is commonly addressed through increased habitat provision or increased habitat provision and stock enhancement (Mann and Powell, 2007; Brumbaugh and Coen, 2009).

Using these principles originally developed for the rehabilitation of biogenic reefs to provide a specific ecosystem service or to enhance a depleted fishery, this study provides a valuable example for the restoration of temperate biogenic reefs of conservation importance. A target of restoring 15 % of damaged ecosystems by 2020 was set by the Convention on Biological Diversity and adopted by the European Union in 2011 (Egoh *et al.*, 2014). Whilst the restoration of temperate marine biogenic reefs provides no quick fixes and is unlikely to contribute significantly to the targets adopted by the EU, it is hoped that this study provides a pragmatic set of examples and a guide for future research. The study also shows that recruitment enhancement the second step of any restoration project is an achievable and realistic goal in areas of physically disturbed biogenic reef.

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