Into the Wild

Documenting and Predicting the Spread of Pacific Oysters (*Crassostrea gigas*) in Ireland

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(B.Sc., M.Sc.)

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Doctor of Philosophy

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Summary

Biological invasions by alien species are causing widespread environmental changes that threaten biodiversity and ecosystem services. Environmental and economic damage has been caused in many ecosystems and management efforts to counteract bioinvasions are steadily increasing. Identifying the factors promoting or inhibiting establishment and spread of invasive species can underpin strategies to control their expansion and improve capacity to predict further spread. The Pacific oyster (*Crassostrea gigas*) has been introduced for aquaculture in many parts of the world and invasive populations have developed, causing significant changes to many coastal ecosystems. In Ireland, aquaculture of Pacific oysters started in the early 70's, however, whether feral populations are established is not known. This thesis aimed to assess the status of Pacific oysters in Ireland by characterizing their distribution and abundance, testing factors associated with their spread and assessing the genetic relationship between feral and aquaculture oyster populations.

A repeatable, cost-effective sampling programme that combined semi-quantitative and quantitative approaches was designed to assess the current distribution of feral Pacific oysters in Ireland and identify factors associated with their presence. Oysters were found at 18 out of 69 sites, with densities ranging from single individuals to nine individuals per m^2. Analysis of size-frequency distributions revealed that several recruitment events have occurred, probably within the last 6-10 years. Logistic regression indicated that feral oysters were positively associated with hard substrata or biogenic reef, long residence times of embayments and large intertidal areas. A tendency for oysters to occur disproportionately in bays with aquaculture, but > 500 m from it was also found. Small-scale analysis within sites showed that oysters were exclusively attached to hard substrata and mussels. The approach taken provides a rigorous repeatable methodology for future monitoring and a detailed basis for the prediction of further spread of Pacific oysters.

Biotic interactions can play a key role in promoting or inhibiting spread of invasive species. The influence of predation and macroalgae on growth and survival of juvenile Pacific oysters and the relationship between numbers and sizes of predatory crabs and presence of oysters on shores in Ireland was addressed. A field experiment was set up at two intertidal macroalgae-dominated boulder shores where

only single individuals of oysters occur. After 10 months, condition of oysters was not significantly decreased in the presence of macroalgal canopy; however, shell growth was significantly reduced in less than 4 months, but only at one site. Under some circumstances, predation on juvenile oysters had a significant effect on their survival, however, effects were generally limited and large-scale patterns of oyster abundance did not relate to abundance of predatory crabs. The results suggest that pre-settlement processes affect the pattern of oyster distribution to a greater degree than post-recruitment processes.

To reconstruct the recent biological history of feral populations of Pacific oysters in Ireland, temporal genetic variability of farmed and feral oysters from the largest enclosed bay in Ireland was assessed using anonymous and EST-linked microsatellites. EST-markers showed no footprints of selection and were jointly used with anonymous markers resulting in 13 different markers for statistical analyses. Relatively high genetic differentiation was found between aquaculture and feral oysters and between different year classes of oysters from aquaculture. A ten-fold higher effective population size (N_e) – and a high number of private alleles – in wild oysters suggest an established feral population that is likely to be self-recruiting and demographically independent from the current aquaculture activities in the estuary.

Using a large-scale survey, field experiments and molecular techniques, this is the first study to have quantified the establishment and distribution of Pacific oysters in Ireland. Results can be used to directly inform strategies and be applied in management and conservation. Compared to the situation in other countries where this species forms extensive reefs and already dominates intertidal habitats, control might be still feasible in Ireland, especially if efforts are focused on areas with higher abundances of wild Pacific oysters. A further cooperation between aquaculture operators, scientists and regulatory and development bodies is urgently needed to allow the development of the aquaculture sector without compromising ecosystem stability.

Statement of Original Authorship

I hereby certify that the submitted work is my own work, was completed while registered as a candidate for the degree stated on the Title Page, and I have not obtained a degree elsewhere on the basis of the research presented in this submitted work.

Dublin, 22nd August 2012

Judith Kochmann

Collaborations

Role of J Kochmann (JK) and collaborators in Chapter 2

JK took a leading role in conception of study and design and development of sampling protocol. Advice received from co-authors (T Crowe and F O'Beirn) at each stage. JK completed 80% of sampling and supervised remaining 20% - provided protocol and advice to samplers as coordinator of collaborative sampling programme.

JK entered data and completed analyses with advice from T Crowe and J Yearsley.

Additional data (environmental explanatory variables) provided by M Burrows and T Dabrowski. Acknowledged, but contribution did not constitute grounds for co-authorship.

JK was lead author of the writing. Prepared full draft and received comments from co-authors (F O'Beirn, T Crowe and J Yearsley) to improve structure and clarity.

Role of J Kochmann (JK) and collaborators in Chapter 4

JK took a leading role in conception of study and design and development of sampling protocol. Received advice from co-authors at each stage.

JK completed all sampling and lab work.

JK entered data and completed analyses with advice from J Carlsson and S Mariani.

JK was lead author of the writing. Prepared full draft and received comments from co-authors (J Carlsson, S Mariani and T Crowe) to improve structure and clarity.

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Finally, I thank my parents who encouraged me throughout my life. I dedicate this thesis to them.

Kinvara shore, painted by Korol

“Oysters … a kind of sessile sheep, that are moved from pasture to pasture in the sea.” (Charles S. Elton, 1958)

Chapter 1 General Introduction

1.1 Bioinvasions

Invasive alien species have been recognized as a major threat to biodiversity alongside habitat change, climate change, pollution and overexploitation of resources (Millennium Ecosystem Assessment 2005). They can cause significant changes to ecosystems and pose large risks to ecosystem services (Worm et al. 2006; Pejchar and Mooney 2009). For example, alien species' introductions lead to the global homogenization of ecosystems (Vitousek et al. 1997), they threaten the genetic integrity of native species through hybridization (for examples see Mooney and Cleland 2001), they can outcompete native species (Holway et al. 2002) or, as in the well-known case of the brown treesnake (*Boiga irregularis* Merrem, 1802) lead to extinctions of native species (Fritts and Rodda 1998). Effects on ecosystem services are known from all ecosystems, e.g., invasive alien woody plants increase fire frequencies and erosion of topsoil and affect hydrological services; feral pigs (*Sus scrofa* Linneaus, 1758) damage crops and trails on Hawaii, the giant hogweed (*Heracleum mantegazzianum* Sommier & Levier, 1895) causes allergies and skin damage, and zebra mussels (*Dreissena* polymorpha Pallas, 1771) clog freshwater pipes which caused a damage of $ 69,070,780 from 1989-1995 in the Great Lakes (USA), with up to $ 460,000 spent every year for the control of large water users (Kettunen et al. 2009; Pejchar and Mooney 2009). In 2005, alien invasive species were estimated to have caused overall economic losses of U$120 billion in agriculture, forestry, and public health in the USA (Pimentel et al. 2005). Annual costs of €9.6 billion for the damage caused by invasive alien species and €2.8 billion for their control were estimated for Europe (Kettunen et al. 2009). However, as the authors of these reports caution, figures are probably significant underestimations and, in reality, many additional invasive alien species cause socio-economic effects but their effects have not been estimated in monetary terms.

1.1.1 Legislation

In response to the threat posed by invasive species, over fifty international and regional conventions, codes of practices and other instruments have been developed that deal directly or indirectly with the spreading of alien species (SCBD 2001; Shine

2007). For example, the aims of the Convention on Biological Diversity (CBD) include the prevention of alien species introduction and the control or eradication of those alien species that threaten ecosystems, habitats or species. Another more specific and comprehensive document on alien species is the European Strategy on Invasive Alien Species (Council of Europe 2007). Other legislative drivers addressing alien species in Europe are the EU 2020 Biodiversity Strategy and the EU Habitats Directive.

Under the EU Marine Strategy Framework Directive, 'Good Environmental Status' (GES) of the EU's marine waters should be achieved by 2020. This also means "non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems". The management of ballast water will become mandatory for the member States of the International Maritime Organization under the Convention for the Control and Management of Ships' Ballast Water and Sediments in order to minimize the introduction of harmful aquatic organisms and pathogens (IMO 2004).

Nevertheless, international and regional Directives need to be implemented through national legislation which can be challenging, e.g. in the case of coordinating existing monitoring programmes, identifying responsible governmental bodies or prioritizing research regarding invasive species. Effective implementation of any environmental legislation is made much more likely if there is a good degree of scientific understanding of relevant patterns and processes.

1.1.2 Defining key concepts in invasion biology

Colautti and MacIsaac (2004) listed more than 30 different terms that are commonly and often interchangeably used in the field of invasion biology today. Many terms refer to the biogeographic origin, e.g., *native*, *indigenous*, *exotic*, *common* or *resident* species opposed to *non-native*, *non-indigenous*, *novel* or *alien* species and have been used as an organizing principle in the field of conservation and restoration ecology (Hall 2003). The problem with these terms is that they have to be defined in each context and involve unavoidably arbitrary spatio-temporal choices (Davis and Thompson 2000; Shrader-Frechette 2001; Warren 2008). *Natural* range of distribution usually means *recognized* range of distribution and evolutionary time scales can be differentiated into large, i.e. glacial and postglacial period, and smaller

time scales, e.g. 6000 BP. A less ambiguous term that refers solely to the movement of organisms mediated or facilitated by humans is *introduced*.

Besides the terms describing origin and the general criticisms towards the use of metaphorical language and value-afflicted terms that evoke anthropogenic concepts (for discussion see Simberloff 2003; Brown and Sax 2004), terminological controversy about what should really be called an *invasion* has also beset the research of bioinvasions. Various concepts and definitions of invasion exist; all have emphasized the process of *dispersal* whereas the *origin* of the species and *impact* have been inconsistently used when defining *invasion* (Richardson et al. 2011).

For some authors, an invasive species must overcome a major geographical barrier, traverse a great distance and arise through human-mediated extra-range dispersal; they call a spreading beyond a former range without direct human assistance *range expansion* (Richardson et al. 2000; Colautti and MacIsaac 2004; Davis 2009; Wilson et al. 2009). Others argue that native species can also become invasive and consider invasion more as an ecological occupation process with community and habitat interactions regardless of biogeographic origin (Davis and Thompson 2000; Reise et al. 2006; Davis et al. 2011). From their point of view, range expansions are a form of invasion where native species undergo demographic explosions, expanding their natural range and rapidly conquer new adjacent or nearby habitats or areas after a change in the environment, e.g., by human activity.

Most of the global strategic programmes consider the *impact* of species and relate to invasive species as introduced species causing enormous damage, e.g. the Convention on Biological Diversity defines invasive alien species as “species whose introduction and/or spread outside their natural past or present distribution threatens biological diversity”. However, others urge against including impact in the definition of invasion as the scale of impact is often not defined and depends on the human perspective (Colautti and MacIsaac 2004). Furthermore, rapidly spreading species and impact, e.g. on native ecosystems, are not necessarily correlated (Ricciardi and Cohen 2007).

To avoid the biogeographic and impact criterion, Valéry et al. (2008) proposed a more mechanistic definition of biological invasion which includes dispersal and geographic range expansion as a more easily recognizable phenomenon, whether

with or without human interference: “A biological invasion consists of a species’ acquiring a competitive advantage following the disappearance of natural obstacles to its proliferation, which allows it to spread rapidly and to conquer novel areas within recipient ecosystems in which it becomes a dominant population.”

After all, the time-scale of an invasion is not fixed and the permanence of ecological dominance and thus, of invasive populations cannot be guaranteed (Simberloff and Gibbons 2004; Reise et al. 2006). Instead of dichotomously classifiying a species as invasive or not invasive, an approach that recognizes biological invasion as a process or the end product of a series of stages has been adopted in the field of invasion biology.

1.1.3 Stages of invasion

A unified framework for biological invasions was recently proposed by Blackburn et al. (2011) (Figure 1.1) merging numerous single frameworks that considered different taxa and environments (Williamson and Fitter 1996; Richardson et al. 2000; Kolar and Lodge 2001; Colautti and MacIsaac 2004; Occhipinti-Ambrogi 2007). The terminology used to describe the ‘Stage’ of an invasion is similar to that used by Reise et al. (2006) (Figure 1.2). However, after Reise et al. (2006), native and non-native species can become invasive, thus, the first stage is simply described as ‘Arrival’ and not separated into ‘Transport’ and ‘Introduction’ which implies human interference. The subsequent stages are described as ‘Establishment’ and ‘Spread’ (or ‘Expansion’), the latter including “boom-and-bust” phenomena (Simberloff and Gibbons 2004) which fall under ‘Adjustment’ in the scheme by Reise et al. (2006). After Blackburn et al. (2011), the term ‘Invasive’ can be used for a population once a population is self-sustaining, however, ‘Naturalized’ or ‘Established’ are equally valid. Thus, as pointed out by the authors, the terminology might still need subsequent debate. The progressing from one stage to the next depends on various conditions or ‘Barriers’ that need to be overcome by individuals (A to C1) and populations (C2 – E). These ‘Barriers’ are indicated in purple (Figure 1.1). As examples (further details can be found in the original paper), C0 describes “individuals that were released into the wild (i.e. outside of captivity or cultivation) in a location where it has been introduced, but which are incapable of surviving a significant period”; E is used for “fully invasive species, with individuals dispersing, surviving and reproducing at

multiple sites across a greater or lesser spectrum of habitats and extent of occurrence".

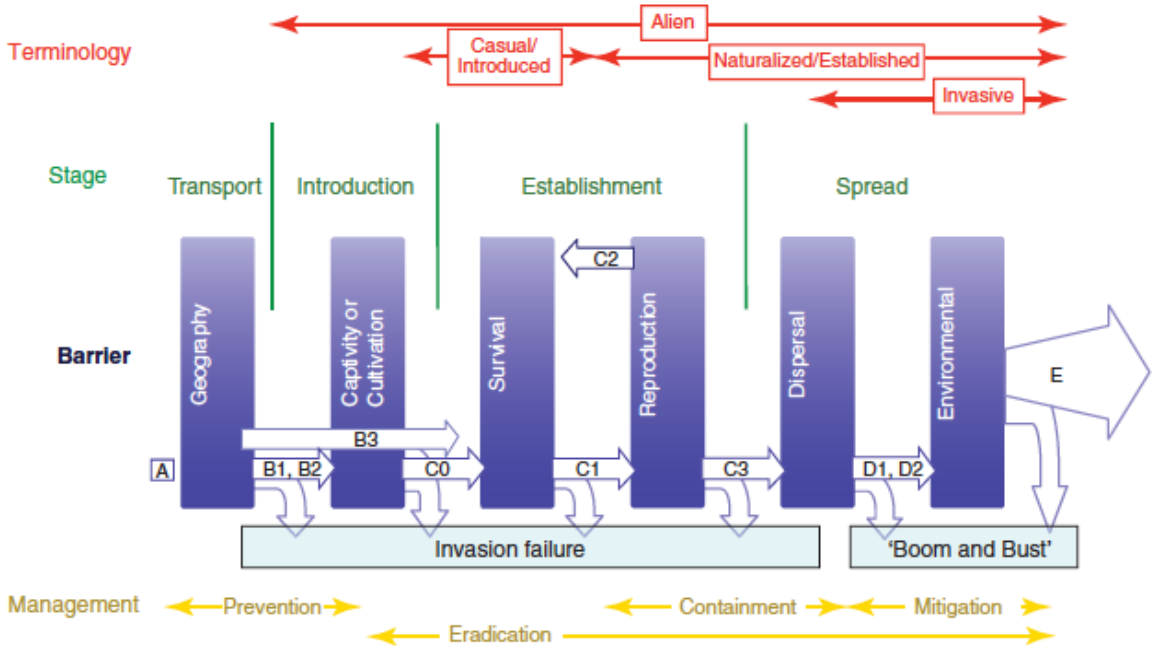


Figure 1.1 Proposed unified framework by Blackburn et al. (2011). For codes see the original paper.

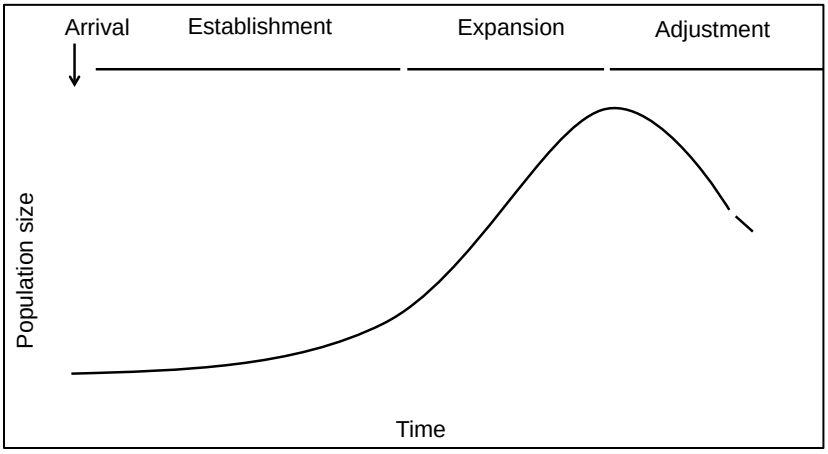


Figure 1.2 Phases of invasion after Reise et al. (2006).

1.1.4 Factors affecting invasion success

Many different factors can influence whether barriers or filters, such as those depicted in Figure 1.1, will be passed during the invasion process and the importance of those factors can vary depending on the stage of invasion (Kolar and Lodge 2001; Marchetti et al. 2004). Therefore, a major challenge for research, undertaken in

different habitats and across different taxa, is the identification and quantification of these factors, which will also help to make predictions and decisions in management.

Propagule pressure plays a major role during early stages of an invasion and is defined as the release of mature adult organisms or early life-history stages into an area where they are not indigenous (Carlton 1996a). It is determined by the number of individuals (propagule size) and the number of introduction events (propagule number) and represents the potential for introduction rather than a realized introduction. Although research in terrestrial and freshwater systems indicates a positive relationship between propagule pressure and invasion success (Lockwood et al. 2005), understandings in marine systems are less advanced and could profit from insights in "supply-side ecology" (Johnston et al. 2009). The analysis of pathways, also covered under the framework of *vector science* introduced by Carlton and Ruiz (2005), can assist the identification of propagule pressure. The six principal pathways that have been described are: 'release', 'escape', 'contaminant', 'stowaway', 'corridor' and 'unaided' and differ in their gradients of intentionality (Hulme et al. 2008). In the case of vertebrates, major pathways of introductions are generally deliberate releases, e.g. the introduction of deer as game animals in Australia (Booth 2008), or escapes from captivity, e.g. in the case of the red fox in California (Lewis et al. 1999). Intentional introductions as commodities are common for plants (Williams and Cameron 2006), but also for invertebrates, e.g. oysters (Ruesink et al. 2005), or vertebrates, e.g. fish (Gross 1998). Unintentional escapes are also very common (Foxcroft et al. 2008; Piccolo and Orlikowska 2012), and in marine environments, ship traffic and aquaculture have been recognized as a major source of unintentional alien species introduction (Wolff and Reise 2002; Haydar and Wolff 2011).

The physical-chemical suitability or resource availability determines whether barriers of survival and reproduction can be overcome during the process of invasion and invasibility is expected to increase with an increase in the availability of *limiting* resources (Davis 2009). This has been shown, for example, for the mussel *Musculista senhousia* (Benson, 1842) (Allen and Williams 2003) and for terrestrial plants (Thompson et al. 2001; but see also Funk and Vitousek 2007). Another aspect is the removal of physiological barriers, e.g. climatic constraints, which can lead to an increased number of species in regions in which they previously could not survive and reproduce (Frenot et al. 2005; Aronson et al. 2007). Milder winters and warmer

summers possibly led to an increase in population growth and a decrease in mortality and promoted the spreading of the Australian barnacle *Austrominius modestus* (Darwin, 1854) (Witte et al. 2010), the slipper limpet *Crepidula fornicata* (Linneaus 1758) (Thieltges et al. 2003) as well as the cordgrass *Spartina anglica* (C.E. Hubbard) (Löbl et al. 2006). Similarly, latitudinal and altitudinal expansion has been observed in the winter pine processionary moth *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775), possibly explained through the increase of feeding hours and lower mortality during warmer winters (Battisti et al. 2005). Propagule pressure might also be increased by climate warming through the alteration of transport patterns (e.g. decrease in shipping time in northern oceans through receding of summer Arctic ice cover) and will affect earlier stages of invasions (Walther et al. 2009).

As described under Grinell's *fundamental niche* concept, a species occurs wherever the environmental conditions are suitable, however, after Hutchinson's view, species can be excluded from a part of their fundamental niche by biotic interactions, resulting in the *realized niche* (for discussion of niche concepts see Guisan and Thuiller 2005). The release from predators, herbivores or parasites commonly served as an explanation for the success of introduced species, however generalizations have been questioned (Colautti et al. 2004). Indeed, many examples for predation on introduced species by native species indicate that predation pressure exists also in the introduced range (Siemann et al. 2006; Carlsson et al. 2011; Dumont et al. 2011). Other biotic or community interactions that have widely been discussed to play a role in the invasion process are mutualism, facilitation and competition, however, they are also case-specific and difficult to generalize (for further discussion and case studies see Lockwood et al. 2007; Davis 2009). Although some authors found strong evidence for the prevention of establishment of non-native species through competition (Simberloff et al. 2002; Green et al. 2004) or predation (Lake and O'Dowd 1991; Hunt and Yamada 2003) on a local scale, a prevention of establishment through biotic interaction is generally difficult to be observed directly possibly due to the fact that failed attempts are unlikely to be detected (Lockwood et al. 2007). However, biotic interactions are known to limit the spread of a species after its establishment (Levine et al. 2004; deRivera et al. 2005), but more studies are needed to provide new insights into the mechanisms underlying

variations in the strength and direction of species interactions, especially in marine environments (Bulleri 2009; Olyarnik et al. 2009).

Understanding of the dynamics of invasion is not only necessary to improve theory, but also crucial to undertake risk assessments which will help to prevent a significant rise in economic costs in the future and counteract significant changes on ecosystems. Risk assessments and scoring systems that differentiate between economic impacts and ecological effects of species, the former generally being easier to assess in monetary terms, can help public policy makers better deal with changes and define priority actions (Thieltges et al. 2006; Olenin et al. 2007; Pejchar and Mooney 2009).

1.1.5 Considerations for management

Once a species has been detected, different management options exist, with a distinct relevance and applicability depending on the stage of invasion, the species and the ecosystem (McNeely et al. 2001; Lodge et al. 2006; Reise et al. 2006). The two broad categories among these options, prevention and mitigation, can be broadly divided into four different management strategies: prevention, early detection, eradication, and control (McNeely et al. 2001; Lodge et al. 2006; Reise et al. 2006). For example, the reduction of propagule pressure through risk screenings will be important at the early stages to prevent introductions and continuous management efforts can reduce the propagule pressure at a site (Jeschke and Strayer 2005). However, these efforts could also be ineffective if the identification of all sources of introduction remains incomplete (Davis 2009). Monitoring programmes can help early detection and will allow early eradication. However, once the establishment of a species is deemed irreversible, eradication will be very difficult, especially in aquatic environments (Bax et al. 2001; but see Culver and Kuris 2000). Control options to contain subsequent spread of a species include mechanical control, chemical control, biological control, habitat management, and integrated approaches using a combination of the various control options (Wittenberg and Cock 2001). Overall, the prevention of introductions has been suggested to be the most effective and also least costly management strategy (Leung et al. 2002; Lodge et al. 2006; Keller et al. 2007). Yet, it might still be an expensive option depending on the type of preventive action undertaken as well as the species.

1.2 Methodologies for characterising and predicting invasions

1.2.1 Models, observations and experiments

In addition to the factors discussed above, many studies have tried to relate species' traits or characteristics to invasion success (e.g. Kolar and Lodge 2001; Alonso and Castro-Díez 2008; Troost 2010). The most common characteristics found across taxa include r-selected life history with a short generation time, high fecundity and high growth rates (Sakai et al. 2001). However, traits are difficult to generalize among different taxa and many studies have been biased by propagule pressure (see meta-analyses Colautti et al. 2006; Hayes and Barry 2008) or focused on plants (e.g. Rejmánek et al. 2005). The extent to which a species has been invasive in other places (Kolar and Lodge 2001; Marchetti et al. 2004) and more recently phenotypic plasticity (Chown et al. 2007; Edgell and Hollander 2011) have been used to predict invasion success. However, predicting invasions based on one factor alone has only met with moderate success and identifications of potential invaders based on a combination of factors with emphasis on climate/habitat matching, invasion history (former successes) and propagule pressure is recommended for risk assessments (Ricciardi and Rasmussen 1998; Hayes and Barry 2008).

Models are a valuable tool to anticipate which non-native species will spread and where. They allow the incorporation of many factors simultaneously, which is an advantage in the case of ecological processes where a full understanding is often missing or uncertain. Species Distribution Models (SDM) are widely used to estimate current and potential distributions of species (Elith et al. 2006; Franklin and Miller 2009) and mechanistic models relate the distribution of species to their physiological needs, e.g. temperature or salinity tolerance. However, as described above, there are many reasons why a species is confined to an area that is smaller than its physiological tolerances would predict.

The native range of a species has been used to anticipate the potential geographic course of an invasion (Peterson 2003), but models based on the realized native niche might provide misleading forecasts for areas that can potentially be invaded since many areas suitable for colonization may lack appropriate vectors to transmit the species to these locations (Herborg et al. 2007; Therriault and Herborg 2008). Predictions based on native occurrence have also been criticized as they do

not take account of release from predators, competitors or parasites which could allow non-native species to occupy a wider ecological niche than in their native ranges (Herborg et al. 2007). Therefore, using correlative models based on observed presence and/or absence data from non-native habitats in which the species is becoming established, rather than only from native habitats, can help to identify areas that will be susceptible to further invasion. The best hypothesis of the potential distribution of the species would then combine information from native and invaded ranges and use predictors linked to the species' physiological requirements (Jiménez-Valverde et al. 2011).

Surveys and observational studies can help to detect patterns of invasion (Cohen and Carlton 1998; Stachowicz and Byrnes 2006; Fridley et al. 2007). They can serve as appropriate descriptions of ecological patterns as long as careful logical thought goes into the planning, collection and interpretation of observations (Underwood et al. 2000). Yet, underlying processes, i.e. cause-and-effect, cannot be determined from patterns or correlation analysis (Lonsdale 1999; Stohlgren 2002). Manipulative experiments enable the importance of mechanistic process to be tested; however, they require careful design recognizing relevant spatial and temporal scales (Underwood 1997). Therefore, an integration of field experiments and monitoring programmes or surveys should enhance the ability to connect patterns and processes in invasion biology (Stachowicz et al. 2002; Stachowicz and Byrnes 2006; Fridley et al. 2007) and is recommended for strategic and applied research programmes (Thompson et al. 2002; Olyarnik et al. 2009).

1.2.2 Genetic tools

As stated above, the identification of propagule pressure and pathway analyses can help to predict the potential for invasion. Among this pathway analysis, molecular tools are very valuable to address management questions related to invasive species; they can help to identify source populations and are critical for determining whether populations are self-sustaining or rely on continued reintroductions (Dlugosch and Parker 2008; Sagarin et al. 2009; Geller et al. 2010). Mitochondrial markers have been used to relate introduced species and their introduction sources including native ranges (Kolbe et al. 2004; Simon-Bouhet et al. 2006; Kelly et al. 2006; Möhler et al. 2011). They also proved to be powerful for genealogical and evolutionary

studies and can be used to date phylogeographic events (Avise 2004). However, mitochondrial markers are less powerful than nuclear microsatellites when detecting low levels of genetic differentiation within shorter time-scales, due to the lack of recombination and the fact that mitochondrial DNA is only inherited in the female line (Sunnucks 2000; Zhang and Hewitt 2003). In contrast to mitochondrial DNA markers, microsatellites are inherited both on the female and male line, and recombine. Microsatellites also have very high mutation rates due to their repetitive structure. These properties make microsatellites the marker of choice when the detection of low levels of genetic differentiation is desired (Norris et al. 2000; Liu and Cordes 2004; Carlsson et al. 2006).

1.3 Aquaculture as a vector for invasive species

Since the 1980's, the global production of capture fisheries has stopped growing, while world aquaculture production has grown steadily by an average of 9% per year, now producing almost half of the fish and shellfish consumed by humans (FAO 2012a). Three quarters of total marine aquaculture production, also referred to as mariculture, consists of molluscs, whereas freshwater aquaculture is dominated by finfish (92%). Overall, facing shortages of fresh water in the future, mariculture will play even a greater role in the world's food production in the near future (Duarte et al. 2009). According to the FAO (2012a), the largest production in marine aquaculture is the production of marine molluscs (mostly bivalves such as oysters, mussels, clams, cockles or scallops). However, it declined by 10% from 1990 to 2010, reflecting the rapid growth of finfish culture, especially salmonids. Marine algae or seaweed production increased from 3.8 million tonnes in 1990 to 19 million tonnes by volume in 2010. Note that from here on, the term 'aquaculture' will be used synonymously for marine aquaculture.

Although aquaculture provides considerable economic and social benefit, the use of non-native species for aquaculture purposes is of significant concern. In marine coastal areas, the number of non-indigenous species is particularly high due to a high propagule pressure through aquaculture, which can become accidentally or deliberately a vector for alien species and promote the spreading of species outside their natural range (Carlton 1996b; Naylor et al. 2001). Examples are the Asian kelp (also called Wakame) *Undaria pinnatifida* (Harvey) Suringar, which was deliberately

introduced for aquaculture, e.g. to coastal regions in the northeastern and southwestern Atlantic and the southwestern and northeastern Pacific (Floc'h et al. 1991; Silva et al. 2002). It is now considered one of the worst invasive species (Global Invasive Species Database www.issg.org). Similarly, it was suggested that the tunicate *Styela clava* (Herdman, 1881) has been introduced by shellfish transfer into the Mediterranean (Davis and Davis 2009) and infestations by large clumps of this species are known to be a major problem for the mussel industry (Arsenault et al. 2009). A list of aquatic species and their introduction source in Ireland was given by Minchin (2007a), indicating aquaculture as one of the main introduction sources for non-native species in marine waters.

In addition to the broad legislative frameworks described above, there is also a body of legislation geared specifically towards reducing risk of introductions of invasive species via aquaculture. The International Council for the Exploration of the Sea (ICES) Code of Practice on the Introductions and Transfers of Marine Organisms (ICES 2005; ICES WGITMO Report 2008) and the European Inland Fisheries Advisory Commission (EIFAC) Code of Practice and Manual of Procedures for considerations of introductions and transfers of marine and freshwater organisms (Turner 1988) are existing codes of practice to prevent accidental introductions and guide authorities on decisions about introductions of alien species. In 2007, the European Council enacted a regulation concerning the use of alien and locally absent species in European aquaculture industry (Council of Europe 2007). Although neither of the aforementioned codes or regulations has been transferred into national legislation yet, a voluntary Marine Aquaculture Code of Practice, which is in compliance with the ICES Code of Practice on the Introductions and Transfers of Marine Organisms 2005, has been drafted by the Invasive Species Ireland project.

1.4 Pacific oysters - a case of marine bioinvasion

A species deliberately introduced for aquaculture in many parts of the world is the Pacific oyster (*Crassostrea gigas* Thunberg, 1793). Over the last 100 years, they have been introduced to more than 70 countries (Ruesink et al. 2005) and today, Pacific oysters have become one of the world's main aquaculture species with an estimated global production of 662,513 metric tonnes in 2010 (FAO 2012b).

Although spread outside aquaculture areas was initially considered unlikely, feral populations can be found worldwide today, e.g. in Russia, China, Australia, New Zealand, Argentina, USA, Canada and South Africa (Ruesink et al. 2005 and Global Invasive Species Database: www.issg.org). Invasive populations are known from many countries, where dense populations with up to 600 individuals per m^2 occur in intertidal habitats, such as sandflats, mudflats, mussel beds or rocky shores, e.g. in Europe in Germany (Reise 1998; Diederich et al. 2005; Nehls and Büttger 2007), the Netherlands (Fey et al. 2010) and France (Cognie et al. 2006), but also in the USA (Ruesink 2007) and Argentina (Orensanz et al. 2002). The presence of dense and extensive populations has a wide range of impacts. The most prominent is the alteration of habitat structure through reef formation. Pacific oysters can overgrow resident species and affect their growth (Dubois et al. 2006; Eschweiler and Buschbaum 2011; Eschweiler and Christensen 2011). They can serve as additional substrate for other introduced species, and thus, might promote other biological invasions (Kochmann et al. 2008; Lang and Buschbaum 2010). An increase in biomass of Pacific oysters may lead to an increase of the filtration pressure which could ultimately lead to a decrease of the carrying capacity, especially in bays with intensive aquaculture (Cognie et al. 2006; Troost 2010). Netting problems for fishermen as well as being a potential hazard for tourists are also amongst the negative impacts (see also Table 1.1). Furthermore, Pacific oysters are one of the main anthropogenic vectors responsible for the introduction of many other non-indigenous species along coastlines (Wolff and Reise 2002; Haydar and Wolff 2011), which should make them a priority for control.

Pacific oyster reefs have developed for approximately 40-50 years in Europe. The time that passed from the introduction of first individuals to becoming a species that forms invasive populations, varies between places, and possibly depended on appropriate conditions (Shatkin et al. 1997; Reise 1998; Orensanz et al. 2002; Robinson et al. 2005; Smaal et al. 2009; Troost 2010). A lot of research on Pacific oysters has been undertaken within aquaculture facilities in order to improve growth conditions and reduce mortalities (see Table 1.2 and Dégremont et al. 2007). Thus, it was shown that temperatures above 16-17°C are needed for gonadal development, spawning and larval development (Mann et al. 1991; Ruiz et al. 1992; Castaños et al. 2009; Rico-Villa et al. 2009). However, photoperiod and food availability can

influence quanity of gametes and intensity of spawning as well (Fabioux et al. 2005). In the wild, an increase in the frequency of summers with temperatures above long-term averages, i.e. above the thresholds identified through research of oysters within aquaculture facilities, has been associated with the rapid spreading in their introduced range (Diederich et al. 2005; Dutertre et al. 2010). Evidence to support this has come from a number of locations across Europe such as Denmark and Norway (Wrange et al. 2010), France (Dutertre et al. 2010), Germany (Diederich et al. 2005) and the Netherlands (Wehrmann et al. 2000). Atmospheric and sea surface temperatures have also been used to predict the potential range of oysters (Carrasco and Barón 2010).

Beside the increase in average temperatures, the generalists' characteristics of Pacific oysters, their gregarious behaviour and low predation pressure, have served as explanations for their invasion success (see Table 1.2, Herbert et al. 2012). *C. gigas* is an oviparous bivalve species and produces approximately 1-100 million eggs per female per year and, under good growing conditions, can attain recruitment competence within 1 year (Lapègue et al. 2007). It has a broad salinity range of 11 - 34 psu, plus a low temperature tolerance of -5°C (Buroker 1985; Reise 1998). In natural habitats, it often attaches to shells of conspecifics forming clumps of oysters that can lead to reef formation (Diederich 2005). In its introduced ranges, predation seems to affect small spat, however, mortality rates due to predation are often not very high (Diederich 2006; Ruesink 2007). Parasitic loads in its introduced ranges are also low (Krakau et al. 2006; Elsner et al. 2011). Furthermore, *C. gigas* presents a high phenotypic plasticity in response to changes in the environment. Correlations have been shown for changes in temperature and expression of heat-shock proteins (Hamdoun et al. 2003), turbidity and size of pallial organs (Barillé et al. 2000), and food availability and resource allocation to reproduction, survival and growth (Ernande et al. 2004) which could have equally contributed to their invasion success.

Research to date of populations in the wild has mainly considered temperature (Diederich et al. 2005; Carrasco and Barón 2010), tidal height and substratum (Diederich 2006) and recruitment strength (Diederich 2005). Studies on biotic interactions have mainly focused on the effects of oysters on native species (e.g. Kochmann et al. 2008; Eschweiler and Buschbaum 2011; Eschweiler and Christensen 2011) whereas studies on the effects of native species on the establishment of Pacific

oysters, i.e. *biotic resistance,* have been neglected (but see Diederich 2006; Ruesink 2007). An association with aquaculture was often inferred (Shatkin et al. 1997; Ruesink et al. 2005), but rarely tested using genetic techniques (Smith et al. 1986; Möhler et al. 2011).

Table 1.1 Impacts of introduced Pacific oysters (outside aquaculture).

"Positive"	"Negative"	"Neutral"
Use for coastal defense (Walles et al. 2011)	Netting problem for fishermen (Troost 2010)	Habitat modification through increase in biogenic hard substratum (Kochmann et al. 2008; Reise and van Beusekom 2008)
Harvesting from wild populations	Potential hazard for tourists	
Additional substratum for attachment for resident species (Kochmann et al. 2008; Lang and Buschbaum 2010)	Additional substrate for attachment of introduced species (Kochmann et al. 2008; Lang and Buschbaum 2010)	Change in abundance of biogenic reef-associated species (Kochmann et al. 2008; Markert et al. 2010; Green unpublished)
Numerical increase in species richness	Vector for other alien species (Haydar and Wolff 2011)	
Improvement of water quality (Dumbauld et al. 2009)	May decrease carrying capacity through increase in filtration pressure (Cognie et al. 2006; discussed in Troost 2010)	Faster feeding rate and metabolic efficiency compared to resident oyster species (Bayne 2002)
Sink for parasitic load in native bivalves (Krakau et al. 2006; Thieltges et al. 2009)	Molluscs-feeding birds may encounter decreasing resource availability (Smaal et al. 2005; Scheiffarth et al. 2007)	
	Reduced growth for blue mussels between oysters (Eschweiler and Christensen 2011)	
	Overgrow resident species (Büttger et al. 2008; Krassoi et al. 2008; Eschweiler and Buschbaum 2011)	
	Change in recruitment, size and age structure of *Sabellaria* reefs (Dubois et al. 2006; Green 2012)	

Table 1.2 Life history characteristics and traits of Pacific oysters promoting successful invasions (modified after Troost 2010).

	Traits/Characteristics	Source
ARRIVAL	Association with humans (aquaculture species)	(Shatkin et al. 1997; Ruesink et al. 2005)
	High propagule pressure (size, number)	Germany (Reise 1998) , Netherlands (Drinkwaard 1998; Smaal et al. 2009), Belgium (Kerckhof et al. 2007), France (Cognie et al. 2006), UK (Spencer et al. 1994), Denmark/Norway/Sweden (Wrange et al. 2010), Brazil (Melo et al. 2010), Argentina (Castaños et al. 2009), South Africa (Robinson et al. 2005), Australia, UK (Spencer et al. 1994)
ESTABLISHMENT	r-selected life history: rapid growth rapid sexual maturation high fecundity	(Quayle 1988; Reise 1998; Diederich 2006; Troost 2010)
	Generalists: tolerate wide range of environmental conditions	temperature (Mann et al. 1991; Ruiz et al. 1992; Fabioux et al. 2005; Castaños et al. 2009; Enríquez-Díaz et al. 2009; Rico-Villa et al. 2009); salinity (Mann et al. 1991), food availability (King et al. 2006; Grangeré et al. 2009); seston load (Barillé et al. 1997; Gangnery et al. 2003; Dutertre et al. 2009; Grangeré et al. 2009) salinity (Brown 1988; Brown and Hartwick 1988; Mann et al. 1991)
	tolerate wide range of habitat types	(Ruesink et al. 2005; Cognie et al. 2006; Troost 2010)
	broad diet	(Dupuy et al. 1999, 2000; Dubois et al. 2007)
	gregarious behavior	(Diederich 2005; Tamburri et al. 2007)
	genetic variability and phenotypic plasticity	GxE interactions (Evans and Langdon 2006; Swan et al. 2007); phenotypic plasticity (Barillé et al. 2000; Tanguy et al. 2002; Hamdoun et al. 2003; Ernande et al. 2004); genetic variability (Li et al. 2006)
	Low predation pressure	(Diederich 2006; Ruesink 2007)
SPREAD	Climate change	(Diederich et al. 2005; Miossec et al. 2009; Dutertre et al. 2010)
	Dispersability	(Cardoso et al. 2007)
	Traits of 'Establishment'	

1.5 Outline of study

Facing the expansion of the aquaculture sector in the future, the identification of factors associated with establishment and a better understanding of introduction pathways of non-native species will be crucial to allow preventive actions to be employed, which can be more cost-effective than the long-term control of invasive populations. Furthermore, research in the same ecological system with the specific species of concern will be of great management value and be crucial to prevent further spread. (Leung et al. 2002; Davis 2009; Shine et al. 2010).

Pacific oysters have been grown in aquaculture in Ireland for over 40 years now and they form an important source of income to the Irish economy with an annual production of approximately 6,500 tonnes and a value of €15 million providing almost a quarter to the total value of shellfish aquaculture in 2006 (Browne et al. 2007). *C. gigas* is extensively farmed around the north, the west and south coast in approximately 50 bays. Often other shellfish e.g. native oysters *Ostrea edulis* (Linneaus, 1758), blue mussels *Mytilus edulis* (Linneaus, 1758), clams or scallops are cultured or harvested from the wild in the same bay. Oyster spat is usually obtained from hatcheries in the UK or France and oysters are sold as half grown (~ 10% of production) or larger sizes (~ 85% of production) to France (F O'Beirn, personal comment). Oysters are kept mainly in the intertidal on culture racks (trestles) in mesh bags until they are sold, but can be moved between sites to allow better growth depending on age (B O'Loan, personal comment). At some sites, oysters are also kept subtidally, however, these locations are rather small-scale trials.

Wild oyster populations have developed in other countries soon after aquaculture has been introduced (Brandt et al. 2008; Troost 2010). Considering this history of invasion of Pacific oysters in other countries, there is an urgency to determine their current status in Ireland. Initial observations of feral oysters have been made, however, no large-scale surveys of feral Pacific oysters exist. In this thesis, a combined approach of a large-scale survey (Chapter 2), field experiments (Chapter 3) and molecular techniques (Chapter 4) was used to address the following aims:

In Chapter 2 the aim was to characterize the distribution and abundance of Pacific oysters in Ireland. A large-scale sampling programme was undertaken to

assess the current status of Pacific oysters in Ireland and identify the main environmental factors associated with their presence or absence. Size-frequency distributions and small-scale habitat associations were also determined.

Considering the lack of studies in marine environments on biotic interactions and a focus on impacts rather than *biotic resistance* in research on Pacific oysters, the influence of biotic interactions on survival and growth of Pacific oysters were tested in an experimental approach in Chapter 3. More specifically, the role of predation and macroalgae was tested in order to assess their importance for controlling the establishment of oysters.

There are several microsatellites available for *C. gigas* (e.g. Sauvage et al. 2009), but they have not been used to trace the origins of invasive Pacific oyster populations so far. The third aim (Chapter 4) was therefore to use microsatellites to assess the genetic relationship among aquaculture and feral Pacific oyster populations in Ireland and establish whether *C. gigas* is forming self-sustaining feral populations or populations rely on repeat input of gametes from aquaculture.

In Chapter 5 overall results and general implications for aquaculture practices in Ireland are discussed.

Note for use of terminology: Although Colautti and MacIsaac (2004) pointed out, what we call *invasive species* are really *invasive populations of a species* since very few species are invasive everywhere they are found (in other words: invasiveness should not be regarded as an inherent trait or characteristic of a species), the term *invasive species* will be used in this thesis. Considering the still unresolved terminology (Blackburn et al. 2011), Pacific oysters will be referred to as *invasive* considering their successful invasion in other places even if they might not be at the stage of spread yet in Ireland. The terms *non-native, non-indigenous* and *alien* will be used interchangeably, as will *native* and *resident*. The terms *feral* or *wild* oysters will be used for oysters living outside aquaculture.

Chapter 2

Environmental factors associated with the local establishment of Pacific oysters: modelling occurrence data from a coordinated sampling programme

Under review, Journal *Biological Invasions*: "Environmental factors associated with the local establishment of Pacific oysters: modelling occurrence data from a coordinated sampling programme" by Judith Kochmann, Francis O'Beirn, Jon Yearsley and Tasman P. Crowe

2.1 Abstract

Documenting the establishment and spread of invasive species requires extensive co-ordinated sampling programmes. Identifying the factors promoting or inhibiting the local establishment of an invasive species can improve the capacity to predict further spread and underpin strategies to limit spread. Here, a structured sampling programme was used to assess the current distribution of feral populations of Pacific oysters, *Crassostrea gigas*, in Ireland. Sixty-nine sites were sampled using a standardised protocol combining semi-quantitative and quantitative approaches. Sites were chosen to represent variation in proximity to aquaculture and a range of environmental variables. Oyster populations were found at 18 locations, with densities ranging from single individuals to nine individuals per m^2. The broad size range of oysters found suggests more than one recruitment event over the past years. Logistic regression indicated that feral oysters were positively associated with the presence of hard substrata or biogenic reef, long residence times of embayments and large intertidal areas. There was also a tendency for oysters to occur disproportionately in bays with aquaculture, but > 500 m from it. Small-scale analysis within sites showed that oysters were exclusively attached to hard substrata and mussels. The approach taken here provides a rigorous repeatable methodology for future monitoring and a detailed basis for the prediction of further spread.

Keywords: logistic regression, environmental variables, *Crassostrea gigas*, aquaculture

2.2 Introduction

Improving our knowledge of the distributions of non-native species assists predictions of spread and allows the strategic targeting of management actions for their control (Anderson et al. 2003; Gormley et al. 2011; Simberloff and Rejmánek 2011). Species' distributions are not easy to predict because they are controlled by many factors acting upon different life stages, e.g. hydrodynamics and tides can influence the delivery of spat (Roughgarden et al. 1988; Gaines and Bertness 1992; Dunstan and Bax 2007) whereas habitat availability is important for settlement (Travis and Dytham 1999) and post-settlement mortality can strongly affect recruitment patterns (Connell 1985; Hunt and Scheibling 1997; Jenkins et al. 2009). Furthermore, propagule pressure plays a major role in the early stages of an invasion (Lockwood et al. 2005; Johnston et al. 2009).

Distributions of invasive species are often documented in a fragmented and descriptive way, and data are often collated from a number of sources and can be of mixed quality and resolution (Zaniewski et al. 2002; Elith et al. 2006; Hulme and Weser 2011). Interpreting such data requires synthesis and meta-analysis and does not yield unequivocal tests of hypotheses about factors associated with colonisation by the species. It is recommended to use well-designed survey data and analyse functionally relevant predictors (Elith and Leathwick 2009). Thus, extensive coordinated surveys and monitoring and assessment programmes using carefully standardised protocols and well thought-out designs are preferable as they avoid survey bias and result in balanced comprehensive datasets. When a set of sites has been surveyed and presence/absence or abundance has been recorded, generalised linear models encompassing logistic regression are especially useful as additive combinations of predictors and manually selected interaction terms representing interactions between predictors can be included (Elith and Leathwick 2009; Franklin 2009).

Species distribution models (SDM) estimate the relationship between species and spatial and/or environmental characteristics and are widely used to estimate current and potential distributions of species (Elith et al. 2006; Franklin 2009). They have been widely used in terrestrial ecosystems but applications for distribution of species in marine habitats are sparse (Kelly et al. 2001; Garza-Pérez et al. 2004; Beger and Possingham 2008; Robinson et al. 2011). Additionally, including measures of

introduction effort is important for assessing on-going biological invasions or for identifying areas that are susceptible to invasion (Herborg et al. 2007; Therriault and Herborg 2008).

The Pacific oyster (*Crassostrea gigas* Thunberg, 1793) has been introduced for aquaculture to many parts of the world and has become one of the world's main aquaculture species (FAO 2012b). In many intertidal habitats outside aquaculture areas it has established permanent, self-sustaining and also invasive populations worldwide (Reise 1998; Ruesink et al. 2005; Troost 2010). In Europe, there are invasive populations along the Atlantic and North Sea coasts, e.g. in Germany (Reise 1998; Diederich et al. 2005; Nehls and Büttger 2007), the Netherlands (Fey et al. 2010) and France (Cognie et al. 2006). Invasive oyster populations can have substantial impacts, including saturation of the carrying capacity of estuaries, change in phytoplankton composition and food webs, spatial competition with other species and alteration of habitat heterogeneity (Ruesink et al. 2005; Cognie et al. 2006; Troost 2010). Recent studies indicate that the northern boundaries of distributions of this species are expanding; they have been found in England and Wales (Couzens 2006), Northern Ireland (Guy and Roberts 2010) and Scandinavia (Wrange et al. 2010). Given its rate of spread, there is an urgent need to characterise its pattern of establishment at an early stage and determine which factors are associated with its presence or absence and spread.

Pacific oysters are habitat generalists. Their colonization process generally starts with settlement onto pieces of hard substratum, e.g. shell fragments, stones, mussel beds, aquaculture racks or harbour walls and often results in dense reef formation due to their gregarious behaviour (Reise 1998; Diederich 2005; Nehls and Büttger 2007). They can be found in a wide range of habitat types, from coastal sheltered soft-sediment environments to exposed rocky shores (Ruesink et al. 2005; Cognie et al. 2006; Troost 2010) and they are tolerant of a wide range of environmental conditions (Fabioux et al. 2005; Dridi et al. 2007; Enríquez-Díaz et al. 2009). Growth of oysters occurs between 3-35°C whereas temperatures for spawning range between 16-34°C (Mann et al. 1991; Ruiz et al. 1992) and increased summer temperatures have been associated with the spread of Pacific oysters in Europe (Diederich et al. 2005; Fey et al. 2010).

Aquaculture has been closely associated with the rapid spread of Pacific oysters; in many locations wild oyster populations have established soon after oyster farming had commenced (Brandt et al. 2008; Troost 2010). Aquaculture is often practiced in shallow and almost enclosed bays. A high larval retention in those shallow and enclosed bays will certainly prevent the planktonic larvae drifting away from suitable habitats. Pacific oyster larvae often settle within a few kilometers of their source, although different hydrodynamic conditions and residual currents can change their dispersal and colonization patterns (Wehrmann et al. 2000; Brandt et al. 2008).

Pacific oysters were introduced to Ireland in 1973 for aquaculture and they are now extensively farmed around the north, the west and south coast (Browne et al. 2007). Recently, there have been reports of individuals being found in the wild, but the extent and distribution of these populations is not yet known. In this study, a coordinated national sampling programme was undertaken to document the current distribution of Pacific oysters in Ireland. A cost-effective, but rigorous and repeatable sampling protocol was developed. It was used at selected sites, and enabled the characterisation of factors associated with the presence or absence of oysters to improve the prediction of its future spread.

2.3 Methods

2.3.1 Sampling programme

A sampling programme was undertaken from May until September 2009 at 69 sites around the coast of Ireland (coordinates and manual provided in Appendix 1 and 2). The sites were selected to represent variation in distance from aquaculture, latitude, wave exposure, embayment residence time (also known as flushing time), intertidal area (shore width) and habitat type (Table 2.1 and Appendix 1). Sites ranged in area between approximately 3500 m^2 (narrow rocky shores), 40000 m^2 (mussel beds) and 80000 m^2 (sandflats and mudflats) and salinities ranged between 22.5 and 35 psu.

Sites were visited and sampled during spring low tides. The sampling methodology was designed to be flexible, repeatable and efficient. *Phase 1* of the methodology involved a timed semi-quantitative sample of oysters at each site and a simple characterisation of the habitats available at that site. It could be completed within 40-45 min maximising the number of sites it was possible to visit in the

available time. *Phase 2* was only used at sites where oysters were found. It involved a more detailed quantitative survey of the area of greatest density of oysters to enable more precise statistical comparisons among sites and between present and future surveys. In addition, it also provided the basis for analyses of small-scale associations between oysters and features of the biotic and abiotic environment. Further details of these phases are provided below.

Phase 1: At each site the first 40-45 min were spent identifying the habitat types in the lower intertidal, searching for Pacific oysters within those areas and assessing their abundance using the SACFOR scale (Connor et al. 2004). The abundance categories used were (in individuals per m^2): Superabundant (100-999/m^2), Abundant (10-99/m^2), Common (1-9/m^2), Frequent (0.1-0.9/m^2), Occasional (0.01-0.09/m^2), Rare (<0.009/m^2) and Absent. After the timed search, each site was classified using a modified EUNIS framework of habitat types (Connor et al. 2004) to better describe the types of substratum encountered in the habitats studied here. The modified categories were: bedrock; boulders (25.6 cm – 102.4 cm); cobbles and pebbles (25.6 cm – 1.6 cm); gravel (1.6 cm – 0.4 cm); sand (0.063 mm – 4 mm); mud (< 0.063 mm); mixed sediment; biogenic reef (mussel beds, *Sabellaria* reefs); and macroalgae-dominated substrate (from here onwards referred to as 'macroalgae'). More than one habitat was noted for a site if the type of substratum changed significantly (visual estimation) (see Appendix 1). Coverage by different types of substratum was expressed in % of the area by visual estimate with generally 10% accuracy except in a few cases where 5% were estimated, especially in the lower ranges.

Aquaculture was categorised as absent, close (trestles with Pacific oysters were encountered during the timed search) and far (known to be present in the embayment, but generally > 500 m from the study site) based on licensing information from Bord Iascaigh Mhara, the Irish Sea Fisheries Board. Wave fetch was used as an index of wave exposure; it was defined as the closest distance to the land in 16 angular sectors (average in km), and calculated after the method developed by Burrows et al. (2008). Residence time was determined using the formula developed by Hartnett et al. (2011): $T_r[days] = 8.65 \,/\, TPR - 2.45 \times B_0 + 0.59 \times L - 5.05$. TPR is the tidal prism ratio, which was derived from the volume of water between low water and high water [m^3] divided by the volume of the embayment at high water

[m^3]; B_0 is the width of the mouth of the embayment [km]; and L is the length of the embayment along the longitudinal axis [km]**.** Each site's intertidal width was categorized into 1 = 0-50 m, 2 = 51-100 m, 3 = 101-150 m, 4 = >151 m, based upon measurements from high water line to the lowest water line. Each site was classified according to each of the variables described above with up to three habitats per site (see Table 2.1 and Appendix 1 for details).

Phase 2: When oysters were present at overall densities greater than 0.1 individuals/m^2 (i.e. abundance category Frequent or above), two transects of 30 m x 1 m were randomly placed in the habitat of greatest oyster density. In each transect, the numbers of oysters, the sizes of oysters to the nearest mm (Vernier callipers) and substrata to which they were attached were recorded. If more than 100 oysters were found in the first transect, only counts and attachments to substrata but no further size measurements were recorded in the second transect. On mussel beds, 17 random quadrats of 1 m x 1 m were taken in each transect as densities were too high to account for every single oyster within a transect line. Conversely, in the Shannon Estuary, extended transects were run on two rocky shores to ensure that sufficient length measurements were collected for size frequency analysis.

To estimate substratum availability, substrata were recorded quantitatively along two 10 m tapes placed haphazardly in the habitat where oysters occurred. The distances along the tape at which the substratum changed from one type to another were recorded, and these distances were converted into estimates of the percentage area covered by different substrata. These data were used in conjunction with the data collected on oysters and the substratum they were attached to. This enabled tests of small-scale associations between oysters and biotic and abiotic features of habitat.

Teams of researchers from the different institutions were trained in the use of the protocols by the coordinator of the project, who also accompanied each team on its first sampling trip to ensure consistency of methodology. Each team was assigned a number of specific sites to survey in a sequence that ensured minimal temporal and observer bias with respect to the site variables described above. Each team surveyed a maximum of two sites on each day, with pairs of sites selected to be in close proximity to each other. Each site visit was timed such that the low shore could be

visited within 20 minutes of a spring low tide. In any given day, a *Phase 1* survey was initiated one hour before predicted low water, with the timed search gradually progressing down the shore in step with the receding tide. If oysters were found at that site, the *Phase 2* survey was completed during the incoming tide. If no oysters were found, the team moved on to the second site for the day and completed a *Phase 1* survey during the incoming tide.

2.3.2 Data Analysis

Logistic regression allows multiple explanatory variables, and their interactions to be included in a single model (Vittinghoff et al. 2005). Here, logistic regression was used to find a set of environmental variables that could be used to predict oyster presence/absence. In the 69 sites visited during the sampling programme, 127 habitats were identified (*Phase 1*) and classified for presence/absence of oysters and the environmental conditions encountered, including proximity of aquaculture (see Appendix 1). The model was based on this set of 127 observations.

Prior to running the model, Spearman rank correlations (ρ) were calculated among all pairs of environmental variables. When a Spearman rank correlation exceeded an absolute value of 0.35, one of the pair of variables was omitted from the model to avoid co-linearity (Dormann et al. 2012). The following pairs of variables had $|\rho| > 0.35$: Macroalgae with Rest ($\rho = -0.63$), Latitude with Residence ($\rho = 0.46$), Rest with Width ($\rho = 0.46$) and Macroalgae with Hardreef ($\rho = -0.35$), where 'Rest' refers to the EUNIS categories sand, gravel, mixed sediment and mud, 'Hardreef' refers to bedrock, cobble, pebble and biogenic reef, 'Residence' refers to residence time and 'Width' refers to shore width. Latitude, Rest and Macroalgae were therefore omitted from the model. The variables used in the full model were % cover of bedrock, cobble, pebble and biogenic reef (called Hardreef), proximity to aquaculture (called Aquaculture with levels: absent, far and close), residence time (called Residence), wave fetch (called Fetch) and shore width (called Width with levels < 50 m and ≥ 50 m)(see also Table 2.1). The full logistic model used a logit link function and a model equation:

Oysters~1+Width+Fetch+Aquaculture+Residence+Hardreef+Hardreef:(Fetch+Width + Residence+Aquaculture)+Fetch:Width

The variable Oysters is 1 if oysters are present and zero otherwise, the other variables are explained in Table 2.1 and ':' indicates an interaction between two variables. All interactions between Hardreef and the other variables are included in this full model because oysters attach almost exclusively on hard substrata. Additionally, an interaction between Fetch and Width was included because the extent of the shore is not considered in the calculation of wave fetch and can be important when shores are wider than 100 m (see Burrows et al. 2008). Starting from this full model, backward stepwise selection was used with Akaike's Information Criterion (Akaike 1974) to arrive at a 'best fit' model. The performance of the 'best fit' model to correctly classify oyster presence/absence at a habitat unit was quantified using ROC curves and their AUC values (Fielding and Bell 1997). Additionally, a probability threshold that gave a classifier that weighed omission errors (false negatives, where oysters are incorrectly predicted to be absent) more than commission errors (false positives, where oysters are incorrectly predicted to be present) was selected as this type of classifier is mostly desirable for invasive species (Gormley et al. 2011).

χ^2 analysis (goodness-of-fit test) was used to test hypotheses about small-scale associations between oysters and different types of substratum (using data obtained during *Phase 2*).

To estimate the number of oyster cohorts at each individual site the lowest number of modes for each site's oyster length-distribution was estimated using the method of Manly (1996). As oysters usually recruit only once per year in Europe (e.g. Diederich 2005), one would expect the mean size of oysters from different age classes to occur at separate peaks in a size frequency distribution. Manly's method uses a kernel density estimation and smoothed bootstrap re-sampling to find the lowest number of modes that best fits the data. The function fits a distribution to the data with the "h" attribute controlling the "smoothing" of that distribution. The function slowly decreases h from a large value and stops at the exact value where the distribution changes from having one mode to having two modes. A dataset of a similar size is then randomly sampled 1000 times and compared to the one being tested from within the distribution. A distribution is then fitted to the random dataset using the h value being tested. If the number of modes is greater than the number being tested less than 50 out of 1000 times, then the distribution fitted by the h value

is the optimum distribution. If not, h will be further decreased and the steps above will be repeated.

Logistic model calculations were performed with R, using the MASS and pROC packages (R Development Core Team 2011). For calculations of residence time and wave fetch detailed descriptions can be found in the original papers (Burrows et al. 2008; Hartnett et al. 2011).

2.4 Results

2.4.1 Distribution, densities and sizes of feral Pacific oysters

Pacific oysters occurred at 18 of the 69 sites (Figure 2.1). No oysters were found at sites in the south. Most oysters were found in the large estuaries of Lough Swilly, Lough Foyle and the Shannon, with many sites scored Common or Frequent for the abundance of oysters. Oysters were Occasional or Rare at five sites in Galway Bay and single individuals of oysters were found at one site in Tralee Bay and another site in Ballynakill Harbour, which therefore scored Rare on the SACFOR scale.

Oyster densities in the different habitats varied from single individuals (ind.) to 8.5 ind./m^2 (Table 2.2). Sites in Lough Swilly and Lough Foyle had the highest densities whereas sites in the Shannon Estuary, Galway Bay, Tralee Bay and Ballynakill Harbour oysters were found in lower densities (Table 2.2). Pacific oysters were mostly found in the lower intertidal. During an exceptionally low spring tide, a subtidal mussel bed could be accessed at Rathmelton in Lough Swilly, where densities were estimated at 12.5 ind./m^2 (not listed in the Table 2.2).

At all of the sites with oysters > 0.1 ind./m^2, the range of sizes of oysters found exceeded 120 mm (Figure 2.2). In Lough Swilly, oyster sizes ranged from 13.8 mm – 125.7 mm (n = 147) on a mussel bed and from 25.3 mm – 135.0 mm (n = 182) on a rocky shore. Similar sizes of oysters from 23.0 mm – 135.5 mm (n = 182) were also measured on a mussel bed in Lough Foyle. In the Shannon Estuary slightly larger oysters were found, with the smallest and largest oyster measuring 43.4 mm and 146.2 mm (n = 125) respectively at Loghill. At Glin, oyster sizes ranged from 40.4 mm – 123.0 mm (n = 101). Four, six and eight modes were found in the size distributions except on mussel beds in Lough Foyle and Lough Swilly where only one mode was identified (Figure 2.2).

2.4.2 Associations between oysters and environmental variables at the scale of sites (*Phase 1*)

127 different habitats were identified at the 69 sites of which there were 27 with oysters present and 100 where no oysters were found (Figure 2.3). Four variables (Aquaculture, Width, Hardreef and Residence) and no interactions were retained in the 'best-fit' logistic regression model (Table 2.3). Comparing the best-fit model's predictions against the oyster presence/absence data gave an AUC of 0.9. Applying a

classification probability threshold of 0.1 to this model (corresponding to the threshold that maximised the sum of specificity and sensitivity) gave 25 true positives, 71 true negatives, 29 false positives and 2 false negatives for the oyster presence/absence data in this study. Oyster presence was positively associated with Hardreef (bedrock, boulders, cobbles, pebbles or biogenic reef), Residence and Width ≥ 50 m. Aquaculture far was also positively associated with oyster presence, with Aquaculture far showing a weakly significant increase in the probability of oyster presence compared to Aquaculture close ($p = 0.035$, Table 2.3). There was no detectable reduction in oyster presence when aquaculture was absent, indicated by a high standard error on the regression coefficient (Table 2.3). It is important to note that Latitude, Rest and Macroalgae, which were omitted from the full model due to co-linearity, could equally well underlie the same associations as the terms that were left in the model in their place (i.e. Residence, Width and Hardreef respectively). Thus, Latitude might equally be positively associated with oyster presence whereas Macroalgae or a low % cover of Rest might be negatively associated with oyster presence. Fetch was in the full model but was not kept in the best-fit model owing to its low explanatory power.

2.4.3 Small-scale associations between oysters and habitat within sites (*Phase 2*)

Oysters were disproportionately associated with hard substrata (boulders, cobbles, pebbles and mussels) given their availability relative to that of macroalgae, sand, *Sabellaria* tubes and mud (Table 2.4). At sampling sites in the Shannon Estuary as well as on a rocky shore in Lough Swilly significantly more oysters were found on boulders, cobbles or pebbles than on mud, sand or macroalgae than expected by chance given the availability of those substrata. At Ballybagley in Lough Swilly, all oysters were found on mussels, boulders or cobbles. This was also true for oysters found in Lough Foyle where 100% of the oysters were found attached to mussels. On a mussel bed in Lough Swilly, most oysters were observed on mussels whereas on mud and macroalgae no oysters were found.

Tables

Table 2.1 Categories of environmental variables and aquaculture. The number of habitats with oysters present and absent is only shown for categorical variables. Note that categories for Width with the same superscript letters were combined for the logistic regression. Latitude was not included in the model and Hardreef was the only substratum cover used in the full model. More details of the variables are provided in the text.

Variable	Category	Oysters present	Oysters absent
Latitude	low: N51°- N52.3°	1	41
	medium: N52.3°- N54°	12	38
	high: N55°	14	21
Aquaculture	absent	1	12
	close	5	52
	far	21	36
Width	0-50 m [a]	5	28
	51-100 m[b]	10	31
	101-150 m[b]	5	12
	>151 m[b]	7	29
Fetch	$\log_{10}$(km) transformation, continuous		
Substratum cover (Hardreef)	%, arcsine transformation, continuous		
Residence	days, continuous		

Table 2.2 Density of Pacific oysters estimated by transects (2 x 30 m^2) or random quadrats (2 x 17 x 1 m^2 on mussel beds) in intertidal areas with the highest density of oysters at each site at which oysters were found. The locations of sites can be seen in Figure 2.1. At sites scored rare or occasional on the SACFOR scale (see methods), no transects were used as densities were too low. In those cases, the SACFOR values are given in the table as Occasional = 0.01-0.09/m^2 or Rare = < 0.009/m^2.

Location	No. of oysters m^{-2} (± SD)
Lough Foyle	
Muff, mussel bed	5.35 (0.42)
Longfield	0.38 (0.04)
Ball's Point	0.38 (0.37)
Moville	rare
Lough Swilly	
Rathmelton, rocky shore	6.32 (0.31)
Rathmelton, mussel bed	8.53 (0.17)
Inch Island	0.76 (0.17)
Ballybagley	0.85 (0.07)
Galway Bay	
Ballynacorty	rare
Dunbulcaun	rare
Parkmore	rare
Finvarra	occasional
Ballyvelaghan	rare
Shannon Estuary	
Glin	0.72 (0.49)
Loghill	0.68 (0.31)
Tarbert	rare
Tralee Bay	
Black Rock, Spa	rare
Ballynakill Harbour	
Letterfrack	rare

Table 2.3 Coefficients, Standard Errors and p-values from the 'best fit' logistic regression model. The intercept corresponds to Width ≥ 50 m, Aquaculture close, Residence = 0, Hardreef = 0.

	Estimate	Standard Error	p-value
Intercept	-4.63	0.86	<0.001
Width < 50 m	-2.28	0.93	0.010
Aquaculture far	1.41	0.67	0.035
Aquaculture absent	-0.37	1.31	0.780
Residence	0.06	0.02	0.001
Hardreef	2.69	0.85	0.002

Table 2.4 Observed and expected numbers of oysters depending on availability of substrata at sites where transects or quadrats were sampled (*Phase 2* of the protocol). Chi² goodness-of-fit test was used and p-values were simulated when expected values were smaller than 5.

		No. of oysters			
Location	Available substratum (%)	Obs.	Exp.	*Chi²*	*p*
Shannon Estuary					
Glin	mud, sand (60%)	0	60.60	171.97	<0.001
	boulders or cobbles (37%)	101	37.37		
	macroalgae (3%)	0	3.03		
Loghill	boulder, cobble (80%)	125	100.00	31.25	<0.001
	mud (10%)	0	12.50		
	macroalgae (10%)	0	12.50		
Lough Swilly					
Rathmelton, mussel bed	mussels (47%)	267	126.90	292.11	<0.001
	boulder or cobbles (10%)	3	27.00		
	mud (28%)	0	75.60		
	macroalgae (15%)	0	40.50		
Rathmelton, rocky shore	boulder, cobbles, pebbles (52%)	373	196.56	330.07	<0.001
	Sabellaria (26%)	5	98.28		
	mud (22%)	0	83.16		
Inch Island	mussels (78%)	22	17.16	6.21	<0.050
	mud (22%)	0	4.84		
Ballybagley	mussels (35%)	26	17.85	98.28	<0.001
	boulder or cobbles (11%)	25	5.61		
	mud (13%)	0	6.63		
	macroalgae (41%)	0	20.91		
Lough Foyle					
Muff, mussel bed	mussels (90%)	156	140.40	17.33	<0.001
	mud (10%)	0	15.60		
Ball's Point	mussels (50%)	8	4.00	8.00	<0.010
	mud(50%)	0	4.00		
Longfield	mussels (92%)	13	11.96	1.13	>0.050
	sand (8%)	0	1.04		

Figures

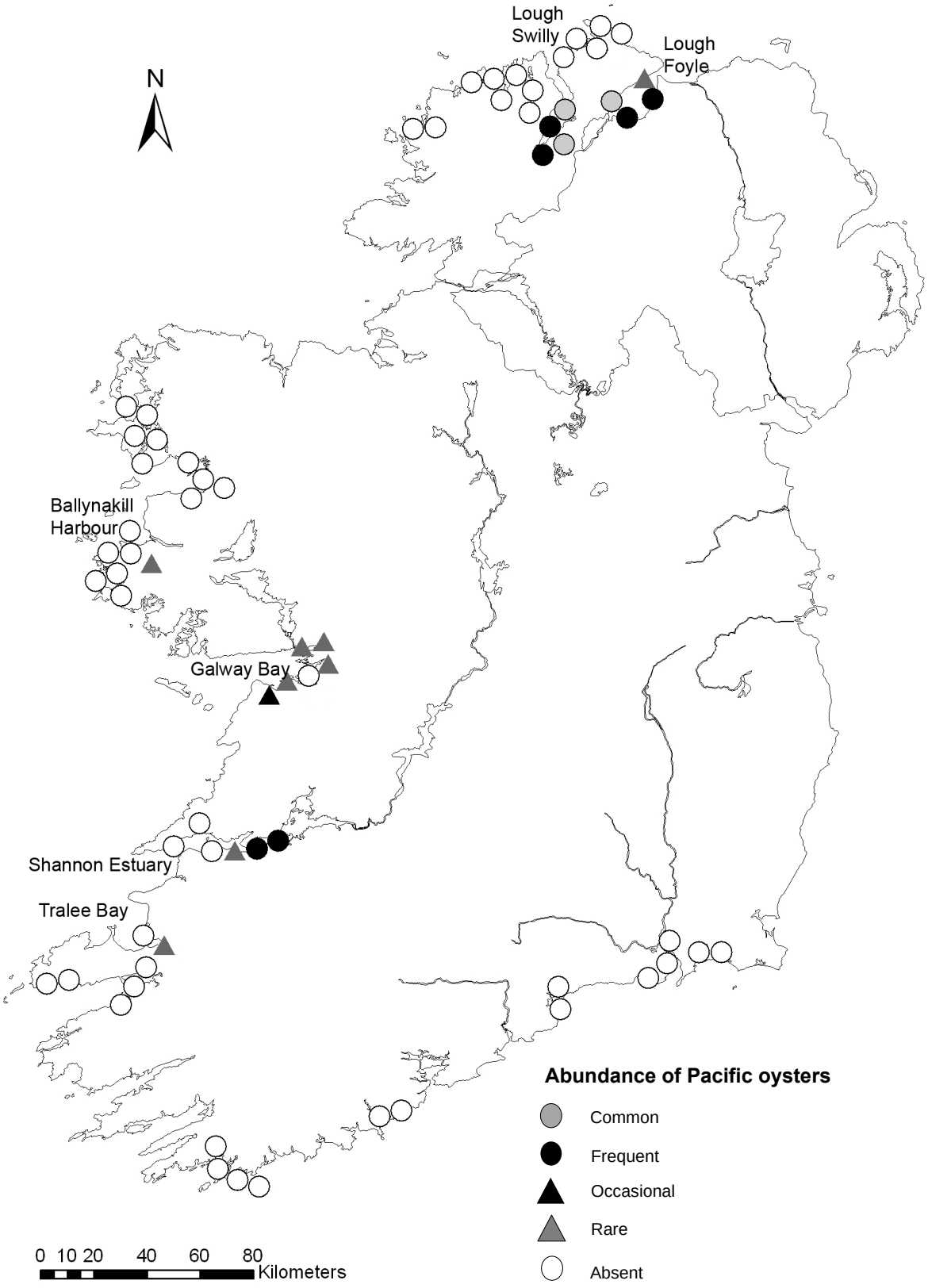


Figure 2.1 Sampling sites and abundance of feral Pacific oysters in Ireland in 2009. Sites are categorised on the SACFOR scale on the basis of timed searches (see methods) by symbols. Names of embayment where oysters were found are given.

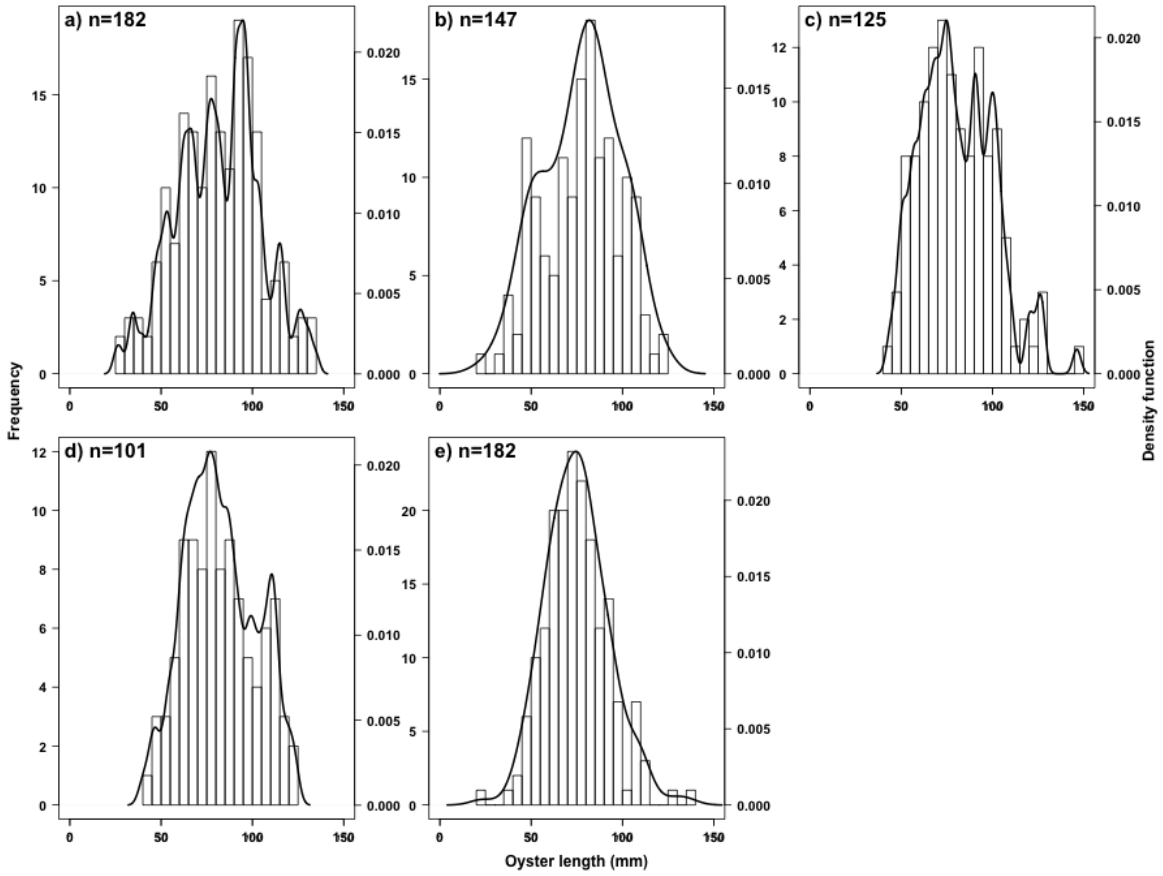


Figure 2.2 Size-frequencies of Pacific oysters in 5 mm size intervals at different locations and kernel density estimates (second y-axis) for the size distributions. A combination of kernel density estimation and smoothed bootstrap re-sampling was used to test for the modality of the oyster size distributions at each site. a Lough Swilly, Rathmelton, rocky shore (8 modes), b Lough Swilly, Rathmelton, mussel bed (1 mode), c Shannon Estuary, Loghill (6 modes), d Shannon Estuary, Glin (4 modes), e Lough Foyle, Muff, mussel bed (1 mode). Measurements were taken from transects and number of modes identified after significance level testing (see method), n = number of oysters.

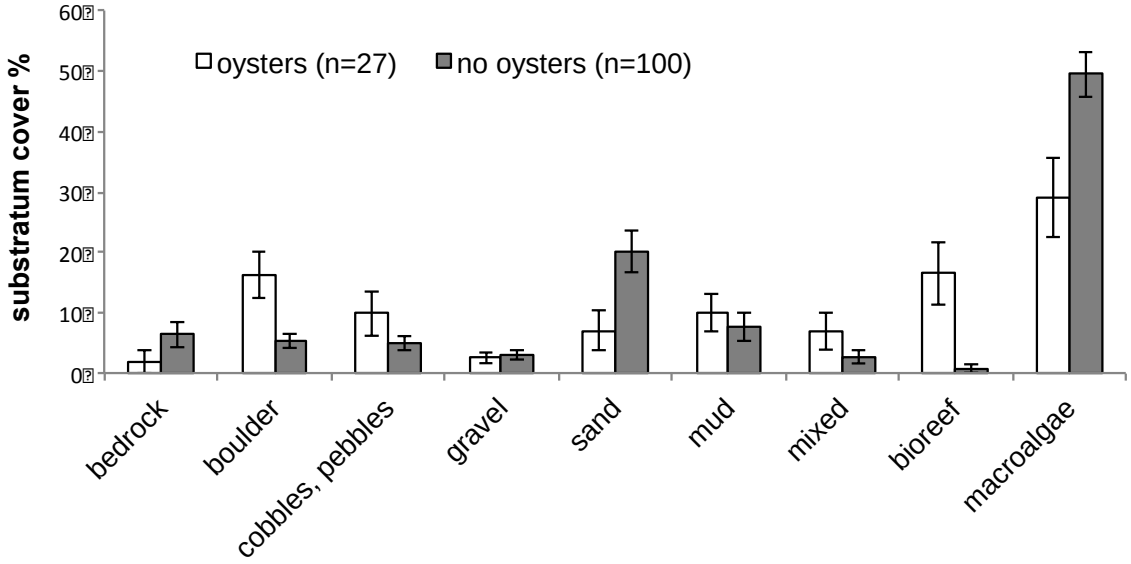


Figure 2.3 Visualized results of estimated types of substratum from all collected habitats with and without oysters. Shown are means (± standard errors). The number of habitats with and without oysters is given in brackets.

2.5 Discussion

This study confirms that feral populations of Pacific oysters have become established in intertidal habitats in Ireland. Pacific oysters were found at 18 of 69 sites. Densities at those sites ranged from single individuals to 8.5 ind./m^2 in the intertidal; they were also observed at higher densities in the shallow subtidal (J Kochmann, personal observation) and are also known to occur in subtidal areas in Loughs Foyle and Swilly (McGonigle et al. 2011; Marine Institute and BIM 2012). At sites where transects were sampled, the sizes of oysters always exceeded 120 mm, which, together with the mode analysis, strongly suggests a successful recruitment of Pacific oysters in more than one year in several bays in Ireland.

Comparably low densities of 0.01-42.44 ind./m^2 were found e.g. in Sweden and Denmark (Wrange et al. 2010), the Wadden Sea (Reise 1998; Wehrmann et al. 2000; Diederich et al. 2005) or Argentina (Orensanz et al. 2002) in the early stages of invasion. In the Wadden Sea, Pacific oysters usually reach 20-50 mm in the first year and 30-80 mm in the second year on mussel beds (Reise 1998; Schmidt et al. 2008; Fey et al. 2010) which are the lower size ranges also found in this study. Guy and Roberts (2010) found densities of 1 ind./m^2 in Northern Ireland with the largest oysters reaching lengths of 155 mm. Based on their analysis of age-size relationships in Strangford Lough (Northern Ireland), the largest oysters found in this study were approximately 6 years old. However, age-size relationships of *C. gigas* can vary among sites (references in Diederich 2006), so this inference is tentative.

The broad size distribution found at all the locations, with a range > 100 mm, suggests several years of recruitment at all sites. One to eight modes were identified in the size frequency distributions of oysters sampled at different sites. However, the statistical method used is a rather conservative way of looking for gaps in size distributions and the modes are unlikely to correspond to the number of recruitment years. For example, some of the modes are only 10 - 20 mm different, and well within the range of variation for oysters of a single cohort. On mussel beds, only one mode was identified, but, it is very unlikely that individual oysters from the same year of recruitment would vary ~100 mm in total length. Similar 'spurious' normal-distributions of oysters have been observed on mussel beds in the North-Frisian Wadden Sea, however, the authors observed different size-frequency distributions in

subsequent years (Büttger et al. 2011). New recruitment (oysters < 15 mm length) has also been observed in the intertidal in 2011 (J Kochmann, pers. obs).

In determining factors associated with the occurrence of oysters the 'best fit' model (Table 2.3) included no interaction terms but hard substrata and biogenic reef, residence time and shore width (> 50m). Weaker but detectable effects of aquaculture were also detected. The AUC of 0.9 indicates a high discriminatory ability of the model. AUC summarises a model's classification performance over all possible thresholds (Fielding and Bell 1997). However, when false negatives should be given greater weight than false positives (e.g. in the case of invasive species) individual thresholds are preferred (Liu et al. 2005; Lobo et al. 2008). The best individual classification threshold for the model was 0.1, resulting in 2 false negatives and 25 true positives. Another approach to estimate model performance is Cohen's kappa (Cohen 1960). Cohen's kappa is maximised for the model here at a threshold of 0.48. However, classification performance with this threshold had less true positives and more false negatives and was therefore not considered as the best classifier.

The same data were used to fit the model and to calculate model performance, which is not an independent validation of the model. Moreover, several sites were sampled within individual embayments, and several habitats were sometimes sampled within sites, thus sampling locations were spatially clustered and may lack independence. This might have led to spatial autocorrelation, which can cause p-values to be underestimated and therefore increase Type I errors. However, initial results from mixed-model logistic regression that correct for spatial autocorrelation with a random effect of site on the intercept did not change results, i.e. estimated coefficients remained qualitatively the same as in the logistic model. The predictive performance of the model could be tested easily elsewhere as oyster populations have been found in places worldwide outside their native range for at least 40 years (Ruesink et al. 2005; Carrasco and Barón 2010).

Recruitment and spreading of Pacific oysters has often been observed in areas where they have been introduced for aquaculture before (Diederich et al. 2005; Schmidt et al. 2008; Melo et al. 2010). Despite the fact that only one single individual of Pacific oyster was found in a bay without aquaculture, oysters were not significantly associated with the presence of aquaculture (Fisher's exact test with 69

sites: p = 0.67). However, most of the sites visited were located in bays where aquaculture of Pacific oysters was present (aquaculture present in 62 of the 69 sites). It will be important to sample more bays where aquaculture is absent to draw more detailed and robust conclusions about associations of aquaculture and Pacific oysters in the wild and exclude other vectors of introduction. Yet the arrival of Ireland's feral Pacific oysters from other areas where they are already established (e.g. UK, French coast or Wadden Sea) is highly unlikely as the relevant coastal currents either do not reach the Irish coast or the current velocity is too small to allow sufficient time for dispersal before the settlement of larvae (Brown et al. 2003; Fernand et al. 2006). As recently suggested for Pacific oyster populations in the Wadden Sea, an initial, sporadic or continuous input of larvae from local oyster farms can be inferred (Möhler et al. 2011). However, feral populations can also be self-recruiting and be decoupled from the closest aquaculture (Chapter 4).

Pacific oysters show gregarious behaviour, and naturally form into reefs. They often recruit close to conspecifics and also on unused and abandoned structures of oyster racks and trestles (Diederich et al. 2005; Cognie et al. 2006; Tamburri et al. 2007). Thus, more oysters were expected in close proximity to oyster racks and aquaculture sites. However, a tendency of oysters to be present more frequently far from aquaculture was found (p = 0.035, Table 2.3). Differences in dispersal can result in differences in spatial patterns of distribution and abundance of species and wide dispersal can decouple propagule supply from local conditions (Kinlan and Gaines 2003). The dispersal of larvae away from aquaculture sites and thus, their subsequent settlement at sites further away from conspecifics might play a larger role than their gregarious behaviour. Typical drift distances of *C. gigas* larvae range between 5-15 km and can be strongly influenced by hydrographic conditions (Brandt et al. 2008). In the northern areas of the distribution of Pacific oysters, reduced oocyte sizes have been observed and larval dispersal distances are increased due to a longer development time (Cardoso et al. 2007). A significantly lower mean biomass of wild Pacific oysters on highly exploited racks than on low exploited or unused racks was observed by Cognie et al. (2006). It was suggested that farmers' upkeep activities might explain those patterns and disturbance activities possibly played a role in the observations here with a negative association of close proximity to aquaculture and oyster presence. However, as the significant difference between Aquaculture far and

Aquaculture close in the 'best fit' model was close to 5%, proximity to aquaculture is not a good predictor of oyster presence or absence.

Even if larvae don't necessarily behave as passive particles (e.g. Knights et al. 2006), flushing characteristics of coastal waterbodies such as residence times can help in the identification of areas likely to retain larvae (see Dyer and Orth 1994). Indeed, limitations in larval supply resulting from the interactions between spawning location and local hydrodynamics may impede the proliferation of introduced species in bays (Dunstan and Bax 2007; Brandt et al. 2008; Rigal et al. 2010). The planktonic larvae of Pacific oyster can spend 3-4 weeks in the water column before they reach competence to settle (Quayle 1988). Thus, enhanced oyster settlement could be expected to occur in bays exceeding the residence time of 21 days as larvae may be entrained for the duration of their planktonic phase. Except for two bays, Ballynakill and Tralee Bay, where single individuals of oysters were found, oysters were present in bays with residence times of more than 21 days.

Wave exposure can play a role in abundance patterns of Pacific oysters on rocky shorelines, with propagule pressure being a likely driver of variation in different oyster density among sites (Ruesink 2007). However, in this study, wave exposure (quantified by wave fetch) played no role in the selected model of oyster presence. As shown by Burrows et al. (2008), the extent of the shore is not considered in the calculation of wave fetch and can be important when shores extend > 100 m. This might be especially important when extensive intertidal areas offer some kind of hard substratum for oyster attachment, e.g. mussel beds. Thus, intertidal width was additionally used as a proxy for settlement area and the model selection showed that intertidal width was a better predictor of oyster presence with a shore width smaller than 50 m being negatively associated with oyster presence. The positive correlation of Width with Rest might be an indication of the characteristics of the larger intertidal areas, which were often extensive intertidal sand or mud flats. Although wave fetch explained 50% of variation in rocky shore communities in the study by Burrows et al. (2008), the authors acknowledged the importance of other factors, such as bathymetry, habitat complexity, and variation in recruitment or food supply. Likewise in this study, in which the presence of aquaculture, hard substrata, shore width and residence time better explained oyster presence than wave fetch.

Another factor that explained oyster presence was the availability of hard substrata. It is widely known that oysters start colonization with a few individuals settling onto pieces of hard substratum, e.g. shell fragments, stones, mussel beds or rocks (Reise 1998; Escapa et al. 2004; Diederich 2005; Nehls et al. 2006). Similarly, in the current study oysters were always found attached to bare boulders, cobbles, pebbles or biogenic reef (live or dead material) and were rarely found at sites with extensive cover of macroalgae. Due to the co-linearity of Macroalgae and Hardreef, the positive association of Hardreef with oyster presence could also be a negative association with the % cover of macroalgae. However, when both factors were kept in the full model, Hardreef was kept in the 'best-fit' model indicating it as a better predictor than Macroalgae. Similarly to the logistic model, the small-scale analysis of associations of oysters with different substrata revealed an expected positive association between oysters and hard substrata. Oysters were never found attached to macroalgae (with one single exception (J Kochmann, personal observation) and in the semi-quantitative sampling (*Phase 1*) were very rarely found under macroalgae. This is in agreement with a study by Diederich (2005) in a soft-sediment intertidal mussel bed, where significantly fewer recruits of Pacific oysters were found under *Fucus* than on bare hard substrata. Field studies with barnacle larvae have shown that algal fronds can inhibit settlement on their surfaces by exuding metabolites (Brock et al. 2007) and that macrophyte canopies could prevent larvae from settling on rocks underneath them because they sweep the surface and limit access to the substratum (Jenkins et al. 1999). These effects might also play a role in the settlement patterns of Pacific oysters. A study on the Eastern oyster *Crassostrea virginica* (Gmelin, 1791) showed that macroalgae can negatively affect recruitment and increase mortality (Thomsen and McGlathery 2006). Smothering of Pacific oysters was observed underneath dense layers of *Ulva* (Cadée 2004a). Observations during the timed searches (*Phase 1*) suggested that the green shore crab *Carcinus maenas* (Linnaeus, 1758) was common under macroalgal canopies. Information about predation on feral Pacific oysters in Europe is scarce (but see Diederich 2006), however, it is known that shore crabs are able to handle and feed on them (Walne and Davies 1977; Mascaró and Seed 2001a). Thus, predation by shore crabs might be another factor controlling the abundance and survival of oysters on intertidal habitats in Ireland, especially on shores dominated by macroalgae.

Both spawning and settlement of Pacific oysters take place only above a temperature threshold of 16°C (Mann et al. 1991; Ruiz et al. 1992; Rico-Villa et al. 2009). It is therefore widely assumed that temperature limits the spread of the species, and indeed there is considerable evidence in support of this contention (e.g. the link between warm years and large recruitment events in the Wadden Sea – Diederich et al. 2005) and temperature has been used to predict its potential geographic range (Carrasco and Barón 2010). If latitude were considered a broad proxy for temperature, the finding of greater densities of feral Pacific oysters in northern sites than southern sites might be considered surprising. In fact, local temperatures and biogeographic patterns cannot simply be predicted by latitude (Helmuth et al. 2002; Dutertre et al. 2010) and temperature data from the Irish Environmental Protection Agency (EPA 2010), which was available for some bays, suggests that averages of maximum summer temperatures cannot be simply characterized by latitude but vary between bays. Thus, the high correlation of residence time and latitude suggests that any influence of latitude in the current study might be more related to bay features than to temperature. Certainly, bay features can be related to temperature and part of the reason for a positive association between residence time and oyster occurrence in the current study may be that extensive shallow bays tend to be warmer at certain times than smaller deeper ones. As various authors have suggested, a possible climate change involving warmer summer water temperatures or higher frequencies of hot summers could result in a higher propagule pressure through a continuous input of larvae which will increase the possibility of a range expansion and further proliferation of Pacific oysters northward in shallow European bays, including those used for aquaculture farming (Diederich et al. 2005; Miossec et al. 2009; Dutertre et al. 2010).

Repeated monitoring using the protocol established here could be used to establish rates of spread and to determine the phase or stage of invasion (Reise et al. 2006; Blackburn et al. 2011) in Ireland. The time-scales from the first introduction to the first sightings of Pacific oysters vary between locations, e.g. 5 years on the island of Sylt (Reise 1998) to 30-50 years in the UK or South Africa (Robinson et al. 2005; Couzens 2006). In Ireland, almost 40 years have passed since the introduction of Pacific oysters in 1973. It is very likely that Pacific oyster cultivation has been initiated later in individual bays; however, experimental trials were common since

the 70's. The numbers of oysters found here are similar to those found in other introduced ranges during their early establishment (Reise 1998; Wehrmann et al. 2000; Orensanz et al. 2002; Diederich et al. 2005; Wrange et al. 2010). Time lags characterize the invasion process; they can vary substantially between species and locations but are fundamental for the management of invasive species (Crooks 2005; Reise et al. 2006). A four to six year phase of establishment of Pacific oysters has been described in the southern North Sea which was followed by a rapid increase of the population (Schmidt et al. 2008). It is expected that Pacific oysters in Ireland, particularly in the bays were densities are above 0.01 ind./m^2, are likely to expand and increase in densities, with stronger spatfalls expected in years with high water temperatures during summer. Even if breeding will occur only sporadically, the longevity allied with high individual gamete production of Pacific oysters will likely allow them to recover or increase from low densities (Reise 1998). Although the survey did not cover subtidal areas, Pacific oysters have been found on shallow subtidal native oyster (*Ostrea edulis*) beds in Lough Swilly and sporadically in Galway Bay (Marine Institute and BIM 2012). With 5.64 million feral Pacific oysters estimated from these habitats in Lough Swilly during a dredging survey in November 2011 (Marine Institute 2012), Pacific oysters are expected to form self-sustaining populations even if aquaculture ceases in this bay. Evidence was recently found for self-recruitment of feral Pacific oysters in Lough Foyle (Chapter 4).

The abundance of oysters at individual sites certainly cannot be explained exclusively by single factors. Beside sources of introduction, e.g. aquaculture, a comprehension of the early life history of Pacific oysters requires a broad understanding of abiotic and biotic factors. Large-scale dynamics affect pelagic larvae and benthic juveniles, and biological, small-scale interactions affect its survival and recruitment to the benthos. Experiments are needed to test causal mechanisms and further understand processes promoting and limiting spread. Recently, surface seawater and atmospheric temperature records were used to predict the potential geographic range of the Pacific oyster in South America (Carrasco and Barón 2010). However, the authors averaged monthly near-coast temperatures over several years and acknowledged that in some locations, especially in estuaries and tidal flats, their predictions of oyster occurrence did not match the real situation, most likely because of a mismatch between local and near-coastal temperature regimes. Predictor

variables such as residence time and habitat availability might increase the effectiveness for spatial predictions, particularly to discriminate among sites with similar temperature regimes. On the basis of this study, it should be anticipated that the sites most likely to develop populations of oysters would (a) be in embayments with temperature regimes allowing for oyster spawning and larval development and with long residence times and (b) have hard substrata, e.g. mussel beds and rocky shores and (c) not have extensive cover of macroalgae and d) have large intertidal areas. In the current study, oysters only occurred in bays with aquaculture, but further sampling of bays without aquaculture is needed to characterise the association more fully.

Unlike the situation in many other European countries, no dense intertidal reefs of Pacific oysters are established yet in Ireland, despite extensive aquaculture. However, an increase in abundance is expected and subtidal occurrences have also been confirmed in three large bays. The structured framework and sampling protocol here was used in cooperation with relevant state agencies in Ireland. Its cost-effectiveness and repeatability make it valuable and widely applicable for future assessments, allowing rigorous analysis of the extent of spread of oysters to new sites and habitats. Investigations describing the population dynamics at an early stage of marine bioinvasion are extremely valuable to gain insights into early stages of establishment, to improve prediction of further spread and directly inform strategies to reduce the risk of invasion.

Chapter 3

Effects of predation and macroalgae on survival and growth of juvenile Pacific oysters (*Crassostrea gigas*)

3.1 Abstract

Biotic interactions can play a key role in promoting or inhibiting spread of invasive species. Here, the influence of predation and macroalgae on growth and survival of juvenile Pacific oysters (*Crassostrea gigas*) and the relationship between numbers and sizes of predatory crabs and presence of oysters on shores in Ireland was addressed. A field experiment was set up in July 2011 at two intertidal macroalgae-dominated boulder shores where only single individuals of oysters occur. After 10 months, condition of oysters was not significantly decreased in the presence of macroalgal canopy; however, at one site shell growth was significantly reduced over a period of less than 4 months (from 6 mm per month to 7 mm per month on average). Although predation had a strong negative effect on oyster survival in a pilot experiment conducted in July 2010, no effect was detected in the present study. Trapping of shore crabs (*Carcinus maenas*), which are considered one of the main potential predators of Pacific oysters in their introduced range, revealed the presence of significantly larger crabs at sites where oysters were absent. More crabs (> 35 mm carapace width) were found at shores where oysters were rare but numbers were not significantly different from those on other shores. Although spatial variation in survival and growth of juvenile oysters was found, the results suggest that pre-settlement processes might better explain abundance patterns of Pacific oysters in intertidal habitats than post-recruitment processes.

Keywords: biotic resistance, predation, *Crassostrea gigas*, macroalgae, condition index

3.2 Introduction

A successful invasion requires different barriers or filters to be overcome (Kolar and Lodge 2001; Sakai et al. 2001; Blackburn et al. 2011). Besides understanding the pathways of introduction, the environmental conditions prevailing in the invaded area can play an important role in the success or failure of an invasion (Lonsdale 1999; Nehring 2006).

Abiotic factors play a major role in the survival, growth and reproduction of a species and a range of abiotic factors, such as salinity (Dethier and Hacker 2005; Jaspers et al. 2011), temperature (Diederich et al. 2005; Löbl et al. 2006; Witte et al. 2010), nutrient availability (Funk and Vitousek 2007) or the amount of spatial resources (Stachowicz and Byrnes 2006) has been associated with the invasion of introduced species.

However, the extent to which introduced species can exploit potentially suitable areas does not only depend on abiotic factors but also on their interactions with the native biota. Native species can provide suitable habitat for introduced species (Diederich 2005; Eschweiler and Buschbaum 2011) but can also compete with them for space or resources (Levine et al. 2004). They can serve as food resources for introduced species (Savidge 1987; Kats and Ferrer 2003) or directly prey on them, preventing their establishment (Lake and O'Dowd 1991; Hunt and Yamada 2003) or limiting their abundance and spread (Reusch 1998; deRivera et al. 2005; Shinen et al. 2009). Understanding variation in the nature and intensity of biotic interactions can greatly improve predictions of where and when species will become invasive.

Interactions between native and introduced species may not only vary depending on the stage of an invasion, but also on a species' life-stage. The survival of early life stages is necessary for the success of any species, hence, an understanding of the early recruitment success of invasive species can help to underpin effective management strategies. In marine benthic species, recruitment patterns are influenced by pre- and early post-settlement processes (Connell 1985; Hunt and Scheibling 1997; Jenkins et al. 2009). However, density-dependent and habitat-dependent growth and survival may modify initial patterns of recruit abundance and

thus, contribute significantly to determining adult density patterns (Roughgarden et al. 1988; Aguirre and McNaught 2011).

Macrophyte canopies modify the physical and biological environment and can also modify recruitment patterns and survival of intertidal benthic species (Menge 1978; Bertness et al. 1999; Hancock and Petraitis 2001). Macrophytes can alter pre-settlement processes of pelagic larvae by attenuating water currents and concentrating pelagic larvae (Roughgarden et al. 1988; Eckman et al. 1989; Leonard 1999) or act as barriers, limiting the supply and settlement of larvae (Jenkins et al. 1999; Jenkins and Hawkins 2003). However, initial patterns of abundance may be modified by habitat complexity with greater growth and survival in algae habitats than on barren grounds (Aguirre and McNaught 2011). In very sheltered areas, a dense canopy cover can reduce current velocities leading to increased sedimentation (Albrecht and Reise 1994), which is known to be disadvantageous for shellfish growth (Barillé et al. 1997). Inside eelgrass beds, reduced food availability has been shown to negatively affect growth of non-native mussels (Reusch and Williams 1999; Allen and Williams 2003).

In sheltered and vegetated habitats, such as those provided by macrophyte canopies, predation pressure and densities of predators can be high (Menge 1978; Bertness et al. 2004; Amaral et al. 2009). One of the most conspicuous predators in the intertidal is the green shore crab *Carcinus maenas.* While juveniles tend to accumulate inside intertidal structured habitats (Moksnes et al. 1998), adults usually undergo migrations from the subtidal to the intertidal and mainly forage during high tides (Hunter and Naylor 1993). However, both life stages might find refuge from predation and desiccation under dense macroalgae canopies. Thus, structural complexity and predation can be interrelated and/or interact on the survival of prey species, however, effects might be taxa-specific and complex (e.g. Heck and Thoman 1981; Savino and Stein 1989; Moreno 1995; Anderson 2001).

Surveys are valuable to describe patterns of invasions and correlative analyses can identify possible mechanisms of invasions. However, the only possible tool for testing whether mechanisms are indeed capable of explaining the observed pattern are manipulative experiments that are carefully designed and recognize relevant spatial and temporal scales (Underwood 1997). The design and objectives of later

surveys can then be guided by the outcome of these experiments. Therefore, integrating field experiments and large scale observations or surveys enhances the ability to connect patterns and processes in invasion biology (Stachowicz et al. 2002; Stachowicz and Byrnes 2006; Fridley et al. 2007).

Pacific oysters have formed invasive populations in many parts of the world, with extensive populations in intertidal habitats (Reise 1998; Ruesink et al. 2005; Troost 2010) and occurrences known from subtidal areas (Smaal et al. 2009). Under favourable conditions they can spread rapidly, causing significant changes in habitat structure and abundance of associated species (Kochmann et al. 2008; Green 2012).

An extensive survey in 2009 revealed that Pacific oysters are at an early stage of establishment in Ireland but have the potential to become invasive with self-sustaining populations (Chapter 2, 4 and see Guy and Roberts 2010). A positive association of oysters with hard substrata and a negative association with macroalgae were revealed. Up to 6 individuals per m^2 were found on some rocky shores, but only single individuals were found on shores dominated by macroalgae (Chapter 2). Shore crabs were observed under heavy macroalgae canopies at several sites and can be more abundant at sheltered locations with a higher population density in vegetated habitats than in non-vegetated habitats (Amaral et al. 2009; Silva et al. 2010).

Different studies showed that crabs, fish and birds are preying on Pacific oysters in their introduced range (Anderson and Connell 1999; Mascaró and Seed 2001a; Cadée 2008; Troost 2010) and evidence from a pilot study (for description see Appendix 3) suggests that a high predation pressure on juvenile oyster exists (Appendix 4 and 5). Even if life-history traits of *C. gigas* might allow the species to persist in the face of high per capita predation rate, predators might control their populations, especially at an early stage of invasion when abundances are relatively low.

This study aimed to assess the potential impact of biotic interactions on the establishment of *Crassostrea gigas.* It was tested whether macroalgae and predators can influence growth and survival of juvenile Pacific oysters and thus, can contribute to explain differences in abundance of oysters at different shores in Ireland. More specifically, the following hypotheses were tested:

1) The presence of macroalgae canopy decreases survival and growth of juvenile oysters

2) Predation decreases oyster survival on macroalgae-dominated shores

3) Macroalgae and predation interact to affect survival and growth of oysters

4) The influence of macroalgae and predation varies among sites

5) Given experimental evidence for the influence of predators on oysters in experimental plots, we also tested whether high abundances of crabs were associated with low abundances of oysters at the scale of whole shores.

The first four hypotheses were tested experimentally. The experiment was initiated at two different sites 2011. It ran for a total of ten months. The fifth hypothesis was tested with a programme of crab trapping in 2011.

3.3 Methods

3.3.1 Experimental design, set up and sampling

The experiment was run from 3rd July 2011 to 6th May 2012 (an overview of the timing is given in Appendix 6) at two sites in Galway Bay: Ballynacourty (N 53°12'41.37'' W 008°57'28.44'') and Rinville (N 53°14'37.87'' W 008°58'15.09''). Both sites were dominated by boulder, cobbles and *Fucus serratus* in the low intertidal. The sediment was a mixture of maerl, sand and gravel. *Mytilus edulis* were attached to boulders in the lower intertidal area at Rinville. Wave fetch, used as an index of wave exposure, was estimated after Burrows et al. (2008) and is slightly larger at Ballynacourty with 1.07 (log_{10} wave fetch(km)) than at Rinville with 0.98 (log_{10} wave fetch(km)). Oysters are cultured in intertidal trestle systems close to the sites and single oyster individuals (> 70 mm shell length) had been found attached to boulders during a survey in 2009, both of which indicate that the sites are suitable for oysters.

The experiment was a fully crossed, 2-factor design (Figure 3.1) with macroalgae and cage as independent variables and survival, growth and condition of oysters as the response variables. Macroalgae had two levels: with and without macroalgae. Cage had three levels: full cage (to exclude predators), no cage (full access of predators) and a cage control (a cage cut partly on sides and top as a control for cage artefacts). Suitable plots distributed along the lower shore in a band 10 m wide and 150 m long were marked and randomly allocated to treatments. Plots were all established on the tops of boulders at least 530 cm^2 in area, 15-30 cm high and at least 4 m apart. All plots were covered by at least 40% cover of macroalgae (mainly *Fucus serratus*).

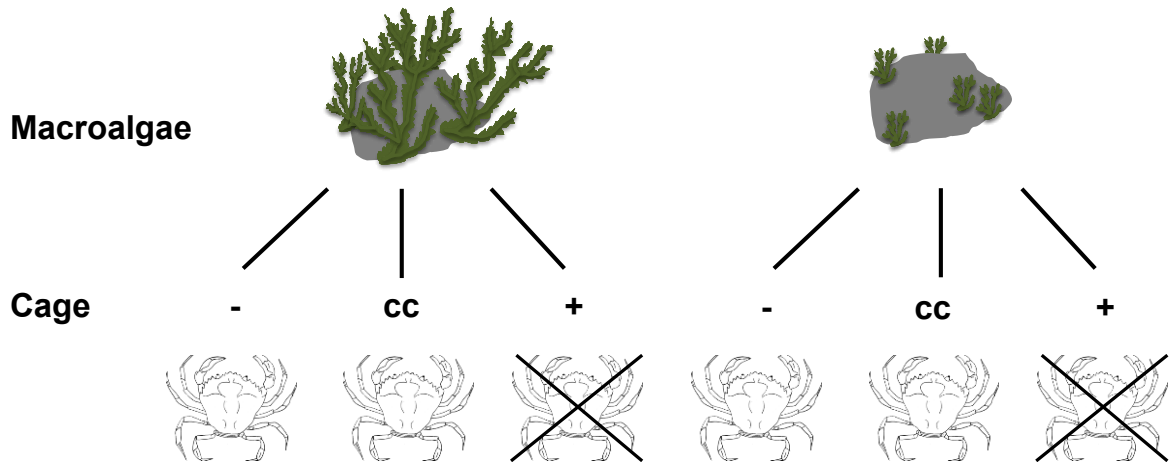


Figure 3.1 Experimental design (predators are represented by crab icons, but other predators could have also been involved). Abbreviations used are – = without cage, cc = cage control, + = with cage.

For plots allocated to treatments without macroalgae, all canopy algae within a 1 m radius were removed by cutting them as close to the ground or boulder as possible. Canopy algae were kept cropped for the duration of the study. Cages (L 23 cm x H 10 cm x W 23 cm) were made out of plastic mesh bags. The average mesh size was 10 mm, with 1.7 mm and 3.45 mm at the thinnest or thickest part of the strand respectively. This kind of mesh material is used in intertidal oyster culture on trestles to contain oysters and exclude predatory organisms such as crabs and other large predators (e.g. birds, fish, whelks)(Dare et al. 1983; Mascaró and Seed 2001a). Cages for the partial cage treatment were manipulated by cutting out half of each side of the cage and cutting a hole of 100 cm^2 on top.

Ten individual diploid oysters (mean shell length of 22 mm) were attached to red, unglazed ceramic tiles (14 cm x 14 cm) with Super Glue (Gorilla™), allowing maximum space between single individuals and towards edges. After permitting 3 hours for the glue to dry, the tiles were kept in aerated seawater. In total, 60 tiles were prepared, randomly allocated to plots and set up on the two shores over four days. Initially, all tiles were deployed in full cages to protect them from predation while they acclimated to field conditions, whereas macroalgae were already manipulated. The initial deployment of full cages also allowed oysters to attach more securely to the substrate, which better simulated a natural interaction between oysters and potential predatory organisms.

Based on a pilot study done in 2010, it was anticipated that removing the cages would lead to substantial mortality due to predators. Additional plots were therefore

allocated to the 'cage' treatment in which cages were retained throughout the study, to maximise the number of replicates, and therefore the power, for analyses of growth and condition with and without macroalgae. Thus, at each site, nine plots were allocated to the 'cage' treatment, three to 'no cage' and three to 'partial cage', in each of the with/without macroalgae conditions. Cages were manipulated on the 13th/14th September 2011 (for timing see Appendix 6).

Sites were visited on a monthly basis (except during winter months, see Appendix 6) to count the number of oysters attached on the tiles and measure shell lengths of oysters. Measurements of shell lengths were based on pictures of the plots taken with a digital camera (Casio Exlim-H15). A wooden frame was used to ensure that the camera was always correctly oriented and at a fixed distance from the plots. The lengths of individual oysters were derived by measuring the greatest distance (mm) between the hinge and the outer shell edge using ImageJ freeware 1.45s (Abramoff et al. 2004). Oyster survival was estimated by counting the number of oysters (with upper shell present) still attached on the tiles. For estimations of growth, only oysters kept inside full cages from the beginning were considered and growth was estimated as the difference between the individual shell lengths measured at the start of the experiment in July 2011 and the new shell lengths measured (for all different dates separately). Only individuals that survived the duration of the whole experiment were taken into account for growth measurements. The final growth measurement of oysters in May 2012 was made using a digital Vernier calliper.

Reduction in survival of bivalve recruits has been shown for *C. virginica* when plots are stressed by sediment (Thomsen and McGlathery 2006). In this study, shores with heavy macroalgae canopy and boulders covered with sediment were avoided. Nevertheless, caging has potential to induce sedimentation, but plots were cleared monthly of sediment (except during the first two months) and heavy sedimentation of tiles was never observed. Tiles were brushed and cleared of any sediment or *Ulva* during each visit after cage manipulation to ensure clarity of photographs. Before the manipulation of cages in September 2011, the number of barnacles was counted on each tile and the percentage cover with *Ulva* was estimated visually to the nearest 5% as a potential aid to interpreting variation in growth among tiles.

As somatic growth of *C. gigas* can be higher in spring and summer (Honkoop and Bayne 2002), the month of May was chosen to determine condition index of oysters as potential differences were expected to be more prominent than during winter months. Oysters were brought back to the lab to enable analysis of their condition, removed from the tile and any attached epifauna were cleaned off. Oysters were then opened after boiling them in water (~10 seconds) and the tissue was removed and dried in an oven to constant weight (~48 hours) at 75°C. Air-dried shells and the dried tissue were weighed on a torsion balance with a precision of 0.001 g. Condition index was estimated after Walne and Mann (1975) using the formula CI = Dry meat weight (g)*1000/dry shell weight (g) which quantifies the balance between metabolism directed towards calcification processes and metabolism focused towards somatic and gametic processes.

3.3.2 Crab trapping 2011

Fifteen sites were visited along the western and northern coast of Ireland to estimate abundance and sizes of crabs as potential predators of oysters in the intertidal. Sampling was undertaken from 21st July - 3rd October 2011, as the activity of crabs in the intertidal is greatest during these months (F O'Beirn, personal comment, Aargaard et al. 1995; Silva et al. 2010). Sites were chosen from a survey in which the abundance of oysters was assessed (Chapter 2). There were five sites with no oysters, five sites with single individuals of oysters (called rare) and five sites with > 0.1 oysters/m^2 (called frequent). Trapping was done during neap tides so that crabs could be captured during high water at night, when their activity is greatest (Silva et al. 2010). The timing of high water during spring tides would not have permitted crab trapping at night. A maximum of two shores was visited in one day in a random sequence over the sampling period. At each shore, four crayfish traps were installed at the lowest possible intertidal shore height, within approximately 1 hour before or after low tide. The traps were cylinders of mesh shape (mesh size 2 mm) with two funnelled openings of 5 cm. Traps measured 60 cm in total length and 24 cm in maximal height and width. Traps were separated by 40 m to ensure independence (Silva et al. 2010). Ten adult mussels (> 30 mm) were crushed and used as bait in a mesh bag and placed inside each trap. Traps were left for 12 hours (during high tide) and collected at the next low tide. The number of crabs, their carapace widths and

lengths were noted. Crabs smaller than 35 mm were considered as juveniles (Crothers 1967) and not measured but only counted.

3.3.3 Data analysis

Survival data (%) were analysed using three-way ANOVA. Factors macroalgae (two levels) and cage (three levels) were orthogonal and fixed and site was treated as an orthogonal, random factor. For analysis of survival, three replicates were used for each treatment level. Growth (mm) and condition index (CI) were taken as mean values (± standard error (SE)) for each tile and only analysed with respect to the factors macroalgae and site, using data from oysters that grew within cages (n = 9). The influence of cage structure on growth of oysters was analysed using three replicates of partial cages, plots without cages and full cage. This analysis was only undertaken at one site (Rinville) where sufficient replicates of all treatments were present at the end of the experiment.

Before analyses of variance, Cochran's test for homogeneity of variances was applied and data were transformed in case of heterogeneity. After analysis, significant terms were analysed using post-hoc Student-Newman-Keuls (SNK) procedure. The software GMAV5 for Windows (Underwood and Chapman 1998) was used for computations.

The relationship between the start size of oysters and their growth was estimated using Pearson's correlation coefficient and calculated using R (R Development Core Team 2011). To estimate whether *Ulva* might have influenced oyster growth, an additional analysis of the oyster increment measured from the end of August 2011, i.e. from the point when plots were always cleaned of *Ulva*, was undertaken.

Carapace widths and numbers of crabs obtained during the trapping were analysed with one-way ANOVA with five replicates for each level of oyster abundance. Although it is known that juvenile shore crabs (< 35 mm carapace width) can prey on oysters, especially on the smaller size ranges < 24 mm (Mascaró and Seed 2001b), they were excluded from the analysis to allow comparison between sites independent of recruitment events over the summer.

3.4 Results

3.4.1 Survival

No significant effect of cage or macroalgae on oyster survival was found two weeks after the manipulation of cages in September 2011 or at the end of the experiment in May 2012 (Table 3.1). Plots with macroalgae and no cage had the lowest percentage survival of oysters at the end of the experiment, with 33% ± 17% at Ballynacourty and 83% ± 3% at Rinville (Figure 3.2). Overall, lower percentages of oysters survived at Ballynacourty than at Rinville (Figure 3.2, Table 3.1, $p < 0.05$).

3.4.2 Growth and Condition Index

Although the overall analysis of oyster shell growth revealed no significant effect of macroalgae nor a significant interaction at the end of the experiment in May 2012 (Table 3.2), an additional analysis of data only from Rinville revealed significantly greater growth of oysters in plots without macroalgae than in plots with macroalgae, with means of 34.58 mm ± 1.25 and 30.89 mm ± 1.02 respectively (Figure 3.3, $F_{1,16} = 5.22$, $p = 0.036$). The mean length that oysters reached in Rinville was 56.27 mm ± 1.27 in plots without macroalgae and 52.60 mm ± 1.08 in plots with macroalgae (Table 3.3). In Ballynacourty, almost no difference in growth was measured at the end of the experiment with 27.46 mm ± 1.46 in plots with macroalgae and 27.37 mm ± 1.10 in plots without macroalgae (Figure 3.3). Mean length of oysters was 49.44 mm ± 1.39 in plots without macroalgae and 48.89 mm ± 1.08 in plots with macroalgae (Table 3.3).

Except for September 2011, oyster growth was always significantly larger in plots without macroalgae than in other plots at Rinville, whereas in Ballynacourty a significant difference between treatments was only found in September and October 2011 with greater oyster growth in plots with macroalgae (Figure 3.3, Table 3.2 – significant macroalgae x site interactions September 2011 until April 2012, SNK procedure). Almost no growth occurred between October 2011 and March 2012 at either of the sites (Figure 3.3).

There was no correlation between the initial size of oyster individuals and their growth in the first two months (July until September 2011) or their growth over 10 months (July 2011 until May 2012) (Pearson's correlation coefficient r = 0.04, $p = 0.524$, n = 292 and r = 0.19, $p = 0.001$, n = 292).

No significant difference in oyster condition was found at the end of the experiment between treatments ($p > 0.05$, Table 3.2). In Ballynacourty, the mean condition index of oysters was 61.10 ± 2.50 in plots with macroalgae and 61.01 ± 0.76 in plots without macroalgae. These values were not significantly larger than those from Rinville (57.9 ± 0.74 and 58.72 mm ± 1.37 in plots with macroalgae and without macroalgae respectively).

3.4.3 Supplementary analyses

Analyses of cage controls revealed that the cage structure had no significant effect on oyster growth on any date or on oyster condition estimated at the end of the experiment (two-way ANOVA, $p > 0.05$, Appendix 7 and 9).

Two months after the experiment had been set up, plots cleared of macroalgae had developed 100% cover of *Ulva* whereas cover in plots with macroalgae was at least 50% less than this (Appendix 7 and 8, significant site x macroalgae interaction). This was expected as *Ulva* is an opportunistic species and similar effects have been shown before, especially in the absence (due to caging) of grazers (Underwood 1980). The analysis of oyster growth, taking oyster lengths measured at the end of August 2011 as starting sizes to avoid potential influences of *Ulva* on oyster growth during the first two months, revealed the same pattern as taking oyster increments from July 2011. There was no significant difference in oyster growth between macroalgae treatments at the end of the experiment and no interaction of site and macroalgae was found except in October 2011 (two-way ANOVA, $F_{1,32} = 6.16$, $p = 0.019$), when there was a significantly larger growth of oysters in plots without macroalgae in Rinville and no significant difference between treatments in Ballynacourty.

Barnacles were counted before the manipulation of cages, and significantly more barnacles (mainly *Austrominius modestus* Darwin, 1854) were found at Ballynacourty in treatments with macroalgae than without macroalgae (31.2 ± 13.60 and 4.4 ± 1.67 respectively) but no difference in the number of barnacles was found between treatments at Rinville with 57.47 ± 16.29 barnacles in plots without macroalgae and 36.66 ± 10.46 barnacles in plots with macroalgae (Appendix 7 and 8). Barnacles seemed to accumulate at oyster edges.

3.4.4 Crab trapping 2011

No significant difference in the number of adult crabs was found between sites with different oyster abundance (one-way ANOVA, $F_{2,12} = 1.24$, $p = 0.325$). Mean numbers of crabs per trap ranged from 16.55 (± 2.68) at shores where oysters are rare, 12.9 (± 1.79) at shores where oysters are absent and 12.7 (± 0.18) where oysters occur frequently (Figure 3.4). However, adult crabs found at sites where oysters were absent were significantly larger (mean carapace width of 59.93 mm ± 3.01) than crabs at sites where oysters were frequent (48.5 mm ± 2.95) or rare (46.08 mm ± 1.75) (one-way ANOVA, $F_{2,12} = 7.87$, $p = 0.007$, SNK test). In total, 564 male crabs (mean carapace width of 53.83 mm ± 0.49) and 278 female crabs (mean carapace width of 45.05 mm ± 0.69) were captured (Figure 3.5).

Tables

Table 3.1 Results of ANOVA comparing oyster survival within the different treatments at two sites in September 2011 (2 weeks after cage opening) and May 2012. Significant effects are in bold. n= 3.

Source of variation	df	MS	F	*p*	MS	F	*p*
		September 2011			May 2012		
Site	1	2627.34	6.83	**0.015**	9319.68	17.94	**0.000**
Macroalgae	1	577.90	7.64	0.221	23.68	0.21	0.728
Cage	2	438.39	1.33	0.430	2041.05	1.83	0.353
Site x Macroalgae	1	75.65	0.20	0.661	114.49	0.22	0.643
Site x Cage	2	330.03	0.86	0.437	1115.60	2.15	0.139
Macroalgae x Cage	2	233.10	5.40	0.156	467.60	8.84	0.102
Site x Macroalgae x Cage	2	43.20	0.11	0.894	52.89	0.10	0.904
Residual	24	384.73			519.41		
Transformation		ArcSin (%)			none		
Cochran' s test		0.4424 ($p<0.05$)			0.2781 (n.s.)		

Table 3.2 Results of ANOVA comparing oyster growth from August 2011 until May 2012 and condition index in May 2012 between macroalgae treatments at two sites. Significant effects are in bold, n = 9 (all caged).

Source of variation	df	MS	F	*p*	MS	F	*p*
		August 2011			September 2011		
Site	1	35.07	8.14	**0.008**	74.76	13.21	**0.001**
Macroalgae	1	7.98	0.48	0.614	0.35	0.01	0.943
Site x Macroalgae	1	16.55	3.84	0.059	43.65	0.22	**0.009**
Residual	32	4.31			5.66		
Cochran' s test		0.3322 (n.s.)			0.3671 (n.s.)		
		October 2011			January 2012		
Site	1	93.06	14.40	**0.000**	141.42	17.84	**0.000**
Macroalgae	1	1.25	0.02	0.917	1.12	0.02	0.920
Site x Macroalgae	1	72.70	11.25	**0.002**	69.84	8.81	**0.006**
Residual	32	6.46			7.93		
Cochran' s test		0.2746 (n.s.)			0.3719 (n.s.)		
		March 2012			April 2012		
Site	1	191.65	17.16	**0.000**	334.58	26.70	**0.000**
Macroalgae	1	5.87	0.09	0.815	3.61	0.05	0.865
Site x Macroalgae	1	65.99	5.91	**0.021**	77.34	6.17	**0.018**
Residual	32	11.17			12.53		
Cochran' s test		0.3300 (n.s.)			0.3445 (n.s.)		
		May 2012			Condition Index		
Site	1	254.70	19.03	**0.000**	67.93	3.26	0.080
Macroalgae	1	29.20	0.52	0.515	1.21	0.65	0.568
Site x Macroalgae	1	32.15	2.40	0.131	1.86	0.09	0.767
Residual	32	13.38			20.83		
Cochran' s test		0.3573 (n.s.)			0.6763 (sign.) no transformation		

Table 3.3 Mean oyster lengths (mm ± standard errors) at different dates in plots with and without macroalgae at Rinville and Ballynacourty, n = 9 (all caged).

	Oyster length (mm) ± SE with macroalgae		Oyster length (mm) ± SE without macroalgae	
Rinville				
July 2011	21.61	0.58	21.63	0.42
August 2011	36.69	0.95	36.95	0.95
September 2011	42.74	1.18	44.71	0.65
October 2011	45.36	0.98	48.55	0.95
January 2012	46.33	0.96	49.44	0.87
March 2012	47.28	1.35	50.77	1.18
April 2012	50.82	1.34	54.36	1.32
May 2012	52.60	1.08	56.27	1.27
Ballynacourty				
July 2011	20.85	0.37	21.87	0.29
August 2011	35.75	0.78	34.07	0.93
September 2011	41.73	0.95	34.07	0.93
October 2011	44.66	0.97	42.87	1.10
January 2012	44.82	1.19	43.07	1.19
March 2012	45.05	1.37	43.83	1.15
April 2012	47.33	1.50	45.71	1.25
May 2012	48.85	1.56	49.44	1.39

Figures

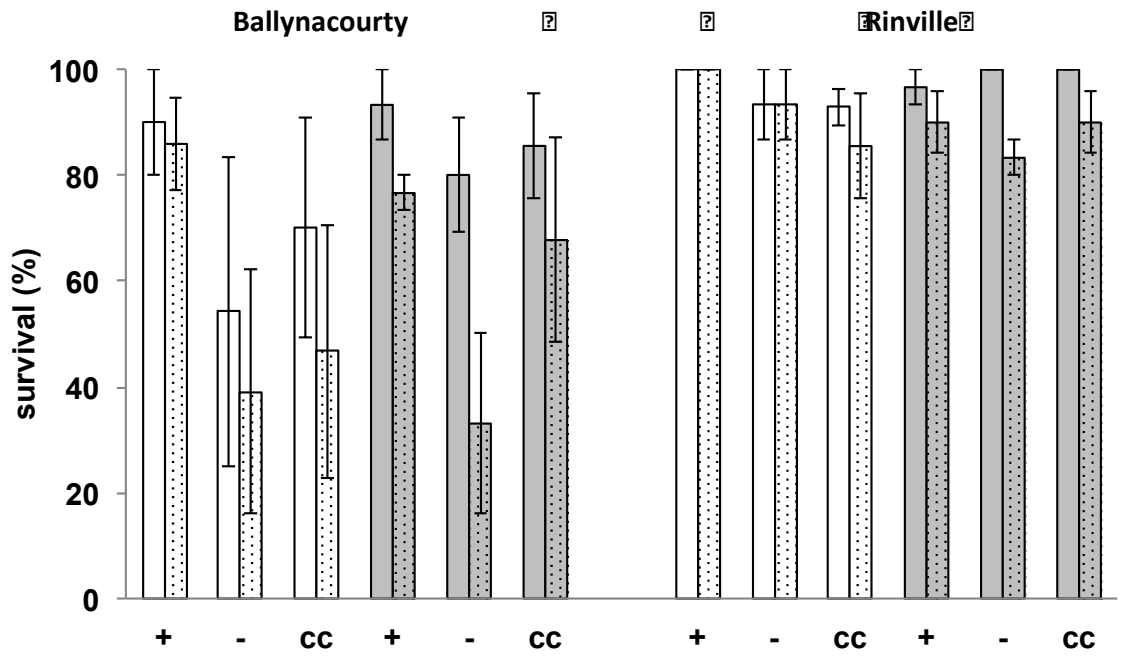


Figure 3.2 Mean percentage survival of oysters ± standard errors in each treatment for the two locations. Coding for treatments: grey colour (with macroalgae), white colour (without macroalgae), bars without pattern: September 2011, bars with pattern: May 2012, with cage (+), without cage (-), cage control (cc), n = 3.

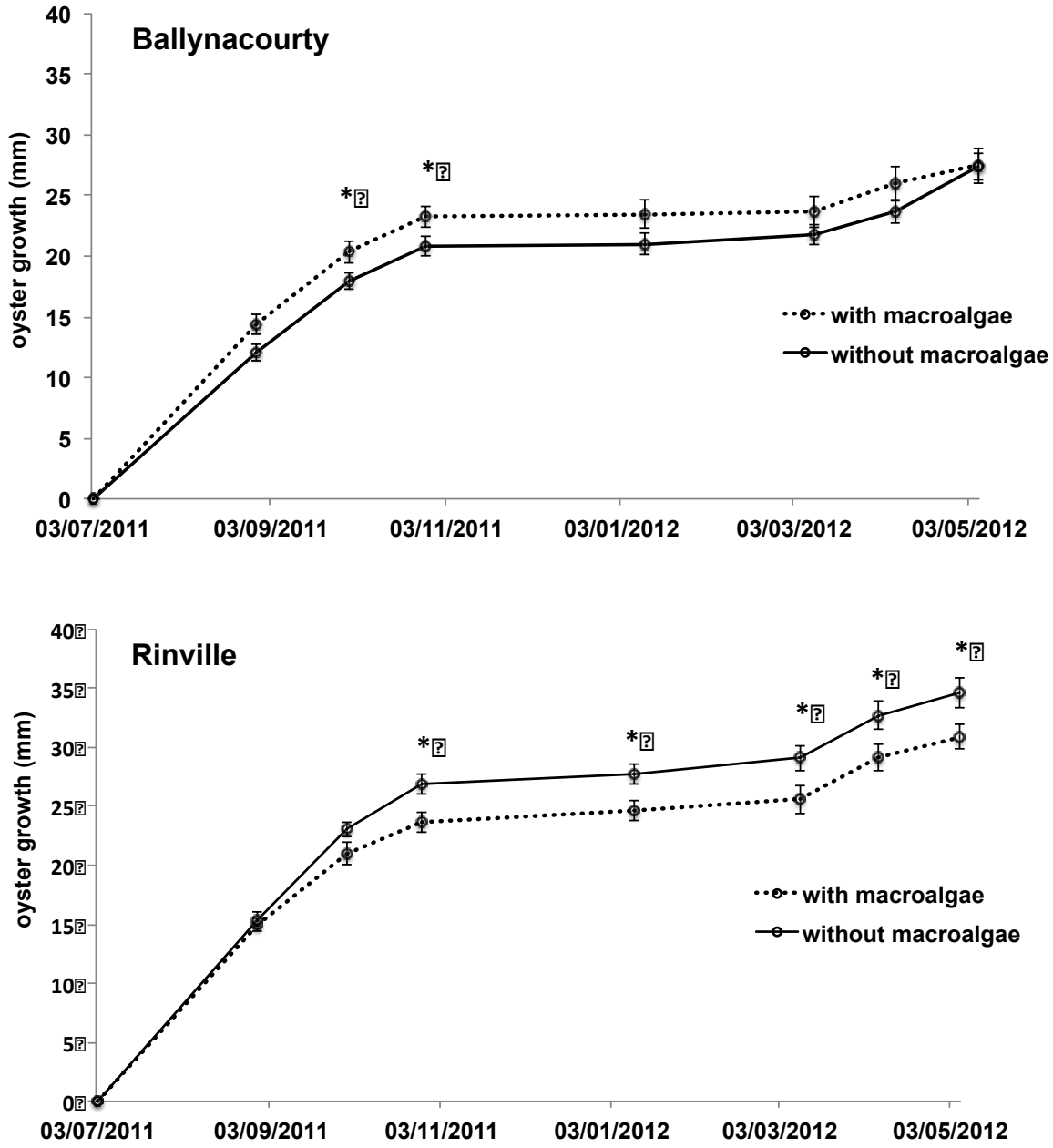


Figure 3.3 Growth of oysters (all caged) at the two sites from the beginning of the experiment in July 2011 until May 2012, shown are means ± standard errors, * indicate outcomes of SNK tests for significant interaction effects. Note that no significant interaction of macroalgae and site occurred in the end (Table 3.2), but mean oyster growth was still significantly different between treatments in Rinville when tested separately, n = 9.

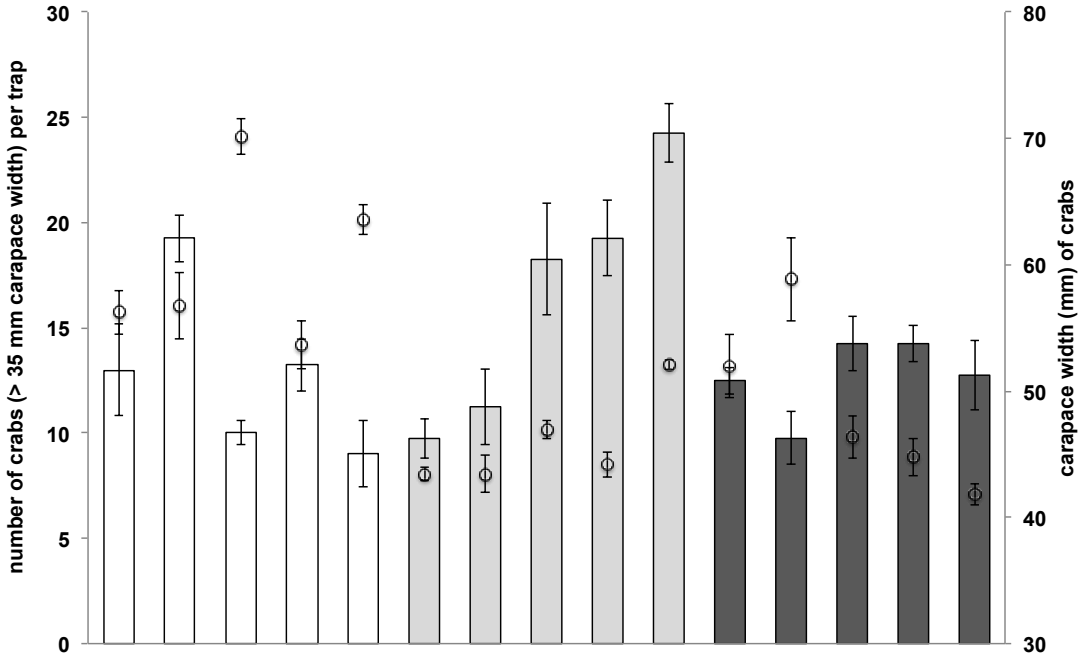


Figure 3.4 Number of adult crabs (bars) and carapace width of crabs (circles) captured per trap. Shown are means ± standard errors, n = 4. Locations where oysters are absent (in white), rare (< 0.01 individuals/m^2 in grey) or frequent (> 0.1 individuals/m^2 in dark grey).

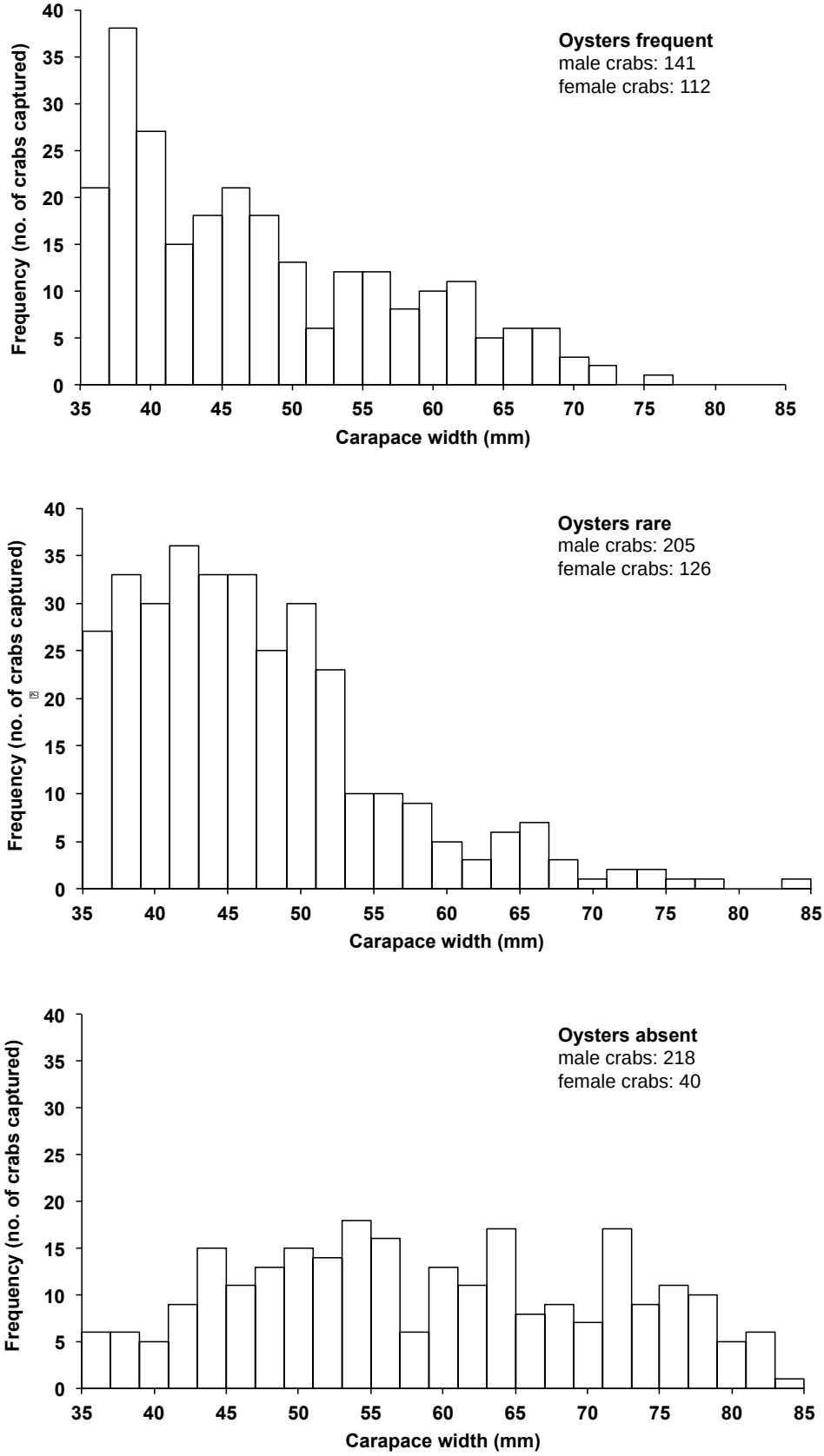


Figure 3.5 Size frequency distribution of shore crabs with carapace width > 35 mm captured at shores where oysters were frequent, rare or absent (4 traps per shore), n = 5.

3.5 Discussion

In this study, potential impacts of biotic interactions on juvenile Pacific oysters were addressed. Under some circumstances, predation on juvenile oysters had a significant effect on their survival, but effects were generally limited. Macroalgae did not affect condition of the oysters and effects on linear shell growth were not consistent among sites. The results suggest that factors other than predation or the macroalgae themselves might be causing the small abundances of *C. gigas* on macroalgae-dominated shores.

3.5.1 Predation

Although the pilot study revealed a strong effect of predation (Appendix 4 and 5), no effect of predation on oyster survival was detected in the study reported here. At the time of manipulation of the cages (September 2011), oysters had a mean length of 35.5 mm ± 0.39 which is within the size range oysters can reach during their first year (e.g. Diederich 2006). Although no field data for these sizes exists, laboratory studies have shown that shore crabs, *Carcinus maenas*, are able to prey on Pacific oysters from > 5 mm until 40 mm shell length (Dare et al. 1983; Mascaró and Seed 2001a) and chipped shells are an indication of shell damage by crabs (Dare et al. 1983). Spat of *C. gigas* ranging from 5.6 mm to 10.6 mm in size have been shown to be affected by predation on the East Pacific coast in Canada (Ruesink 2007), but results were not consistent and varied among sites with unknown predator identity. However, the author speculated that crabs and seastars as well as whelks could have been involved as predators. In the pilot study undertaken in July 2010, with mean oyster lengths of 16 mm, predation caused > 80% mortality within 2 weeks (Appendix 4). Predators could not be observed directly but chipped shells, crushed shells and shells with holes were found, with birds, crabs or dog whelks being potentially involved. Although the smaller sizes of the oysters used in the pilot study is one possible explanation for the different effects of predation between the pilot study and the main experiment, it is also possible that differences between the sites at which the pilot and main experiments were run and the times of year may be responsible. Also, in the pilot experiment, spat were attached with glue and cages were manipulated immediately. Thus, those oysters did not fully mimic the shape of oysters which had been allowed to attach naturally to hard substrata before exposure to predators in the experiment reported here.

A complete absence of crabs from the sites the current experiment was set up seems unlikely as shore crabs were found during the crab trapping at several sites and are known to occur in the intertidal in Ireland during these months (F O'Beirn, personal comment). Given that shore crabs were the main crab species present on the shores (see section on crab trapping), are known to consume Pacific oysters, and are also considered as a potential predator of the Pacific oysters in its introduced range (Troost 2010, Diederich unpublished), they were considered the predators most likely to be influential in this experiment.

The prey value or profitability is important in the selection of prey species and differs between size classes of shore crabs (Mascaró and Seed 2001b). As previously described in studies for mussels, choosing smaller, suboptimal (with regard to the energy gain) sizes of prey can be explained by the claw damage risk, which might affect mating success (Juanes 1992; Smallegange and van der Meer 2003). Other studies showed that *C. maenas* has a strong reluctance to feed on, and more difficulty grasping, flat oysters, *Tiostrea lutaria* and *Ostrea edulis*, which indicates that a relationship between crab chelae and prey shell shape in determining feeding habits and prey preferences is likely (Dare et al. 1983; Richardson et al. 1993; Mascaró and Seed 2001a). Similarly, it has been suggested that Pacific oysters may be less susceptible to predators (crabs, shrimp, starfish), presumably due to their firm attachment and flat shape when small and a sturdy shell when larger (Elsner et al. 2011). Moreover, Silva et al. (2008) described shell width and attachment force as critical factors for *C. maenas* that can influence the vulnerability of prey species to predation by these crabs and might explain the absence of a predation effect on oysters, especially when grown flat to a surface.

In a study by Silva et al. (2010) the gut contents of three crab species were not related to the abundance of prey on the shore. The authors acknowledged accessibility of prey, habitat refuges, prey preferences or sizes as drivers of spatial foraging patterns. At Rinville, mussel clumps were found attached on boulders whereas at Ballynacourty limpets, dogwhelks and periwinkles were present. Prey preference behaviour towards these species might be another explanation for the absence of a predation effect. It is known that *C. maenas* with a carapace width of over 40 mm select mussels when offered mussels and Pacific oysters simultaneously

(Mascaró and Seed 2001a, Diederich unpublished) and can forage on limpets on rocky intertidal shores (Silva et al. 2008).

Dogwhelks (*Nucella lapillus*) and oystercatchers (*Haematopus ostralegus*) were also observed on the shore but neither of them seemed to have an effect on the survival of the oysters; drilled shells as an indication of the activity of dog whelks were not observed in 2012. Although the American oystercatcher (*Haematopus palliates)* preys on native oyster *C. virginica* in the U.S. (Hand et al. 2010), in Europe, the Eurasian oystercatcher (*Haematopus ostralegus*) appears to be unable to open shells of live individuals of *C. gigas* and is more likely to prey on gaping or desiccated oysters washed ashore (Cadée 2008). Usually, observations on birds handling oysters describe handling of loose oysters (K Troost, personal observation) rather than attached oysters. The small size and the fact that oysters were attached on tiles might explain the absence of predation by this bird species. Predation by fish (toadfish) is known from Australia but oysters seem to have a predation refuge once sizes over 30 mm are reached and although fish can greatly reduce numbers of juvenile recruits, consistent recruitment of oysters is unlikely to be counteracted by fish predation (Anderson and Connell 1999).

Considerable variation occurred among plots at Ballynacourty indicated by the large error bars, especially in treatments without a cage or control cages. On two plots all oysters were missing whereas on other plots up to 80% were present at the end of the experiment. It has been shown that the distribution of species can be very patchy at small scales - over a few metres on the shore (Underwood and Chapman 1996; Chapman 2002; Fraschetti et al. 2005). Thus, a patchy distribution of predators may have decreased oyster survival in some plots but overall effects of predation might be negligible when larger scales (or several sites) are considered. The numbers of shore crabs captured in traps did not differ significantly between sites of different oyster abundance, again suggesting that there is little or no influence of predation by this species on the establishment of oysters at the scale of whole shores.

Silva et al. (2010) recently showed that the distribution of crabs in the intertidal varies depending on wave exposure and shore level. They found more and larger shore crabs in the lower intertidal at sheltered locations. A negative correlation between wave fetch and number of crabs (Pearson's $r = -0.495$, $p = 0.06$, $n = 15$) and

between wave fetch and crab size (Pearson's r = -0.228, $p = 0.41$, n = 15) was also found here. However, wave fetch was not significantly different between sites with different levels of oyster abundances (one-way ANOVA, $F_{2,12} = 2.67$, $p = 0.110$ and $F_{2,9} = 1.08$, $p = 0.380$, Appendix 10). Wave fetch was not a good predictor of the presence of oysters in a survey of Pacific oysters (Chapter 2). Other factors, such as residence time and hard substrata, better explained the presence of oysters and might also possibly better explain different oyster densities than the negatively correlated wave fetch and crabs.

Similar results suggesting that predation might not be an important factor controlling the abundance of oysters in intertidal areas were shown by Ruesink (2007) with no significant difference in predation for Pacific oyster spat between wave-exposed sites where oysters are rare or wave-protected sites where they are commonly found. Although, in the current study, significantly larger sizes of crabs were found at shores where oysters were absent, this is unlikely to lead to a higher predation pressure than at the other sites as juvenile oysters will possibly be no less vulnerable to smaller crabs than to larger crabs.

Dense macroalgae canopies can decrease the effect of fish grazing (e.g. Hoey and Bellwood 2011) and can restrict movement of urchins and star fish (Gagnon et al. 2003, 2004). Although larger crabs might have difficulties maneuvering between large macrophyte canopy during high and/or low tide, similar numbers of crabs were found at rocky shores and macroalgae-dominated shores during the crab trapping. Thus, a physical effect of the presence of macroalgae in this study is also unlikely to explain the absence of a predation effect, especially because an effect of predation was also absent in cleared plots without macroalgae, i.e. no interaction between macroalgae and cage on oyster survival was detected.

3.5.2 Growth and Condition Index

Variation in oyster growth rates between sites is common (Robinson and Horton 1987; Diederich 2006; Cardoso et al. 2007) and was confirmed in this study, with significantly slower growth of oysters in Ballynacourty than in Rinville. Environmental conditions, such as food availability, turbidity, seston concentration, temperature and salinity, are important factors for oyster growth (Brown and Hartwick 1988; Quayle 1988; King et al. 2006; Cassis et al. 2011) and might vary

between sites. Furthermore, pollution can decrease filtration activity and lead to inhibition of growth in adult oysters (Pridmore et al. 1990; Claude 1991; Encomio and Chu 2000). As oyster aquaculture is present in close proximity to the sites studied here, pollution is expected to be at levels not negatively affecting oyster growth; however, pollution levels were not determined. Wave-exposure can also affect oyster growth (Ruesink 2007), but, due to similar wave exposure occurring at Rinville and Ballynacourty, is unlikely to explain the differences in growth observed here. Almost no growth was detected during the winter months and has been observed before for wild oysters in Europe (Diederich 2006). It has been suggested that temperature (Gangnery et al. 2003) and phytoplankton dynamics (Grangeré et al. 2009) might drive seasonal and year-to-year variability of growth patterns.

Oyster shape is known to vary with environmental factors such as density and type of substrate, possibly to escape suffocation (Quayle 1988; Diederich 2006) and enhanced shell growth of oysters was found underneath *Fucus* canopy in the Wadden Sea without a corresponding increase in condition index (Diederich 2006). However in this study, a significantly smaller shell growth of oysters in plots without macroalgae but not in condition index was found, but only at one site. Final measurements in May 2012 did not show any effect of macroalgae on oyster growth, survival or condition. However, an interaction of site and macroalgae occurred from September 2011 until April 2012 with significantly reduced growth of oysters in plots with macroalgae in Rinville and no significant difference between plots with or without macroalgae at Ballynacourty after October 2011. The significantly greater growth of oysters in plots with macroalgae in Ballynacourty detected in October 2011 was absent in January 2012 and March 2012 although almost no difference in total mean growth occurred. This could have been caused by a higher variability of oyster growth between periods of measurements, also indicated by a higher standard error. Although the interaction suggests that an effect of macroalgae might spatially vary, this is only true when shell growth or oyster length is considered.

Uncoupling of shell and somatic growth has been found in studies of oysters (Brown and Hartwick 1988; Honkoop and Bayne 2002) and the ratio of dry tissue to dry shell weight is a better indicator of oyster performance than the very variable growth and shape of oyster shell alone. Therefore, the results of the condition index were considered a more reliable indicator of the influence of algae. Although oysters

were collected at the time when biggest difference in condition index might have been expected, no effect of macroalgae on oyster condition was found at the end of the experiment in May 2012. Estimates of condition was within the range measured from similar field studies of oysters growing in their natural setting in their introduced range (Diederich 2006) and growth and condition of oysters was not affected by cage structure and therefore, estimations are likely to reflect natural patterns.

While the two sites were chosen for an approximately equal cover of macrophytes, a higher density of *Fucus* with patches of *Ascophyllum* was generally observed at Ballynacourty. This might have also been reflected in the significantly higher recruitment of barnacles in the presence of macroalgae in Ballynacourty, leading to an enhanced barnacle recruitment in the absence of whiplash (due to the cage structure) (Leonard 1999). Whether varying densities or patch sizes of macrophytes have different effects on oyster growth is not known and studies on density effects or patch size of macrophytes are rare (Irlandi 1996; Reusch and Williams 1999). In the pilot study undertaken in 2010, at a site with very dense cover of macroalgae, an effect of macroalgae on growth was present from October 2010 until March 2011 with a bigger growth of oysters in treatments with macroalgae (Appendix 4 and 5), suggesting again that the effect of macroalgae on shell growth might not be consistent among sites. However at the end of that experiment, no differences in oyster growth and condition index were detected.

The effect of *Ulva* can be severe for oysters and suffocation can occur under large bundles of *Ulva* (Cadée 2004b). However, plots were cleared monthly and no significant mortality was observed after the first two months in plots with a high cover of *Ulva.* The analysis of oyster increment measured from the end of August 2011, i.e. from the point when plots were always cleaned of *Ulva*, revealed the same pattern as taking oyster growth from the start of the experiment in July 2011, which indicates that *Ulva* was unlikely to have had an effect on oyster growth in the current study.

Although under some circumstances, predation on juvenile oysters had a significant effect on their survival, effects were generally limited and large-scale patterns of oyster abundance did not relate to abundance of predatory crabs.

Macroalgae did not directly affect survival and appeared to have limited and variable effects on growth and condition. Furthermore, processes other than predation and those due to macroalgae seem to generate differences between sites, indicated by the overall smaller growth and survival of oysters at Ballynacourty regardless of the experimental treatments imposed. Overall, abundance patterns might be more likely driven by other factors, e.g. propagule pressure, larval dispersal and settlement processes. *Fucus* is highly successful in scouring the substratum (Jenkins et al. 1999) negatively affecting settlement of barnacle larvae through a whiplash effect (Hancock and Petraitis 2001; Brock et al. 2007) and poor oyster recruitment underneath algal canopies has been observed on mussel beds (Diederich 2005). High survival of spat in the intertidal was observed in the German Wadden Sea by Diederich (2006). The author suggested that post-settlement predation pressure in the intertidal might be rather low for juvenile *C. gigas,* with a fast growth rate potentially enabling them to grow into an early size refuge. The results of this study similarly suggest that the importance of native predators on Pacific oysters might be very low and is unlikely to counteract their proliferation and spreading in the introduced range. However, to examine the possibility that the influence of macroalgae and predation may be greater for oysters at different life history stages and sizes, research into settlement processes as well as experiments using juvenile oysters of a range of sizes would be needed.

Chapter 4

Genetic evidence for the uncoupling of local aquaculture activities and a population of an invasive species – a case study of Pacific oysters (*Crassostrea gigas*)

Accepted for publication in *Journal of Heredity*: "Genetic evidence for the uncoupling of local aquaculture activities and a population of an invasive species – a case study of Pacific oysters (*Crassostrea gigas*)" by Judith Kochmann, Jens Carlsson, Tasman P. Crowe, Stefano Mariani

4.1 Abstract

Human-mediated introduction of non-native species into coastal areas via aquaculture is one of the main pathways that can lead to biological invasions. To develop strategies to counteract invasions it is critical to determine whether populations establishing in the wild are self-sustaining or based on repeated introductions. Invasions by the Pacific oyster (*Crassostrea gigas*) have been associated with the growing oyster aquaculture industry worldwide. In this study, temporal genetic variability of farmed and wild oysters from the largest enclosed bay in Ireland was assessed to reconstruct the recent biological history of the feral populations using seven anonymous microsatellites and seven microsatellites linked to expressed sequence tags (ESTs). There was no evidence of EST-linked markers showing footprints of selection. Allelic richness was higher in feral than in aquaculture samples ($p = 0.003$, paired t-test). Significant deviations from Hardy-Weinberg equilibrium (HWE) due to heterozygote deficiencies were detected for almost all loci and samples, most likely explained by the presence of null-alleles. Relatively high genetic differentiation was found between aquaculture and feral oysters (largest pairwise multilocus F_{ST} 0.074, $p < 0.01$) and between year classes of oysters from aquaculture (largest pairwise multilocus F_{ST} 0.073, $p < 0.01$), which was also confirmed by the strong separation of aquaculture and wild samples using Bayesian clustering approaches. A ten-fold higher effective population size (N_e) – and a high number of private alleles – in wild oysters suggest an established feral population that is likely to be self-recruiting and demographically independent from the current aquaculture activities in the estuary. Alternative pathways of introduction pathways are discussed.

Keywords: aquaculture, anonymous microsatellites, *Crassostrea gigas*, EST-linked microsatellites, invasive species

4.2 Introduction

Biological invasions are considered a major threat to biodiversity (Millennium Ecosystem Assessment 2005) and can cause substantial ecological and economic impacts (McGeoch et al. 2010; Simberloff and Rejmánek 2011). Coastal marine ecosystems are particularly prone to biological invasions due to an increased, deliberate or accidental, transfer of species mediated by human activities across natural barriers of dispersal (Carlton and Geller 1993; Molnar et al. 2008), e.g. via shipping, trading and aquaculture activity (Naylor et al. 2001; Minchin 2007b). Invasive populations are the focus of considerable management effort (McGeoch et al. 2010) and for the purpose of control, it is critical to determine whether populations establishing in the wild are self-sustaining or based on repeated introductions (Dlugosch and Parker 2008; McGeoch et al. 2010; Geller et al. 2010).

The genetic diversity of a species can lend information about the mechanisms of the invasion processes, e.g. population of origin, inbreeding, adaptation to local environmental conditions or range expansion (Sakai et al. 2001; Geller et al. 2010). Anonymous microsatellites and microsatellites linked to expressed sequence tags (ESTs) have been employed to test ecological and population genetic hypotheses. Putatively neutral DNA markers occur in the non-coding regions of the genome and are widely used to unravel neutral evolution (Zane et al. 2002; Avise 2004). More recently, due to the interest in studying adaptive genetic variation, EST-linked microsatellites, which are physically linked to coding DNA regions, have been used to detect footprints of selection (Nielsen et al. 2009). Furthermore, EST-linked microsatellites are believed to show a reduced number of null-alleles (mismatches in the primer binding region) due to their functional constraints (Ellis and Burke 2007). A combined approach using anonymous and EST-linked genetic markers might reveal differences between the two marker types and could allow a more thorough assessment of the population-level consequences of multiple evolutionary forces (Kirk and Freeland 2011; Coscia et al. 2012).

The Pacific oyster (*Crassostrea gigas* Thunberg, 1793) has been introduced for aquaculture purposes in many locations worldwide outside its natural range and wild (feral) populations have established few years after oyster farming commenced (Brandt et al. 2008; Troost 2010). This species has also established permanent, self-sustaining invasive populations in many intertidal habitats outside aquaculture areas

(Reise 1998; Ruesink et al. 2005). Recently, Möhler et al. (2011) found a significant increase of genetic differentiation with distance between aquaculture and wild populations, however, only in the Northern part of the Wadden Sea. The authors suggested an on-going supply of genetic material from divergent mitochondrial lineages from aquaculture breeding stocks into invasive feral populations of *C. gigas* in the European Wadden Sea based on the use of a mitochondrial DNA marker.

Pacific oyster farming in Ireland started in 1973, and individuals of multiple cohorts have been recently found during an extensive survey in various habitats outside aquaculture settings (Chapter 2). Until now, it is not known whether populations are established in the wild, i.e. self-recruiting, or depend on repeated introductions presumably from aquaculture operations. In Ireland, numerous oyster aquaculture operations are often licensed simultaneously in one bay with multiple sources of seed oysters; this will hinder the study of introduction sources. However, in Lough Foyle, a large shallow bay sharing borders between the Republic of Ireland and Northern Ireland, oyster farming activity started in 1998/99 and a sole hatchery from the Channel Islands has been continuously used as the only source of oyster seed.

In this study, the genetic variability of wild and farmed oysters within Lough Foyle was assessed, using a suite of microsatellite markers that included both anonymous and EST-linked loci. The aims were: i) to assess the genetic relationship between local feral oysters and their potential aquaculture source within the same bay, ii) to evaluate the effectiveness of EST-linked and anonymous microsatellites in detecting different evolutionary processes (e.g. geneflow, genetic drift, selection, adaptation) and iii) to attempt to reconstruct the recent biological history of the feral populations using population genetic approaches.

4.3 Methods

4.3.1 Sample collection

Pacific oysters were randomly collected from an aquaculture site in the Lough Foyle estuary, Northern Ireland (N 55°06.161’ W 007°13.215’) and from an adjacent mussel bed. Additionally, samples were obtained from an aquaculture operator in Bangor, Menai Strait, Wales (N 53°09.970’ W 004°14.990’), whose oyster seed is provided by the same hatchery that supplies the Lough Foyle farm. Oysters from the mussel bed

were separated into three size classes of 50 individuals, with lengths (mean ± standard deviation (SD)) of 60 mm ± 6 mm (Feral 1), 80 mm ± 6 mm (Feral 2) and 116 mm ± 12 mm (Feral 3). Those size classes did not necessarily represent separate cohorts, as a real age-length relationship is not established for Pacific oysters in the estuary. However, the age-size relationship of Pacific oysters established by others at European coasts (Diederich 2006; Guy and Roberts 2010) suggests that at least two age classes are present. Fifty oysters from each of two year classes (5 years (AQF 1) and 3 years (AQF 2)) were provided by aquaculture operators in Lough Foyle. Fifty 10-year-old individuals (AQB) of stocks used during the launch of oyster aquaculture in Lough Foyle were obtained from the Menai Strait aquaculture operator as this year class was not present in Lough Foyle at the time of the study.

Muscle tissue samples were stored in pure ethanol (100%) and kept in the fridge until DNA was extracted.

4.3.2 Microsatellite data collection and analyses

Genomic DNA was isolated using the chloroform/isoamyl alcohol method after Miller et al. (1988) and quality and concentration of the DNA samples was assessed using a spectrophotometer (NANODROP 1000). 7 anonymous microsatellite (naSSR) and 7 Expressed-Sequence-Tag-linked microsatellite (EST-SSR) loci were selected from previous studies. The naSSR that were used were: CG108, CG49 and CG44 (Magoulas et al. 1998), L10 (Huvet et al. 2000), Crgi 50, Crgi 26 and Crgi10 (Sekino et al. 2003). The EST-SSR were: CGE007 (Yu and Li 2007), Cgsili43, Cgsili46, Cgsili39, Cgsili4, Cgsili50 and Cgsili29 (Sauvage et al. 2009). Three multiplex PCR reactions were arranged according to the annealing temperatures of primers to maximise the number of loci suitable for simultaneous analysis. Fluorescent dyes (NED, PET, VIC, 6-FAM) for one primer of each primer pair were also used to allow non-ambiguous genotyping among loci with overlapping allelic ranges.

The protocol of Li et al. (2010) was slightly modified for PCR multiplex amplifications using the Multiplex PCR Kit (Qiagen, Hilden, Germany); 10µl reaction volume contained 5µl of the Multiplex PCR Master Mix (2x) (including Taq® DNA Polymerase, Microsatellite PCR buffer with 6mM $MgCl_2$ and dNTPs), 0.05-0.40µl of primers, 1µl of genomic DNA (10ng) and RNase-free dH_2O to a total volume of 10µl. Amplification started with an initial activation step at 95°C for 15 min, followed by 30

cycles with denaturation at 94°C for 30 s, T_a (55°C, 58°C or 60°C, depending on the multiplex) for 90 s, extension at 72°C for 90 s and final extension at 60°C for 30 min. The PCR products were diluted and loaded in the ABI3130xl Genetic Analyser with 9µl Hi-Di formamide and 0.2µl of ladder (GeneScan™ 600 LIZ® Size Standard). Fragment length was estimated using GeneMapper 3.7 (Applied Biosystem©) and 10% of the samples were rerun to confirm repeatability of PCR and allele scoring. For 32 individuals from aquaculture sources three alleles were observed possibly identifying triploid oysters and were excluded from further analysis (Note: It is known that the seed supplier (oyster hatchery) also produces triploid *C. gigas*). Potential triploids were never observed in the wild samples.

4.4 Statistical analyses

MICRO-CHECKER v2.2.3 (Van Oosterhout et al. 2004) was used to identify genotyping errors, i.e. stuttering, large allele drop-outs and null-alleles, within the dataset by performing 1000 randomizations for each sample. General variability measures (e.g. number of alleles, allelic richness, observed and expected heterozygosities) and deviations from Hardy-Weinberg expectations (HWE) were calculated and tested using GENEPOP v4.0.10 (Rousset 2009). MICROSATELLITE ANALYSER (MSA) v4.05 (Dieringer and Schlötterer 2003) was used to estimate and test significance of Weir and Cockerham's (1984) unbiased estimator of Wrights' F-statistics, F_{ST} (10000 permutations) and to estimate allelic richness per locus and sample. Null-alleles often occur in oysters but may not be necessarily detrimental for F_{ST} and individual assignment analyses (Carlsson 2008). However, besides carrying out all the analyses retaining all loci, the Brookfield 1 correction was applied (refer to supplementary Appendix 12) and tested for correlation between F_{ST} matrices for corrected and uncorrected allele frequencies. Furthermore, F_{ST} analyses were conducted using only the 7 loci that did not exhibit or only showed a low frequency (maximum average of 7% across all populations) of null alleles, to check whether the levels and the patterns of differentiation were consistent across outputs. Visual comparisons were made through the construction of alternative non-metric Multidimensional Scaling (MDS) plots, using the software PAST v1.82b (Hammer et al. 2001). The same software was used to test for a correlation between EST-SSR and naSSR matrices using the Mantel test. The software LOSITAN (Beaumont and Nichols 1996; Antao et al. 2008) was

used to identify loci potentially affected by selection, using the F_{ST}-outlier method with '"neutral" mean F_{ST}' and 'Force mean F_{ST}' using 20000 simulations with both the infinite allele and the stepwise mutation models. Significance levels for multiple tests were adjusted using the Sequential Bonferroni technique (Rice 1989). Estimates of effective population sizes (N_e) – defined as the size of an idealized population exhibiting the same rate of random genetic drift as the population under consideration (Wright 1931) – were generated using the software LDNe v1.0 (Waples and Do 2008). The software COLONY v2.0 (Jones and Wang 2010) was used to assess family sizes and sibling numbers within samples. Clustering within and among samples was tested using STRUCTURE v2.2.3 (Pritchard et al. 2000) admixture model with 50000 burn-in and 300000 MCMC repetitions, with K = 1-10 and 10 replicates for each K. The number of clusters (K) was chosen where Delta K was largest (Evanno et al. 2005). The STRUCTURE repeat runs at the most likely K were combined and visualised using CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007) using 1000 repeats and the greedy option. The CLUMPP analysis had an average pairwise matrix similarity (G) of 0.9964 (SD = 0.0005).

4.5 Results

4.5.1 Genetic variability

Across all loci the number of alleles ranged from 5-18 in aquaculture samples and from 7-20 in feral samples (Table 4.1). Allelic richness per locus and sample (R_s) varied from 5.13-18.00 and was significantly different between pooled aquaculture and pooled feral samples (paired t-test, p = 0.003). However, without pooling of aquaculture samples, significant differences were only detected between pooled feral samples and AQB (p = 0.007, paired t-test corrected for 6 multiple comparisons) and pooled feral samples and AQF 2 (p = 0.043, paired t-test corrected for 6 multiple comparisons). Observed heterozygosity ranged from 0.217 – 1.000 and expected heterozygosity from 0.668 – 0.932. Many deviations from Hardy-Weinberg occurred (Table 4.1), those being all heterozygote deficiencies (except locus CG108 in sample AQB, which showed an excess of homozygotes). This was possibly caused by null-alleles as shown in the MICRO-CHECKER analysis with three EST-SSRs and one naSSR showing null-alleles among all samples (refer to supplementary Appendix 11). Locus CG44 was affected by stuttering and excluded from further analysis. High numbers of

private alleles at frequencies < 5% occurred in the feral samples, 23 being at naSSR and 23 at EST-SSR. Up to two private alleles at frequencies > 5% occurred in the aquaculture samples at EST-SSR and naSSR, respectively (Figure 4.1).

4.5.2 Population differentiation

Values of global multilocus and pairwise multilocus F_{ST} are separately shown for naSSR, EST-SSR, the 13 markers combined, and also for the 7 loci without null alleles in Table 4.2 (for corrected F_{ST} after Brookfield 1 refer to Appendix 12). Global multilocus F_{ST} were all significantly different from zero and values ranged between 0.033 (7 loci), 0.0359 (naSSR), 0.0383 (13 loci) and 0.0403 (EST-SSR). The F_{ST} matrices for corrected and uncorrected allele frequencies were highly correlated (R = 0.99, p = 0.002, Mantel test). Using only EST-SSR all feral samples were significantly differentiated (after sequential Bonferroni correction) from the aquaculture samples with pairwise multilocus F_{ST} ranging from 0.036 -0.0786. Bangor Aquaculture (AQB), which contained the oldest oysters, was differentiated from the younger year classes from Foyle Aquaculture, AQF 1 and AQF 2 (F_{ST} = 0.0584 and 0.0789, p = 0.0001), which themselves were differentiated from one another (F_{ST} = 0.0136, p = 0.0101). There was no significant differentiation between the three feral cohort samples (F_{ST} = -0.0004, 0.0026 or 0.0065, p > 0.05). Using only naSSR, differences between Feral 1 and Feral 3 samples (F_{ST} = 0.0152, p = 0.0001) and between AQF 1 and AQF 2 samples (F_{ST} = 0.0298, p = 0.0001) were detected. These differences were also present when all 13 markers were combined, while a marginal difference between Feral 2 and Feral 3 (F_{ST} = 0.0057) was observed using 7 loci only. A comparison of EST and anonymous marker matrices were visualized on a MDS plot (Figure 4.2) and the Mantel test (R = 0.95, p = 0.003) indicated highly correlated matrices of EST and anonymous markers. LOSITAN results did not indicate any evidence of footprints of selection. Thus, EST-linked loci could not be used as indicators of selection or adaptation in this study and were employed as additional anonymous markers for further statistical analyses.

The estimated effective population sizes (N_e) differed between samples from aquaculture and feral samples, indicating that the number of breeding individuals is about one order of magnitude greater in the feral oysters than in the aquaculture oysters (Figure 4.3). The effective population size of the feral samples using 0.05 as the lowest allele frequency ranged from 138.4 (C.I. 91.5-261.9) for Feral 1 to 229.8

(C.I. 122.9-1121.2) for Feral 2 and 202.4 (C.I. 113.0-728.3) for Feral 3 samples. Foyle Aquaculture had an effective populations size of N_e = 13.8 (C.I. 11.7-16.4) for sample AQF 1 and N_e = 17.4 (C.I. 14.9-20.5) for sample AQF 2. The Bangor Aquaculture sample showed a value of N_e = 11.8 (C.I. 10.3-13.5). Pooling the samples gave similar estimations (Figure 4.3).

COLONY analyses showed that the largest observed family with full siblings in Feral samples was never more than 2 (observed in five families). In Foyle Aquaculture samples, 2 siblings (11 families), 3 siblings (three families) and 4 siblings (one family) were detected, whereas in Bangor Aquaculture the family sizes ranged from 2 siblings (four families), 3 (one family), 5 (one family), 6 (one family) to a maximum of 7 (one family) siblings. No individuals from different geographical locations were siblings.

Clustering within and among samples using STRUCTURE gave the most likely value of k = 4 using the admixture model (Figure 4.4), with the most dramatic change in Delta K (213.9658) occurring at a mean LnP(D) of -13813.89. Fifteen individuals from AQB, two individuals from AQF 2 and ten individuals from AQF 1 were assigned to the same cluster as the feral samples (80% cut-off point, Figure 4.5).

Tables

Table 4.1 Summary statistics for 14 microsatellite loci among Pacific oyster collections (left box: EST-linked loci; right box: anonymous loci).

	Locus														
Sample	CGE007	Cgsili43	Cgsili46	Cgsili39	Cgsili4	Cgsili29	Cgsili50	Crgi50	L10	CG44	CG49	CG108	Crgi10	Crgi26	Average across loci
Feral 1															
n	50	47	50	45	50	45	50	48	48	46	50	49	50	49	
a	9	16	11	11	14	17	8	9	18	10	15	15	7	14	12.43
a (private)	0	1	0	1	0	1	0	1	0	0	1	0	0	3	
Rs	8.20	14.52	9.18	10.29	13.51	16.10	7.57	8.60	16.12	9.55	12.61	13.39	6.60	13.13	11.38
as	98-150	205-337	178-212	357-389	241-311	336-406	200-222	164-190	111-165	260-288	130-178	118-162	162-216	184-224	
H_e	0.815	0.898	0.795	0.867	0.916	0.932	0.796	0.828	0.920	0.869	0.827	0.909	0.810	0.888	0.86
H_o	**0.440**	**0.702**	0.720	**0.378**	0.820	**0.644**	0.800	**0.583**	0.896	**0.217**	**0.360**	0.816	0.760	0.878	0.64
HW	**0.000**	**0.000**	0.324	**0.000**	0.039	**0.000**	0.586	**0.000**	0.635	**0.000**	**0.000**	0.340	0.533	0.629	
Feral 2															
n	49	49	50	48	50	43	50	48	50	47	49	49	50	50	
a	7	18	11	10	17	16	9	8	18	11	11	15	9	14	12.43
a (private)	0	2	0	0	2	0	2	0	2	2	1	0	0	0	
Rs	6.67	15.37	9.57	9.24	14.92	14.62	8.42	7.64	15.23	9.93	10.20	13.38	7.79	13.09	11.15
as	98-150	205-337	178-212	357-389	233-311	336-406	198-222	166-190	113-175	266-296	130-178	120-162	162-216	184-222	
H_e	0.769	0.899	0.817	0.828	0.913	0.893	0.792	0.774	0.900	0.835	0.853	0.903	0.769	0.856	0.84
H_o	**0.510**	**0.633**	0.800	**0.354**	0.940	**0.465**	0.820	**0.521**	0.900	**0.255**	**0.551**	1.000	0.800	0.920	0.68
HW	**0.000**	**0.000**	0.068	**0.000**	0.223	**0.000**	0.958	**0.000**	0.148	**0.000**	**0.000**	0.054	0.653	0.770	
Feral 3															
n	49	48	50	49	50	44	50	48	50	48	44	50	50	49	
a	7	13	12	9	17	18	7	7	20	11	14	15	9	14	12.36
a (private)	0	1	1	0	1	2	0	0	2	1	1	0	1	1	
Rs	6.75	11.35	10.73	8.39	15.60	15.98	6.62	6.96	17.16	10.52	13.01	14.15	8.18	13.24	11.33
as	98-150	205-337	178-212	357-375	217-311	336-406	200-222	166-178	111-173	260-288	130-174	120-162	162-218	184-222	
H_e	0.724	0.844	0.744	0.819	0.924	0.906	0.819	0.750	0.913	0.834	0.896	0.904	0.784	0.854	0.84
H_o	**0.429**	**0.646**	**0.600**	**0.347**	0.900	**0.432**	0.820	**0.458**	0.920	**0.333**	**0.455**	0.920	0.760	0.898	0.64
HW	**0.000**	**0.000**	**0.002**	**0.000**	0.142	**0.000**	0.658	**0.000**	0.616	**0.000**	**0.000**	0.275	0.650	0.871	
AQF 1															
n	29	30	31	28	31	29	31	31	31	27	31	30	31	31	
a	8	11	9	10	13	12	7	8	18	9	11	14	8	9	10.50
a (private)	0	0	0	0	0	0	0	1	0	0	2	0	0	0	
Rs	8.00	11.00	9.00	10.00	13.00	12.00	7.00	8.00	18.00	9.00	11.00	14.00	8.00	9.00	10.50
as	98-150	207-289	178-208	357-391	235-273	338-406	200-222	166-190	111-181	266-302	120-162	118-162	174-216	184-208	
H_e	0.792	0.866	0.809	0.881	0.898	0.889	0.696	0.723	0.923	0.856	0.861	0.905	0.771	0.871	0.84
H_o	**0.345**	0.733	0.774	**0.679**	0.968	**0.690**	0.548	**0.581**	**0.774**	**0.185**	0.677	0.900	0.710	0.806	0.67
HW	**0.000**	**0.002**	0.063	**0.000**	0.102	**0.000**	**0.000**	0.100	**0.000**	**0.000**	0.042	0.025	0.029	0.084	
AQF 2															
n	37	37	38	34	38	37	38	38	38	32	38	38	37	36	
a	6	16	8	10	13	14	6	5	15	9	7	13	8	6	9.71
a (private)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Rs	5.78	14.82	7.78	9.94	12.89	13.33	5.81	4.99	14.49	8.93	6.97	12.66	8.00	6.00	9.46
as	98-148	207-337	178-200	357-391	229-275	336-406	200-212	166-180	111-181	264-302	128-162	120-162	174-216	184-206	
H_e	0.772	0.860	0.814	0.874	0.871	0.854	0.729	0.667	0.876	0.813	0.748	0.905	0.849	0.763	0.81
H_o	**0.297**	0.865	**0.368**	**0.412**	0.921	**0.649**	0.711	**0.500**	0.868	**0.281**	0.579	0.947	**0.649**	0.694	0.62
HW	**0.000**	0.016	**0.000**	**0.000**	0.013	**0.000**	0.028	0.027	**0.000**	**0.000**	**0.007**	**0.006**	**0.001**	0.153	
AQB															
n	49	49	49	47	49	44	49	49	49	43	49	48	45	48	
a	7	13	8	11	16	11	8	6	17	8	12	15	7	12	10.79
a (private)	0	0	0	2	1	0	0	0	0	0	0	0	0	1	
Rs	6.97	11.09	7.37	9.35	14.00	10.49	7.48	5.13	15.03	7.45	10.60	13.71	6.07	10.06	9.63
as	98-150	205-337	178-204	355-387	229-311	336-406	200-222	168-178	111-165	260-302	128-174	118-162	162-216	184-214	
H_e	0.764	0.815	0.793	0.801	0.890	0.865	0.801	0.475	0.903	0.581	0.838	**0.890**	0.720	0.832	0.78
H_o	**0.592**	**0.673**	0.735	**0.340**	0.980	**0.523**	0.878	0.367	0.939	**0.186**	**0.653**	1.000	0.756	0.833	0.68
HW	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	0.040	0.016	**0.000**	**0.000**	**0.000**	**0.000**	0.291	0.119	

Feral 1, 2, 3 = Pacific oysters of different mean sizes from a mussel bed, AQF 1, 2 = two different year classes of Pacific oysters from one aquaculture operator in Lough Foyle, AQB = One year class of Pacific oysters from one aquaculture operator in Bangor, n = number of individuals; a = number of alleles; Rs = allele richness per locus and sample; as = allele size range in base pairs; H_e = expected heterozygosity; H_o = observed hetereozygosity; HW = probability values of concordance with Hardy-Weinberg expectations; values in bold type are significant probability estimates after sequential Bonferroni correction.

Table 4.2 Global multilocus and pairwise multilocus, top: F_{ST} values for 7 EST-linked (upper right diagonal) and six anonymous (lower left diagonal) microsatellite loci separated. Bottom: F_{ST} values for 13 loci combined (upper right diagonal) and 7 loci with a low % of null-alleles (lower left diagonal). F_{ST}-estimates found to be significantly different from zero in bold type after Bonferroni correction. Note: 13 and 7 loci without Bonferroni correction (same result as with Bonferroni correction).

anonymous \ EST	Feral 1	Feral 2	Feral 3	AQF 1	AQF 2	AQB
Feral 1		-0.0004	0.0064	**0.0378**	**0.0688**	**0.0403**
Feral 2	0.0054		0.0026	**0.0360**	**0.0697**	**0.0376**
Feral 3	**0.0152**	0.0046		**0.0428**	**0.0786**	**0.0502**
AQF 1	**0.0322**	**0.0226**	**0.0315**		**0.0136**	**0.0584**
AQF 2	**0.0623**	**0.0566**	**0.0692**	**0.0298**		**0.0789**
AQB	**0.0486**	**0.0277**	**0.0392**	**0.0484**	**0.0660**	

global F_{ST}/p	anonymous loci: 0.0359/0.0001
global F_{ST}/p	EST loci: 0.0403/0.0001

7 loci \ all 13 loci	Feral 1	Feral 2	Feral 3	AQF 1	AQF 2	AQB
Feral 1		0.0023	**0.0105**	**0.0352**	**0.0659**	**0.0441**
Feral 2	0.0035		0.0035	**0.0299**	**0.0638**	**0.0331**
Feral 3	**0.0105**	**0.0057**		**0.0375**	**0.0743**	**0.0452**
AQF 1	**0.0374**	**0.0307**	**0.0305**		**0.0211**	**0.0539**
AQF 2	**0.0628**	**0.0578**	**0.0580**	**0.0187**		**0.0731**
AQB	**0.0348**	**0.0246**	**0.0345**	**0.0506**	**0.0586**	

global F_{ST}/p	13 loci: 0.0383/0.0001
global F_{ST}/p	7 loci: 0.0331/0.0001

Figures

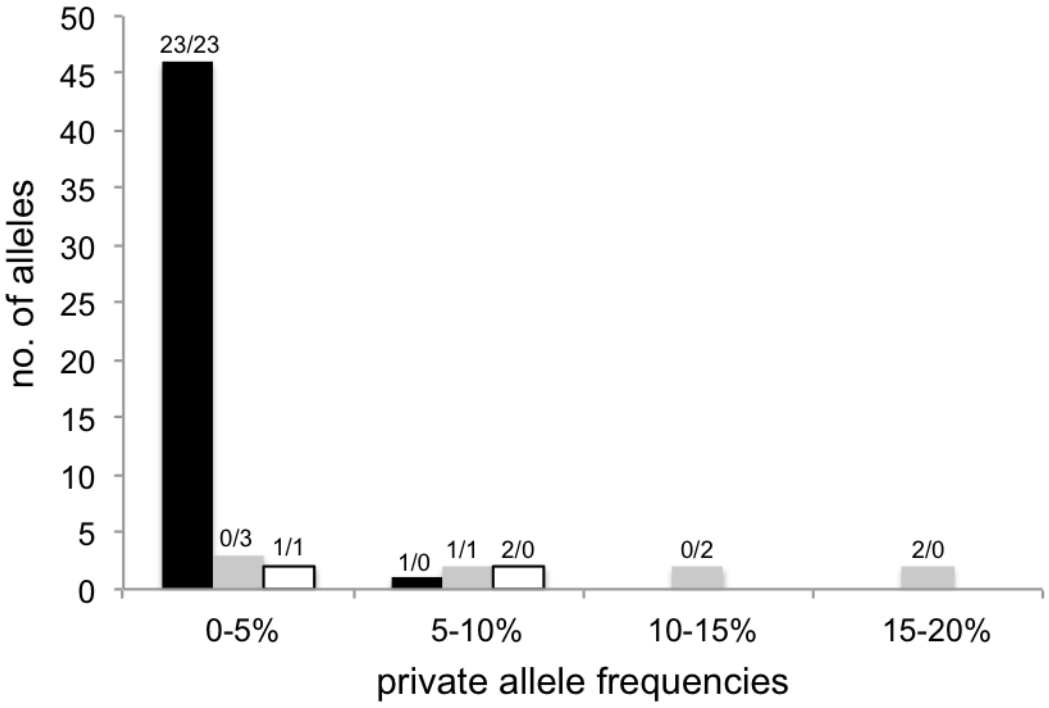


Figure 4.1 Private allele frequencies of feral oysters (black), aquaculture oysters Foyle (grey) and aquaculture oysters Bangor (white); numbers above bar plots indicate number of private alleles of EST linked loci (1. number) and anonymous loci (2. number).

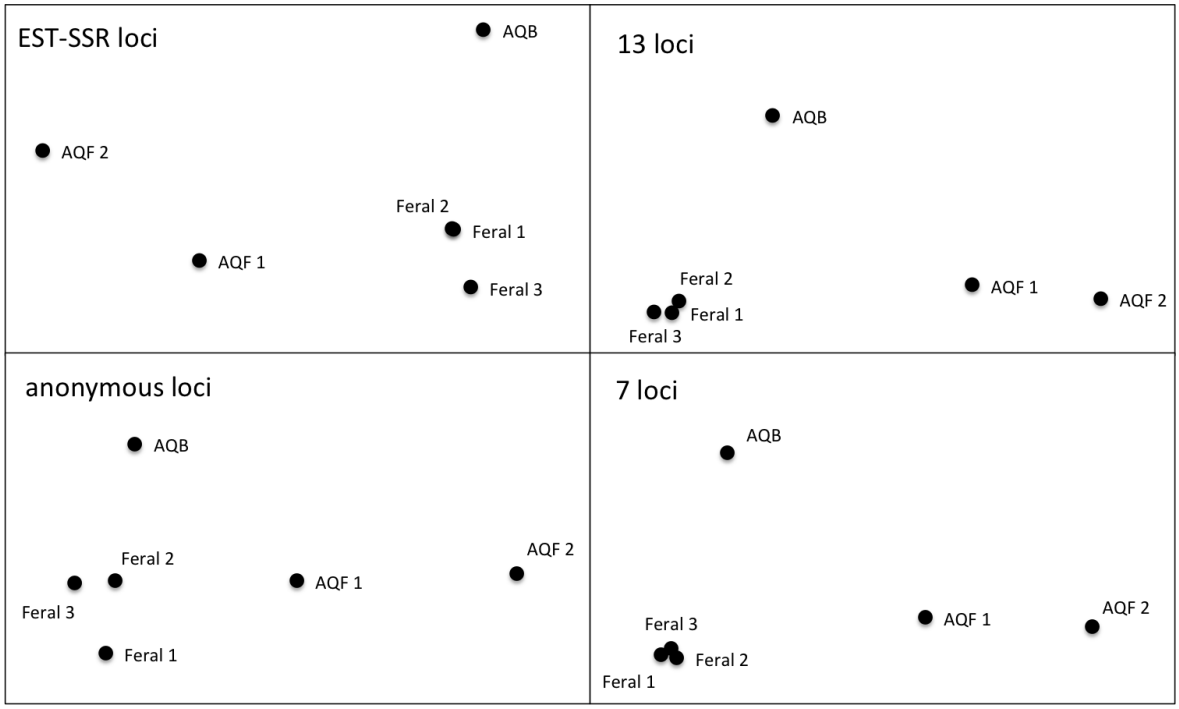


Figure 4.2 MDS plot based on F_{ST} of the six different samples (Feral 1, 2, 3, Aquaculture Foyle 1, 2 and Aquaculture Bangor) using only 7 EST-linked loci, only six anonymous loci, all 13 loci and 7 loci only.

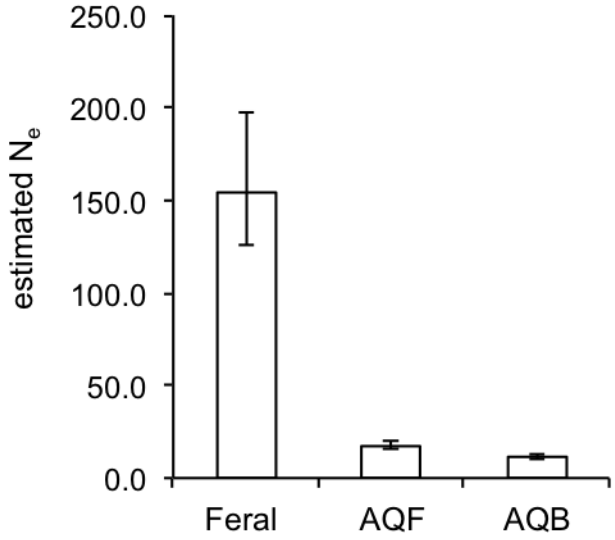


Figure 4.3 Estimates of effective population sizes (N_e) and 95% Confidence Intervals of pooled samples.

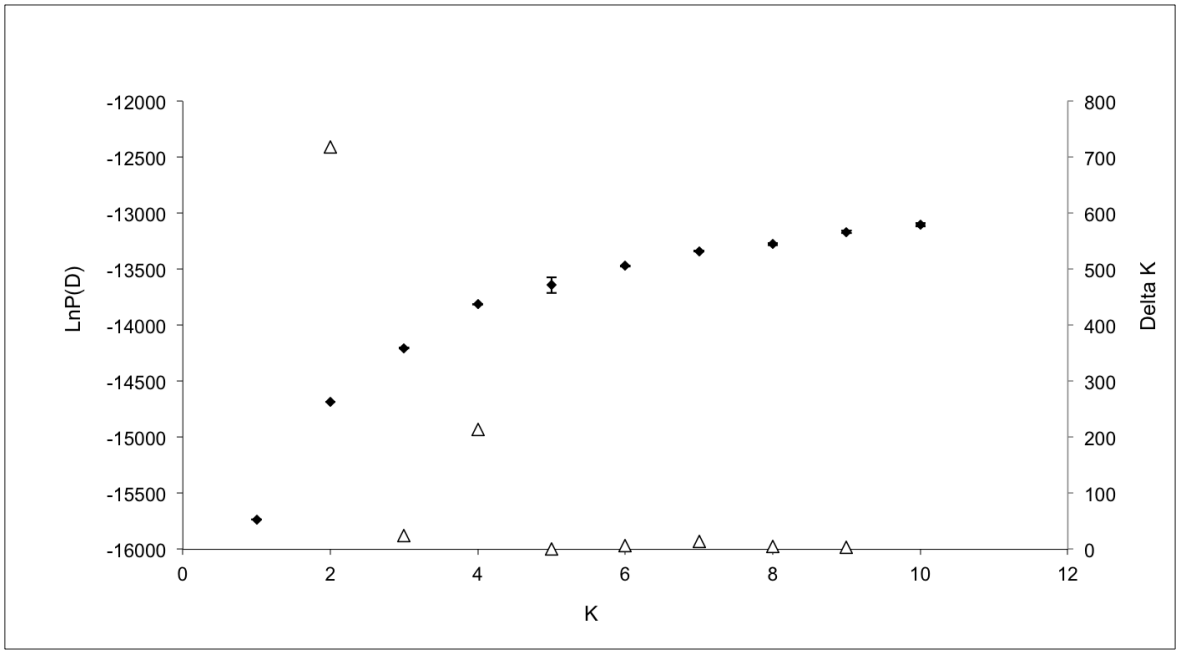


Figure 4.4 Mean LnP(D) values (black) (± SD) of 10 replicates for each K value and Delta K (white triangle).

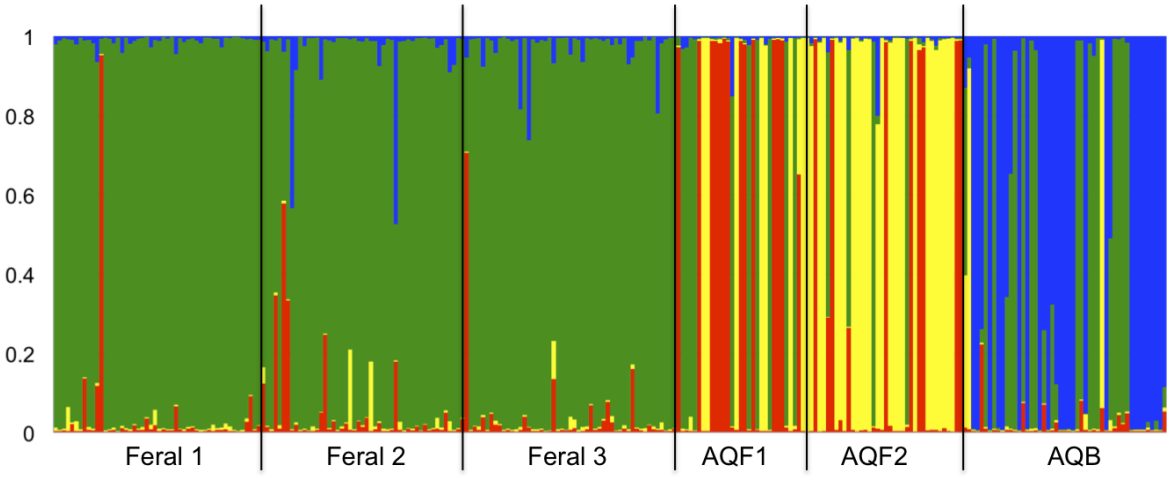


Figure 4.5 STRUCTURE output for all samples (k=4), visualised using CLUMPP. Each vertical line represent one individual, each colour represents the proportion of assignment to the different clusters and black lines separate different samples.

4.6 Discussion

This study has shown that the feral oysters established on a mussel bed in Lough Foyle are relatively high differentiated and more diverse than those oysters kept adjacent to them in intertidal aquaculture trestle systems.

It has been suggested that the functional constraints of EST-SSR make them less prone to null-alleles (Ellis and Burke 2007) and this might be advantageous for studying oysters as null alleles are particularly common in this species (e.g. (McGoldrick et al. 2000; Hedgecock et al. 2004). Recent studies on *C. gigas* microsatellites detected low frequencies of null-alleles using EST markers (Sauvage et al. 2009; Li et al. 2010). However, using the same EST markers as in those studies, null-alleles occurred at higher frequencies, although not significantly (paired t-test, $p = 0.078$), in EST-linked loci than in the anonymous loci in this study and did not conform to the general assumption. EST-linked markers behaved as additional anonymous markers in this study: none showed potentially non-neutral behaviours and collectively they provided structuring patterns that were highly correlated with anonymous markers. Similar results using both types of markers were observed before in other species (Woodhead et al. 2005; Kim et al. 2008) which confirms that only a small percentage of genes show evidence of a positive selection even when closely associated with functional regions (Ellis and Burke 2007).

An excess of homozygotes leading to deviations from Hardy-Weinberg expectations was observed among almost all loci and samples except one (Crgi26) and this is likely due to the presence of null-alleles. Null-alleles might overestimate population differentiation due to a reduction of genetic diversity within populations (Chapuis and Estoup 2007). However, as shown by Carlsson (2008) null-alleles are not necessarily problematic for genetic differentiation and assignment testing, especially when a reasonably large number of loci are used. Even if F_{ST} estimates were slightly overestimated due to the presence of null-alleles, tests conducted with Brookfield-corrected allelic frequencies, or using the only 7 loci without null alleles, showed unchanged patterns.

In a comparison between aquaculture and feral oyster samples, pairwise multilocus F_{ST} values using 13 loci ranged from 0.030 – 0.074 ($p < 0.01$). Significant genetic differentiation was also found between the three year classes of oysters from

aquaculture, spanning 10, 5, and 3 years (F_{ST} = 0.054 – 0.074, p = 0.0001, Table 4.2), suggesting great temporal variation in origin or numbers of parents used for producing seed. Möhler et al. (2011) similarly suggest temporal variation in oysters used for stocking or crossing schemes within oyster hatcheries. The significant F_{ST} between Feral 1 and Feral 3, and the one detected between Feral 2 and Feral 3, using 7 loci, suggest also a genetic difference among these samples, but this result was not confirmed using adjusted genotypes after Brookfield correction for null-alleles (refer to supplementary Appendix 12) nor did the individuals differently cluster. Hence, a genetic difference between Feral 1 and Feral 3 is unlikely and in its place, the results more likely indicate self-recruitment of feral oysters.

Despite the similar but still significantly different level of diversity between feral and farmed oysters, the high number of private alleles in feral samples and the higher frequencies (5-20%) of individual private alleles in aquaculture samples equally suggest an established feral population, genetically differentiated from the aquaculture populations. If the aquaculture populations were the source of the feral oysters, their private alleles (particularly considering their sometimes very high frequencies, i.e. 20%) would be found in the feral population. Similarly, the larger effective population size of feral oysters indicates a feral population that is independent of the current oysters in aquaculture. The small number of full siblings in the feral population and the fact that no siblings were found across individuals of feral and aquaculture populations supports this. As a common practice in aquaculture, few breeders are usually kept in aquaculture leading to a small N_e, which has been found in *C. gigas* used in aquaculture in other countries (Hedgecock and Sly 1990; Appleyard and Ward 2006) and was observed in the aquaculture samples in this study. The significantly higher N_e observed in the feral population, argues against a direct link between the feral and farmed stocks.

The observed genetic differentiation among cultured and feral oysters is strongly supported by the results of the STRUCTURE analysis, which suggests four different clusters. In total, 27 individuals out of 150 samples from aquaculture were assigned to the same cluster as the feral samples. However, it is unlikely that those individuals can be considered the source of the feral population as the effective population size of those individual oysters pooled together was still too low (N_e = 24.8, C.I. 19.5-32.7) to be the source population of the feral ones which had a ten

times higher effective population size. In addition, the cluster predominant in aquaculture was absent in feral oysters. Similar results were obtained by using only 7 loci that had a low frequency of null-alleles (Appendix 13). A translocation from the wild mussel beds into the aquaculture cannot serve as an explanation because oyster farmers did not collect oysters from the wild for this purpose (C McGonigle, Lough's Agency, personal comment). Although a sequential use of different strains in the hatchery seems likely, as indicated by the differentiation of aquaculture oyster year classes, non-sampled year classes from aquaculture containing the strain from which the feral oysters originated, should be dismissed based on the principle of parsimony. The most likely explanation might be that those single individuals from aquaculture may have originated from spawners collected from a wild population that is demographically connected with the oysters found on the mussel bed. If oyster farmers did not supplement their stocks with those oysters, the hatchery might have done so.

Regular aquaculture with Pacific oysters started in 1998/99 in Lough Foyle, with juvenile seed oysters imported from one British hatchery every year and then grown to market size in the open water in intertidal trestle systems (C McGonigle, Lough's Agency, personal comment). Some stocks from other Irish bays have been moved to aquaculture areas in Lough Foyle in 2009 and these may have been of French origin. Nonetheless, the samples were collected in May 2010 and are unlikely to be the progeny of those oysters due to their large size spectrum. However, and albeit impossible to verify, there is a possibility that the current feral oysters may have originated from experimental tests for growth conditions conducted three decades ago in the estuary.

Aquaculture has been closely associated with the rapid spread of Pacific oysters and in many locations, wild oyster populations have established few years after oyster farming had commenced (Ruesink et al. 2005; Brandt et al. 2008; Troost 2010). Recently, a study comparing aquaculture and wild oyster populations in the Wadden Sea did not find pronounced genetic differentiation between any of their populations using a mitochondrial DNA marker (Möhler et al. 2011). However, the authors suggested that a repeated input of genetic material from a changing breeding stock from aquaculture can influence invasive natural populations in close proximity. On the contrary, including temporal variation and sampling the genetic material of

oysters used in aquaculture in three of the last 10 years, no significant indication that oyster farming currently affects population dynamics of the feral oysters was found.

Iterative planktonic dispersal with the main current systems has been invoked to explain the current spreading of oysters along the east coast of the North Sea (Brandt et al. 2008; Wrange et al. 2010). However, this seems to be highly unlikely in the case of Lough Foyle: even if oyster reefs are found now along the UK coasts (Couzens 2006), the water current circulation pattern does not support larval drift from those areas into the bay (Brown et al. 2003; Fernand et al. 2006). The introduction and subsequent spreading of species can often be traced back to ships and their ballast water (Carlton 1999). If vessels contained oyster larvae from areas where Pacific oysters are established, e.g., UK, France, Denmark, Germany or the Netherlands, those larvae might have been introduced into the estuary and successfully settled. However, this cannot be followed, as Northern Ireland Port Movements data are limited in geographic content. Mussel or native oyster fisheries might more likely be associated with the appearance of *C. gigas* on the mussel beds, through the unintentional transfer of juveniles from other adjacent estuaries or bays where oysters are already established. Indeed, the continuous movement of vessels (mussel and native oyster dredgers) between Lough Foyle and Lough Swilly, the estuary adjacent to Lough Foyle and with known establishment of oysters, could be a vector of introduction.

In summary, the origin of feral oysters in Lough Foyle can only be reliably inferred through comparisons with several other established populations in Ireland and northwestern Europe. What is apparent from the present investigation is that feral oysters in Foyle are demographically uncoupled from the oysters currently used in aquaculture in the bay, and that management strategies in the area should look beyond the short-term activities of the local farm as the source of individuals maintaining populations of feral Pacific oysters. Although aquaculture has been originally responsible for the spread of the Pacific oyster in Europe, the findings of this study suggest that portions of northeast Atlantic coastal ecosystems now include a complex network of established *C. gigas* populations, whose fate no longer depends on their farmed counterparts. The challenge for conservation biologists and environmental managers will now be to identify a more ecologically comprehensive solution for this conspicuous invader.

Chapter 5 General Discussion

Different aspects of the establishment of Pacific oysters in Ireland were investigated in the previous chapters. Chapter 2 focused on the current range and status of Pacific oysters in Ireland as well as on the environmental factors associated with their presence or absence. It was shown that *C. gigas* is established in several bays in Ireland with evidence of several recruitment events. A positive association with hard substrata and biogenic reefs, residence times of embayments and large intertidal areas, and also aquaculture, was found. Experimental tests in Chapter 3 showed that the influence of predation was variable and could depend on the sizes of the oysters or on other factors associated with particular sites or times. In addition, condition of oysters was not significantly decreased in the presence of macroalgal canopy; however, shell growth was significantly reduced, but only at one site. Results suggest that other factors influencing dispersal and early recruitment success of oysters might underpin the small abundances of *C. gigas* found on macroalgae-dominated shores. Aquaculture as a potential vector for Pacific oysters was investigated in Chapter 4 using microsatellites. It was revealed that feral Pacific oyster populations are demographically uncoupled from aquaculture populations in Lough Foyle and that a self-recruitment of oysters is very likely.

The results described above will be integrated here in order to create a broader picture of the relevant factors that led to the successful establishment of *C. gigas* and may affect its further spread in Ireland and elsewhere. Possible effects on the recipient community and general implications for aquaculture practices and management of invasive populations of *C. gigas* will be highlighted and discussed.

5.1 Factors promoting invasion of *C. gigas*

5.1.1 Temperature

Although not explicitly considered in this thesis, temperature plays an important role for the timing and magnitude of population growth through its influence on reproduction and survival of planktonic stages as well as on the synchronized release of gametes among males and females, which is crucial to overcome the 'Allee effect' (Crooks and Soulé 1999; Drake and Lodge 2006). Thus, unfavourable temperature regimes are among the ecological mechanisms that may lead to a 'prolonged lag phase' from the time between first sightings and rapid expansion in invasive species (Crooks and Soulé 1999). The establishment of Pacific oysters in the wild in Ireland was reached approximately 40 years after its introduction. Whether this can be really seen as a late establishment compared to other areas or might be rather a result of failed early detection remains unresolved (for densities recorded in other areas see Table 5.1).

Table 5.1 Some examples of Pacific oyster introduction and densities

Country	1st introduction 1st sighting	ind./m² (establishment) ind./m² (expansion)
Germany (Reise 1998; Nehls et al. 2006)	1986 1991	0.1-2.2 (1993-1995) 290-600 (2004)
Netherlands (Drinkwaard 1998; Fey et al. 2010)	1964 1975/1976	~ 50 (2003) > 500 (2006)
Sweden (Wrange et al. 2010)	1973-1976 few sightings until 2007	1 (2007) na
UK (Couzens 2006; Natural England 2009; Guy and Roberts 2010)	1965 1994	1 - 10 (2009) na
Argentina (Orensanz et al. 2002)	1982 1987	2 (1995) 120 (1998)

Temperature is not only important for gametogenesis and spawning of oysters but also for oyster growth and might, together with phytoplankton dynamics, i.e. food supply, drive seasonal and year-to-year variability of growth patterns (Gangnery et al. 2003; Grangeré et al. 2009). Almost no growth of oysters occurred during the winter months (Chapter 3) and maximal linear shell growth in summer and minimal or

absent shell growth in winter has been shown before for oysters in aquaculture (Gangnery et al. 2003) and in wild populations (Diederich 2006).

Strong recruitment of Pacific oysters has been observed in summers with temperatures above long-term averages and the occurrence of Pacific oysters has been correlated with warmer summer water temperatures. Evidence to support this has come from a number of locations across Europe (Wehrmann et al. 2000; Diederich et al. 2005; Dutertre et al. 2010; Wrange et al. 2010). Along the European coast, a general trend with average water temperatures decreasing with increasing latitude is observed which might potentially limit the northern distribution of Pacific oysters (Cardoso et al. 2007).

In Ireland, temperature records show that water temperatures vary between bays but do not necessarily decrease with latitude (EPA 2010). Furthermore, temperature data obtained from satellites reflecting near-coastal locations can differ significantly from local temperatures within bays which may lead to mismatches in predictions of oysters and real occurrence data (Helmuth et al. 2002; Carrasco and Barón 2010; Dutertre et al. 2010). Thus, it can be argued that latitude should not be generally taken as a proxy for temperature.

Latitude correlated highly with residence time in the survey undertaken in Chapter 2, which indicates that an influence of latitude might be more related to features of the bays than to temperature. Shallow bays often show localized heating and oyster spawning might be enhanced in these bays. However, average depth of a bay alone might not be a good predictor for oyster presence. Even if temperature regimes are favourable for oyster spawning, strong tidal currents might carry the larvae out of a bay before they attain competence to settle. Thus, residence time, which encompasses the tidal volumes, width of the mouth and length of a bay, was considered more relevant to the different life-stages of oysters than average depth only and used in the model. Nevertheless, temperature and residence time could be correlated, however, this could not be tested in the model because temperature records were not available for all bays. Records of maximum water temperatures measured by the EPA between 2007-2009 suggest that temperature conditions were indeed sufficient for oyster spawnings, recruitment and larval settlement in the bays

where oysters were found (except for Ballynakill Harbour), assuming that spawning can occur over 16°C (Ruiz et al. 1992).

Even if temperature could be included in future surveys and might serve as a good predictor, decisions on what data would be suited best, i.e. average water temperature, maximum water temperature, SST (Sea Surface Temperature), AT (Atmospheric Temperature) and over which time period, would have to be made and could indeed be critical (see Carrasco and Barón 2010). Different results could be obtained using either mean water temperatures or maximum water temperatures. For example, in Lough Swilly, maximum temperatures of 21.42°C and 18.55°C in the Shannon Estuary were recorded and differ substantially from the median records of 15.96°C and 16.1°C.

As recently shown for *C. gigas* in aquaculture in two Irish bays, spawning might not occur even if temperatures are favourable (Mag Aoidh 2012) and it is known that photoperiod can influence quantity of gametes and intensity of spawning as well (Fabioux et al. 2005). Likewise, many other environmental factors are important, not only for gametogenesis but also for subsequent life stages, such as food availability (King et al. 2006; Grangeré et al. 2009; Rico-Villa et al. 2009), turbidity and seston load (Barillé et al. 1997; Gangnery et al. 2003), salinity (Brown 1988; Brown and Hartwick 1988; Mann et al. 1991) and tidal elevation (Ruesink 2007; Cassis et al. 2011). Furthermore, Pacific oysters show a high phenotypic plasticity and changes in their eco-physiological processes might lead to a decrease of the minimum threshold temperature required to facilitate spawning (Hamdoun et al. 2003). Therefore, risk assessments or predictions of oyster presence based on temperature alone might not be as valuable as integrating several environmental factors that are related to different life-stages of this species (see Chapter 2).

5.1.2 Larval dispersal

For marine species with planktonic larval stages, the duration of the larval stage determines the length of time that the larvae are subject to movements by currents and exposed to sources of mortality (Pechenik 1999; Pineda et al. 2007). Active swimming behaviour of larvae and flow related stratification of larvae may influence dispersal distances (Knights et al. 2006). To minimize advection by mean currents and facilitate retention, a high reproductive rate, spawning in multiple seasons or

years with an increase in larval dispersal variability due to interannual variation of currents and a shorter development time are beneficial traits (Byers and Pringle 2006). These traits are inherent to Pacific oysters. As suggested by Diederich et al. (2005), an enhanced larval retention is likely to have facilitated population growth of Pacific oysters in the Wadden Sea.

Oyster presence was positively associated with residence time (Chapter 2) and residence time of bays where oysters were found in numbers larger than > 0.09 ind./m^2 was approximately in the range of the duration of the mean lifetime (3-4 weeks) of pelagic larvae of *C. gigas* (Quayle 1988). It could be argued that attention and priority in monitoring of Pacific oysters in the future has to be given to bays in which residence times are at least equal to the mean lifetime of oyster larvae. However, changes in temperature can modify larval development times and patterns of dispersal and thus, might affect the importance of residence time as a predictor for oyster presence in the future.

Longer planktonic larval stage duration at lower temperatures is found in invertebrate species and a greater maximum dispersal distances for larvae in colder waters than for larvae in warmer waters was predicted by O'Connor et al. (2007). Indeed, a smaller oocyte of more northern populations of *C. gigas* has been found by Cardoso et al. (Cardoso et al. 2007) suggesting an increased larval dispersal with further population expansions towards northern latitudes due to an extended development time for small oocytes. Yet, predatory losses might increase and counteract the wider dispersal in case of longer periods spent in the water column. On the contrary, with a trend towards warmer temperatures, a shorter time of ontogenetic development is expected (Gillooly et al. 2002).

Overall, an interaction of residence time and temperature might affect the dispersal of Pacific oyster populations; a successful spawning and a possibly shorter planktonic larval duration of Pacific oysters due to increased temperatures could lead to recruitment of oysters even in bays with lower residence times than observed during the survey in 2009. However, temperature thresholds or the dynamics of these interactions cannot easily be foreseen and although an increase in sea surface temperatures of 1-3°C is predicted for Ireland (Boelens et al. 2005), predictions often suffer high uncertainties (Latif 2011). Furthermore, increases of temperature

calculated for coastal areas might not directly translate into the same changes within bays.

Connectivity of oyster population through larval drift has been suggested for the Wadden Sea and a rapid increase in oyster abundance with an eastward expansion away from the source of introduction within less than 10 years was observed (Wehrmann et al. 2000; Brandt et al. 2008; Wrange et al. 2010). To predict the speed of the invasion in Ireland, local hydrodynamics within and between bays have to be considered as they can determine maximal dispersal range and influence the proliferation of a species (Brandt et al. 2008; Rigal et al. 2010). The possibility of oysters spreading along the Irish coast and between bays within the mean lifetime of 3-4 weeks of the pelagic larvae stage cannot be completely excluded. However, considering the strong northward flow along the West coast, from the north of the Cornish coast to Malin Head in the summer (Fernand et al. 2006), a transport of larvae with the current would only be likely in the direction of southern to northern locations. The same would apply for oysters drifting from other areas to Irish coastal habitats. Larvae tracking, further genetic analysis and sampling of different oyster populations would be needed to resolve questions of connectivity and origin. A sampling of different *C. gigas* populations along the coast of the UK, Ireland and France is currently being undertaken in the SEAFARE project. Using genetic techniques, the aim of this project is to better establish connectivity and dispersal among feral oyster populations which will help to understand pathways of spreading.

5.1.3 Recruitment strength and substratum

Although not explicitly studied here, pre- and early post-settlement processes have been shown to influence recruitment patterns of a species (see review Hunt and Scheibling 1997; Jenkins et al. 2009). Peters (2009) suggested that a consensus on oysters' larvae settlement and metamorphosis is still missing within the literature. Her meta-analysis on oyster settlement processes showed that many studies are predominantly observational or have poor experimental design. However, even if the underlying mechanisms have still not been fully identified, it is known that Pacific oysters attach on various substrata such as blue mussels (live and empty), rock, shell fragments, other biota, and also on jetties and dikes (Escapa et al. 2004; Nehls et al. 2006; Melo et al. 2010; Eschweiler and Buschbaum 2011). This was confirmed in this

study (Chapter 2). However, macroalgae never served as a substratum for attachment, except for one single case (own observation), and during small-scale transects, oysters occurred disproportionately less on macroalgae. In this study, cover of macroalgae was not a useful predictor of Pacific oyster occurrence. This might be explained by the fact that the *Phase 1* surveys which fed the model were not at the right scale to examine the influence of macroalgae as oysters still show up as present at the site despite a high cover with macroalgae, with oysters attached on boulders or cobbles underneath macroalgae.

As abundances could not be incorporated or tested in the model in this study, an experimental approach was chosen to investigate processes affecting abundance of oysters more fully (Chapter 3). Although oyster shell growth was reduced in the presence of macroalgae at one site, condition index, a better estimator for oyster performance of oysters than linear shell growth, was not significantly reduced in the presence of macroalgae. Although it is impossible to exclude that effects on condition index could become more prominent at a later stage and could lead to differences in growth of oysters in the presence of macroalgae, the same result was obtained using larger oysters (mean size 45 mm at the start) in a study by Diederich et al. (2006). Thus, a biotic interaction with macroalgae at the post-recruitment stages of oysters cannot explain the low densities of oysters at macroalgae-dominated shores compared to mussel beds or rocky shores. Instead, pre-settlement processes might be more important and it can be speculated that a high % of macroalgal cover at a shore might cause restrictions of settlement of oyster larvae onto substrata underneath macroalgae with only few oyster larvae succeeding to permanently attach. A lower recruitment of Pacific oysters underneath *Fucus* and overall lower numbers of adult individuals on mussel beds is known from the Wadden Sea (Reise 1998; Diederich 2005) and would suggest that removal of macroalgae could increase oyster recruitment.

However, apart from substrata, other small-scale processes acting at the scale of site but not tested in the model might influence recruitment strength, e.g. larval dispersal, settlement behaviour but also temperature, salinity or food availability that can influence spawning intensity (for discussion see Fabioux et al. 2005). These smaller-scale processes might equally explain the differences in oyster growth noted between sites during the experiment (Chapter 3).

As highlighted by Truscott et al. (2008) in the case of a riparian plant invasion, separating occurrence (yes/no), patch number, area and density is important to understand factors that drive invasions. From a management point of view, however, the most cautious approach is certainly the one that considers factors predicting presence or absence of invaders. Oyster abundances at shores dominated by macroalgae were generally lower than on boulder shores and less oysters were generally observed underneath macroalgae compared to boulders without macroalgae. This suggests substrate type being a factor related to recruitment strength. The collection of data on macroalgae cover is therefore still recommended for future surveys as it would help to disentangle the potentially important effect of macroalgae for establishment and spread of Pacific oysters.

5.1.4 Biotic interactions - predation and other sources of mortality

Predation has long been recognized as an important factor controlling the structure of communities and mobile predators have been shown to limit distribution of prey in the intertidal zone (Paine 1974; Robles 1987). In the context of invasions, native predators can control introduced species and limit their geographical spread after its establishment (deRivera et al. 2005; Shinen et al. 2009). Bishop and Peterson (2006) suggested that allocation of resources to rapid growth and development rather than to predator avoidance may promote early stages of invasion but prevent dense adult populations. In laboratory studies, they showed that the oyster *C. ariakensis* has a greater susceptibility to predatory blue crabs (*Callinectes sapidus*) due to a weaker shell than the native oyster species, the Eastern oysters *C. virginica*. Although they used "cultchless" oysters that might suffer a higher total mortality from predation as crabs can more easily crush the oyster shell in the absence of the protection offered by a shell piece, the same results were obtained using attached spat (Newell et al. 2007).

The main experiment reported in the current study suggests that predation pressure on Pacific oysters might be rather low and that predation is unlikely to have a significant effect on juvenile oyster survival (Chapter 3). However, predation could play a more important role when oysters are smaller as mortality rates are often higher during early post-settlement (Hunt and Scheibling 1997). In a pilot study (Appendix 3), smaller oyster spat was highly susceptible to predators, however,

oysters were not naturally attached to the substratum and the natural field situation was therefore not closely mimicked. However, predation on smaller naturally attached *C. gigas* spat (~10 mm) has been shown in the Wadden Sea and North America indicating that oyster spat is susceptible to predators in the field when attached to a shell (Diederich 2006; Ruesink 2007). However, a high survival of 70% of spat on intertidal mussel beds was observed in the German Wadden Sea by Diederich (2006) suggesting that post-settlement predation pressure might be rather low for juvenile *C. gigas*. The author also suggested that in subtidal habitats, predation pressure might be only important directly after settlement affecting initial recruitment patterns but not post-recruitment survival. Dense subtidal oyster populations have already established in the Netherlands (Smaal et al. 2009) which suggests that predation does not form a strong barrier to their establishment or spread. As shown during the crab trapping (Chapter 3), abundances of the potential main predator *Carcinus maenas* were not significantly different between sites of different oyster densities, which suggests that shore crabs might not directly control abundance patterns of oysters.

However, interactions between recruitment strength and predation might be another important factor that could explain the abundance patterns of oysters and would need further consideration. Knights et al. (2012) recently showed that recruitment intensity of *C. virginica* can vary depending on flow regimes and that the effect of predators is dependent on recruit density. In the case of *C. virginica* they showed that predatory effects might be absent when densities fall below 2000 recruits/m^2. Furthermore, they also noted that predators never consumed recruits to the point of local extinction (over a period of 14 weeks). To disentangle the influence of predation and recruitment strength in density patterns of Pacific oysters further experimental studies in different habitats are needed. Experiments could be set up and replicated on shores where oysters have been found in different densities. For these experiments, enclosed, partly enclosed (cage control) and open settlement plates could be used on mussel beds and rocky shores where higher densities of oysters are found, and macroalgae-dominated shores where oysters are rare. The number of recruits surviving (counted at different time intervals after the setting of larvae onto the plates) would then be compared between sites and treatments. Similar studies already exist on Pacific oysters in the Wadden Sea or on *C. virginica* in

the United States (Diederich unpublished, O'Beirn et al. 1996). As shown by Hollebone and Hay (2007) for the porcelain crab *Petrolisthes armatus* (Gibbes, 1850), large settlement events are able to swamp biotic resistance by native species and intense episodes of recruitment of Pacific oysters may similarly swamp oyster predators.

Beside predation, other sources of mortality might however become important and potentially restrict (both directly or indirectly) the spreading of Pacific oysters. For many years, a high mortality during summer has been observed in aquaculture oyster spat (Dégremont et al. 2007; Soletchnik et al. 2007; Malham et al. 2009), but could not be exclusively related to one environmental factor. Recently, the ostreid herpesvirus (OsHV-1 µvar) has been detected causing high mortalities in Pacific oyster spat (Schikorski et al. 2011) which led to the prohibition of oyster spat from infected areas. Whether the virus or the high summer mortalities will also lead to mortalities among natural oyster spat is not known. Increased mortalities of juvenile oysters during winter were recorded in the Wadden Sea depending on period length of freezing air temperatures and low water temperatures (Reise 1998; Diederich 2006; Büttger et al. 2011). This mortality could be related to the halt of filtration activity below 2°C water temperature (Averdung 2009), ice shear causing mechanical disturbance or intraspecific competition in high densities stocks. Parasitic load by mytilicolid copepods was tested in Pacific oyster populations in the Wadden Sea but seemed to play a minor role (Elsner et al. 2011). An infestation with trematode parasites was found in *C. gigas*, but was lower compared to native mussels (Krakau et al. 2006). In Ireland, *Mytilicola orientalis* (Mori, 1935) occurs in Pacific oysters but does not harm its host at low intensities (Steele and Mulcahy 2001). More common is the parasitic worm *Polydora* sp. which can induce a decrease in oyster growth with sufficient infestation intensity (Chambon et al. 2007).

Whether a combination of the different sources of mortality might restrict further spreading of oysters in Ireland is not known. However, the high longevity and high individual gamete production of Pacific oysters will generally allow them to recover or increase, even from low densities (Reise 1998).

Competition as another form of biotic resistance seems unlikely to stop establishment or limit the spread of Pacific oysters. In Australia, *C. gigas* has a

competitive advantage over the Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) due to faster rates of feeding and greater metabolic efficiencies of both feeding and growth (Bayne 2002). In the northeast Pacific, neighbour species can reduce growth of juvenile oysters but also improve survival (Ruesink 2007). The high filtration activity of oysters and enhanced flow rates on elevated reefs generally maximise growth and survival (Schulte et al. 2009; Troost 2010). A high survival and growth rate in intertidal and subtidal habitats in the Wadden Sea and the possible absence of density-dependent growth reduction suggest that *C. gigas* might have a competitive advantage in the long term, e.g. over blue mussels (Diederich 2006). Further information about differences in habitat use with native oysters *Ostrea edulis* will be discussed in a subsequent section.

5.2 Aquaculture as a vector for *C. gigas*

5.2.1 Aquaculture as a predictor for presence of oysters

Beside the factors discussed above that are associated with the presence of Pacific oysters in the wild, aquaculture is considered to be an important vector for Pacific oysters; a spreading of feral oysters was often observed in bays of oyster aquaculture (Brandt et al. 2008; Troost 2010). However, studies that link presences of aquaculture facilities or their distances to feral populations are missing. Therefore, aquaculture was tested, first as a predictor for oyster presence in a model (Chapter 2), but also as a direct introduction source (Chapter 4).

In the model approach, the two categories 'Aquaculture close' and 'Aquaculture far' were kept in the model, with a tendency for oysters to occur in bays with aquaculture, but more likely far from aquaculture installations than close to them. The fact that oysters were found at one site in the absence of aquaculture, although they were only single individuals (SACFOR category 'rare'), together with the low total number of bays sampled without aquaculture, might explain why a reduction in oyster presence in the absence of aquaculture was not detected. Nevertheless, even if more data could be easily obtained in the future to validate the model in Ireland or elsewhere, the use of aquaculture as a strong single predictor for oyster presence is highly questionable given the inclusion of other predictor variables in the model, i.e. hard substrata and biogenic reef, residence time and intertidal area and the fact that not all bays with aquaculture had populations of wild oysters.

Although Colautti et al. (2006) suggested that management options should focus on reducing propagule pressure, results from a study by Mag Aoidh (2012) suggest that aquaculture does not necessarily represent a good proxy for propagule pressure as spawning might not be initiated. The current evidence of established, self-sustaining feral oyster populations decoupled from aquaculture stocks equally suggests that the presence of aquaculture stocks alone might not necessarily serve as a good predictor for oyster presence. For future surveys or monitorings, the presence (and/or distances) of established wild populations should be taken into account and tested as another predictor for the presence or absence of oysters.

Geller et al. (2010) cautioned that temporal and geographic sampling for genetic studies is often inadequate, in particular if native ranges have not been sufficiently sampled. The authors suggest that in most cases, inferences of multiple introductions and sources of invasive populations should be offered as hypotheses warranting further exploration. The approach undertaken here (Chapter 4) focused solely on one bay, Lough Foyle, where only one source of introduction is known for aquaculture oysters. Thus, the possibility that the sampling strategy could miss the source of origin did not arise and three different year classes, including individuals of stocks that were used at the beginning of known aquaculture activities in the bay ~10 years ago, were sampled to include some of the temporal variability of the hatchery oyster stocks.

The relatively high genetic differentiation and significantly different allelic richness between feral and aquaculture oysters, the high number of private alleles found in the feral and aquaculture oysters, and the alleles absent in feral oysters but only present in aquaculture argues against aquaculture oysters as the source of the feral populations on the mussel bed in Lough Foyle. Aquaculture oyster populations showed small effective population sizes compared to the feral population and it is unlikely that aquaculture oysters from a non-sampled year would be the source of the feral oyster population in Lough Foyle and provide the unique genetic variability found in the feral populations. Still, there is a chance that individuals from aquaculture, e.g. from a year that was not sampled, could have been involved in the establishment of the feral population on the mussel bed.

5.2.2 Aquaculture practices

The results from the genetic analysis here clearly showed that hatchery/aquaculture stocks and their genetic input into yearly production of oyster spat is very variable. Reasons for this can only be speculated, as no information on hatchery practice was available. Breeding stocks of Pacific oysters for Europe were introduced from the native range from the Sea of Japan and the Pacific coasts of the Japanese Islands in the 1960's but also from British Columbia where this species has been introduced earlier in the 1920's (Shatkin et al. 1997). Today, hatcheries keep their own breeding stocks, possibly without introducing adult individuals from the native source regions, and various methods are used to collect spat also from natural spatfalls in its introduced range, e.g. in France (Lapègue et al. 2007). The very small effective population size of ~30 found here (Chapter 4) is rather typical for hatcheries (Hedgecock and Sly 1990; Appleyard and Ward 2006). However, the significant genetic differentiation found between oysters of different years from aquaculture was not expected but was also recently found in another study on *C. gigas* (D. Lallias, personal comment). A strong variance in reproductive success among potential breeders is known from hatchery breeding stocks of *Ostrea edulis* (Lallias et al. 2010). For this study, it would mean that, if hatcheries did not change their breeding stocks from year to year, only a small number of progenitors contributed to the next generation. Thus, the observed high F_{ST} between aquaculture populations in this study could have been caused by a low number of different successful spawners in each year, making genetic drift more pronounced. If mass spawning is practiced, then the breeding stock is likely kept in a big tank and larvae are collected with no control of parentage. Ideally, to get a good representation of a hatchery's breeding stock, pairwise crosses through stripping of gonads should be done but this type of sampling was not feasible in this study. From a management perspective it will certainly be interesting to increase the sampling effort to include more feral oysters from other locations within the same bay to exclude the aquaculture stocks used in the bay as an introduction source. In the SEAFARE project, different natural populations within Europe are currently compared to establish a larger demographic population picture.

Triploid oysters were found in all aquaculture samples (Chapter 4). Spontaneous triploids can occur in diploid oysters stocks (Guo et al. 1992). However, the large amount of triploids identified in the aquaculture samples is unlikely to be

solely explained by the spontaneous formation of triploids. Whether those individuals were mixed within diploid spat in the hatchery, which is known to produce triploids, or later in the oyster mesh bags, cannot be resolved. It certainly indicates a need for better control of practices if monitoring of diploid and triploid oyster performance, e.g. mortalities, growth or susceptibility to infections is intended without the need of genetic tools. Particularly in the case of restricting licenses to one type of oyster, this might become an important issue in the future.

5.2.3 Conflicts with native species – *Ostrea edulis*

The European flat oyster *Ostrea edulis* is the native oyster species in Ireland and although it was very abundant throughout the nineteenth century in Europe, numbers have significantly declined due to overexploitation, habitat degradation and infections with the parasitic disease bonamiosis (Drinkwaard 1998; Culloty and Mulcahy 2007). Natural populations of native oysters almost went extinct in the Wadden Sea and only small numbers are sporadically found today (Reise 1982; Drinkwaard 1998). In these areas, the Pacific oyster is rather seen as a substitute for the formerly present reef-building oyster and a large overlap with other filter-feeding species occurs only with blue mussels. However, Ireland has one of the last remaining native oyster fisheries in Europe and native oyster beds can be found in Tralee Bay, Galway Bay, Kilkieran Bay, Blacksod Bay, Lough Swilly and Lough Foyle (Marine Institute and BIM 2012). Small, dispersed beds are present in Clew Bay going north to Achill Island and Belmullet, and Mannin Bay. Although aquaculture production (i.e. dredging from these areas of native oyster beds) was only 360 tonnes compared to the Pacific oyster production of 6,511 tonnes in 2006 (Browne et al. 2007), they are still a highly valued, unique natural resource. In the aforementioned areas, the introduced oyster species might now exert another pressure to it.

Ostrea edulis is listed in the OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic (species and habitat protection) and biogenic reefs are recognized as a habitat for protection under Natura 2000 by the European Union. However, no special consideration under Irish legislation exists, most likely due to the fact that they are still fished as a commercial species. As recently proposed by Beck et al. (2011) the extent of oyster reef habitat loss worldwide would justify a more explicit recognition in protected areas policies. The authors suggest that native

oyster reefs of *O. edulis* should be clearly identified and elevated to a priority habitat type given their functional extinction throughout much of Europe. The status of Pacific oysters, a non-native species establishing in these habitats needs to be addressed.

O. edulis can be found in intertidal areas in Ireland and the UK (Kennedy and Roberts 1999), but most extensive native oyster in Ireland beds are found in the subtidal. During a scientific survey in November 2011, Pacific oysters were also found on subtidal native oyster beds with sizes up to 194 mm (Marine Institute and BIM 2012). It will be important to assess whether both species will be able to coexist in the long term, particularly in bays where Pacific oysters are established and native oyster beds are present, such as Lough Foyle, Lough Swilly and Galway Bay. A comparison of life-history traits of *C. gigas* (oviparity, 1-100 million eggs per female per year, with a broad salinity range of 11-34 psu, plus a low temperature tolerance of -5°C and a recruitment competence with 1 year) and *O. edulis* (larviparity, 0.1-1.5 million larvae per female per year, with a narrow salinity range 25-34 psu, a low temperature tolerance of -1.5°C and recruitment competence generally with 2-3 years) (Buroker 1985; Reise 1998) suggest that *C. gigas* might become the dominant oyster species. However, Pacific oysters tend to be dominant in intertidal areas and shoreward of native oysters with the latter becoming more common at the edge of channels and in the shallow sub-tidal (Marine Institute and BIM 2012). This suggests that, although the two species overlap in their distribution, coexistence through separate niche formation might occur.

A study from the West coast of the USA suggested that native oyster (*Ostrea lurida*) industry is unlikely to recover in the presence of Pacific oysters. They argued that although settlement of native oyster larvae on shells of *C. gigas* was the same in subtidal and intertidal areas in experimental trials, the lack of shell accumulations in the subtidal will lead to an increased settlement in the intertidal where *C. gigas* acts as a 'recruitment sink' due to an increased mortality suffered by the native oyster at short emersion times (Trimble et al. 2009). In Ireland, larvae attachment in the subtidal is given, as shells of oysters, native or non-native, are still present and a 'recruitment sink' for native oysters is therefore unlikely.

Dredging activities on mussel beds or native oyster beds could be used as tool for reworking the sediment and might give native oyster larvae opportunity to settle as additional surfaces might become available in areas which were covered by sediment before. However, the timing has to be carefully chosen to avoid disturbance of competent spawning or breeding adults. From an economic point of view a common framework is needed to evaluate the importance of native oyster fisheries and develop strategies, which can strongly enhance the recruitment of native oysters, e.g. fishing under a certain size limit needs to be strongly regulated to prevent any further loss of potential breeders.

Other conflicts might arise in areas where Pacific oysters overlap with blue mussel and *Sabellaria* spp. reefs (Chapter 2), species that have a similar protection status as the native oyster, and different studies have addressed questions regarding their coexistence and competition with Pacific oysters (see also Table 1.1). For example, Pacific oysters have been shown to negatively impact the establishment of reef forming *Sabellaria* species (Dubois et al. 2006; Green 2012). On the other hand, it was shown that although Pacific oysters have been present for 20 years in the Wadden Sea and mostly replaced beds of *Mytilus edulis*, mussels are still settling in *C. gigas* reefs (Diederich 2005; Kochmann et al. 2008). However, a trade-off between increased survival and reduced growth for *M. edulis* living on Pacific oyster reefs was recently found suggesting a change in overall fitness, a smaller size and altered population performance in blue mussels (Eschweiler and Christensen 2011). Overall, changes to communities and ecosystem processes in different habitats should be investigated further and need to consider oyster densities as a source of variation in the nature and strength of impacts (Kochmann et al. 2008; Green 2012).

5.3 Recommendations for management to prevent, control and monitor oyster spreading

5.3.1 Options of control in aquaculture

A high priority for resource managers, conservationists and the aquaculture industry is to minimize the escapes of non-native aquaculture species (Cook et al. 2008). While the presence of aquaculture oysters does not necessarily lead to the formation of feral oyster populations, attention still needs to be paid to new introductions or importations of aquaculture oyster stocks, especially in enclosed bays with long

residence times. As Möhler et al. (2011) recently showed, aquaculture can play a role in shaping the genetics of wild *C. gigas* populations. Thus, an increase in the production of aquaculture oysters is likely to increase the risk of oysters spreading outside aquaculture. However, the monitoring of gonadal stages of aquaculture oysters (Mag Aoidh 2012) as well as taking samples from recent spatfalls from the wild could further help detect and ascertain whether inputs from aquaculture to wild populations occur.

The introduction of non-native species for aquaculture can also function as a vector for other non-native species. Examples for the unintentional introduction associated with Pacific oyster aquaculture are the common slipper limpet *Crepidula fornicata* (Linnaeus, 1758) and the Japanese wireweed *Sargassum muticum* (Yendo) Fensholt. Both species have successfully invaded European coasts (Critchley et al. 1990; Blanchard 1997). In relation to the introduction of Pacific oyster spat from hatcheries, less precautionary and preventive measures are needed than for imports of oyster spat from native ranges or areas where other potentially invasive species occur.

Due to increased mortalities of aquaculture oysters during summer periods, selective breeding of resistant strains of oysters in aquaculture has been undertaken (Dégremont et al. 2007, 2010). Even if the environment of cultured spat inside hatcheries, open waters (intertidal trestles) and natural habitats have different characteristics and differ in selection pressure and rates of survival, cultivating oysters of a resistant strain in the intertidal still bears the risk of a potential spreading of resistant strains into the wild. To prevent any further input from aquaculture without compromising the growth of the sector, the use of triploid oysters might be the best approach. The reproductive potential of a triploid oyster population in hatcheries can be very low (2% of normal diploids) and likely to be even lower in a natural situation with only a fraction of the germinal cells maturing to the gametic stage (Guo and Allen 1994; Normand et al. 2009). Although triploid oysters are not completely sterile and automatic reversals into diploid form can occur (Nell 2002), they have greatly reduced reproductive potential compared with diploid stocks and the use of triploids in aquaculture would reduce the amount of new oyster introductions significantly (Gong et al. 2004). The triploid chemical induction method is less efficient and involves the use of toxic chemicals on oysters used on the market.

Therefore, mating between diploid and tetraploid broodstock is now the most common method of triploid production (Guo et al. 1996; McCombie et al. 2005). Ireland's aquaculture practice is currently being reviewed and triploid Pacific oysters already introduced are being used in some bays, e.g. in Lough Foyle most aquaculture involves diploid oysters but new licenses were only given for triploid oysters from 2009/2010 onwards (C McGonigle, personal comment).

5.3.2 Other options for control

Aquaculture of Pacific oysters is likely to carry a high risk of introduction of this non-native species into new areas. However other ways of introduction such as transport in ballast water, natural dispersal from areas where oysters are established (Brandt et al. 2008) or contamination of fishing gear also needs to be considered in risk assessments or screenings as well (see Chapter 4). Although oysters were detected early in many places, no eradication or control efforts were undertaken and eradication was deemed unfeasible after several strong recruitment events (e.g. Reise 1998; Diederich et al. 2005). An attempt to prevent an expansion phase has been made in Strangford Lough (Northern Ireland) by Guy and Roberts (2010) by manually eradicating oysters at low densities from intertidal rocky shores. Such attempts are perhaps unlikely to be successful in the long term, especially when oyster aquaculture remains productive in the same estuary or inaccessible subtidal populations exist. A complete eradication would possibly become a continual project demanding high economic costs. On the other hand, harvesting of feral populations through dredging from subtidal and intertidal areas is currently undertaken in Lough Swilly (F O'Beirn, personal comment) and might contribute to their control. Similar methods were already successfully applied to reduce population densities of slipper limpets in France (Blanchard 2009). Certainly, to target any measures for control, surveys and monitoring of *C. gigas* should be continued.

Other eradication methods for controlling bivalves, e.g. species-specific pellets "BioBullets" (Aldridge et al. 2005), are only useful for closed facilities but will not be applicable in open environments. Ballast water as a vector of oyster larvae introduction has not been described yet, however, recent approaches of ship design (with water continually flowing through tubes while the ship is moving, thus, releasing only local water in the arrival ports), ballast water treatment, harbour

regulations and international policies will even further decrease the likelihood of introduction through this vector (IMO 2004).

5.3.3 Surveys and distribution maps

Surveys are often portrayed as a complex coordination challenge, especially if they involve a range of different actors (Nixon 1996; Leibenath et al. 2010). Different goals and motivations of managers and scientists can act as barriers to communication (Shaw et al. 2010). Therefore, project-based cooperation relies on positive-sum outcomes to keep the different actors involved (Roux et al. 2006; Leibenath et al. 2010). Those include local co-ordination, facilitation of national execution and options for future regional cooperation (Tuan and Pernetta 2010). Moreover, the involvement of 'citizen scientists' can be a vital component for the success of monitoring the spread of invasive species (Delaney et al. 2008). The sampling programme developed in Chapter 2 involved different actors working under a unified framework. A cost-effective protocol was developed collaboratively. In addition to providing baseline data, the work provides a basis for rigorous repeated sampling to assess future changes in oyster distribution in Ireland. Further cooperation between aquaculture operators, scientists and regulatory and development bodies will allow the results to directly inform strategies and to be applied in management and conservation.

Online distribution maps for invasive species, marine and terrestrial, are now widely available (e.g. www.nobanis.org, www.issg.org, www.aquamaps.org, www.europe-aliens.org) and global environmental data is easily accessible for terrestrial climates via online repositories such as WorldClim or for open-ocean data (Valavanis et al. 2008) which allows the development of models and predictions of geographic distributions. However, the temporal and spatial resolution of such environmental data often varies and can be inadequate, especially for intertidal benthic species. Furthermore, online databases rarely incorporate the stage of invasion in a consistent way. Thus, until the stages of invasion or the status of an alien species are unified under a common framework, e.g. as proposed by Blackburn et al. (2011), and widely accepted, more objective measures, such as presence/absence or abundances of a species, will certainly provide valuable information on species' distribution (alien or native). According to the terminology used by Blackburn et al.

(2011), Pacific oysters in Ireland should be considered *invasive*. However, individuals have to survive at a significant distance from the original source of introduction to be considered in a phase of spread. As introduction sources are usually not easily identified, this definition seems to be insufficient and inadequate. Follow-up monitoring that records changes in abundance and population sizes is needed to estimate rates of spread. Thus, a definition based on *dominance* and *range expansion* as proposed by other authors (Reise et al. 2006; Valéry et al. 2008) might be more applicable compared to the identification of introduction sources. Generally, as suggested by Davis (2009) for the use of language in invasion biology, rather than using metaphors or a hybrid language that mix values with scientific concepts, a descriptive language, e.g. SPRED (SPecies REDistribution) should be preferred and would equally allow following the change of abundance and location of species.

Existing databases and more specifically for Ireland the Biodiversity Data Centre and Invasive Species Ireland project, can help to collate data on species distribution. However, improvements can be made as online distribution maps usually cover only records of presences and rarely include recorded absences. More importantly, detailed semi-quantitative (e.g. SACFOR) or quantitative information on abundances will help to define the status of a species and will allow its spread to be followed. Clear information about the extent of surveys and the methods will benefit the description of the status of a species in a country or a region. Overall, the use of standardized methods that integrate data on environmental factors, abundance or distribution of *C. gigas* from regional and national datasets, e.g. Trilateral Wadden Sea Cooperation, and more recently the UK (Natural England 2009), and a further coordination and commitment to improve databases is needed to facilitate rapid responses and improve the management of *C. gigas*.

5.4 Future research

In this thesis, through the combined use of a large-scale survey, experimentation and genetic tools, the role of different environmental variables as well as aquaculture in the establishment of Pacific oysters was assessed.

Compared to numbers from other areas, dense populations of Pacific oysters in the intertidal are not yet in Ireland and control might still be possible. However, it

will be important to collect quantitative data from subtidal areas as well to allow a better estimation of the biomass of Pacific oysters in each bay.

Repeated monitoring using the protocol established here could be used to validate the dataset as well as follow rates of Pacific oyster spread in Ireland, but also other potentially invasive species, e.g. the slipper limpet *Crepidula fornicata*, recently recorded in Northern Ireland (McNeill et al. 2010) or the tunicate *Didemnum vexillum.* The latter might be of specific importance as they can directly interfere with fishing, and aquaculture. Increased fouling of manmade structures such as docks and boat can occur and this species has been recently recorded in UK waters (Griffith et al. 2009), but presences are also known from Irish waters. Microsatellites have been recently developed for *Didemnum vexillum* (Hess et al. 2009), thus, even the genetic approach used in this thesis would be applicable to identify populations and sources of first colonizers. It could however become more challenging when different introduction sources of origin are likely but are unknown to the researcher, thus the identification of the source of origin could easily be missed.

Species that are spreading will have records that mix environmental preferences with spatial dispersal limitations; thus, many invasive species have had insufficient time in their new range to correlate current range and environmental factors and give accurate definitions of the potential range (Richardson and Pyšek 2008). Models that adjust for variation in propagule pressure (e.g. from aquaculture or wild populations) and the spatial process of dispersal will help to disentangle these effects (Guisan and Thuiller 2005). These drawbacks are however more related to predictions of abundances and dispersal ranges but less related to predictions of presences, which is certainly the most cautious approach for the management of invasive species. Until more detailed information on spreading is available further monitoring efforts will improve datasets and predictions.

On a large scale, the establishment of a 'European Ocean Observing System', recently suggested by European member states in the Ostend Declaration 2010, would certainly help to meet future research challenges, especially with regard to the spread of non-native species. Currently, temperature and salinity are monitored in 17 bays by BIM around Ireland to better assess conditions for oyster growth. However, if only temperature and salinity would be considered, many waters would be deemed

suitable for oysters and potentially other marine invaders (Therriault and Herborg 2008). Documented occurrences together with other environmental variables can provide more informative projections for predictions of geographic ranges.

Oyster growth rates are known to vary substantially between locations (Cardoso et al. 2007; Diederich 2006; Guy and Roberts 2010). To further underpin risk assessments of further spread of oysters in Ireland and to confirm the number of oyster cohorts (Chapter 2), it would be necessary to identify growth rates and ascertain the age-length relationship for oysters in different habitats in Ireland. To examine the possibility that the influence of macroalgae and predation may be greater for oysters at different life history stages and sizes, research into settlement processes as well as experiments using juvenile oysters of a range of sizes (particularly smaller than 20 mm) would be needed.

A sustainable management of ecosystems helps to ensure that policy frameworks support continuing economic development without compromising the integrity of species (Ruckelshaus et al. 2008; Weinstein 2008). In several bays, a Co-ordinated Local Aquaculture Management System (C.L.A.M.S.) has been established by BIM to promote sustainable aquaculture and coastal zone management. Energy and nutrient budgets and carrying capacity of estuaries have been modelled for estuaries worldwide (Dame and Prins 1997; Cognie et al. 2006; Ferreira et al. 2008; Dumbauld et al. 2009). Those carrying capacity models might need to include wild populations of Pacific oysters as well in the future and will become important in Irish estuaries with intensive aquaculture.

Aquaculture is becoming an important global food-producing sector that will help to meet growing food demands (Duarte et al. 2009). It will be important to allow the development of this sector and weigh the economic value of Pacific oysters, but without compromising ecosystem stability. Moreover, when goals and priorities for the management and restoration of native fisheries, especially blue mussels and native oysters, are considered, the focus should lie on their ecological effects and impacts rather than on their non-native status.

References

Aargaard A, Warman CG, Depledge MH (1995) Seasonal and tidal changes in foraging activity of the shore crab *Carcinus maenas* in the weakly tidal littoral zone of Kerteminde Fjord, Denmark. Marine Ecology Progress Series 122:165–172

Abramoff MD, Magalhaes PJ, Ram SJ (2004) Image Processing with ImageJ. Biophotonics International 11:36–42

Aguirre JD, McNaught DC (2011) Habitat modification affects recruitment of abalone in central New Zealand. Marine Biology 158:505–513

Akaike H (1974) A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716–723

Albrecht A, Reise K (1994) Effects of *Fucus vesiculosus* covering intertidal mussel beds in the Wadden Sea. Helgoländer Meeresuntersuchungen 48:243–256

Aldridge DC, Elliott P, Moggridge GD (2005) Microencapsulated BioBullets for the control of biofouling zebra mussels. Environmental Science and Technology 40:975–979

Allen BJ, Williams SL (2003) Native eelgrass *Zostera marina* controls growth and reproduction of an invasive mussel through food limitation. Marine Ecology Progress Series 254:57–67

Alonso A, Castro-Díez P (2008) What explains the invading success of the aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca)? Hydrobiologia 614:107–116

Amaral V, Cabral H, Jenkins S, Hawkins S, Paula J (2009) Comparing quality of estuarine and nearshore intertidal habitats for *Carcinus maenas*. Estuarine, Coastal and Shelf Science 83:219–226

Anderson TW (2001) Predator responses, prey refuges, and density-dependent mortality of a marine fish. Ecology 82:245–257

Anderson MJ, Connell SD (1999) Predation by fish on intertidal oysters. Marine Ecology Progress Series 187:203–211

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. BMC Bioinformatics 9:323

Appleyard SA, Ward RD (2006) Genetic diversity and effective population size in mass selection lines of Pacific oyster (*Crassostrea gigas*). Aquaculture 254:148–159

Aronson RB, Thatje S, Clarke A, Peck LS, Blake DB, Wilga CD, Seibel BA (2007) Climate Change and Invasibility of the Antarctic Benthos. Annual Review of Ecology, Evolution, and Systematics 38:129–154

Arsenault G, Davidson J, Ramsay A (2009) Temporal and spatial development of an infestation of *Styela clava* on mussel farms in Malpeque Bay, Prince Edward Island, Canada. Aquatic Invasions 4: 189–194

Averdung M (2009) Filtrationsraten der heimischen Miesmuschel *Mytilus edulis* und der eingewanderten Auster *Crassostrea gigas* bei winterlichen Temperaturen.

Diploma thesis, Universität Hamburg

Avise JC (2004) Molecular markers, natural history, and evolution, 2nd edn. Sinauer Associates, Sunderland

Barillé L, Haure J, Cognie B, Leroy A (2000) Variations in pallial organs and eulatero-frontal cirri in response to high particulate matter concentrations in the oyster *Crassostrea gigas*. Canadian Journal of Fisheries and Aquatic Sciences 57:837–843

Barillé L, Prou J, Héral M, Razet D (1997) Effects of high natural seston concentrations on the feeding, selection, and absorption of the oyster *Crassostrea gigas* (Thunberg). Journal of Experimental Marine Biology and Ecology 212:149–172

Battisti A, Stastny M, Netheŕer S, Robinet C, Schopf A, Roques A, Larsson S (2005) Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. Ecological Applications 15:2084–2096

Bax N, Carlton JT, Mathews-Amos A, Haedrich RL, Howarth FG, Purcell JE, Rieser A, Gray A (2001) The control of biological invasions in the world's oceans. Conservation Biology 15:1234–1246

Bayne BL (2002) A physiological comparison between Pacific oysters *Crassostrea gigas* and Sydney Rock oysters *Saccostrea glomerata*: food, feeding and growth in a shared estuarine habitat. Marine Ecology Progress Series 232:163–178

Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. Proceedings of the Royal Society B: Biological Sciences 263:1619–1626

Beck MW, Brumbaugh RD, Airoldi L, Carranza A, Coen LD, Crawford C, Defeo O, Edgar GJ, Hancock B, Kay MC, Lenihan HS, Luckenbach MW, Toropova CL, Zhang G, Guo X (2011) Oyster reefs at risk and recommendations for conservation, restoration, and management. BioScience 61:107–116

Beger M, Possingham HP (2008) Environmental factors that influence the distribution of coral reef fishes: modeling occurrence data for broad-scale conservation and management. Marine Ecology Progress Series 361:1–13

Bertness MD, Leonard GH, Levine JM, Schmidt PR, Ingraham AO (1999) Testing the relative contribution of positive and negative interactions in rocky intertidal communities. Ecology 80:2711–2726

Bertness MD, Trussell GC, Ewanchuk PJ, Silliman BR, Crain CM (2004) Consumer-controlled community states on Gulf of Maine rocky shores. Ecology 85:1321–1331

Bishop MJ, Peterson CH (2006) When r-selection may not predict introduced-species proliferation: predation of a nonnative oyster. Ecological Applications 16:718–730

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. Trends in Ecology and Evolution 26:333–339

Blanchard M (1997) Spread of the slipper limpet *Crepidula fornicata* (L. 1758) in Europe: current state and consequences. Scientia Marina 61:109–118

Blanchard M (2009) Recent expansion of the slipper limpet population (*Crepidula fornicata*) in the Bay of Mont-Saint-Michel (Western Channel, France). Aquatic Living Resources 22:11–19

Boelens R, Minchin D, O'Sullivan G (2005) Climate change implications for Ireland's marine environment and resources. Marine Foresight Series No. 2, Marine Institute, Galway

Booth C (2008) A deer mistake: the Victorian government's proposal to promote recreational deer hunting on farms. Invasive Species Council Australia

Brandt G, Wehrman A, Wirtz KW (2008) Rapid invasion of *Crassostrea gigas* into the German Wadden Sea dominated by larval supply. Journal of Sea Research 59:279–296

Brock E, Nylund GM, Pavia H (2007) Chemical inhibition of barnacle larval settlement by the brown alga *Fucus vesiculosus*. Marine Ecology Progress Series 337:165–174

Brown JR (1988) Multivariate analyses of the role of environmental factors in seasonal and site-related growth variation in the Pacific oyster *Crassostrea gigas*. Marine Ecology Progress Series 45:225–236

Brown J, Carrillo L, Fernand L, Horsburgh KJ, Hill AE, Young EF, Medler KJ (2003) Observations of the physical structure and seasonal jet-like circulation of the Celtic Sea and St. George's Channel of the Irish Sea. Continental Shelf Research 23:533–561

Brown JR, Hartwick EB (1988) Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas*: II. Condition index and survival. Aquaculture 70:253–267

Brown JH, Sax DF (2004) An essay on some topics concerning invasive species. Austral Ecology 29:530–536

Browne R, Deegan B, O'Carroll T, Norman M, Ó'Cinnéide M (2007) Status of Irish Aquaculture 2006. Marine Institute, Bord Iascaigh Mhara and Taighde Mara Teo

Bulleri F (2009) Facilitation research in marine systems: state of the art, emerging patterns and insights for future developments. Journal of Ecology 97:1121–1130

Buroker NE (1985) Evolutionary patterns in the family Ostreidae: larviparity vs. oviparity. Journal of Experimental Marine Biology and Ecology 90:233–247

Burrows MT, Harvey R, Robb L (2008) Wave exposure indices from digital coastlines and the prediction of rocky shore community structure. Marine Ecology Progress Series 353:1–12

Büttger H, Asmus H, Asmus R, Buschbaum C, Dittmann S, Nehls G (2008) Community dynamics of intertidal soft-bottom mussel beds over two decades. Helgoland Marine Research 62:23–36

Büttger H, Nehls G, Witte S (2011) High mortality of Pacific oysters in a cold winter in the North-Frisian Wadden Sea. Helgoland Marine Research 65:525–532

Byers JE, Pringle JM (2006) Going against the flow: retention, range limits and

invasions in advective environments. Marine Ecology Progress Series 313:27–41

Cadée GC (2004) Japanese oesters *Crassostrea gigas* gestikt onder algenbedekking in 2003. Het Zeepard 64:110–114

Cadée GC (2008) Scholeksters en Japanese oesters. Natura 105:6–7

Cardoso JFMF, Langlet D, Loff JF, Martins AR, Witte JI, Santos PT, Veer HW van der (2007) Spatial variability in growth and reproduction of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) along the west European coast. Journal of Sea Research 57:303–315

Carlsson J (2008) Effects of microsatellite null alleles on assignment testing. Journal of Heredity 99:616 –623

Carlsson NOL, Bustamante H, Strayer DL, Pace ML (2011) Biotic resistance on the increase: native predators structure invasive zebra mussel populations. Freshwater Biology 56:1630–1637

Carlsson J, Morrison CL, Reece KS (2006) Wild and aquaculture populations of the Eastern oyster compared using microsatellites. Journal of Heredity 97:595–598

Carlton JT (1996a) Biological invasions and cryptogenic species. Ecology 77:1653–1655

Carlton JT (1996b) Marine bioinvasions: The alteration of marine ecosystems by nonindigenous species. Oceanography 9:36–43

Carlton JT (1999) Scale and ecological consequences of biological invasions in the world`s oceans. In: Sandlund OT, Schei PJ, Viken Å (eds) Invasive species and biodiversity management. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 195–212

Carlton JT, Geller J (1993) Ecological roulette - the global transport of nonindigenous marine organisms. Science 261:78–82

Carlton JT, Ruiz GM (2005) Vector science and integrated vector management in bioinvasion ecology: conceptual frameworks. In: Mooney HA, Mack RN, McNeely JA, Neville LE, Schei PJ, Waage JK (eds) Invasive alien species: a new synthesis. Island Press, Washington, DC, p 36–58

Carrasco MF, Barón PJ (2010) Analysis of the potential geographic range of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) based on surface seawater temperature satellite data and climate charts: the coast of South America as a study case. Biologial Invasions 12:2597–2607

Cassis D, Pearce CM, Maldonado MT (2011) Effects of the environment and culture depth on growth and mortality in juvenile Pacific oysters in the Strait of Georgia, British Columbia. Marine Ecology Progress Series 1:259–274

Castaños C, Pascual M, Camacho AP (2009) Reproductive biology of the nonnative oyster, *Crassostrea gigas* (Thunberg, 1793), as a key factor for its successful spread along the rocky shores of Northern Patagonia, Argentina. Journal of Shellfish Research 28:837–847

Chambon C, Legeay A, Durrieu G, Gonzalez P, Ciret P, Massabuau J-C (2007) Influence

of the parasite worm *Polydora* sp. on the behaviour of the oyster *Crassostrea gigas*: a study of the respiratory impact and associated oxidative stress. Marine Biology 152:329–338

Chapman MG (2002) Patterns of spatial and temporal variation of macrofauna under boulders in a sheltered boulder field. Austral Ecology 27:211–228

Chapuis M-P, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24:621 –631

Chown SL, Slabber S, McGeoch MA, Janion C, Leinaas HP (2007) Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. Proceedings of the Royal Society B: Biological Sciences 274:2531–2537

Claude A (1991) Environmental problems caused by TBT in France: assessment, regulations, prospects. Marine Environmental Research 32:7–17

Cognie B, Haure J, Barillé L (2006) Spatial distribution in a temperate coastal ecosystem of the wild stock of the farmed oyster *Crassostrea gigas* (Thunberg). Aquaculture 259:249–259

Cohen J (1960) A coefficient of agreement for nominal scales. Educational and Psychological Measurement 20:37 –46

Cohen AN, Carlton JT (1998) Accelerating invasion rate in a highly invaded estuary. Science 279:555–558

Colautti RI, Grigorovich IA, MacIsaac HJ (2006) Propagule pressure: a null model for biological invasions. Biological Invasions 8:1023–1037

Colautti RI, MacIsaac HJ (2004) A neutral terminology to define “invasive” species. Diversity and Distributions 10:135–141

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecology Letters 7:721–733

Connell JH (1985) The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. Journal of Experimental Marine Biology and Ecology 93:11–45

Connor DW, Allen JH, Golding N, Howell KL, Lieberknecht LM, Northen KO, Reker JB (2004) The marine habitat classification for Britain and Ireland. Internet Version 04/05. JNCC, Peterborough

Cook EJ, Ashton G, Campbell M, Coutts A, Gollasch S, Hewitt C, Liu H, Minchin D, Ruiz G, Shucksmith R (2008) Non-native aquaculture species releases: implications for aquatic ecosystems. In: Holmer M, Black K, Duarte CM, Marbà N, Karakassis I (eds) Aquaculture in the Ecosystem. Springer Netherlands, p 155–184

Coscia I, Vogiatzi E, Kotoulas G, Tsigenopoulos CS, Mariani S (2012) Exploring neutral and adaptive processes in expanding populations of gilthead sea bream, *Sparus aurata* L., in the north-east Atlantic. Heredity 108:537–546

Council of Europe (2007) Council Regulation (EC) No 708/2007 of 11 June 2007 concerning use of alien and locally absent species in aquaculture. OJ L 168, 28.6.2007, p 1-17

Couzens G (2006) The distribution and abundance of the non-native Pacific oyster, Crassostrea gigas, in West Devon - a result of climate change? In: Shellfish News:5–7

Critchley AT, Farnham WF, Yoshida T, Norton TA (1990) A bibliography of the invasive alga *Sargassum muticum* (Yendo) Fensholt (Fucales; Sargassaceae). Botanica Marina 33:551–562

Crooks JA (2005) Lag times and exotic species: The ecology and management of biological invasions in slow-motion. Ecoscience 12:316–329

Crooks JA, Soulé ME (1999) Lag times in population explosions of invasive species: causes and implications. In: Sandlund OT, Schei PJ, Viken Å (eds) Invasive Species and Biodiversity Management. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 103–125

Crothers JH (1967) The biology of the shore crab *Carcinus maenas* (L.) 1. The background - anatomy, growth and life history. Field studies 2:407–434

Culloty SC, Mulcahy MF (2007) Bonamia ostreae in the native oyster *Ostrea edulis*. Marine Environment and Health Series No. 29, Marine Institute, Galway

Culver CS, Kuris AM (2000) The apparent eradication of a locally established introduced marine pest. Biological Invasions 2:245–253

Dame R, Prins T (1997) Bivalve carrying capacity in coastal ecosystems. Aquatic Ecology 31:409–421

Dare PJ, Davies G, Edwards DB (1983) Predation on juvenile Pacific oysters (*Crassostrea gigas* Thunberg) and mussels (*Mytilus edulis* L.) by shore crabs (*Carcinus maenas* (L.)). Fisheries Research Technical Report No. 73, Ministry of Agriculture, Fisheries and Food Directorate of Fisheries Research, Lowestoft

Davis MA (2009) Invasion Biology. Oxford University Press, New York

Davis MA, Chew MK, Hobbs RJ, Lugo AE, Ewel JJ, Vermeij GJ, Brown JH, Rosenzweig ML, Gardener MR, Carroll SP, Thompson K, Pickett STA, Stromberg JC, Tredici PD, Suding KN, Ehrenfeld JG, Philip Grime J, Mascaro J, Briggs JC (2011) Don't judge species on their origins. Nature 474:153–154

Davis MH, Davis ME (2009) *Styela clava* (Tunicata, Ascidiacea) – a new threat to the Mediterranean shellfish industry? Aquatic Invasions 4: 283–289

Davis MA, Thompson K (2000) Eight ways to be a colonizer; two ways to be an invader: a proposed nomenclature scheme for invasion ecology. Bulletin of the Ecological Society of America 81:226–230

Dégremont L, Ernande B, Bédier E, Boudry P (2007) Summer mortality of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). I. Estimation of genetic parameters for survival and growth. Aquaculture 262:41–53

Dégremont L, Soletchnik P, Boudry P (2010) Summer mortality of selected juvenile Pacific oyster *Crassostrea gigas* under laboratory conditions and in comparison with field performance. Journal of Shellfish Research 29:847–856

Delaney DG, Sperling CD, Adams CS, Leung B (2008) Marine invasive species: validation of citizen science and implications for national monitoring networks. Biological Invasions 10:117–128

deRivera CE, Ruiz GM, Anson H. Hines, Jivoff P (2005) Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. Ecology 86:3364–3376

Dethier MN, Hacker SD (2005) Physical factors vs. biotic resistance in controlling the invasion of an estuarine marsh grass. Ecological Applications 15:1273–1283

Diederich S (2005) Differential recruitment of introduced Pacific oysters and native mussels at the North Sea coast: coexistence possible? Journal of Sea Research 53:269–281

Diederich S (2006) High survival and growth rates of introduced Pacific oysters may cause restrictions on habitat use by native mussels in the Wadden Sea. Journal of Experimental Marine Biology and Ecology 328:211–227

Diederich S, Nehls G, Justus E.E. van Beusekom, Reise K (2005) Introduced Pacific oysters (*Crassostrea gigas*) in the northern Wadden Sea: invasion accelerated by warm summers? Helgoland Marine Research 59:97–106

Dieringer D, Schlötterer C (2003) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes 3:167–169

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17:431–449

Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Marquéz JRG, Gruber B, Lafourcade B, Leitão PJ, Münkemüller T, McClean C, Osborne PE, Reineking B, Schröder B, Skidmore AK, Zurell D, Lautenbach S (2012) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography:1–20

Drake JM, Lodge DM (2006) Allee effects, propagule pressure and the probability of establishment: risk analysis for biological invasions. Biological Invasions 8:365–375

Dridi S, Romdhane MS, Elcafsi M (2007) Seasonal variation in weight and biochemical composition of the Pacific oyster, *Crassostrea gigas* in relation to the gametogenic cycle and environmental conditions of the Bizert lagoon, Tunisia. Aquaculture 263:238–248

Drinkwaard A (1998) Introductions and developments of oysters in the North Sea area: a review. Helgoland Marine Research 52:301–308

Duarte C, Holmer M, Olsen Y, Soto D, Marbà N, Guiu J, Black K, Karakassis I (2009) Will the oceans help feed humanity? BioScience 59:967–976

Dubois S, Commito JA, Olivier F, Retière C (2006) Effects of epibionts on *Sabellaria alveolata* (L.) biogenic reefs and their associated fauna in the Bay of Mont Saint-Michel. Estuarine, Coastal and Shelf Science 68:635–646

Dumbauld BR, Ruesink JL, Rumrill SS (2009) The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries. Aquaculture 290:196–223

Dumont CP, Gaymer CF, Thiel M (2011) Predation contributes to invasion resistance of benthic communities against the non-indigenous tunicate *Ciona intestinalis*. Biological Invasions 13:2023–2034

Dunstan PK, Bax NJ (2007) How far can marine species go? Influence of population biology and larval movement on future range limits. Marine Ecology Progress Series 344:15–28

Dutertre M, Beninger PG, Barillé L, Papin M, Haure J (2010) Rising water temperatures, reproduction and recruitment of an invasive oyster, *Crassostrea gigas*, on the French Atlantic coast. Marine Environmental Research 69:1–9

Dutertre M, Beninger PG, Barillé L, Papin M, Rosa P, Barillé A-L, Haure J (2009) Temperature and seston quantity and quality effects on field reproduction of farmed oysters, *Crassostrea gigas*, in Bourgneuf Bay, France. Aquatic Living Resources 22:319-329

Dyer KR, Orth RJ (1994) Changes in fluxes in estuaries: implications from science to management. Olsen and Olsen, Fredensborg

Eckman JE, Duggins DO, Sewell AT (1989) Ecology of under story kelp environments. I. Effects of kelps on flow and particle transport near the bottom. Journal of Experimental Marine Biology and Ecology 129:173–187

Edgell TC, Hollander J (2011) The evolutionary ecology of European Green crab, *Carcinus maenas*, in North America. In: Galil BS, Clark PF, Carlton JT (eds) In the wrong place - alien marine crustaceans: distribution, biology and impacts. Springer Netherlands, Dordrecht, p 641–659

Elith J, H. Graham C, P. Anderson R, Dudík M, Ferrier S, Guisan A, J. Hijmans R, Huettmann F, R. Leathwick J, Lehmann A, Li J, G. Lohmann L, A. Loiselle B, Manion G, Moritz C, Nakamura M, Nakazawa Y, McC. M. Overton J, Townsend Peterson A, J. Phillips S, Richardson K, Scachetti-Pereira R, E. Schapire R, Soberón J, Williams S, S. Wisz M, E. Zimmermann N (2006) Novel methods improve prediction of species' distributions from occurrence data. Ecography 29:129–151

Elith J, Leathwick JR (2009) Species distribution models: ecological explanation and prediction across space and time. Annual Review of Ecology, Evolution and Systematics 40:677–697

Ellis JR, Burke JM (2007) EST-SSRs as a resource for population genetic analyses. Heredity 99:125–132

Elsner NO, Jacobsen S, Thieltges DW, Reise K (2011) Alien parasitic copepods in mussels and oysters of the Wadden Sea. Helgoland Marine Research 65:299–307

Encomio, Chu FL (2000) The effect of PCBs on glycogen reserves in the eastern oyster *Crassostrea virginica*. Marine Environmental Research 50:45–49

Enríquez-Díaz M, Pouvreau S, Chávez-Villalba J, Pennec M (2009) Gametogenesis, reproductive investment, and spawning behavior of the Pacific giant oyster *Crassostrea gigas*: evidence of an environment-dependent strategy. Aquaculture International 17:491–506

EPA (2010) Water Quality in Ireland 2007-2009. Report No. 978-1-84095-387-9

Ernande B, Boudry P, Clobert J, Haure J (2004) Plasticity in resource allocation based life history traits in the Pacific oyster, *Crassostrea gigas*. I. Spatial variation in food abundance. Journal of Evolutionary Biology 17:342–356

Escapa M, Isacch JP, Daleo P, Alberti J, Iribarne O, Borges M, Santos E Dos, Gagliardini DA, Lasta M (2004) The distribution and ecological effects of the introduced Pacific oyster *Crassostrea gigas* (Thunberg, 1793) in Northern Patagonia. Journal of Shellfish Research 23:765–772

Eschweiler N, Buschbaum C (2011) Alien epibiont (*Crassostrea gigas*) impacts on native periwinkles (*Littorina littorea*). Aquatic Invasions 6:281–290

Eschweiler N, Christensen HT (2011) Trade-off between increased survival and reduced growth for blue mussels living on Pacific oyster reefs. Journal of Experimental Marine Biology and Ecology 403:90–95

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611–2620

Fabioux C, Huvet A, Lesouchu P, Lepennec M, Pouvreau S (2005) Temperature and photoperiod drive reproductive internal clock. Aquaculture 250:458–470

FAO (2012a) The State of World Fisheries and Aquaculture 2012. Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations, Rome

FAO (2012b) Fisheries and Aquaculture Fact Sheets. Fisheries and Aquaculture Fact Sheets, http://www.fao.org/fishery/culturedspecies/Crassostrea_gigas/en, accessed 2012-04-21

Fernand L, Nolan GD, Raine R, Chambers CE, Dye SR, White M, Brown J (2006) The Irish coastal current: a seasonal jet-like circulation. Continental Shelf Research 26:1775–1793

Ferreira JG, Hawkins AJS, Monteiro P, Moore H, Service M, Pascoe PL, Ramos L, Sequeira A (2008) Integrated assessment of ecosystem-scale carrying capacity in shellfish growing areas. Aquaculture 275:138–151

Fey F, Dankers N, Steenbergen J, Goudswaard K (2010) Development and distribution of the non-indigenous Pacific oyster (*Crassostrea gigas*) in the Dutch Wadden Sea. Aquaculture International 18:45–59

Fielding AH, Bell JF (1997) A review of methods for the assessment of prediction errors in conservation presence/absence models. Environmental Conservation 24:38–49

Floc'h JY, Pajot R, Wallentinus I (1991) The Japanese brown alga *Undaria pinnatifida* on the coast of France and its possible establishment in European waters. Journal du Conseil: ICES Journal of Marine Science 47: 379–390

Foxcroft LC, Richardson DM, Wilson JRU (2008) Ornamental plants as invasive aliens: problems and solutions in Kruger National Park, South Africa. Environmental Management 41:32–51

Franklin J, Miller JA (2009) Mapping species distributions: spatial inference and prediction. Cambridge University Press, Cambridge

Fraschetti S, Terlizzi A, Benedetti-Cecchi L (2005) Patterns of distribution of marine assemblages from rocky shores: evidence of relevant scales of variation. Marine Ecology Progress Series 296:13–29

Frenot Y, Chown SL, Whinam J, Selkirk PM, Convey P, Skotnicki M, Bergstrom DM (2005) Biological invasions in the Antarctic: extent, impacts and implications. Biological Reviews of the Cambridge Philosophical Society 80:45–72

Fridley JD, Stachowicz JJ, Naeem S, Sax DF, Seabloom EW, Smith MD, Stohlgren TJ, Tilman D, Holle B Von (2007) The invasion paradox: reconciling pattern and process in species invasions. Ecology 88:3–17

Fritts TH, Rodda GH (1998) The role of introduced species in the degradation of island ecosystems: a case history of Guam. Annual Review of Ecology and Systematics 29:113–140

Funk JL, Vitousek PM (2007) Resource-use efficiency and plant invasion in low-resource systems. Nature 446:1079–1081

Gagnon P, Himmelman JH, Johnson LE (2004) Temporal variation in community interfaces: kelp-bed boundary dynamics adjacent to persistent urchin barrens. Marine Biology 144:1191–1203

Gagnon P, Wagner G, Himmelman JH (2003) Use of a wave tank to study the effects of water motion and algal movement on the displacement of the sea star *Asterias vulgaris* towards its prey. Marine Ecology Progress Series 258:125–132

Gangnery A, Chabirand J-M, Lagarde F, Gall P Le, Oheix J, Bacher C, Buestel D (2003) Growth model of the Pacific oyster, *Crassostrea gigas*, cultured in Thau Lagoon (Mediterranee, France). Aquaculture 215:267–290

Garza-Pérez JR, Lehmann A, Arias-González JE (2004) Spatial prediction of coral reef habitats: integrating ecology with spatial modeling and remote sensing. Marine Ecology Progress Series 269:141–152

Geller JB, Darling JA, Carlton JT (2010) Genetic perspectives on marine biological invasions. Annual Review of Marine Science 2:367–393

Gillooly JF, Charnov EL, West GB, Savage VM, Brown JH (2002) Effects of size and temperature on developmental time. Nature 417:70–73

Gong N, Yang H, Zhang G, Landau BJ, Guo X (2004) Chromosome inheritance in triploid Pacific oyster *Crassostrea gigas* Thunberg. Heredity 93:408–415

Gormley AM, Forsyth DM, Griffioen P, Lindeman M, Ramsey DS, Scroggie MP, Woodford L (2011) Using presence-only and presence-absence data to estimate the current and potential distributions of established invasive species. Journal of Applied Ecology 48:25–34

Grangeré K, Ménesguen A, Lefebvre S, Bacher C, Pouvreau S (2009) Modelling the influence of environmental factors on the physiological status of the Pacific oyster *Crassostrea gigas* in an estuarine embayment; The Baie des Veys (France). Journal of Sea Research 62:147–158

Green D (2012) The impacts of non-indigenous oysters on biodiversity and ecosystem functioning. PhD thesis unpublished, University College Dublin

Green PT, Lake PS, O'Dowd DJ (2004) Resistance of island rainforest to invasion by alien plants: influence of microhabitat and herbivory on seedling performance. Biological Invasions 6:1–9

Griffith K, Mowat S, Holt R, Ramsay K, Bishop J, Lambert G, Jenkins S (2009) First records in Great Britain of the invasive colonial ascidian *Didemnum vexillum* Kott, 2002. Aquatic Invasions 4:581–590

Gross MR (1998) One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and in aquaculture. Canadian Journal of Fisheries and Aquatic Sciences 55:131–144

Guisan A, Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. Ecology Letters 8:993–1009

Guo X, Allen SK (1994) Reproductive potential and genetics of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). Biological Bulletin 187:309–318

Guo X, Cooper K, Hershberger WK, Chew KK (1992) Genetic consequences of blocking polar body I with cytochalasin B in fertilized eggs of the Pacific oyster, *Crassostrea gigas*: I. Ploidy of resultant embryos. Biological Bulletin 183:381–386

Guo X, DeBrosse GA, Allen SK (1996) All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture 142:149–161

Guy C, Roberts D (2010) Can the spread of non-native oysters (*Crassostrea gigas*) at the early stages of population expansion be managed? Marine Pollution Bulletin 60:1059–1064

Hall M (2003) Editorial: The native, naturalized and exotic—plants and animals in human history. Landscape Research 28:5–9

Hamdoun AM, Cheney DP, Cherr GN (2003) Phenotypic plasticity of HSP70 and HSP70 gene expression in the Pacific oyster (*Crassostrea gigas*): implications for thermal limits and induction of thermal tolerance. Biological Bulletin 205:160–169

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Paleontologia Electronica 4:1-9

Hancock KM, Petraitis PS (2001) Effects of herbivorous snails and macroalgal canopy on recruitment and early survivorship of the barnacle *Semibalanus balanoides* (L.). Journal of Experimental Marine Biology and Ecology 257:205–218

Hand CE, Sanders FJ, Jodice PGR (2010) Foraging proficiency during the nonbreeding season of a specialized forager: are juvenile American oystercatchers "bumble-beaks" compared to adults? The Condor 112:670–675

Hartnett M, Dabrowski T, Olbert A (2011) A new formula to calculate residence times of tidal waterbodies. Proceedings of the ICE - Water Management 164:243–256

Haydar D, Wolff WJ (2011) Predicting invasion patterns in coastal ecosystems: relationship between vector strength and vector tempo. Marine Ecology Progress Series 431:1–10

Hayes K, Barry S (2008) Are there any consistent predictors of invasion success? Biological Invasions 10:483–506

Heck KL Jr, Thoman TA (1981) Experiments on predator-prey interactions in vegetated aquatic habitats. Journal of Experimental Marine Biology and Ecology 53: 125–134

Hedgecock, Li G, Hubert S, Bucklin K, Ribes V (2004) Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, *Crassostrea gigas*. Journal of Shellfish Research 23:379–385

Hedgecock D, Sly F (1990) Genetic drift and effective population sizes of hatchery-propagated stocks of the Pacific oyster, *Crassostrea gigas*. Aquaculture 88:21–38

Helmuth B, Harley CDG, Halpin PM, O'Donnell M, Hofmann GE, Blanchette CA (2002) Climate change and latitudinal patterns of intertidal thermal stress. Science 298:1015–1017

Herbert RJH, Roberts C, Humphreys J, Fletcher S (2012) The Pacific oyster (*Crassostrea gigas*) in the UK: economic, legal and environmental issues associated with its cultivation, wild establishment and exploitation. Report for the Shellfish Association of Great Britain

Herborg L-M, Rudnick DA, Siliang Y, Lodge DM, MacIsaac HJ (2007) Predicting the range of Chinese mitten crabs in Europe. Conservation Biology 21:1316–1323

Hess JE, Swalla BJ, Moran P, others (2009) New molecular markers to genetically differentiate populations of *Didemnum vexillum* (Kott, 2002) - an invasive ascidian species. Aquatic Invasions 4:299–310

Hoey AS, Bellwood DR (2011) Suppression of herbivory by macroalgal density: a critical feedback on coral reefs? Ecology Letters 14:267–273

Hollebone AL, Hay ME (2007) Propagule pressure of an invasive crab overwhelms native biotic resistance. Marine Ecology Progress Series 342:191–196

Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ (2002) The causes and consequences of ant invasions. Annual Review of Ecology and Systematics 33:181–233

Honkoop PJC, Bayne BL (2002) Stocking density and growth of the Pacific oyster (*Crassostrea gigas*) and the Sydney rock oyster (*Saccostrea glomerata*) in Port Stephens, Australia. Aquaculture 213:171–186

Hulme PE, Bacher S, Kenis M, Klotz S, Kühn I, Minchin D, Nentwig W, Olenin S, Panov V, Pergl J, Pyšek P, Roques A, Sol D, Solarz W, Vilà M (2008) Grasping at the routes of biological invasions: a framework for integrating pathways into policy. Journal of Applied Ecology 45:403–414

Hulme PE, Weser C (2011) Mixed messages from multiple information sources on invasive species: a case of too much of a good thing? Diversity and Distributions 17:1152–1160

Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. Marine Ecology Progress Series 155:269–301

Hunt CE, Yamada SB (2003) Biotic resistance experienced by an invasive crustacean in a temperate estuary. Biological Invasions 5:33–43

Hunter E, Naylor E (1993) Intertdial migration by the shore crab *Carcinus maenas*. Marine Ecology Progress Series 101: 131–138

Huvet A, Boudry P, Ohresser M, Delsert C, Bonhomme F (2000) Variable microsatellites in the Pacific Oyster *Crassostrea gigas* and other cupped oyster species. Animal Genetics 31:71–72

ICES (2005) ICES Code of Practice on the Introductions and Transfers iof Marine Organisms 2005. Code de Conduite du CIEM pour les Introductions and Transferts d'Organismes Marins 2005. ICES, Copenhagen

ICES WGITMO Report (2008) Report of the Working Group on Introductions and Transfers of Marine Organisms (WGITMO), 12-14 March 2008, Copenhagen

IMO (2004) International Convention for the Control and Management of Ships' Ballast Water and Sediments. International Maritime Organization, www.imo.org

Irlandi EA (1996) The effects of seagrass patch size and energy regime on growth of a suspension-feeding bivalve. Journal of Marine Research 54:161–185

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806

Jaspers C, Møller LF, Kiørboe T (2011) Salinity gradient of the Baltic sea limits the reproduction and population expansion of the newly invaded comb jelly *Mnemiopsis leidyi*. PLoS ONE 6(8):e24065

Jenkins SR, Hawkins SJ (2003) Barnacle larval supply to sheltered rocky shores: a limiting factor? Hydrobiologia 503:143–151

Jenkins SR, Marshall D, Fraschetti S (2009) Settlement and Recruitment. In: Wahl M (ed) Marine Hard Bottom Communities. Springer Berlin Heidelberg, p 177–190

Jenkins SR, Norton TA, Hawkins SJ (1999) Settlement and post-settlement interactions between *Semibalanus balanoides* (L.) (Crustacea: Cirripedia) and three species of fucoid canopy algae. Journal of Experimental Marine Biology and Ecology 236:49–67

Jeschke JM, Strayer DL (2005) Invasion success of vertebrates in Europe and North America. Proceedings of the National Academy of Sciences USA 102:7198–7202

Jiménez-Valverde A, Peterson A, Soberón J, Overton J, Aragón P, Lobo J (2011) Use of niche models in invasive species risk assessments. Biological Invasions 13:2785–2797

Johnston EL, Piola RF, Clark GF (2009) The role of propagule pressure in invasion success. In: Rilov G, Crooks JA, Caldwell MM, Heldmaier G, Jackson RB, Lange OL, Mooney HA, Schulze E-D, Sommer U (eds) Biological invasions in marine ecosystems. Springer Berlin Heidelberg, p 133–151

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551–555

Juanes F (1992) Why do decapod crustaceans prefer small-sized molluscan prey? Marine Ecology Progress Series 87:293–249

Kats LB, Ferrer RP (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Diversity and Distributions 9:99–110

Keller RP, Lodge DM, Finnoff DC (2007) Risk assessment for invasive species produces net bioeconomic benefits. Proceedings of the National Academy of Sciences USA 104:203–207

Kelly NM, Fonseca M, Whitfield P (2001) Predictive mapping for management and conservation of seagrass beds in North Carolina. Aquatic Conservation: Marine and Freshwater Ecosystems 11:437–451

Kelly DW, Muirhead JR, Heath DD, Macisaac HJ (2006) Contrasting patterns in genetic diversity following multiple invasions of fresh and brackish waters. Molecular Ecology 15:3641–3653

Kennedy RJ, Roberts D (1999) A survey of the current status of the flat oyster *Ostrea edulis* in Strangford Lough, northern Ireland, with a view to the restoration of its oyster beds. Biology and Environment: Proceedings of the Royal Irish Academy 99B:79–88

Kerckhof F, Haelters J, Gollasch S (2007) Alien species in the marine and brackish ecosystem: the situation in Belgian waters. Aquatic Invasions 2:243–257

Kettunen M, Genovesi P, Gollasch S, Pagad S, Starfinger U, Brink P ten, Shine C (2009) Technical support to EU strategy on invasive species (IAS) - assessment of the impacts of IAS in Europe and the EU (final module report for the European Commission). Institute for European Environmental Policy (IEEP), Brussels

Kim KS, Ratcliffe ST, French BW, Liu L, Sappington TW (2008) Utility of EST-derived SSRs as population genetics markers in a beetle. Journal of Heredity 99:112 – 124

King JW, Malham SK, Skov MW, Cotter E, Latchford JW, Culloty SC, Beaumont AR (2006) Growth of *Crassostrea gigas* spat and juveniles under differing environmental conditions at two sites in Wales. Aquatic Living Resources 19:289–297

Kinlan BP, Gaines SD (2003) Propagule dispersal in marine and terrestrial environments: a community perspective. Ecology 84:2007–2020

Kirk H, Freeland JR (2011) Applications and implications of neutral versus non-neutral markers in molecular ecology. International Journal of Molecular Sciences 12:3966–3988

Knights AM, Crowe TP, Burnell G (2006) Mechanisms of larval transport: vertical distribution of bivalve larvae varies with tidal conditions. Marine Ecology Progress Series 326:167–174

Knights AM, Firth LB, Walters K (2012) Interactions between multiple recruitment drivers: post-settlement predation mortality and flow-mediated recruitment. PLoS ONE 7(4):e35096

Kochmann J, Buschbaum C, Volkenborn N, Reise K (2008) Shift from native mussels to alien oysters: differential effects of ecosystem engineers. Journal of Experimental Marine Biology and Ecology 364:1–10

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends in Ecology and Evolution 16:199–204

Kolbe JJ, Glor RE, Schettino LRG, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. Nature 431:177–181

Krakau M, Thieltges D, Reise K (2006) Native parasites adopt introduced bivalves of the North Sea. Biological Invasions 8:919–925

Krassoi FR, Brown KR, Bishop MJ, Kelaher BP, Summerhayes S (2008) Condition-specific competition allows coexistence of competitively superior exotic oysters with native oysters. Journal of Animal Ecology 77:5–15

Lake PS, O'Dowd DJ (1991) Red crabs in rain forest, Christmas Island: biotic resistance to invasion by an exotic snail. Oikos 62:25–29

Lallias D, Boudry P, Lapègue S, King J, Beaumont A (2010) Strategies for the retention of high genetic variability in European flat oyster (*Ostrea edulis*) restoration programmes. Conservation Genetics 11:1899–1910

Lang AC, Buschbaum C (2010) Facilitative effects of introduced Pacific oysters on native macroalgae are limited by a secondary invader, the seaweed *Sargassum muticum*. Journal of Sea Research 63:119–128

Lapègue S, Boudry P, Goulletquer P (2007) Genetic effects of domestication, culture and breeding of fish and shellfish, and their impacts on wild populations. Pacific cupped oyster - *Crassostrea gigas*. In: Svåsand T, Crosetti D, García-Vázquez E, Verspoor E (eds). Genetic impact of aquaculture activities on native populations. Genimpact final scientific report (EU contract n. RICA-CT-2005-022802). http://genimpact.imr.no/

Latif M (2011) Klimavaribilität, El Niño/Southern Oscillation, die Nordatlantische und die Atlantsiche Multidekadische Oszillation mit Anmerkungen zur Vorhersagbarkeit. In: Lozán JL, Graßl H, Karbe L, Reise K (eds) Warnsignal Klima: Die Meere - Änderungen und Risiken. Wissenschaftliche Auswertungen, Hamburg

Leibenath M, Blum A, Stutzriemer S (2010) Transboundary cooperation in establishing ecological networks: The case of Germany's external borders. Landscape and Urban Planning 94:84–93

Leonard GH (1999) Positive and negative effects of intertidal algal canopies on recruitment and survival of barnacles. Marine Ecology Progress Series 178:241–249

Leung B, Lodge DM, Finnoff D, Shogren JF, Lewis MA, Lamberti G (2002) An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. Proceedings of the Royal Society B: Biological Sciences 269:2407–2413

Levine J, Adler P, Yelenik S (2004) A meta-analysis of biotic resistance to exotic plant invasions. Ecology Letters 7:975–989

Lewis JC, Sallee KL, Golightly RT (1999) Introduction and range expansion of nonnative red foxes (*Vulpes vulpes*) in California. The American Midland Naturalist 142:372–381

Li R, Li Q, Cornette F, Dégremont L, Lapègue S (2010) Development of four EST-SSR multiplex PCRs in the Pacific oyster (*Crassostrea gigas*) and their validation in parentage assignment. Aquaculture 310:234–239

Liu C, Berry PM, Dawson TP, Pearson RG (2005) Selecting thresholds of occurrence in the prediction of species distributions. Ecography 28:385–393

Liu ZJ, Cordes JF (2004) DNA marker technologies and their applications in aquaculture genetics. Aquaculture 238:1–37

Löbl M, Justus E. E. Beusekom, Reise K (2006) Is spread of the neophyte *Spartina anglica* recently enhanced by increasing temperatures? Aquatic Ecology 40:315–324

Lobo JM, Jiménez-Valverde A, Real R (2008) AUC: a misleading measure of the performance of predictive distribution models. Global Ecology and Biogeography 17:145–151

Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. Trends in Ecology and Evolution 20:223–228

Lockwood J, Hoopes M, Marchetti M (2007) Invasion Ecology. Blackwell Publishing, Oxford

Lodge DM, Williams S, MacIsaac HJ, Hayes KR, Leung B, Reichard S, Mack RN, Moyle PB, Smith M, Andow DA, Carlton JT, McMichael A (2006) Biological invasions: recommendations for U.S. policy and management. Ecological Applications 16:2035–2054

Lonsdale WM (1999) Global patterns of plant invasions and the concept of invasibility. Ecology 80:1522–1536

Mag Aoidh R (2012) Reproduction of *Crassostrea gigas* in Irish waters: an analysis of gametogenesis and condition comparing tidal location and ploidy level. unpublished MSc thesis, University College Dublin

Magoulas A, Gjetvaj B, Terzoglou V, Zouros E (1998) Three polymorphic microsatellites in the Japanese oyster, *Crassostrea gigas* (Thunberg). Animal Genetics 29:69–70

Malham SK, Cotter E, O'Keeffe S, Lynch S, Culloty SC, King JW, Latchford JW, Beaumont AR (2009) Summer mortality of the Pacific oyster, *Crassostrea gigas*, in the Irish Sea: The influence of temperature and nutrients on health and survival. Aquaculture 287:128–138

Manly BFJ (1996) Are there clumps in body-size distributions? Ecology 77:81–86

Mann R, Burreson EM, Baker PK (1991) The decline of the Virginia oyster fishery in Chesapeake Bay: considerations for introduction of a non-endemic species, *Crassostrea gigas* (Thunberg, 1793). Journal of Shellfish Research 10:379–388

Marchetti MP, Moyle PB, Levine R (2004) Alien fishes in California watersheds: characteristics of successful and failed invaders. Ecological Applications 14:587–596

Marine Institute, BIM (2012) Shellfish stocks and fisheries review 2011: An assessment of selected stocks. Marine Institute and Bord Iascaigh Mhara

Markert A, Wehrmann A, Kröncke I (2010) Recently established *Crassostrea*-reefs versus native *Mytilus*-beds: differences in ecosystem engineering affects the macrofaunal communities (Wadden Sea of Lower Saxony, southern German Bight). Biological Invasions 12:15–32

Mascaró M, Seed R (2001a) Choice of prey size and species in *Carcinus maenas* (L.) feeding on four bivalves of contrasting shell morphology. Hydrobiologia 449:159–170

Mascaró M, Seed R (2001b) Foraging behavior of juvenile *Carcinus maenas* (L.) and Cancer pagurus L. Marine Biology 139:1135–1145

McCombie H, Ledu C, Phelipot P, Lapègue S, Boudry P, Gérard A (2005) A complementary method for production of tetraploid *Crassostrea gigas* using crosses between diploids and tetraploids with cytochalasin b treatments. Marine Biotechnology 7:318–330

McGeoch MA, Butchart SHM, Spear D, Marais E, Kleynhans EJ, Symes A, Chanson J, Hoffmann M (2010) Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. Diversity and Distributions 16:95–108

McGoldrick D, Hedgecock D, English L, Baoprasertkul P, Ward R (2000) The transmission of microsatellite alleles in Australian and North American stocks of the Pacific oyster (*Crassostrea gigas*): selection and null alleles. Journal of Shellfish Research 19:779–788

McGonigle C, Cavanagh M, Santiago R (2011) Native oyster stock assessment Lough Foyle. The Loughs Agency (Foyle, Carlingfordand Irish Lights Commission), Londonderry, Carlingford

McNeely JA, Mooney HA, Neville LE, Schei P, Waage JK (Eds) (2001) A global strategy on invasive alien species. IUCN Gland, Switzerland, and Cambridge

McNeill G, Nunn J, Minchin D (2010) The slipper limpet *Crepidula fornicata* Linnaeus, 1758 becomes established in Ireland. Aquatic Invasions 5:S21–S25

Melo CMR, Silva FC, Gomes CHAM, Solé-Cava AM, Lazoski C (2010) *Crassostrea gigas* in natural oyster banks in southern Brazil. Biological Invasions 12:441–449

Menge BA (1978) Predation intensity in a rocky intertidal community. Oecologia 34:17–35

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: biodiversity synthesis. World Resources Institute, Washington, DC

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16:125

Minchin D (2007a) A checklist of alien and cryptogenic aquatic species in Ireland. Aquatic Invasions 2:341–366

Minchin D (2007b) Aquaculture and transport in a changing environment: overlap and links in the spread of alien biota. Marine Pollution Bulletin 55:302–313

Miossec L, Deuff R-M Le, Goulletquer P (2009) Alien Species Alert: *Crassostrea gigas* (Pacific oyster). ICES Cooperative Research Report No. 299, Copenhagen

Möhler J, Wegner KM, Reise K, Jacobsen S (2011) Invasion genetics of Pacific oyster *Crassostrea gigas* shaped by aquaculture stocking practices. Journal of Sea Research 66:256–262

Moksnes, P, Pihl L, van Montfrans, J (1998) Predation on postlarvae and juveniles of the shore crab *Carcinus maenas*: importance of shelter, size and cannibalism. Marine Ecology Progress Series 166: 211–225

Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Frontiers in Ecology and the Environment 6:485–492

Mooney HA, Cleland EE (2001) The evolutionary impact of Iinvasive species. PNAS 98:5446–5451

Moreno CA (1995) Macroalgae as a refuge from predation for recruits of the mussel *Choromytilus chorus* (Molina, 1782) in Southern Chile. Journal of Experimental Marine Biology and Ecology 191:181–193

Natural England (2009) Pacific oyster survey of the North East Kent European marine sites. Natural England Commissioned Report NECR016, Sheffield

Naylor RL, Williams SL, Strong DR (2001) Aquaculture-a gateway for exotic species. Science 294:1655–1656

Nehls G, Büttger H (2007) Spread of the Pacific oyster *Crassostrea gigas* in the Wadden Sea: causes and consequences of a successful invasion. HARABASINS Report for the Common Wadden Sea Secretariat, Wilhelmshaven

Nehls G, Diederich S, Thieltges D, Strasser M (2006) Wadden Sea mussel beds invaded by oysters and slipper limpets: competition or climate control? Helgoland Marine Research 60:135–143

Nehring S (2006) Four arguments why so many alien species settle into estuaries, with special reference to the German river Elbe. Helgoland Marine Research 60:127–134

Nell JA (2002) Farming triploid oysters. Aquaculture 210:69–88

Newell R, Kennedy V, Shaw K (2007) Comparative vulnerability to predators, and induced defense responses, of eastern oysters *Crassostrea virginica* and non-native *Crassostrea ariakensis* oysters in Chesapeake Bay. Marine Biology 152:449–460

Nielsen EE, Hemmer-hansen J, Larsen PF, Bekkevold D (2009) Population genomics of marine fishes: identifying adaptive variation in space and time. Molecular Ecology 18:3128–3150

Nixon SW (1996) Regional coastal research-What is it? Why do it? What role should NAML play? The Biological Bulletin 190:252–259

Normand J, Ernande B, Haure J, McCombie H, Boudry P (2009) Reproductive effort and growth in *Crassostrea gigas*: comparison of young diploid and triploid oysters issued from natural crosses or chemical induction. Aquatic Biology 7:229–241

Norris AT, Bradley DG, Cunningham EP (2000) Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers. Aquaculture 182:73–83

O'Beirn F, Walker RL, Heffernan PB (1996) Enhancement of subtidal oyster, *Crassostrea virginica*, recruitment using mesh bag enclosures. Journal of Shellfish Research 15: 313–318

O'Connor MI, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP, Weiss JM (2007) Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. PNAS 104:1266–1271

Occhipinti-Ambrogi A (2007) Global change and marine communities: Alien species and climate change. Marine Pollution Bulletin 55:342–352

Olenin S, Minchin D, Daunys D (2007) Assessment of biopollution in aquatic ecosystems. Marine Pollution Bulletin 55:379–394

Olyarnik SV, Bracken MES, Byrnes JE, Hughes AR, Hultgren KM, Stachowicz JJ (2009) Ecological factors affecting community invasibility. In: Rilov G, Crooks JA (eds) Biological Invasions in Marine Ecosystems. Springer Berlin Heidelberg, p 215–238

Oosterhout C van, Hutchinson W, Wills D, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538

Orensanz JM, Schwindt E, Pastorino G, Bortolus A, Casas G, Darrigran G, Elias R, Lopez Gappa JJ, Obenat S, Pascual M, al. et (2002) No longer the pristine confines of the world ocean: a survey of exotic marine species in the southwestern Atlantic. Biological Invasion 4:115–143

Paine RT (1974) Intertidal community structure. Oecologia 15:93–120

Pechenik J (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Marine Ecology Progress Series 177:269–297

Pejchar L, Mooney HA (2009) Invasive species, ecosystem services and human well-being. Trends in Ecology and Evolution 24:497–504

Peters K (2009) The role of abiotic factors for the setting of oyster larvae: a metaanalysis. unpublished BSc thesis, Universität Osnabrück

Peterson AT (2003) Predicting the geography of species' invasions via ecological niche modeling. The Quarterly Review of Biology 78:419–433

Piccolo J, Orlikowska E (2012) A biological risk assessment for an Atlantic salmon (*Salmo salar*) invasion in Alaskan waters. Aquatic Invasions 7:259–270

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273–288

Pineda J, Hare J, Sponaugle S (2007) Larval transport and dispersal in the coastal ocean and consequences for population connectivity. Oceanography 20:22–39

Pridmore RD, Roper DS, Hewitt JE (1990) Variation in composition and condition of the Pacific oyster, *Crassostrea gigas*, along a pollution gradient in Manukau Harbour, New Zealand. Marine Environmental Research 30:163–177

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945 –959

Quayle DB (1988) Pacific oyster culture in British Columbia. Canadian Bulletin of Fisheries and Aquatic Sciences 218:1–241

R Development Core Team (2011) R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria

Reise K (1982) Long-term changes in the macrobenthic invertebrate fauna of the Wadden Sea: Are polychaetes about to take over? Netherlands Journal of Sea Research 16:29–36

Reise K (1998) Pacific oysters invade mussel beds in the European Wadden Sea. Marine Biodiversity 28:167–175

Reise K, Beusekom J van (2008) Interactive effects of global and regional change on a coastal ecosystem. Helgoland Marine Research 62:85–91

Reise K, Olenin S, Thieltges D (2006) Are aliens threatening European aquatic coastal ecosystems? Helgoland Marine Research 60:77–83

Rejmánek M, Richardson DM, Pyšek P (2005) Plant invasions and invasibility of plant communities. In: Maarel E Van der (ed) Vegetation Ecology. Blackwell, Oxford, p 332–355

Reusch TBH (1998) Native predators contribute to invasion resistance to the non-indigenous bivalve *Musculista senhousia* in southern California, USA. Marine Ecology Progress Series 170:159–168

Reusch TBH, Williams SL (1999) Macrophyte canopy structure and the success of an invasive marine bivalve. Oikos 84:398–416

Ricciardi A, Cohen J (2007) The invasiveness of an introduced species does not predict its impact. Biological Invasions 9:309–315–315

Ricciardi A, Rasmussen JB (1998) Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. Canadian Journal of Fisheries and Aquatic Sciences 55:1759–1765

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Richardson DM, Pyšek P (2008) Fifty years of invasion ecology - the legacy of Charles Elton. Diversity and Distributions 14:161–168

Richardson DM, Pyšek P, Carlton JT (2011) A compendium of essential concepts and terminology in invasion ecology. In: Richardson DM (ed) Fifty years of invasion ecology, Wiley-Blackwell, p 409–420

Richardson DM, Pyšek P, Rejmanek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6:93–107

Richardson CA, Seed R, Al-Roumaihi EMH, McDonald L (1993) Distribution, shell growth and predation of the New Zealand oyster, *Tiostrea* (=*Ostrea*) *lutaria* Hutton, in the Menai Strait, North Wales. Journal of Shellfish Research 12:207–214

Rico-Villa B, Pouvreau S, Robert R (2009) Influence of food density and temperature on ingestion, growth and settlement of Pacific oyster larvae, *Crassostrea gigas*. Aquaculture 287:395–401

Rigal F, Viard F, Ayata S-D, Comtet T (2010) Does larval supply explain the low proliferation of the invasive gastropod *Crepidula fornicata* in a tidal estuary? Biological Invasions 12:3171–3186

Robinson LM, Elith J, Hobday AJ, Pearson RG, Kendall BE, Possingham HP, Richardson AJ (2011) Pushing the limits in marine species distribution modelling: lessons from the land present challenges and opportunities. Global Ecology and Biogeography 20:789–802

Robinson TB, Griffiths CL, Tonin A, Bloomer P, Hare MP (2005) Naturalized populations of oysters, *Crassostrea gigas* along the South African coast: distribution, abundance and population structure. Journal of Shellfish Research 24:443–450

Robinson A, Horton H (1987) Environmental effects on the growth of sibling Pacific oysters *Crassostrea gigas* (Thunberg) and overwintered spat. Journal of Shellfish Research 6:49–53

Robles C (1987) Predator foraging characteristics and prey population structure on a sheltered shore. Ecology 68:1502–1514

Roughgarden J, Gaines S, Possingham H (1988) Recruitment dynamics in complex life cycles. Science 241:1460–1466

Rousset F (2009) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources 8:103–106

Roux D, Rogers K, Biggs H, Ashton P, Sergeant A (2006) Bridging the science-management divide: moving from unidirectional knowledge transfer to knowledge interfacing and sharing.

Ruckelshaus M, Klinger T, Knowlton N, DeMaster DP (2008) Marine ecosystem-based management in practice: scientific and governance challenges. BioScience 58:53–63

Ruesink JL (2007) Biotic resistance and facilitation of a non-native oyster on rocky shores. Marine Ecology Progress Series 331:1–9

Ruesink JL, Lenihan HS, Trimble AC, Heiman K W, Micheli F, Byers J E, Kay M C (2005) Introduction of non-native oysters: ecosystem effects and restoration implications. Annual Review of Ecology, Evolution and Systematics 36:643–689

Ruiz C, Abad M, Sedano F, Garcia-Martin LO, López JLS (1992) Influence of seasonal environmental changes on the gamete production and biochemical composition of *Crassostrea gigas* (Thunberg) in suspended culture in El Grove, Galicia, Spain. Journal of Experimental Marine Biology and Ecology 155:249–262

Sagarin R, Carlsson J, Duval M, Freshwater W, Godfrey MH, Litaker W, Muñoz R, Noble R, Schultz T, Wynne B (2009) Bringing molecular tools into environmental resource management: untangling the molecules to policy pathway. PLoS Biology 7(3):e1000069

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annual Review of Ecology and Systematics 32:305–332

Sauvage C, Boudry P, Lapègue S (2009) Identification and characterization of 18 novel polymorphic microsatellite makers derived from expressed sequence tags in the Pacific oyster *Crassostrea gigas*. Molecular Ecology Resources 9:853–855

Savidge JA (1987) Extinction of an island forest avifauna by an introduced snake. Ecology 68:660–668

Savino JF, Stein RA (1989) Behavioural interactions between fish predators and their prey: effects of plant density. Animal Behaviour 37, Part 2:311–321

SCBD (2001) Review of the efficiency and efficacy of existing legal instruments applicable to invasive alien species. Secretariat of the Convention on Biological Diversity, Montreal

Scheiffarth G, Ens B, Schmidt A (2007) What will happen to birds when Pacific oysters take over the mussel beds in the Wadden Sea? Wadden Sea Newsletter:10–14

Schikorski D, Renault T, Saulnier D, Faury N, Moreau P, Pépin J-F (2011) Experimental infection of Pacific oyster *Crassostrea gigas* spat by ostreid herpesvirus 1: demonstration of oyster spat susceptibility. Veterinary Research 42:27

Schmidt A, Wehrmann A, Dittmann S (2008) Population dynamics of the invasive Pacific oyster *Crassostrea gigas* during the early stages of an outbreak in the Wadden Sea (Germany). Helgoland Marine Research 62:367–376

Schulte DM, Burke RP, Lipcius RN (2009) Unprecedented restoration of a native oyster metapopulation. Science 325:1124–1128

Sekino M, Hamaguchi M, Aranishi F, Okoshi K (2003) Development of novel microsatellite DNA markers from the Pacific Oyster *Crassostrea gigas*. Marine Biotechnology 5:227–233

Shatkin G, Shumway SE, Hawes R (1997) Considerations regarding the possible introductions of the Pacific oyster (*Crassostrea gigas*) to the Gulf of Maine: a review of global experience. Journal of Shellfish Research 16:463–477

Shaw JD, Wilson JRU, Richardson DM (2010) Initiating dialogue between scientists and managers of biological invasions. Biological Invasions 12:4077–4083

Shine C (2007) Invasive species in an international context: IPPC, CBD, European Strategy on invasive alien species and other legal instruments. EPPO Bulletin 37:103–113

Shinen JS, Morgan SG, Chan AL (2009) Invasion resistance on rocky shores: direct and indirect effects of three native predators on an exotic and a native prey species. Marine Ecology Progress Series 378:47–54

Shrader-Frechette K (2001) Non-indigenous species and ecological explanation. Biology and Philosophy 16:507–519

Siemann E, Rogers WE, Dewalt SJ (2006) Rapid adaptation of insect herbivores to an invasive plant. Proceedings of the Royal Society B: Biological Sciences 273:2763–2769

Silva ACF, Hawkins SJ, Boaventura DM, Brewster E, Thompson RC (2010) Use of the intertidal zone by mobile predators: influence of wave exposure, tidal phase and elevation on abundance and diet. Marine Ecology Progress Series 406:197–210

Silva ACF, Hawkins SJ, Boaventura DM, Thompson RC (2008) Predation by small mobile aquatic predators regulates populations of the intertidal limpet *Patella vulgata* (L.). Journal of Experimental Marine Biology and Ecology 367:259–265

Silva PC, Woodfield RA, Cohen AN, Harris LH, Goddard JHR (2002) First report of the Asian kelp *Undaria pinnatifida* in the northeastern Pacific Ocean. Biological Invasions 4: 333–338

Simberloff D (2003) Confronting introduced species: a form of xenophobia? Biological Invasions 5:179–192

Simberloff D, Gibbons L (2004) Now you see them, now you don't! – Population crashes of established introduced species. Biological Invasions 6:161–172

Simberloff D, Rejmánek M (2011) Encyclopedia of biological invasions. University of California Press, Berkeley and Los Angeles

Simberloff D, Relva MA, Nuñez M (2002) Gringos en el bosque: introduced tree invasion in a native *Nothofagus*/*Austrocedrus* forest. Biological Invasions 4:35–53

Simon-Bouhet B, Garcia-Meunier P, Viard F (2006) Multiple introductions promote range expansion of the mollusc *Cyclope neritea* (Nassariidae) in France: evidence from mitochondrial sequence data. Molecular Ecology 15:1699–1711

Smaal AC, Kater BJ, Wijsman J (2009) Introduction, establishment and expansion of the Pacific oyster *Crassostrea gigas* in the Oosterschelde (SW Netherlands). Helgoland Marine Research 63:75–83

Smaal AC, Stralen MR Van, Craeymeersch J (2005) Does the introduction of the Pacific oyster *Crassostrea gigas* lead to species shifts in the Wadden Sea? In: Dame RF, Olenin S (eds) The comparative roles of suspension-feeders in ecosystems. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 277–289

Smallegange IM, Der Meer J Van (2003) Why do shore crabs not prefer the most profitable mussels? Journal of Animal Ecology 72:599–607

Smith PJ, Ozaki H, Fujio Y (1986) No evidence for reduced genetic variation in the accidentally introduced oyster *Crassostrea gigas* in New Zealand. New Zealand Journal of Marine and Freshwater Research 20:569–574

Soletchnik P, Ropert M, Mazurié J, Gildas Fleury P, Coz F Le (2007) Relationships between oyster mortality patterns and environmental data from monitoring databases along the coasts of France. Aquaculture 271:384–400

Spencer BE, Edwards DB, Kaiser MJ, Richardson CA (1994) Spatfalls of the non-native Pacific oyster, *Crassostrea gigas*, in British waters. Aquatic Conservation: Marine and Freshwater Ecosystems 4:203–217

Stachowicz JJ, Byrnes JE (2006) Species diversity, invasion success, and ecosystem functioning: disentangling the influence of resource competition, facilitation, and extrinsic factors. Marine Ecology Progress Series 311:251–262

Stachowicz JJ, Fried H, Osman RW, Whitlatch RB (2002) Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. Ecology 83:2575–2590

Steele S, Mulcahy MF (2001) Impact of the copepod *Mytilicola orientalis* on the Pacific oyster *Crassostrea gigas* in Ireland. Disease of Aquatic Organisms 47:145–149

Stohlgren TJ (2002) Beyond theories of plant invasions: lessons from natural landscapes. Comments on Theoretical Biology 7:355–379

Sunnucks (2000) Efficient genetic markers for population biology. Trends in Ecology and Evolution 15:199–203

Tamburri MN, Zimmer RK, Zimmer CA (2007) Mechanisms reconciling gregarious larval settlement with adult cannibalism. Ecological Monographs 77:255–268

Therriault TW, Herborg L-M (2008) Predicting the potential distribution of the vase tunicate *Ciona intestinalis* in Canadian waters: informing a risk assessment. ICES Journal of Marine Science: Journal du Conseil 65:788 –794

Thieltges DW, Reise K, Prinz K, Jensen KT (2009) Invaders interfere with native parasite–host interactions. Biological Invasions 11:1421–1429

Thieltges DW, Strasser M, Reise K (2003) The American slipper limpet *Crepidula fornicata* (L.) in the northern Wadden Sea 70 years after its introduction. Helgoland Marine Research 57:27–33

Thieltges D, Strasser M, Reise K (2006) How bad are invaders in coastal waters? The case of the American slipper limpet *Crepidula fornicata* in western Europe. Biological Invasions 8:1673–1680

Thompson RC, Crowe TP, Hawkins SJ (2002) Rocky intertidal communities: past environmental changes, present status and predictions for the next 25 years. Environmental Conservation 29:168–191

Thompson K, Hodgson JG, Grime JP, Burke MJW (2001) Plant traits and temporal scale: evidence from a 5-year invasion experiment using native species. Journal of Ecology 89:1054–1060

Thomsen MS, McGlathery K (2006) Effects of accumulations of sediments and drift algae on recruitment of sessile organisms associated with oyster reefs. Journal of Experimental Marine Biology and Ecology 328:22–34

Trimble AC, Ruesink JL, Dumbauld BR (2009) Factors preventing the recovery of a historically overexploited shellfish species, *Ostrea lurida* Carpenter 1864. Journal of Shellfish Research 28:97–106

Troost K (2010) Causes and effects of a highly successful marine invasion: Case-study of the introduced Pacific oyster *Crassostrea gigas* in continental NW European estuaries. Journal of Sea Research 64:145–165

Truscott A-M, Palmer SCF, Soulsby C, Hulme PE (2008) Assessing the vulnerability of riparian vegetation to invasion by *Mimulus guttatus*: relative importance of biotic and abiotic variables in determining species occurrence and abundance. Diversity and Distributions 14:412–421

Tuan VS, Pernetta J (2010) The UNEP/GEF South China Sea project: lessons learnt in regional cooperation. Ocean and Coastal Management 53: 589–596

Turner GE (1988) Codes of practice and manual of procedures for consideration of introductions and transfers of marine and freshwater organisms. EIFAC/CECPI Occasional paper No. 23

Underwood AJ (1980) The effects of grazing by gastropods and physical factors on the upper limits of distribution of intertidal macroalgae. Oecologia 46:201–213

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge

Underwood AJ, Chapman MG (1996) Scales of spatial patterns of distribution of intertidal invertebrates. Oecologia 107:212–224

Underwood AJ, Chapman MG (1998) A method for analysing spatial scales of variation in composition of assemblages. Oecologia 117:570–578

Underwood AJ, Chapman MG, Connell SD (2000) Observations in ecology: you can't make progress on processes without understanding the patterns. Journal of Experimental Marine Biology and Ecology 250:97–115

Valavanis V, Pierce G, Zuur A, Palialexis A, Saveliev A, Katara I, Wang J (2008) Modelling of essential fish habitat based on remote sensing, spatial analysis and GIS. Hydrobiologia 612:5–20

Valéry L, Fritz H, Lefeuvre J-C, Simberloff D (2008) In search of a real definition of the biological invasion phenomenon itself. Biological Invasions 10:1345–1351

Vitousek PM, D'Antonio CM, Loope LL, Rejmánek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global change. New Zealand Journal of Ecology 21:1–16

Vittinghoff E, Glidden DV, Shiboski SC, McCulloch CE (2005) Regression methods in biostatistics: linear, logistic, survival, and repeated measures models. Springer, New York

Walles B, Ysebaert TJW, Troost K, Herman PMJ, Smaal AC (2011) The use of an ecosystem engineer in coastal defense. In: Book of Abstracts of the 14th International Conference on Shellfish Restoration, Stirling, Scotland, 23-27 August 2011.

Walne PR, Davies G (1977) The effect of mesh covers on the survival and growth of *Crassostrea gigas* Thunberg grown on the sea bed. Aquaculture 11:313–321

Walne PR, Mann R (1975) Growth and biochemical composition in *Ostrea edulis* and *Crassostrea gigas*. In: Barnes H (ed) 9th European Marine Biology Symposium. Aberdeen University Press, Scotland, p 587–607

Walther G-R, Roques A, Hulme PE, Sykes MT, Pyšek P, Kühn I, Zobel M, Bacher S, Botta-Dukát Z, Bugmann H, Czúcz B, Dauber J, Hickler T, Jarošík V, Kenis M, Klotz S, Minchin D, Moora M, Nentwig W, Ott J, Panov VE, Reineking B, Robinet C, Semenchenko V, Solarz W, Thuiller W, Vilà M, Vohl K, Settele J (2009) Alien species in a warmer world: risks and opportunities. Trends in Ecology and Evolution 24:686–693

Waples RS, Do C (2008) LDNe: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8:753–756

Warren CR (2008) Alien concepts: a response to Richardson et al. Progress in Human Geography 32:299–300

Wehrmann A, Herlyn M, Bungenstock F, Hertweck G, Millat G (2000) The distribution gap is closed — first record of naturally settled Pacific oysters *Crassostrea gigas* in the East Frisian Wadden Sea, North Sea. Senckenbergiana maritima 30:153–160

Weinstein MP (2008) Ecological restoration and estuarine management: placing people in the coastal landscape. Journal of Applied Ecology 45:296–304

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Williams P, Cameron E (2006) Creating Gardens: The diversity and progression of European plant introductions. In: Allen RB, Lee WG (eds) Biological invasions in New Zealand. Springer Berlin Heidelberg, p 33–47

Williamson M, Fitter A (1996) The varying success of invaders. Ecology 77:1661–1666

Wilson JRU, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM (2009) Biogeographic concepts define invasion biology. Trends in Ecology and Evolution 24:586

Witte S, Buschbaum C, Beusekom J van, Reise K (2010) Does climatic warming explain why an introduced barnacle finally takes over after a lag of more than 50 years? Biological Invasions 12:3579–3589

Wittenberg R, Cock MJW (Eds) (2001) Invasive alien species: a toolkit of best prevention and management practices. CAB International, Wallingford, Oxon

Wolff WJ, Reise K (2002) Oyster imports as a vector for the introduction of alien species into Northern and Western European coastal waters. In: Leppäkoski E, Gollasch S, Olenin S (eds) Invasive aquatic species of Europe. Distribution, impact and management. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 193–205

Woodhead M, Russell J, Squirrell J, Hollingsworth PM, Mackenzie K, Gibby M, Powell W (2005) Comparative analysis of population genetic structure in *Athyrium distentifolium* (Pteridophyta) using AFLPs and SSRs from anonymous and transcribed gene regions. Molecular Ecology 14:1681–1695

Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, Jackson JBC, Lotze HK, Micheli F, Palumbi SR, Sala E, Selkoe KA, Stachowicz JJ, Watson R (2006) Impacts of biodiversity loss on ocean ecosystem services. Science 314:787–90

Wrange A-L, Valero J, Harkestad LS, Strand Ø, Lindegarth S, Christensen HT, Dolmer P, Kristensen PS, Mortensen S (2010) Massive settlements of the Pacific oyster, *Crassostrea gigas*, in Scandinavia. Biological Invasions 12:1145–1152

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97–159

Yu H, Li Q (2007) EST-SSR markers from the Pacific oyster, *Crassostrea gigas*. Molecular Ecology Notes 7:860–862

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Molecular Ecology 11:1–16

Zaniewski A, Lehmann A, Overton J (2002) Predicting species spatial distributions using presence-only data: a case study of native New Zealand ferns. Ecological Modelling 157:261–280

Zhang D-X, Hewitt GM (2003) Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Molecular Ecology 12:563–584

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1:3-14

Appendix – Supplementary Material

Appendix 1
Table with raw data from oyster survey

Information in columns from left to right with column 1: number of site, 2: number of habitat, 3: name of bay (abbreviated), 4: coordinate of longitude, 5: coordinate of latitude, 6: wave fetch (log_{10} km), 7: SACFOR categories for oyster abundances (C = Common, F = Frequent, O = Occasional, R = Rare, N = Absent), 8: residence time of bay, 9: width of intertidal shore (1 = 0-50 m, 2 = 51-100 m, 3 = 101-150 m, 4 = 151 m), 10: aquaculture (0 = absent, 1 = close, 2 = far), 11,12,13: % cover of substrata.

Site number	Habitat number	Bay	N (long)	W (lat)	Fetch (log_{10} km)	SACFOR oyster	Residence time (days)	Shore width	Aquaculture	Rest (%)	Macroalgae (%)	Hardreef (%)
1	1	Swilly	-7.560	55.043	1.544564	C	33.82	2	2	20	10	70
1	2	Swilly	-7.560	55.043	1.544564	F	33.82	2	2	40	20	40
2	3	Swilly	-7.578	55.021	1.220631	C	33.82	4	2	20	5	75
2	4	Swilly	-7.578	55.021	1.220631	O	33.82	4	2	35	30	35
2	5	Swilly	-7.578	55.021	1.220631	A	33.82	4	2	25	0	75
3	6	Foyle	-7.220	55.103	1.681241	C	37.41	2	1	30	0	70
4	7	Swilly	-7.513	55.260	2.352183	N	33.82	1	2	0	20	80
5	8	Mulroy	-7.786	55.180	0.795185	N	36.70	4	1	100	0	0
5	9	Mulroy	-7.786	55.180	0.795185	N	36.70	4	1	20	80	0
6	10	Mulroy	-7.744	55.174	0.531479	N	36.70	1	1	10	80	10
7	11	Mulroy	-7.696	55.149	1.075547	N	36.70	1	2	5	0	95
8	12	Mulroy	-7.703	55.120	0.832509	N	36.70	1	2	20	0	80
9	13	Foyle	-7.038	55.185	1.817698	R	37.41	1	2	50	0	50
10	14	Foyle	-7.142	55.054	1.746634	F	37.41	4	2	70	0	30
10	15	Foyle	-7.142	55.054	1.746634	R	37.41	4	2	90	0	10
11	16	Trawbeaga	-7.275	55.281	1.766115	N	10.50	4	1	70	30	0
11	17	Trawbeaga	-7.275	55.281	1.766115	N	10.50	4	1	40	55	5
11	18	Trawbeaga	-7.275	55.281	1.766115	N	10.50	4	1	95	0	5
12	19	Trawbeaga	-7.316	55.289	1.021189	N	10.50	3	1	100	0	0
12	20	Trawbeaga	-7.316	55.289	1.021189	N	10.50	3	1	25	70	5
13	21	Trawbeaga	-7.260	55.293	1.059942	N	10.50	4	2	80	0	20
14	22	Trawbeaga	-7.311	55.308	0.823474	N	10.50	4	1	50	0	50
14	23	Trawbeaga	-7.311	55.308	0.823474	N	10.50	4	1	5	80	15
15	24	Foyle	-6.998	55.115	1.698275	F	37.41	4	2	80	0	20
16	25	Swilly	-7.507	55.084	1.493040	C	33.82	3	2	0	0	100
16	26	Swilly	-7.507	55.084	1.493040	F	33.82	3	2	60	0	40
17	27	Kinsale	-8.517	51.695	0.770852	N	27.30	2	1	100	0	0
17	28	Kinsale	-8.517	51.695	0.770852	N	27.30	2	1	15	60	25
18	29	Oysterhaven	-8.433	51.709	2.729456	N	10.90	2	1	0	90	10
18	30	Oysterhaven	-8.433	51.709	2.729456	N	10.90	2	1	15	70	15
18	31	Oysterhaven	-8.433	51.709	2.729456	N	10.90	2	1	50	50	0

Site number	Habitat number	Bay	N (long)	W (lat)	Fetch (log_{10} km)	SACFOR oyster	Residence time (days)	Shore width	Aquaculture	Rest (%)	Macroalgae (%)	Hardreef (%)
19	32	Galway	-8.950	53.208	1.068928	R	25.42	2	1	0	90	10
20	33	Galway	-8.941	53.197	0.886491	R	25.42	2	1	0	90	10
21	34	Galway	-8.969	53.172	1.495822	N	25.42	3	1	0	95	5
21	35	Galway	-8.969	53.172	1.495822	N	25.42	3	1	60	40	0
21	36	Galway	-8.969	53.172	1.495822	R	25.42	3	1	5	90	5
22	37	Roaringwater	-9.433	51.551	0.428135	N	6.05	2	2	0	90	10
23	38	Roaringwater	-9.372	51.488	0.681241	N	6.05	1	1	0	100	0
23	39	Roaringwater	-9.372	51.488	0.681241	N	6.05	1	1	15	80	5
24	40	Shannon	-9.564	52.668	1.011993	N	53.26	2	2	95	5	0
24	41	Shannon	-9.564	52.668	1.011993	N	53.26	2	2	5	90	5
25	42	Shannon	-9.704	52.588	1.504063	N	53.26	2	1	0	70	30
26	43	Shannon	-9.492	52.575	1.262925	N	53.26	2	1	75	15	10
26	44	Shannon	-9.492	52.575	1.262925	N	53.26	2	1	70	15	15
26	45	Shannon	-9.492	52.575	1.262925	N	53.26	2	1	0	40	60
27	46	Shannon	-9.248	52.586	1.275542	F	53.26	2	2	5	40	55
27	47	Shannon	-9.248	52.586	1.275542	O	53.26	2	2	70	25	5
28	48	Tralee	-9.860	52.285	0.593286	N	14.20	3	0	20	70	10
28	49	Tralee	-9.860	52.285	0.593286	N	14.20	3	0	100	0	0
28	50	Tralee	-9.860	52.285	0.593286	N	14.20	3	0	40	30	30
29	51	Tralee	-9.814	52.273	1.452706	R	14.20	2	0	30	10	60
29	52	Tralee	-9.814	52.273	1.452706	N	14.20	2	0	40	20	40
30	53	Castlemaine	-9.892	52.124	1.372544	N	14.68	4	1	10	80	10
30	54	Castlemaine	-9.892	52.124	1.372544	N	14.68	4	1	95	5	0
31	55	Castlemaine	-9.903	52.113	1.535800	N	14.68	3	2	15	50	35
31	56	Castlemaine	-9.903	52.113	1.535800	N	14.68	3	2	95	5	0
32	57	Castlemaine	-9.922	52.065	0.450249	N	14.68	4	1	65	30	5
32	58	Castlemaine	-9.922	52.065	0.450249	N	14.68	4	1	60	40	0
32	59	Castlemaine	-9.922	52.065	0.450249	N	14.68	4	1	10	90	0
33	60	Ventry	-10.365	52.116	1.073352	N	26.40	2	0	0	30	70
33	61	Ventry	-10.365	52.116	1.073352	N	26.40	2	0	0	100	0
34	62	Dingle	-10.261	52.128	0.869232	N	9.00	2	0	100	0	0
34	63	Dingle	-10.261	52.128	0.869232	N	9.00	2	0	0	100	0
34	64	Dingle	-10.261	52.128	0.869232	N	9.00	2	0	0	20	80
35	65	Shannon	-9.367	52.578	1.177825	R	53.26	4	2	15	60	25
35	66	Shannon	-9.367	52.578	1.177825	N	53.26	4	2	100	0	0
36	67	Shannon	-9.158	52.606	1.330008	F	53.26	1	2	0	20	80
36	68	Shannon	-9.158	52.606	1.330008	O	53.26	1	2	0	20	80
37	69	Waterford	-6.979	52.202	2.353070	N	15.70	4	1	100	0	0
38	70	Bannow	-6.803	52.237	1.055378	N	9.80	2	1	40	60	0
38	71	Bannow	-6.803	52.237	1.055378	N	9.80	2	1	5	95	0

Site number	Habitat number	Bay	N (long)	W (lat)	Fetch (log_{10} km)	SACFOR oyster	Residence time (days)	Shore width	Aquaculture	Rest (%)	Macroalgae (%)	Hardreef (%)
39	72	Bannow	-6.773	52.237	0.878522	N	9.80	1	1	15	80	5
39	73	Bannow	-6.773	52.237	0.878522	N	9.80	1	1	75	20	5
39	74	Bannow	-6.773	52.237	0.878522	N	9.80	1	1	10	90	0
40	75	Dungarvan	-7.566	52.05	1.473925	N	5.19	3	1	5	80	15
41	76	Dungarvan	-7.571	52.085	1.442166	N	5.19	1	2	0	20	80
41	77	Dungarvan	-7.571	52.085	1.442166	N	5.19	1	2	0	90	10
41	78	Dungarvan	-7.571	52.085	1.442166	N	5.19	1	2	0	70	30
42	79	Tramore	-7.083	52.153	0.914872	N	8.60	2	0	35	60	5
43	80	Waterford	-6.971	52.239	1.157154	N	15.70	4	2	70	15	15
43	81	Waterford	-6.971	52.239	1.157154	N	15.70	4	2	5	95	0
44	82	Streamstown	-10.069	53.513	0.719331	N	10.30	2	1	0	100	0
45	83	Streamstown	-10.061	53.503	0.683047	N	10.30	1	2	0	100	0
45	84	Streamstown	-10.061	53.503	0.683047	N	10.30	1	2	80	20	0
46	85	Mannin	-10.047	53.463	0.494155	N	20.80	1	2	50	10	40
47	86	Mannin	-10.084	53.467	1.923244	N	20.80	1	2	0	0	100
48	87	Ballynakill	-9.974	53.571	0.414973	N	16.10	1	1	0	100	0
48	88	Ballynakill	-9.974	53.571	0.414973	N	16.10	1	1	0	100	0
48	89	Ballynakill	-9.974	53.571	0.414973	N	16.10	1	1	0	20	80
49	90	Ballynakill	-9.967	53.551	0.614897	R	16.10	2	2	15	85	0
49	91	Ballynakill	-9.967	53.551	0.614897	N	16.10	2	2	5	90	5
50	92	Ballynakill	-10.011	53.554	0.947434	N	16.10	1	1	20	80	0
51	93	Achill	-9.935	53.876	0.523746	N	10.30	1	1	70	20	10
52	94	Achill	-9.954	54.017	1.484869	N	10.30	3	2	0	90	10
52	95	Achill	-9.954	54.017	1.484869	N	10.30	3	2	0	30	70
53	96	Achill	-9.930	53.997	1.410609	N	10.30	1	2	0	80	20
53	97	Achill	-9.930	53.997	1.410609	N	10.30	1	2	0	60	40
53	98	Achill	-9.930	53.997	1.410609	N	10.30	1	2	50	20	30
54	99	Clew	-9.673	53.883	1.219060	N	0.00	4	1	0	100	0
54	100	Clew	-9.673	53.883	1.219060	N	0.00	4	1	30	70	0
54	101	Clew	-9.673	53.883	1.219060	N	0.00	4	1	100	0	0
55	102	Clew	-9.618	53.849	0.865696	N	0.00	2	2	40	60	0
56	103	Clew	-9.561	53.803	1.299725	N	0.00	4	2	40	60	0
57	104	Achill	-9.941	53.965	1.366236	N	10.30	1	1	0	70	30
58	105	Achill	-9.855	53.957	1.006894	N	10.30	2	2	0	75	25
58	106	Achill	-9.855	53.957	1.006894	N	10.30	2	2	0	100	0
59	107	Clew	-9.635	53.788	1.270912	N	0.00	4	1	10	70	20
60	108	Galway	-9.076	53.156	0.649335	R	25.42	2	1	5	90	5
60	109	Galway	-9.076	53.156	0.649335	N	25.42	2	1	95	5	0
60	110	Galway	-9.076	53.156	0.649335	N	25.42	2	1	0	95	5

Site number	Habitat number	Bay	N (long)	W (lat)	Fetch (log_{10} km)	SACFOR oyster	Residence time (days)	Shore width	Aquaculture	Rest (%)	Macroalgae (%)	Hardreef (%)
61	111	Galway	-9.095	53.148	0.526339	O	25.42	1	2	0	0	100
61	112	Galway	-9.095	53.148	0.526339	R	25.42	1	2	10	40	50
61	113	Galway	-9.095	53.148	0.526339	N	25.42	1	2	5	90	5
62	114	Galway	-8.953	53.154	0.912753	N	25.42	2	2	70	30	0
63	115	Sheep	-7.877	55.141	0.904174	N	26.40	4	1	100	0	0
63	116	Sheep	-7.877	55.141	0.904174	N	26.40	4	1	60	30	10
64	117	Sheep	-7.865	55.135	0.976808	N	26.40	4	2	10	90	0
64	118	Sheep	-7.865	55.135	0.976808	N	26.40	4	2	100	0	0
64	119	Sheep	-7.865	55.135	0.976808	N	26.40	4	2	5	70	25
65	120	Inishfree	-8.318	55.022	0.491362	N	8.20	4	0	100	0	0
66	121	Inishfree	-8.400	55.019	0.653213	N	8.20	4	0	100	0	0
67	122	Swilly	-7.566	55.008	1.536306	F	33.82	3	2	20	0	80
67	123	Swilly	-7.566	55.008	1.536306	F	33.82	3	2	20	60	20
68	124	Roaringwater	-9.404	51.497	0.833784	N	6.05	1	1	0	0	100
68	125	Roaringwater	-9.404	51.497	0.833784	N	6.05	1	1	0	0	100
69	126	Roaringwater	-9.425	51.504	1.875293	N	6.05	2	2	0	100	0
69	127	Roaringwater	-9.425	51.504	1.875293	N	6.05	2	2	0	100	0

Appendix 2
Manual for oyster survey.

Phase 1: Initial site surveys

Each site visit should be timed to be within spring low tide +/- 20 minutes and initiated one hour before predicted low water. Two persons will walk 40 - 45 minutes in the lower intertidal gradually progressing down the shore in step with the receding tide and cover most of the habitats available, always in sight with each other. Some waypoints might be taken on the GPS to get the size of the area. While doing this, complete the Phase 1 recording datasheet, indicating the general features of the site, the habitat availability at the site and your rough estimate of oyster abundance (on the SACFOR scale) in each of those habitats.

If oysters are present at the site, you will need to identify the area of greatest density so that the density in that area can be quantified using the Phase 2 protocol below. If the density of oysters changes significantly within the habitats or among them use little flags to mark dense areas so that it is easy to return to the area of highest density.

If no oysters are found during the Phase 1, try to go to a second site and complete Phase 1 there during the first 40-45 minutes of the rising tide.

Phase 2: transects/quadrats to quantify maximal density of oysters and their sizes

Pick the area with the highest density, and do two random transects of 30 m, take the coordinates at start and end point. In each do ~15 quadrats of 1 x 1 m, starting from 0 - 1 m and follow up at 2 – 3 m, right and left of the tape alternating. If the area is very small decrease the length of each transects. In each quadrat measure the size of the oyster to the nearest mm with Vernier callipers and note the substratum the oyster was attached on (e.g. other oyster, mussel shell (inner, outer), mud, cobble...). If you reach a number of 100 individuals, stop measuring each individual but keep counting them.

If you are in an area where the density of oysters is rather low but you still will be able to do a transect do two 30 m transect and walk within 1 m (e.g. by holding a bar) counting **all** oysters along the tape and within the bar, not in quadrats. The

second person will measure the oysters. If the density is still very low you might do more transects like that in order to find 100 individuals for size-frequency histograms. Use a blank sheet of paper for this or the Phase 2 transect sheet without using the quadrat columns.

Explanations for recording sheet

Recorder : surname, first name

Date: xx/yy/zzzz

Locality: name of the closest town, village

low tide: e.g. 13:15 pm tidal level: e.g. 0.52 m

GPS coordinates: take GPS coordinates at parking AND at area with highest oyster density. Make sure to use lat/long and either map datum: WGS84 OR NIG (National Irish Grid)

Time: start about one hour to 40 min before low tide so that you can get a comprehensive idea of the total intertidal area at low tide (walking/searching should take about 40-45 min along the low water tide line (Phase 1), if you find oysters start Phase 2 afterwards. If you don't find oysters, try to go to a second site and complete Phase 1 there during the first 40-45 minutes of the rising tide.

Start walk: e.g. 13:00am End walk: e.g. 13:40 am

Width in m: measure x meter from low water line to high water line with GPS function (waypoints…)

Salinity: take a water sample from the main tidal stream (if possible)

No. of pictures: you can take ~ 4 pictures, labelled: overview of bay or coastline, main habitat type, key substrate or sediment, key species, single oysters

Access: note way of access e.g. via parking lot, slipway, arable field

Visible surface cover (%): Try to identify the main habitat type and describe the SURFACE coverage in % (5% -10% accuracy), just what you can see from ABOVE (in total 100%). If there is more than one habitat type or the lower-mid shore and lower shore are very different, divide habitats in Habitat 1 and Habitat 2 (or even more) and describe them separately. For macrophyte-dominated substrate there is another column. There, you will describe what is UNDER those macrophytes, and again: Imagine a view from ABOVE (but underneath macrophytes), no manifold layers, it should sum up to 100% again.

SACFOR: Give a category for the oyster density (refer to Codes - see below) in the habitat, if more than one habitat then for each separately. Give also a category for the overall site. Categories should be given straight after the 40 min walk in order to find the area with highest oyster density where to do transects/quadrats. IMPORTANT: Take those categories more as a guide and don't try to measure the actual density of oysters in Phase 1. Exact numbers per m^2 will be taken by transects/quadrats in Phase 2.

Bay feature: characterize the bay/shoreline within the 3 different types (better: calculate residence time and wave fetch accurately using methods by Hartnett et al. 2011 and Burrows et al. 2008)

Comments: notes about coastline and shore (shingle, sand…), main substrate or habitat type (e.g. boulderfield, macrophytes-dominated boulderfield, rocky shore, sandflat, mudflat…), freshwater inlet, characteristic species, distance to aquaculture trestles, EVERYTHING what is of any IMPORTANCE from YOUR point of view.

Categories

Substratum (modified EUNIS framework)	Sizes (cm and mm)
Bedrock	
Boulders	25.6 cm – 102.4 cm
Cobbles, pebbles	1.6 cm – 25.6 cm
Gravel	0.4 cm – 1.6 cm
Littoral sand: clean sands and non-cohesive muddy sand	0.063 mm – 4 mm
Littoral mud: cohesive sandy muds and muds	< 0.063 mm (silt/clay)
Littoral mixed sediment: heterogenous mixtures of gravel, sand and mud, shells, maerl	
Littoral macrophyte-dominated sediment	
Biogenic reef (mussel beds, *Sabellaria* reefs,…)	

SACFOR	Density of oysters	cover % per m^2	Oysters per steps
S superabundant	100 - 999/m^2	20 - 39%	every single step
A abundant	10 - 99/m^2	10 - 19%	every 2 steps
C common	1 - 9/m^2	5 - 9%	every 3 steps
F frequent	0.1 - 0.9/m^2	1 - 5% or density	every 5 steps
O occasional	0.01 - 0.09/m^2	< 1 % or density	every ~10 steps
R rare	0.001 - 0.009/m^2		single individuals
absent	0		none

Recording sheet

recorder _____

date _____

locality (name of closest town or village) _____

tidal level _____ **low tide** _____

GPS coord. (lat./long in WGS84) parking: _____

highest oyster density: _____

time start: _____ end: _____

width from low to high water line (m) _____ **salinity** _____

access (road, quay, path)

visible surface coverage (%)	**habitat 1**	**(habitat 2)**	**(habitat 3)**	
bedrock				br
boulders				b
cobbles, pebbles (25.6 cm -1.6 cm)				c, p
gravel (1.6 cm - 0.4 cm)				g
sand				Sa
mud				Mu
mixed sediment				Mx
biogenic reefs (mussel bed, Sabellaria reefs…)				BR
macrophyte-dominated substrate				Mp
length walked (m)				

sediment underneath macrophytes

habitat 1	br:	b:	c/p:	g:	Sa:	Mu:	Mx:	BR:
habitat 2	br:	b:	c/p:	g:	Sa:	Mu:	Mx:	BR:

SACFOR oyster density per m²			

Bay/Coastline feature (tick box)

high energy, wave exposed, very tide swept	
moderately wave exposed or tide-swept	
wave sheltered and weak tidal currents	

comments
(e.g. freshwater inlet, characteristic species)

Appendix 3
Description of pilot experiment.

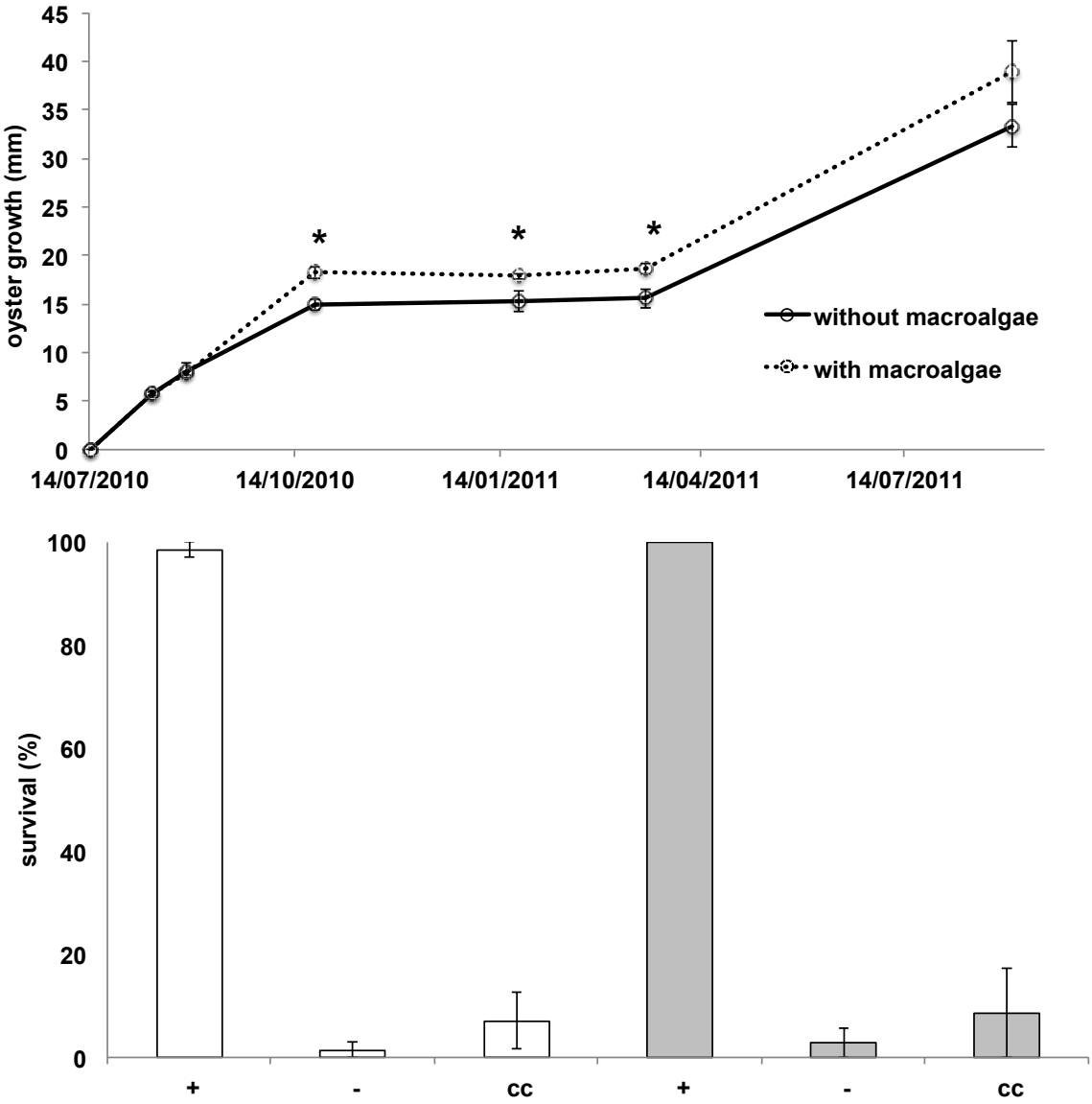
The experimental design and procedures of the pilot study were generally the same as the in the experiment in 2011. Differences are described in the following section.

The pilot study was carried out from 15th and 16th July 2010 to 31st August 2011 at the shore of Parkmore Pier in Kinvarra Bay, County Clare, Ireland (N 53°10'18.92'' W 008°58'9.29''). The mid-lower intertidal was dominated by *Ascophyllum nodosum* and *Fucus serratus* canopy. The sediment was a mixture of maerl, fine sand and mud. Close to the site oysters are cultured in intertidal trestle systems and single oyster individuals have been found attached to boulders during a survey in 2009 indicating suitable growth conditions.

The six different treatments of macroalgae and cage were replicated five times. 14 individuals of oysters (mean shell length of 16 mm) were attached to tiles with Gorilla Super Glue. In total, 30 tiles were prepared over two days and tiles were randomly distributed among the plots and set up on the shore over two days. Cages were manipulated immediately when plots were set up on the shore. Triploid oysters were used for this study. Allen and Downings (1986) suggested that triploidy might be a valuable tool in studies of physiological energetics and although growth can vary between diploid and triploid oysters, this faster growth becomes more pronounced after the first year when diploids become more sexually active (for review see Nell 2002). Thus, similar results were expected for diploid oysters.

After the first two weeks, results for % survival of oysters were obtained (Appendix Figure 1). Length measurements estimated by digital pictures were taken in August 2010, October 2010, January 2011, March 2011. The final growth and condition index was estimated at 31st August 2011 and measured using a digital Vernier calliper (Appendix Figure 1).

Appendix 4
Results of pilot experiment (graphic).



Top: growth of oysters (all caged) in the pilot experiment (Kinvarra shore, Galway Bay) from July 2010 until August 2011, * indicate significant differences; bottom: mean percentage survival of oysters two weeks after experimental set up, with cage (+), without cage (-), control cage cage (cc), shown are means ± standard errors, n = 5.

Appendix 5
Results of pilot experiment (ANOVA table).

Results of one-way ANOVA comparing oyster growth from August 2010 until August 2011 and condition index between macroalgae treatments at Kinvarra shore (pilot experiment) and survival of oysters after 2 weeks within the different treatments. Significant effects are in bold, n = 5.

Source of variation	df	MS	F	*p*	MS	F	*p*
		11th August 2010			26th August 2010		
Macroalgae	1	0.02	0.03	0.8569	0.11	0.06	0.8087
Residuals	8	0.45			1.78		
Cochran's test		0.7927 (n.s.)			0.9193 (sign.), no transformation		
		23rd October 2010			22nd January 2011		
Macroalgae	1	27.71	15.59	0.0042	34.25	5.29	0.0504
Residuals	8	1.78			6.47		
Cochran's test		0.52 (n.s.)			transformation (x^1.1) 0.9043 (n.s.)		
		20th March 2011			31st August 2011		
Macroalgae	1	23.43	7.41	0.0262	77.47	2.07	0.1883
Residuals	8	3.16			37.44		
Cochran's test		0.7657 (n.s.)			0.6691 (n.s.)		
		Condition Index					
Macroalgae	1	30.17	3.95	0.0821			
Residuals	8	7.64					
Cochran's test		0.5329 (n.s.)					
		Survival after 2 weeks					
Macroalgae	1	7.86	0.06	0.8023			
Cage	2	22552.79	183.86	0			
Macroalgae x Cage	2	12.66	0.1	0.9024			
Residuals	24	122.66					
Cochran's test		Transformation (ArcSin (proportion)), 0.455 (n.s.)					

Appendix 6
Timing of activities for the field experiment in 2011/2012.

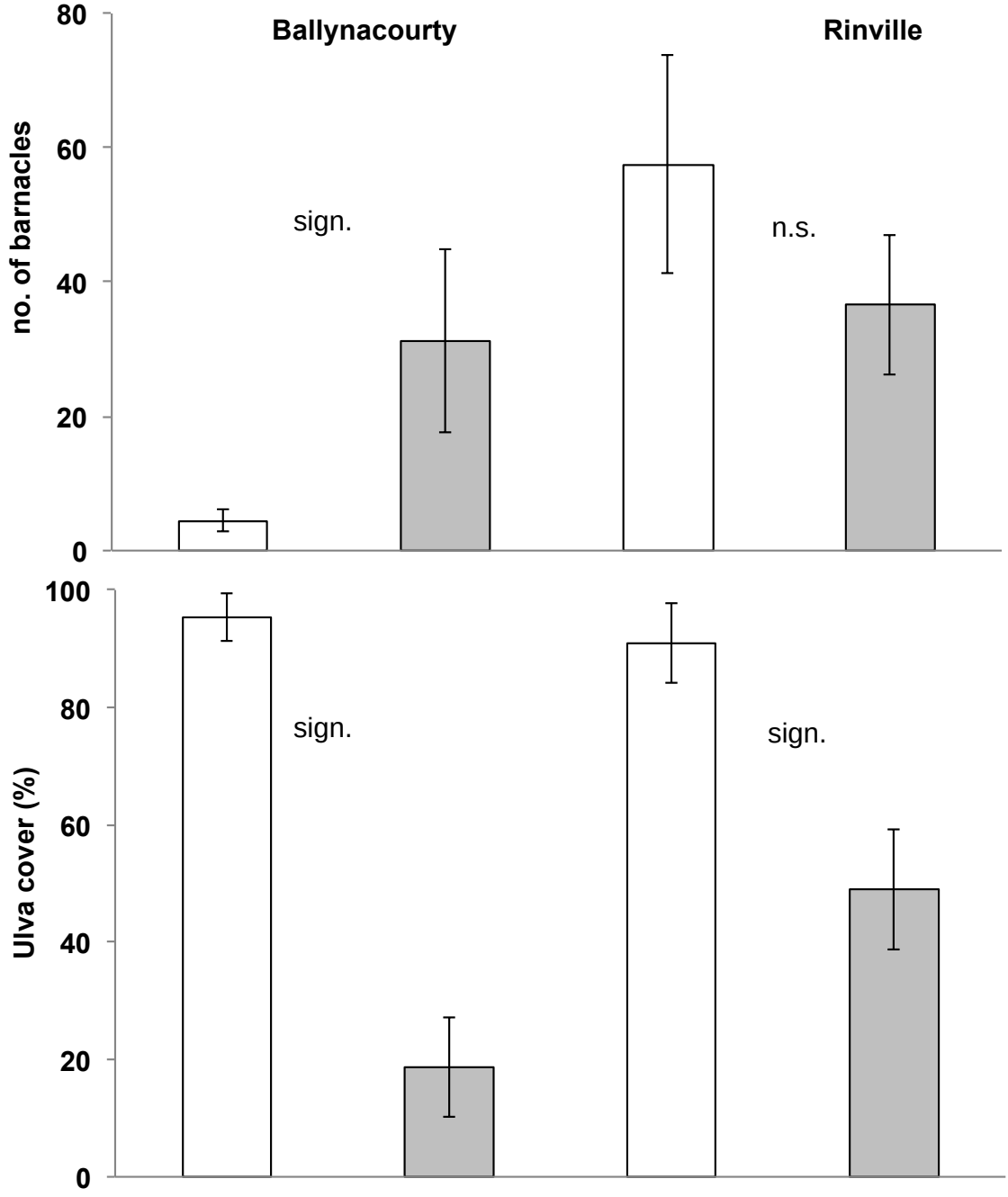
Date	Named as	Activity
3/4 July 2011	Start	Experimental set up, macroalgae manipulation
29/30 August 2011	August 2011	Barnacle count, *Ulva* cover estimation (%), pictures for oyster size measurements
13/14 September 2011	Cage manipulation	Cage manipulation
30 September/1 October 2011	September 2011	
27/28 October 2011	October 2011	From September 2011 until April 2012: pictures taken for size measurements, survival counts
11/12 January 2012	January 2012	
10/11 March 2012	March 2012	
7/8 April 2012	April 2012	
6/7 May 2012	May 2012	End of experiment, condition index and size measurements with Vernier calliper

Appendix 7
Results of two-way ANOVA.

Upper row: % cover with *Ulva* and number of barnacles in macroalgae treatments at the two sites before cage manipulation, n = 15. Lower row: oyster growth (mm) at Rinville measured from the end of August 2011 (after cage manipulation) until May 2012 to test for the effect of cage (results for other dates are not shown but were qualitatively the same) and condition index in all treatments, n = 3. Significant effects are in bold.

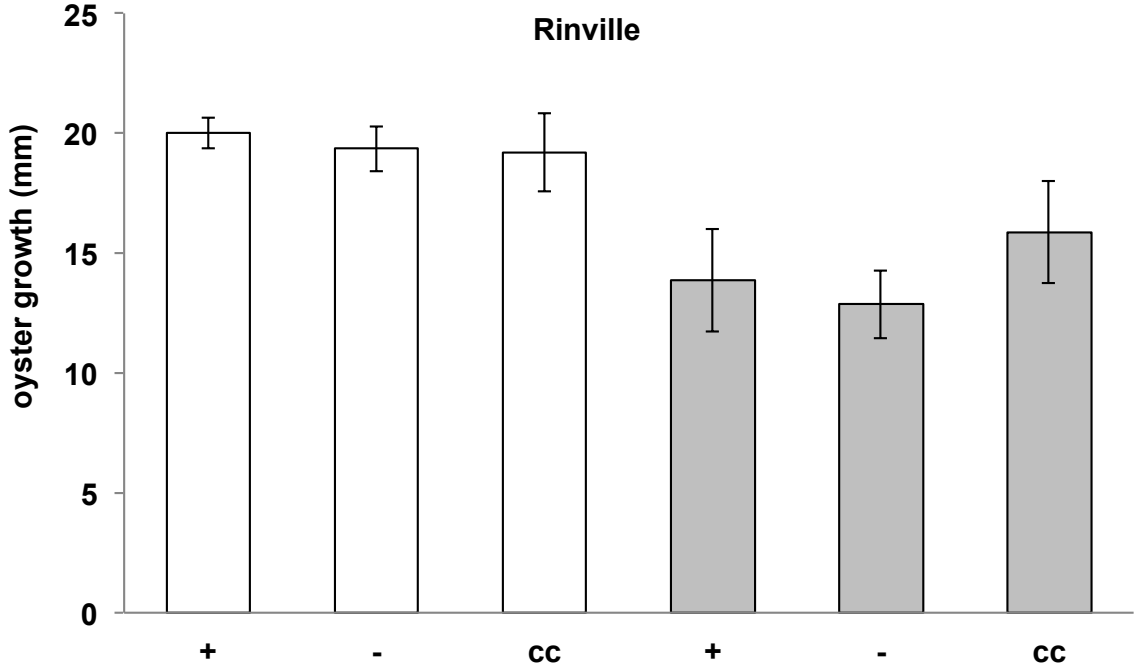
Source of variation	df	MS	F	*p*	MS	F	*p*
		Ulva (%)			No. of barnacles		
Site	1	2535.00	2.84	0.098	113.56	11.61	**0.001**
Macroalgae	1	52806.67	11.72	0.181	6.46	0.13	0.778
Site x Macroalgae	1	4506.67	5.05	**0.029**	48.82	4.99	**0.030**
Residual	56	893.15			9.78		
Transformation					sqrt (x+1)		
Cochran' s test		0.4401 (n.s.)			0.4134 (n.s.)		
		Growth (mm)			Condition Index		
Macroalgae	1	126.96	16.91	**0.001**	34.27	1.11	0.313
Cage	2	3.08	0.41	0.673	45.91	1.49	0.265
Cage x Macroalgae	2	4.34	0.58	0.576	14.01	0.45	0.645
Residual	12	7.51			30.84		
Cochran' s test		0.3046 (n.s.)			0.3124 (n.s.)		

Appendix 8
Number of barnacles and % cover of *Ulva*.



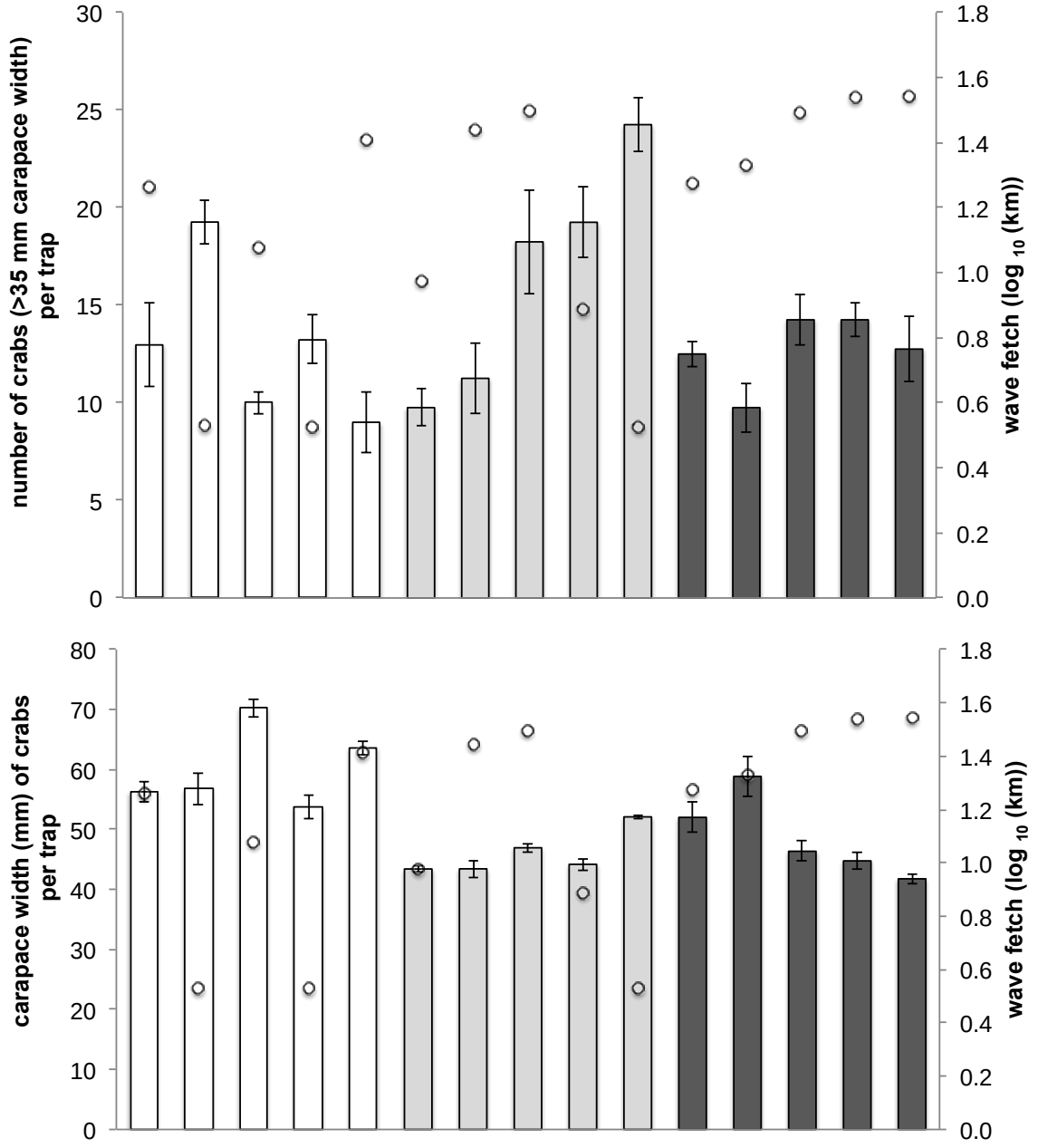
Shown are means ± standard errors, plots with macroalgae (grey) and without macroalgae (white) at the two sites two months after experimental set up (August 2011). Note that all tiles were inside cages, n = 15.

Appendix 9
Test for the effect of cage.



Oyster growth at Rinville measured from the end of August 2011 (after cage manipulation) until May 2012. Shown are means ± standard errors in each treatment. Treatments: without macroalgae (white), with macroalgae (grey), with cage (+), without cage (-), control cage (cc), n = 3.

Appendix 10
Number and sizes of crabs captured and wave fetch at 15 sites.



Number of adult crabs captured (top) and carapace width of crabs (bottom) per trap, represented by bars. Shown are means ± standard errors, n = 4. Open circles indicate wave fetch at the different sites. Locations where oysters are absent (in white), rare (< 0.01 individuals/m^2 in grey) or frequent (> 0.1 individuals/m^2 in dark grey).

Appendix 11
Estimated null-allele frequencies for all samples and loci

Use of Brookfield 1 equation, frequencies < 0.07 = no null-alleles, first 7 loci are EST-SSR, subsequent 7 loci are naSSR, loci with many null-alleles excluded for analysis using only 7 loci are in bold.

	Sample					
Locus	Feral 1	Feral 2	Feral 3	AQF 1	AQF 2	AQB
CGE007	0.203	0.143	0.168	0.244	0.263	0.094
Cgsili43	0.099	0.136	0.103	0.064	-0.009	0.074
Cgsili46	0.037	0.005	0.079	0.012	0.241	0.028
Cgsili39	0.258	0.256	0.256	0.100	0.241	0.252
Cgsili4	0.046	-0.019	0.008	-0.045	-0.033	-0.053
Cgsili29	0.144	0.222	0.245	0.098	0.105	0.179
Cgsili50	-0.007	-0.020	-0.005	0.081	0.005	-0.047
Crgi50	0.130	0.139	0.163	0.077	0.095	0.070
L10	0.008	-0.005	-0.008	0.070	-0.002	-0.024
CG44	0.345	0.313	0.269	0.356	0.288	0.247
CG49	0.252	0.159	0.229	0.092	0.092	0.097
CG108	0.044	-0.056	-0.013	-0.005	-0.029	-0.063
Crgi10	0.023	-0.022	0.009	0.028	0.103	-0.025
Crgi26	0.001	-0.039	-0.029	0.028	0.033	-0.005

Appendix 12
Global multiloci and pairwise multiloci, F_{ST} values.

7 EST (upper right diagonal) and 6 anonymous (lower left diagonal) microsatellite loci separated (top) and also for the 13 loci combined (bottom) after Brookfield 1 correction. F_{ST}-estimates found to be significantly different from 0 in bold type after Bonferroni correction. Note: 13 loci without Bonferroni correction.

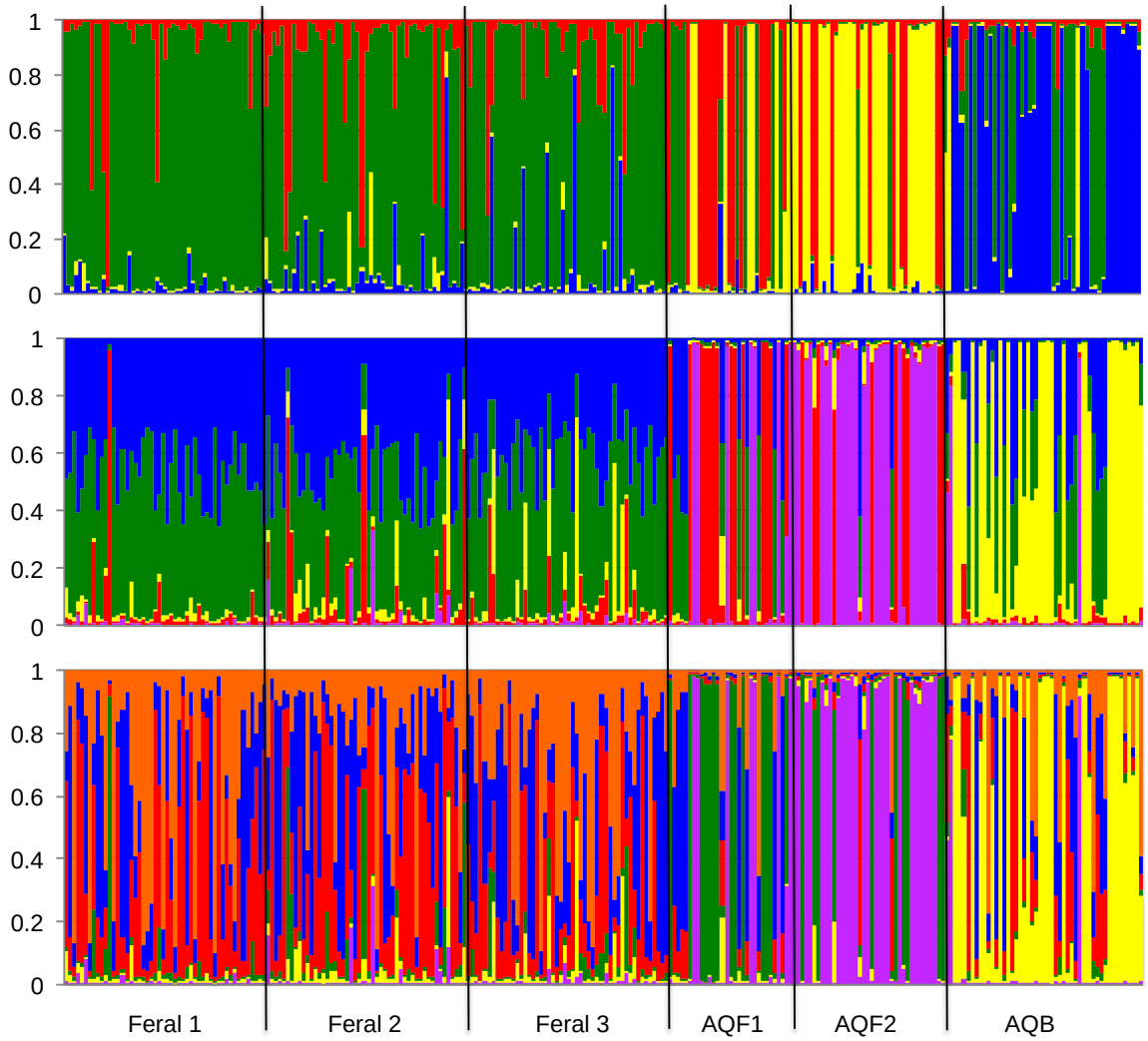
anonymous\EST	Feral 1	Feral 2	Feral 3	AQF 1	AQF 2	AQB
Feral 1		-0.0004	0.0023	**0.0274**	**0.0687**	**0.0363**
Feral 2	0.0054		-0.0031	**0.0233**	**0.0711**	**0.0331**
Feral 3	0.0114	0.0045		**0.0246**	**0.0786**	**0.0438**
AQF 1	**0.0312**	**0.0227**	**0.0236**		0.0101	**0.0445**
AQF 2	**0.0637**	**0.0596**	**0.0639**	**0.0316**		**0.0698**
AQB	**0.0526**	**0.0333**	**0.0531**	**0.0525**	**0.0693**	

global F_{ST}/p	anonymous loci: 0.0319/0.
global F_{ST}/p	EST loci: 0.0286/0.0001

all 13 loci	Feral 1	Feral 2	Feral 3	AQF 1	AQF 2	AQB
Feral 1		0.0023	0.0065	**0.0292**	**0.0664**	**0.0438**
Feral 2			0.0004	**0.0230**	**0.0658**	**0.0332**
Feral 3				**0.0242**	**0.0718**	**0.0481**
AQF 1					**0.0201**	**0.0481**
AQF 2						**0.0696**
AQB						

global F_{ST}/p	13 loci: 0.0301/0.0001

Appendix 13
STRUCTURE output for loci without null-alleles.



STRUCTURE output without loci showing more than 0.07 null-alleles using the same model parameters as with 13 loci (Chapter 4). Visualised using CLUMPP. Top: k = 4 to compare with runs using all 13 loci, middle: k = 5 identified by using Delta k, bottom: k = 6 using mean LnP(D).