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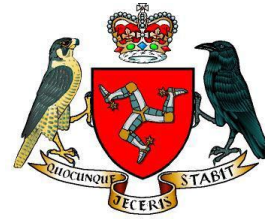
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**AN INVESTIGATION INTO THE SPATIAL
SCALES OF GENETIC AND REPRODUCTIVE
VARIATION IN THE SCALLOP *PECTEN***

MAXIMUS L.

A thesis submitted to Bangor University, Wales in fulfilment of the degree
of Doctor of Philosophy

Natalie Hold, B.Sc, M.Sc

June 2012

School of Ocean Sciences and

The Molecular Ecology and Fisheries Genetics Laboratory, School of Biological
Sciences

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The problem with genetic research is, we're just starting down this path, feeling our way in the dark. We have a small lantern in the form of a gene, but the lantern doesn't penetrate more than a couple of hundred feet. We don't know whether we're going to encounter chasms, rock walls or mountain ranges along the way. We don't even know how long the path is.

-Francis S. Collins

To my husband Danny; for helping to keep the lantern alight, so I didn't completely lose my way.

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ABSTRACT

Understanding the connectivity among populations of marine organisms and the spatial scale at which they interact is essential for the identification of fishery stocks and therefore the successful management of commercially exploited species. A range of approaches are available for the study of connectivity and to define the spatial extent of stocks and population structure, these include; tagging studies, elemental fingerprinting, the use of biophysical coupled oceanographic models and the use of genetic tools to determine population structure.

The current study used a combination of approaches to understand the connectivity and spatial scales of interaction among populations of *Pecten maximus*. Twelve new microsatellite markers were developed and together with four previously published markers these were used to genotype fourteen populations of *P. maximus* across the NE Atlantic. Genetic differentiation analyses identified one large homogenised region around Ireland and the British Isles but with a few isolated populations (Falmouth Bay, Mulroy Bay and to a lesser extent Peel in the Isle of Man). Low but significant structuring was found between the Galicia and the Irish/British region. Norway was significantly differentiated from all other populations with similar magnitudes of structure as that found between the Mediterranean populations of *Pecten jacobaeus* and the populations of *P. maximus*.

A study of spatial variation in reproductive ecology identified small-scale spatial variation (< 5km) in the onset of gonad conditioning and the autumn spawning event in the waters off the Isle of Man. The short-term variation in gonad weight among sampling sites appeared to be driven by the rate of change of temperature in the month prior to sampling, whilst long term variation in gonad weight among sites was affected by the average annual chlorophyll a concentrations and scallop density.

Whilst there is strong genetic connectivity at a regional spatial scale (Ireland and the British Isles), the presence of some isolated populations, low effective population sizes and poor synchronisation in reproduction, suggested that it would be unwise to assume high levels of connectivity in all areas of a region for the purpose of fisheries management. Populations with low effective population size or lower levels of immigration could be at risk of over-exploitation with limited potential for recruitment from neighbouring populations.

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CHAPTER 1: INTRODUCTION

Concern over the removal of spawning stock and the effect of fishing on non-target species dates back to 1884 when Ray Lankester countered Thomas Huxley's famous statement that "probably all great sea-fisheries are inexhaustible". Lankester stated that "it is a mistake to suppose that the whole ocean is one vast store-house" and that fish removed by fishing activities had "a perfectly definite place in the complex interactions of the living beings in their area". Although it is almost 130 years since these concerns were raised by Lankester, the task of sustainably managing fisheries continues to prove difficult. In 2008, 32% of global capture fisheries were producing less than their maximum sustainable potential due to excess fishing pressure (FAO 2010) and 53% were fully exploited and were at, or close to, their maximum sustainable production with no room for further expansion. Only 15% of fisheries were considered able to sustainably produce more than their current catches (FAO 2010). In a study using scientific stock assessments of 166 global fish stocks, 63% had biomass levels which had dropped below the traditional target of maximum sustainable yield (MSY) and needed rebuilding (Worm *et al.* 2009). These figures highlight the need for improved management of commercial fish stocks in order to halt the decline in biomass and to rebuild collapsed stocks so they can reach their maximum production potential. Recently there has been a move to interpret the exploitation rate that achieves MSY as an upper limit compared to the traditional view of it as a management target (Worm *et al.* 2009) in order to decrease exploitation rates and allow collapsed stocks to rebuild. An important step in setting exploitation limits is the delimitation of the stock in question i.e. knowledge of the spatial scale at which a population will react independently from its neighbours to exploitation.

THE IMPORTANCE OF CONNECTIVITY AND SPATIAL SCALE IN FISHERIES MANAGEMENT

Many marine species have life history traits, primarily a pelagic larval phase, which predisposes them to dispersal over large distances. This combined with the perceived lack of boundaries in the ocean and large scale ocean currents potentially connecting geographically distant locations led to the perception that marine populations would be highly connected (e.g. Caley *et al* 1996; Roughgarden *et al* 1986). Some genetic studies (Fevolden *et al* 1986; Rosenblatt and Waples 1995) and passive particle tracking models

have supported the theory of open marine populations. However, recent advancements in genetics have allowed greater power to detect fine scale genetic structure (e.g. Knutsen *et al* 2003; Kenchington *et al* 2006; Mathews 2007) and the coupling of biological and behavioural traits with physical oceanography in particle tracking models have identified local larval retention mechanisms (Cowen *et al* 2006; Metaxas and Saunders 2009; Paris *et al* 2007), both of which suggest that marine organisms may not be as open and there may be less connectivity over large distances than that expected with long pelagic larval phases (Shanks 2009). Understanding the level of connectivity between is an essential step in delimitating stock structure and the sustainable management of fisheries stocks.

Marine protected areas

In the context of an ecosystem based approach to fisheries management Marine Protected Areas (MPAs) are considered to be one of a number of tools used for the protection of biodiversity and fisheries enhancement (Hastings and Botsford 2003). Potentially, protecting an area from fishing reduces mortality of non-target species and habitat destruction, can benefit “spillover” effects to commercially fished areas and may lead to a more resilient ecosystem that supports a more productive fishery. Such supposed benefits of a more productive fishery include; increases in yield outside the reserve through both export of larvae and adult emigration (Gaines *et al.* 2003; Hilborn *et al.* 2004), buffering against environmental and anthropogenic uncertainty (Hilborn *et al.* 2004), protection of genetic diversity of stocks (Bohnsack 1996) and improving age and size structure of a stock as fishing tends to target older and larger individuals resulting in low abundance of these groups (Roberts and Polunin 1991; Beukers-Stewart *et al.* 2005; Baskett *et al.* 2008).

Beneficial changes to target and non-target species have been demonstrated inside reserve boundaries; Increased abundance of target and non-target species (Roberts 1995; Halpern and Warner 2002; Beukers-Stewart *et al.* 2005, 2006; Kaiser *et al.* 2007); increases in local reproductive output (Kaiser *et al.* 2007); increases in the mean size or size at age of fish and invertebrates (Halpern and Warner 2002; Halpern 2003; Beukers-Stewart *et al.* 2005); increased larval recruitment (Howarth *et al.* 2011); and the increased survival and growth of juveniles (Beukers-Stewart *et al.* 2005). High densities of adults can also lead to an increase in the reproductive success of sedentary broadcast spawners, as close proximity to potential mates is essential for fertilisation (Levitan *et al.* 1992). However, whether or not these benefits extend outside reserve

boundaries is less well understood (but see Russ and Alcala 1996; Gell and Roberts 2003; Russ *et al.* 2003; Beukers-Stewart *et al.* 2006).

For benefits of an MPA to extend past its' boundaries there must be connectivity between the closed area and the fished grounds. This connectivity can arise from the movement of adults, that can result in fishers finding higher densities of larger fish close to the boundaries of the reserve (Roberts *et al.* 2001; Bohnsack 2011), but, as many marine species have a planktonic larval phase, the greatest potential for connectivity can be achieved via larval dispersal. This movement of larvae between MPAs and between MPAs and exploited grounds is critical to the success and sustainability of a reserve network and the extent to which it can replenish fished grounds (Carr and Read 1993; Shanks 2009). The extent of connectivity through larval dispersal is important when considering the spacing of MPAs in a network (Lockwood *et al.* 2002; Roberts *et al.* 2010) as the planktonic larval phase can serve to connect populations that lie many kilometres apart (Cowen *et al.* 2006a; Trembl *et al.* 2008; Shanks 2009; Roberts *et al.* 2010). The direct observation of microscopic larvae and their behaviour in situ is difficult to achieve; therefore scientists have often modelled larvae as passive particles in order to estimate their dispersal on currents. However, whilst propagules that remain in the plankton for longer periods have a greater potential to disperse larger distances, this distance is not always realised (Shanks *et al.* 2003; Shanks 2009). This can be due to local oceanographic conditions retraining the larvae in eddies and gyres (Paris *et al.* 2007). In addition larval behaviour affects the distances dispersed compared to those expected of passive particles. For example *Mytilus* spp. in the Irish Sea have been shown to predominate in the water column during the flood tide compared to the ebb tide during which they are found closer to the seabed where the slower current is. This would act to keep them closer to shore but also, in the case of the flood tidal flow in the Irish Sea, transport them in a net northerly direction (Knights *et al.* 2006). When foraging behaviour is taken into account in dispersal models, distances travelled can be 100s km less than when it is not (Woodson and Mcmanus 2007). Vertical movement in the water column affects the direction and distance over which larvae are transported. Where vertical movement is taken into account in dispersal models, the reefs on which coral reef fish settled were closer to their natal reefs than when this behaviour was ignored (Paris *et al.* 2007). The ability of larvae to affect their dispersal means that many models that treat larvae as passive particles may be highly inaccurate. This has led scientists to

improve the biological coupling of oceanographic models (e.g. Emsley *et al.* 2005; Paris *et al.* 2007; Edwards *et al.* 2008) and to seek other indirect methods of measuring dispersal, for example elemental tagging (Becker *et al.* 2009) and genetic connectivity (Kinlan and Gaines 2003).

Another important aspect that determines the outcome of implementing an MPA relates to spatial scale and this has resulted in a number of studies investigating the optimal size of MPAs (e.g. Carr and Read 1993; Halpern 2003; Hastings and Botsford 2003) . An MPA needs to be large enough to afford sufficient protection for the target species and for its associated ecosystem, this is especially true for more mobile species that spend a large proportion of time outside the reserve boundaries. In general the larger the MPA the more species can be afforded conservation benefits from its implementation (Moffitt *et al.* 2011). A single large reserve may be cheaper to enforce than multiple smaller reserves. However, large reserves are less likely to export larvae unless larval dispersal distance is large. Concomitantly, converting large areas of the sea bed and coastline into MPAs means a loss of large amounts of available fishing grounds. A single large reserve will focus the loss of fishing ground on a single area of coast that will impact specific fishers from that part of the coastline who would then have to travel long distances to be able to fish. For this reason, single large MPAs are therefore unlikely to be implemented, particularly in coastal areas. Smaller reserves have been shown to be beneficial for sedentary and less mobile species such as coral reef fishes (Roberts 1995; Russ and Alcala 1996; Russ *et al.* 2003), scallops (Murawski *et al.* 2000; Beukers-Stewart *et al.* 2004, 2005, 2006; Kaiser *et al.* 2007; Howarth *et al.* 2011), lobsters (Rowe 2002) and abalone (Wallace 1999), and small reserves protecting vulnerable life stages of more mobile species could also prove to be beneficial. For example juvenile reef sharks show high site fidelity and could therefore benefit from spatial protection during the young phase of their life cycle (Garla *et al.* 2005). Larval export between MPAs or to exploited grounds has greater potential to occur from smaller reserves than a single large reserve; however they are less likely to show self recruitment and to be self sustaining. Therefore a network of MPAs, close enough together so they can replenish each other via adult or larval connectivity, (Moffitt *et al.* 2011) or maintenance of sufficient spawning stock biomass outside MPAs would be necessary for sustainability. To this end, knowledge of the extent of larval dispersal and connectivity between networks of MPAs and exploited

grounds for the target species is therefore essential for the success of an MPA for fisheries management purposes.

Biologically relevant management units

The degree of connectivity can also affect the appropriate spatial scale at which fisheries managers should apply regulations to the management of a fishery (Fogarty and Botsford 2007). In order to achieve a sustainable yield a manager has to define the quantity of a population that can be harvested without compromising its ability to sustain the population, i.e. total mortality (fishing mortality plus natural mortality) should not exceed recruitment. This requires knowledge of the scale at which a population reacts independently to exploitation from neighbouring populations – the stock concept. There are many definitions of a stock and these generally run along a continuum from the harvest stock (low level of integrity where abundance depends more on recruitment and mortality than immigration and emigration) to a genetic stock (high levels of integrity creating a reproductively isolated unit, genetically different from other stocks) (Carvalho and Hauser 1994). These definitions, although from two ends of a spectrum, are based on biological processes. However, all too often there is a mismatch between these biological management units and the realised management units, that have to include social, economic and political factors (Reiss *et al.* 2009). Consequences of this mismatch can be minimal if a panmictic population is managed as smaller units, however if structured stocks are managed as a single group then there are risks of overharvesting on a local scale (Reiss *et al.* 2009). In a structured stock some subpopulations will be more vulnerable than others to exploitation due to differing levels of connectivity between the subpopulations within the metapopulation. It is therefore important to identify the scale at which subpopulations exist and manage stocks accordingly.

ASPECTS OF SCALLOP BIOLOGY AFFECTING CONNECTIVITY

Pelagic Larval Duration

Oceanographic models that predicted dispersal distances of larvae using passive particles to represent the larvae have a tendency to overestimate dispersal for species with longer pelagic larval duration (PLD) (Shanks 2009). It has been suggested that larval behaviour and life-history traits might be the primary factor in this discrepancy; larvae do not act as passive particles at the mercy of large scale ocean currents but have the ability to affect their dispersal by taking advantage of current changes with depth, tides and local eddies

and gyres. PLD can vary between species but can also vary between intra-specific individuals, with variation in environmental conditions, especially temperature considerably affecting PLD. Temperature is fundamental for larval development, growth and subsequently PLD. PLD is negatively correlated with water temperature across a wide range of taxa (O'Connor *et al.* 2007). Laboratory experiments have shown that low water temperature will increase larval duration in Pectinids (Beaumont and Budd 1982; Beaumont and Barnes 1992; Cragg 2006); species from warm waters show PLD of 10 days (*Chlamys nobilis* at 24.5°C (Ventilla 1982) compared to cold water species such as *Chlamys islandica* (7°C) that has a larval duration of 70 days (Gruffydd 1976). A linear regression of this relationship of PLD with temperature for the family Pectinidae is significant, with an R^2 value of 0.83 (Cragg 2006). *Pecten maximus* experiences high mortality at 8°C and 9°C compared to higher temperatures (Beaumont and Budd 1982; Beaumont and Barnes 1992). The few larvae surviving the low temperatures took a minimum of 65 days to reach metamorphosis and the average PLD at 8°C was estimated to be 78 days (Beaumont and Barnes 1992). The time to metamorphosis decreased to 24 to 35 days at 18°C and the mortality of scallop larvae also decreased (Beaumont and Barnes 1992). The mean larval life span of *P. maximus* larvae from adults spawned in the Baie du St Brieuc and Baie du Brest, France and then reared at 18°C was shown to be just 16 and 19 days respectively (Paulet *et al.* 1988) and the average for hatchery-reared larvae at 18°C (from D-larvae stage, not gamete emission) is 25 days, based on 14 years worth of data (range 18-32 days) (Robert and Gerard 1999). The embryonic phase usually lasts approximately 2 days (Robert and Gerard 1999) so these estimates would be equivalent to 20 -34 days for the entire pelagic phase at 18°C, which is in agreement with the estimates of Beaumont and Barnes (1992). *In situ* estimates of PLD in *P. maximus* range from 18-42 days (Le Pennec *et al.* 2003) but it is not clear at what temperature these estimates were made. These estimates were derived from scallops in the Baie du Brest and Baie du Sainte Brieuc, France. In the Baie du Brest, scallops “trickle spawn” from May to September, whilst in Baie du Sainte Brieuc the main spawning event in this area is triggered when water temperatures reach 15.5 – 16 °C, usually in July (Paulet *et al.* 1988) with the temperature rising throughout the larval phase. These *in situ* estimates for PLD are therefore lower than for populations in the more northerly extent of *P. maximus*' range where the sea temperature is lower. For example, in waters off the Isle of Man, there is a bimodal spawning season with a peak in April / May, small amounts of spawning in June/ July followed by a second peak at

the end of August/ September (Mason 1957; Mason 1958). During this period the water temperature ranges from a low of 8°C in April to a high of 15°C in August (Figure 1) At 8°C larval mortality would be extremely high (Beaumont and Barnes 1992) and the time to metamorphosis around 78 days (Table 1). During this 78 day period the temperature would increase to around 11°C by June (Figure 1), but the time to metamorphosis is still likely to be greater than 50 days (Table 1). During the second peak spawning the temperature will have increased to approximately 14 – 15°C and so PLD will decrease to around 31-44 days and mortality should also decrease. Therefore as well as water temperature varying between geographically distinct populations, spawning events that take place at different times of the year will expose larvae within the same population to different sea temperatures and therefore differences in PLD will be evident throughout the spawning season. Although extended PLD allows for connectivity between more geographically distant populations as the larvae are carried further from their natal habitat there can be a greater chance that they will not encounter suitable settlement habitat in their settlement time window (Siegel et al. 2008). Longer PLD also increases the time exposed to predators and therefore mortality rates increase.

Table 1.1 Estimates of Pelagic Larval Duration for *Pecten maximus* at varying water temperatures.

Temperature (°C)	Pelagic Larval Duration (Days)	Reference
9	78	(Beaumont and Barnes 1992)
12	40-51	(Beaumont and Barnes 1992)
15	31-44	(Beaumont and Barnes 1992)
16	33-38	(Gruffydd and Beaumont 1972)
17	32	(Beaumont and Budd 1982)
18	24-35 / 20-34	(Beaumont and Barnes 1992) / (Robert and Gerard 1999)

Larval Behaviour

Pectinid larvae have been shown to exhibit diel vertical migration (DVM) both in mesocosm experiments (Gallager *et al.* 1996; Manuel *et al.* 1996a,b) and in the field (Tremblay and Sinclair 1990a). *P. maximus* larvae occurred in higher densities near the surface of 4 m deep culture bags when light intensity decreased and this suggested DVM (Kaartvedt *et al.* 1987). Ontogenetic vertical migration (OVM) has been demonstrated for *Placopecten magellanicus* in mesocosms with smaller larvae (< 200 µm) being found above the thermocline and only the larger larvae being able to penetrate the thermocline (Gallager *et al.* 1996). Alteration in swimming behaviour leading to changes in vertical

distribution at different larval stages has been demonstrated in laboratory experiments for *P. maximus*. Early veligers swim vertically upwards, later stage veligers alternate swimming and sinking behaviours but remain in the water column and pediveligers spend increasing amounts of time exploring the bottom of culture tanks (Cragg 1980). Spending time close to the seabed will decrease the distance dispersed as the currents are diminished exponentially towards the boundary layer. Manuel *et al.* (1997) carried out experiments on tidal vertical migrations in *P. magellanicus* where mesocosms were used to look at vertical migrations on both diel (24 hour) and tidal (12.42 hour) temporal cycles. Manuel *et al.* (1997) studied larvae from two populations of scallops and found that the behavioural response to tidal cues was different for the larvae from the two populations. Given the differences in current and tidal regimes that occur at the two locations where the populations occurred, modelling showed that the differences in larval behaviours between the two locations resulted in larval retention in the local area in both cases rather than being dispersed on strong tidal currents (Manuel and O’Dor 1997). Tremblay and Sinclair (1990b) showed that *P. magellanicus* larvae showed vertical tidal migration. These observations were made in a stratified water column where the depth of the pycnocline changed with tidal flow and the larvae were often found to be aggregated above the pycnocline which is a zone of high primary productivity. However in the same study in a weakly stratified or well mixed water column there was no evidence of vertical stratification of larvae (Tremblay and Sinclair 1990a; Tremblay and Sinclair 1990b). Therefore it is important make a distinction between migration in response to tidal flow or in response to changes in the occurrence or position pycnoclines and thermoclines that occur with the tide, as models including such behaviour would depend on the water stratification specific to the modelled sites.

Metamorphosis, settlement and juvenile habitat

Following the pelagic phase Pectinid larvae metamorphose to become benthic juveniles. Juvenile habitat varies between species but many tend show a stage of attachment to benthic epifauna, even if their later life stages are free living. For example *Argopecten irradians* have been shown to settle on seagrasses (Eckman 1987), *Chlamys islandica* have a preference to settle on the perisacs of the dead hydroid *Tubularia larynx* (Harvey *et al.* 1993, 1995), *Aequipecten opercularis* shows a preference for pristine live maerl over dead maerl, sand or gravel in mesocosm experiments (Kamenos *et al.* 2004) and have been found in large numbers on *Laminaria sacharina* (Mason 1958) and the bryozoan *Cellaria fistulosa* in the field (Brand *et al.* 1980). Post-larva *P. maximus* have

proven to be much more cryptic. Mason (1958), although finding large numbers of *A. opercularis*, found only two *P. maximus* spat during a two year study. These individuals were found attached to algae of *Desmarestia* sp. Brand *et al* (1980) found large amounts of *A. opercularis* over a two year sampling period and were able to identify substratum preferences and settlement periods, however during the same study only small numbers of *P. maximus* were found and these were insufficient to identify settlement habitats or patterns. It has been suggested that the delicate shells of post-larval and small juvenile *P. maximus* makes dredging and other destructive sampling methods unsuitable for this species as the shells are destroyed during sampling (Brand *et al.* 1980). During video and photography sampling post-larvae (<2mm) *P. maximus* were seen attached to colonial hydroids in the western English Channel and larger spat (7-15mm) were found attached to algae and free-living on gravels within 0.5km of the coast by divers (16-22m depth) (Dare and Bannister 1987). However, no *P. maximus* spat less than 20mm were found free-living on the sea-bed in the offshore fishing grounds so it appears that the spat settle in shallower inshore areas possibly attached to algae, hydroids or other benthic epifauna, where they will be silt free, and then have to re-disperse to the adult offshore grounds. How the juvenile scallops achieve this is not clear in the literature. One possibility is byssus drifting where by the scallop, once in the water column, secretes a byssus thread in order to slow down its sinking rate and increase the time it is influenced by ocean currents and thereby enhancing dispersal. *A. opercularis* has demonstrated this behaviour in laboratory experiments up to a size of 1.4mm and so could be a mode of transport from early post-larval settlement to juvenile and adult habitats (Beaumont and Barnes 1992). However, in the same study *P. maximus* only demonstrated this behaviour up to a size of 0.5mm, and as much larger spat have been found attached to benthic epifauna (Dare and Bannister 1987) then either the scallops are capable of byssus drifting for a longer period than was demonstrated in the laboratory experiments or another mode of dispersal would be needed. However, studies on other bivalve species have shown byssus drifting occurring up to at least 2.5mm (Baldur Sigursson *et al.* 1976). Byssus drifting increases drag by a factor of three and in some cases up 20 times (Baldur Sigursson *et al.* 1976), such that a current of only 1 cm s⁻¹ is required to re-suspend a small bivalve, therefore despite the lack of direct evidence for *P. maximus*, suggests byssus drifting may represent an effective dispersal method.

POPULATION GENETICS

The definition of a population or stock is broad and includes a wide range of features (Carvalho and Hauser 1994; Waples and Gaggiotti 2006). As discussed previously these definitions can vary along a continuum from a harvest stock which has relatively low integrity up to the genetic stock which has very high integrity (Carvalho and Hauser 1994). The identification of genetic stocks can therefore provide fisheries managers with information about the stock structure at one end of this continuum.

There are a variety of interacting evolutionary forces which drive the patterns of genetic variation that are considered in this thesis; some of these forces will act to link populations together thereby homogenising genetic variation whilst others will drive genetic differentiation between populations.

Hardy-Weinberg Equilibrium

In 1908 G.H. Hardy and Wilhelm Weinberg both independently discovered a generalised principle to describe the allele frequencies in a randomly mating population; The Hardy-Weinberg Equilibrium (HWE). The principle states that:

“single-locus genotypic frequencies after one generation of random mating can be represented by a binomial (with two alleles) or multinomial (with multiple alleles) function of the allelic frequencies” (Hedrick 1985).

So for a population with alleles A and B that are found in the proportions p and q respectively, after one generation of random mating the three possible genotypes, AA, BB and AB, will be in the proportions p^2 , q^2 and $2pq$ respectively. In contrast to most evolutionary equilibria, HWE can be achieved in a single generation making it a very useful principle in population genetics (Crow 1986). The HWE principle can be extended from the two allele example above to multiple alleles by the following rules (Crow 1986);

“The frequency of any homozygous genotype is the square of the corresponding allele frequency”

“The frequency of any heterozygous genotype is twice the product of the two component allele frequencies”

The assumptions of HWE are that there is random mating; mates are chosen without regard to genotype **at the locus under consideration** (Crow 1986) and the allele frequencies in the parent population are the same in both sexes. There should also be an absence of factors which can alter allele frequencies; natural selection, mutation, migration and genetic drift.

If there are several isolated geographical populations, each of which mates at random, then, although each individual population may exhibit HWE, the total population will not. This is called the **Wahlund effect**. The same phenomenon occurs if the allele frequencies vary between age groups (Crow 1986) as can happen if there is an influx of migrants of a particular age or with sweepstake recruitment.

Random genetic drift

Random, chance changes in allele frequencies can occur from generation to generation in all finite populations. These chance events are emphasised in small populations due to the fact that individuals are much more likely to be related to each other when the population is small, even if random mating occurs (Crow 1986). These chance changes lead to fluctuations in the allele frequencies and even the loss of some alleles and fixation of others. This is called **random genetic drift**. With random genetic drift there is a loss of heterozygosity from generation to generation that would imply that, given enough generations, a population would eventually become homozygous. In nature populations do not become completely homozygous. This is due to new mutations adding to the heterozygosity of the population and eventually the rate of loss of heterozygotes will reach an equilibrium with the rate of gain due to mutation; **Mutation Drift Equilibrium**. A general equation for the loss of heterozygosity was first derived by Sewall Wright:

$$Het_t = \left(1 - \frac{1}{2N_e}\right) Het_{t-1}$$

Where Het_t = heterozygosity at time t , N_e is the effective population size and Het_{t-1} is the heterozygosity in the parental generation. This equation can be altered to include a mutation rate term to estimate the expected heterozygosity of a population in mutation drift equilibrium (Crow 1986):

$$H \approx \frac{4Ne\mu}{4Ne\mu+1}$$

Where μ is the mutation rate. It should be noted that these equations assume that each mutant is a new allele and that the alleles are selectively neutral, this may not be strictly true for real populations. Also, note the inclusion of effective population size rather than actual population size. According to Crow (1986);

“The assumption that a gene is equally likely to come from any parent does not mean that each parent produces exactly the same number of progeny, for there will be random variability, but that each parent has the same expected number. In this idealised population the number of progeny per parent will have a binomial distribution. Most actual populations depart from this ideal. Therefore we define the effective population number, N_e , as the size of an idealised population that has the same probability of identity as the actual population being studied”

Important factors that affect the effective population size include the ratio of reproductively successful males to females, and an imbalance decreases the effective population size. An imbalance in the reproductive success of one individual *c.f.* another will decrease the effective population size. This is particularly important in broadcast spawning marine invertebrates for which variation in environmental variables can affect both the fertilisation success and the survival rate of larvae such that a few individuals can be reproductively highly successful compared to others (Hedgewood 1994).

Migration

When an organism is subdivided into different sub-populations, the connectivity levels between them due to migration determine whether their allele frequencies are homogenised or differentiate due to mutation and genetic drift. As mutation rates are generally very low the amount of differentiation is generally driven by the amount of genetically effective migration or **gene flow**. Gene flow is the amount of migration that is associated with the exchange of alleles of genes. High levels of gene flow will homogenise allele frequencies and the total population approximates a single panmictic unit, whilst in sub-populations where there is little gene flow their allele frequencies may

be quite different (Crow 1986). The amount of gene flow needed to homogenise allele frequencies is actually very small, anything more than one migrant per generation will lead to very little differentiation (Crow and Kimura 1970). This assumes that the immigrating genes are completely different to the local population. So if migration occurs between neighbouring populations, for which the genes are expected to be similar, then this number will likely need to be higher to maintain panmixia (Crow and Kimura 1970).

Mutation

In loci where multiple alleles are possible (e.g. microsatellites) then mutation will tend to increase the number of alleles whilst random genetic drift will tend to decrease the number of alleles (Hedrick 1985) and mutation drift equilibrium can be reached as discussed above. At mutation drift equilibrium (under the infinite allele model) the proportion of heterozygotes can be given by:

$$H = \frac{4Ne\mu}{4Ne\mu + 1}$$

where μ is the mutation rate. In general, if $Ne\mu$ is > 1 then mutation determines the amount of heterozygosity in a population, whereas if $Ne\mu$ is < 1 then random genetic drift determines heterozygosity and H will tend to be low (Hedrick 1985).

Molecular markers

A molecular marker is a portion of DNA or protein that has variation in its DNA sequence and consequently has at least two variants or alleles. In diploid eukaryotic organisms, individuals will be genotyped as homozygous (identical alleles) or heterozygous (two different alleles) for each marker. They can therefore be used to reveal genetic differences or similarities. If molecular markers are neutral (not subject to natural selection) then these differences can be used to analyse speciation and population structure as, exchange of genes through migration homogenises allele frequencies (Balloux and Lugon-Moulin 2002) whereas isolation allows differentiation of allele frequencies due to genetic drift. Loci subject to natural selection make it very difficult to analyse population structure (Hellberg *et al.* 2002) as differences in allele frequencies may be due to adaptation to different environments even though migration is occurring.

Microsatellites

Microsatellites are short tandem mono-, di-, tri-, tetra- or penta-nucleotide repeats. Primers or oligonucleotides, usually around 19-22 bases in length, are designed for the sequence in the regions flanking either end of the repeat unit. These can then amplify the microsatellite using the Polymerase Chain Reaction (PCR). Microsatellite alleles appear as differences in the length of the repeat unit and can be highly polymorphic. The size of the alleles can be identified using gel electrophoresis or capillary electrophoresis. Microsatellites are codominant (both alleles can be visualised) and plentiful in the nuclear genome. Microsatellites are thought to be primarily neutral but some trinucleotides have been associated with human diseases (Sutherlands and Richards 1995) and microsatellites have also been found to occur within protein-coding genes and the untranslated region where mutations may cause loss of gene function or change in gene expression (Li *et al.* 2004). Microsatellites are thought to mutate primarily via slippage at DNA replication whereby the repeat unit is lengthened or shortened by one unit (stepwise mutation model –SMM)(Balloux and Lugon-Moulin 2002). Replication slippage occurs much more frequently than point mutations and as such microsatellites are estimated to have mutation rates in the order of 10^{-5} - 10^{-2} mutations per locus per generation (Jarne and Lagoda 1996). However studies have shown that larger mutational steps are possible where more than single repeat units can be added or subtracted (Weber and Wong 1993). Under this K-allele model different probabilities can be given to the different step lengths such that the probability of mutations of a single repeat unit is more likely than a mutation of multiple units. However, point mutations in the flanking regions or within the microsatellite itself, causing it to be broken in two or “interrupted” mean that microsatellites rarely follow either of these two models strictly and the infinite allele model, where mutation to any allele is equally probable, performs well in most cases (Balloux and Lugon-Moulin 2002; Estoup *et al.* 2002). Microsatellites have become the marker of choice for many studies in population ecology. The higher mutation rates not only illuminate shallower divergences but also provide higher levels of variation giving improved statistical power in population structure studies (Hauser and Seeb 2008).

The main difficulties associated with microsatellites are the occurrence of null alleles and homoplasmy. Mutations in the primer region can cause the loci to fail to amplify and cause null alleles. In this situation a homozygote null allele will fail to amplify and a heterozygote will appear to be a homozygote for the non-null allele. This will cause a deviation from HWE due to an apparent excess of homozygotes. This can cause a loss of

confidence and power in many of the statistical analyses for which HWE is a core assumption. However, it appears that some methods can be fairly robust to null alleles (Carlsson 2008). Homoplasy occurs when two alleles that are identical in length are not identical by descent i.e. they occurred by independent mutation events. This would lead to an underestimation of population differentiation, however the high levels of variability at microsatellite markers generally compensates for size homoplasy in population differentiation studies (Estoup *et al.* 2002).

AIMS AND THEMES OF THE THESIS

Connectivity and the spatial scale of population dynamics are important for defining the scale at which fisheries management should occur. Defining appropriate management units is a difficult task and although genetic data can provide useful insights into the population structure of marine organisms it is not a panacea and genetic tools should not be used in isolation but integrated with other data for best results, especially when the underlying genetic structure is weak (Selkoe *et al.* 2008). This study aims to gather data on the connectivity and spatial structuring of the commercially important Bivalve, *Pecten maximus*. The intention is to shed light on the scale at which reproductively isolated populations reside and also biological data on the spatial scale at which variation in the reproductive schedules occurs. The main aims and objectives of the thesis are:

1. To evaluate the small scale spatial variation in the reproductive conditioning of *Pecten maximus*: This was achieved by scallop sampling from ten sites around the Isle of Man in the Irish Sea and examining reproductive conditioning. Environmental drivers of spatial differences are also examined. The results are presented in chapter two.
2. To develop a suite of microsatellite markers for *Pecten maximus*: This development methodology is described briefly in chapter three and in more detail in the supplementary information.
3. To examine the population structure of *Pecten maximus* at a variety of spatial scales throughout Europe: Sampling locations are located at a small spatial scale within the Irish Sea, then at a larger spatial Scale around the UK, increasing to European wide and finally to include the sister species *Pecten jacobaeus* in the Mediterranean. The results are presented in chapter three.

Genetic analyses are expensive, so as much information as possible was analysed from the genotyped microsatellite data. This gave rise to some objectives that were not directly associated with the main theme of connectivity and spatial scale issues but can still provide useful information for fisheries managers:

4. To estimate effective population sizes, demographic events and levels of inbreeding and relatedness in populations of *Pecten maximus*: This data are presented alongside the population genetic data in chapter three.
5. To give advice on the genetic consequences of stock enhancement using hatchery seed: Samples from the wild populations of *Pecten maximus* from the Isle of Man were compared to those obtained from a hatchery. The results are presented in chapter four.

CHAPTER 2: SPATIAL VARIATION IN THE GONAD CONDITION OF THE SCALLOP *PECTEN MAXIMUS* L.

ABSTRACT

Small spatial scale variation in the gonad conditioning and the onset of spawning of *Pecten maximus* was inferred from interviews with scallop fishers and subsequently verified by empirical observations. The potential drivers (environmental and fishing) of these spatial patterns were then investigated using a generalised additive modelling approach. The gonad wet weight, gonad observation index, shell size and scallop ages were recorded for scallops collected from ten different sites around the Isle of Man (IOM). The gonad conditioning Index was calculated from this data. Environmental data was collected from NEODAAS satellite images and from modelled physical data. Fishing intensity was estimated from vessel monitoring system data. In general the

fishers' observations were accurate and validated by the current study. Statistically significant differences in the gonad condition of scallops over small spatial scales (< 5 km) were found but these spatial patterns varied temporally over 13 months. The within site variation in gonad condition was also high, indicating a “bet hedging” reproductive strategy. Long term drivers of gonad weight were identified as average annual chlorophyll a concentration, scallop density, stratification index and shell size. The rate of change in temperature over the month prior to sampling was identified as the short term driver of gonad wet weight associated with the autumn spawning event. The GAM explained 42.8% of the deviance in gonad weight. An increase in shell length from 100mm to 110mm equated to an increase of approximately 20% in gonad weight. In the IOM protecting scallops from fishing mortality until 110mm (age 4) compared to 100mm (age 3) may lead to an overall increase in lifetime reproductive output of 3.4x. The “bet hedging” reproductive strategy can decrease the chance of fertilisation especially at low densities found on commercial scallop grounds and therefore, areas protected from fishing where scallop densities can increase may help to buffer against reproductive failure and the allee effect.

2.1. INTRODUCTION

Local Ecological Knowledge (LEK) or Traditional Ecological Knowledge (TEK) has increasingly been used in ecology, conservation and fisheries management. Examples include estimates of abundance (Anadón *et al.* 2009), ecology and reproduction in fish (Silvano and Begossi 2005), essential fish habitat (Hilborn *et al.* 2004), tidal and lunar movements and aggregations of fish (see Johannes, Freeman, & Hamilton (2008)) and the location and depletion of spawning aggregations of Grouper (Serranidae) (Johannes *et al.* 1999; Hamilton *et al.* 2005). The use of LEK can also help to focus scientific effort to the appropriate sampling locations and seasons, and thereby improve efficiency (Davis, Hanson, Watts, & MacPherson, 2004; Johannes *et al.*, 2008), avoid missing important biological and ecological changes (Johannes *et al.* 2008) and help develop hypotheses.

The king scallop, *Pecten maximus* is a functional hermaphrodite of commercial importance, found in European waters from Norway to Spain with landings of

approximately 60,000 tonnes in 2009 (FAO 2012). Scallops are the third most valuable fishery in the UK (MMO 2012) of which the main commercial species is *P. maximus*. In the Isle of Man *P. maximus* accounts for over 65% of all fishery income (Beukers-Stewart *et al.* 2003). Previous work has demonstrated considerable variability in the timing and synchronisation of spawning over large spatial scales (100s of kms) (Mason 1958; Paulet *et al.* 1988; Cochard and Devauchelle 1993; Mackie and Ansell 1993). Although one might expect less variation in reproductive schedules at smaller scales, fishers will often target a particular scallop ground with good quality roe until the scallops spawn and then move on to another ground just tens of kms away which are yet to spawn (Fishers, IOM, Pers. Comm.). The higher market value of good quality roe ensures that fishers are sensitive to the gonad maturation state and therefore their knowledge on reproductive schedules could be valuable for scallop and fisheries biologists. If reproductive variation is present on such a small spatial scale this provides challenges for effective management of the stock and understanding this variation and the parameters driving it is essential and periods of spatially favoured fishing at a time of gonad ripening could also make the fishery more susceptible to collapse.

P. maximus first show signs of gametogenic maturation in the second year of growth (Mason 1958; Devauchelle and Mingant 1991), although an adult pattern of spawning may not develop until their fourth year of growth (Mason 1958). *P. maximus* is reported to exhibit several patterns of spawning: continual partial spawning from spring until early autumn; bimodal peaks in spawning (spring and autumn) or a single synchronised spawning event (Mason 1958; Paulet *et al.* 1988; Pazos *et al.* 1996). These strategies seem to be genetically mediated (Cochard & Devauchelle 1993, Mackie & Ansell 1993) although environmental conditions appear to alter the timing and onset of maturity and spawning (Magnesen and Christophersen 2008). Understanding reproduction in *P. maximus* is important for a number of reasons. Market value increases with high quality roe attached hence the timing of harvesting affects the profitability of the fishery. Variation in reproductive schedules, driven by climatic variation and hydrographic conditions, can affect the consistency of recruitment (Orensanz *et al.* 2006). Therefore management of recruitment-limited fisheries, such as the Isle of Man king scallop (Beukers-Stewart *et al.* 2003), requires in-depth knowledge of factors that influence reproductive ecology.

Explanatory variables for the onset of spawning

Changes in water temperature or temperature threshold are potential triggers for the onset of gonad maturation and spawning in different Pectinid species (Paulet *et al.* 1988; Pazos *et al.* 1996). In many populations, spawning coincides with different temperatures and with both rising temperatures in the spring and falling temperatures in the autumn (Mason 1958; Pazos *et al.* 1996). Hence, it is probable that change in temperature *per se*, rather than absolute temperature is a key factor in the onset of the rate of spawning.

Microalgae are the main energy source that are required for scallop growth, including gonad maturation (Farías and Uriarte 2006), and food availability has been suggested as a major driver of reproductive cycles in marine invertebrates. Starr, Himmelman, & Therriault (1990) found that there was a direct coupling of spawning and phytoplankton concentration in green sea urchins and blue mussels. Mussels and urchins spawned progressively earlier with increasing phytoplankton concentrations. The timing of phytoplankton blooms is dependent on a number of different interacting environmental variables beyond simple increases in water temperature. The timing of spawning in *P. maximus* has been linked to blooms of phytoplankton in Galicia, Spain (Pazos *et al.* 1996). However, energy reserves stored in the adductor muscle and digestive gland can also be used for gonad conditioning in the absence of food (Sastry and Blake 1971; Pazos *et al.* 1997).

Another driver of energy allocation is fishing pressure. It has been shown that scallops exposed to physical disturbance from regular commercial fishing have lower gonad weights in relation to shell size than their counterparts in protected waters (Beukers-Stewart *et al.* 2005; Kaiser *et al.* 2007). Scallops in fished areas may have to allocate energy to repairing tissue and shell damage caused by contact with the towed gear and hence there is less energy for the production of reproductive tissue in closed areas (Beukers-Stewart *et al.* 2005; Kaiser *et al.* 2007).

The present study aimed to i) understand, from fishers' observations, the spatial patterns of spawning around the Isle of Man ii) to test the validity of these observations using independent scientific sampling iii) to explain these observations in terms of key environmental and fishery drivers.

2.2. METHODS AND MATERIALS

2.2.1 Fishers questionnaires

A questionnaire was designed to capture fishers' knowledge of the timing of and patterns in the spawning of *P. maximus*. Questions were administered by face to face interviews. A total of 8 out of 26 (31%) full time Manx scallop fishers completed questionnaires. Each questionnaire consisted of five closed answer questions with tick boxes for answer choices, one question for which fishers were asked to label a map showing timing of spawning in different areas, and a finally an open comments section. One of the closed questions included a picture to visualise a particular gonad maturation stage (recovering stage IV).

2.2.2 Study Site and sampling

In the present study, ten commercial scallop beds in the waters around the Isle of Man, Irish Sea were sampled across a period of 13 months (Figure 2.1): Bradda Inshore (BRI), Bradda Offshore (BRO), Chickens (CHK), East of Douglas (EDG), Laxey (LAX), Peel (PEL), south of Port St Mary (PSM), Ramsey (RAM), South East Douglas (SED), Targets (TAR). These sites vary in their depths (21m - 69m), oceanographic regime (mixed and stratified water columns) and fishing intensity.

Sampling took place in mid October 2009 and 2010. The timing of the sampling was chosen to coincide with the autumn period of gonad maturation and spawning. Sampling was also carried out in June 2009 and 2010 to provide data during a non-spawning period. Scallops were sampled as per Murray *et al* (In review) which was an adaptation of the methods used by Beukers-Stewart *et al* (2003). For each tow of 20 minutes duration, the start and end GPS points were recorded, this data, along with the width of the dredges, allowed calculation of the area swept. The total number of scallops from each dredge, in each tow, was weighed and a subsample of 40 scallops were weighed and measured. An estimate of the abundance was made using the subsample weight to extrapolate to the total weight for each dredge. Density was then calculated using the abundance and area swept data (width of dredge x length of tow). A sample of at least 50 scallops were set aside for gonad maturation analysis from each site and where possible 50 scallops from each of the age groups three years, four years and five years plus were sampled. Scallops younger than three years are considered virgin or juvenile (Mason 1958).



Figure 2.1. Survey sites around the Isle of Man in the Irish Sea. Site Codes: Bradda Inshore (BRI), Bradda Offshore (BRO), Chickens (CHK), East of Douglas (EDG), Laxey (LAX), Peel (PEL), South of Port St Mary (PSM), Ramsey (RAM), South East Douglas (SED), Targets (TAR)

2.2.3 Gonad Maturation State

Scallops were sampled from each of the 10 sites. Scallops were dissected on the day of capture and the gonad frozen for wet weight determination back at the laboratory ($\pm 0.01\text{g}$). Shell height ($\pm 1\text{mm}$) was recorded on board the research vessel. The Gonad Observation Index (GOI), as described by Mason (1958), was also recorded. This index categorises a scallop gonad into one of seven stages. Stages one and two relate to virgin

scallops, stage three is the first stage of recovery following spawning, stage four and five are filling, stage six is full and stage seven is a spent gonad. We also calculated the Relative Gonad to shell Height (RGH) index which standardises the gonad weight in relation to the size of the scallop:

$$\text{RGH} = (\text{gonad weight (g)} / \text{Shell Height (mm)}) * 100 \quad \text{Eq. 1}$$

2.2.4 Environmental Covariates

The samples of scallops examined for gonad condition were sampled from specific sites around the Isle of Man. The condition and maturity state of the scallops at each site will reflect the circulation history of food availability at that site (Chlorophyll a concentration (Chla)) and exposure to physiological drivers (e.g. water temperature). To understand how spawning may be influenced by these overlying hydrographic conditions, monthly composite sea surface temperature (SST) and Chla data was obtained from NEODAAS for the period of November 2008 to October 2010. The NEODAAS data was in the format of geo-referenced raster files spatially resolved at a scale of 1km² (SST) and 500m² (Chla). Sedentary organisms will be influenced by a body of overlying water at a scale of a least one tidal excursion. Therefore the value for each environmental variable, in each month, for each site was extracted from the raster layer as the average across an area that corresponded to that of a tidal ellipse. This was carried out using ArcMAP v9.3. The ellipses were drawn as a polygon layer in ArcGIS v10. The depth-averaged, principle lunar semi – diurnal (M2) current vectors (ms⁻¹) (north and east components) and the angle of the major vector were obtained from a model of the Irish Sea (Neill *et al* 2008) for the point nearest to each site reference position. These vectors were converted to distances (m) for the semi-major and semi-minor axes of an ellipse using the tidal excursion time of 12 hours 26 minutes and oriented to the angle of the major current vector. Within the area of this ellipse the average SST for the months of September and October 2009 and 2010 was calculated which allowed calculations of the temperature change over the month prior to sampling of the scallops. The average chlorophyll concentration in each tidal ellipse, for each month in the year prior to sampling were extracted using ArcGIS. This allowed calculation of the mean chlorophyll a concentration for each site for the 12 months prior to scallop sampling. It was anticipated that change in temperature would relate to short-term responses in gonad

condition associated with the spawning event whereas average annual Chla concentration would relate to a longer term response.

Scallops are benthic species, hence a stratification index (SI) was also calculated (Eq 2). In a mixed water column SST will accurately represent bottom temperatures but in stratified waters the bottom temperature will be lower than SST.

$$SI = \text{Log}_{10}(u/h) \quad \text{Eq. 2}$$

Where u is the modelled, M2, depth averaged tidal current (ms^{-1}) (Neill *et al* 2007) and h is depth (m).

Water depth was recorded at the time of sampling scallops and was corrected for tidal state using WXTide32 version 4.7 (Hopper 2007) using data for the closest reference location for each site (Douglas, Peel and Ramsey).

2.2.5 Fishing Intensity

Vessel monitoring system (VMS) data for all vessels fishing in the waters off the Isle of Man was used to create a raster layer of fishing intensity at 1km^2 resolution for the period of the scallop fishing open season 2008/2009 and 2009/2010. VMStools, a package for use in R (Hintzen *et al.* 2012), was used to filter the data for duplicates and to remove records recorded at speeds (recorded to the nearest 0.2 nautical miles per hour) of less than 1 knot and greater than 3.5 knots. Vessels moving at speeds of 1 to 3.5 knots are considered to be a good estimate of scallop dredging activity as this is the main type of bottom fishing in IOM waters (Murray *et al.* 2011). VMStools was then used to make straight-line interpolation between pings from the same vessel. An additional ten points between the original two hourly pings were generated along the straight line interpolation to improve the resolution of modelled fishing intensity. This interpolated data was then used to create a raster layer of fishing pressure that was imported into ArcGIS, using “VMStools”. A layer containing a polygon for each site was created using the site reference position as a centre point and creating a 2km^2 square polygon. The total fishing intensity for this area was then calculated for each site and fishing season.

3. DATA ANALYSIS

2.3.1 Validating fishers' observations

Spatial variation in gonad maturation state as described by the fishers was investigated using the data collected from the survey on board RV Prince Madog. A two way ANOVA to examine RGH variation with site and year was used to investigate the fishers observation that scallop gonads condition varies among sites. In order to meet the assumptions of homogeneity of variance and normality it was necessary to \log_{10} transform RGH. Tukey's HSD was used to investigate post hoc pairwise differences among sites within a single year. The percentage of individuals categorised in each stage of the GOI were calculated for each site. The GOI percentages were then compared to the maps on which fishers had listed sites that they believed spawned the earliest and the latest. For example, a site that is believed to spawn early may have high percentages of scallops with stage seven and stage three gonads, whereas a later spawning site may have scallops with stages five and six. The claim of the fishers that spawning is occurring later in more recent years was examined by comparing the GOI patterns in the current study to the work of Mason (1958).

To test the Fishers hypothesis that spawning was synchronised to the extent that most of the scallops at a single site spawned within a day or two of each other the coefficient of variation (CV) of the gonad maturation index would be calculated. As CV is a standardised index it could be compared to a previous study that used CV to compared synchronicity among sites that had reproductive schedules varying from "trickle" spawning to a single synchronised spawning event (Mackie and Ansell 1993).

CV = standard deviation / mean

2.3.2 Modelling drivers of gonad maturation state

Data exploration followed the protocol of Zuur and Ieno (2010) to identify outliers, collinearity, independence of variables, homogeneity of variance and identification of problems with interaction terms. Normality of the data was not considered as, due to the nature of the dependant variable (no negative values allowed), a gamma distribution would be used for statistical analysis. Covariates were transformed using either a square root or a \log_{10} transformation to stabilise variance.

Gonad weight was used as the response variable with shell height as a covariate in the model to avoid any loss of resolution which is inevitable when using an index such as the RGH.

Spatial autocorrelation in all covariates and the response variable was investigated using Moran's I in the "ape" package in R. The data was divided into two separate analyses for the east and west of the Isle of Man to avoid the problem of the distance matrix containing pairwise distances that cross the island. The division of data into an east and west grouping was based upon the known oceanography - shallow tidally mixed water column in the east compared with the west with lower tidal mixing and seasonal thermal stratification.

The "mgcv" package in R was used to apply generalised additive models (GAM) to the data. Thin plate regression splines were used and generalised cross validation (GCV) was implemented for automatic optimisation of the smoothing parameter (Zuur *et al* 2009). To avoid over-fitting of the smoothers, as can sometimes happen with GCV, penalisation was increased by setting $\gamma = 1.4$ rather than the default of 1. A gamma distribution was used with a log link function.

The start model contained all parameters and a backward stepwise selection was used to obtain a preferred model. Model parameters were checked for significance using the estimated p-values given in the output summary. Non-significant terms were dropped from the model, which was re-run, and the Akaike Information Criterion (AIC) values compared. Changes of 2 AIC points or more were considered significant (Zuur *et al* 2009) and the model that minimised the AIC was chosen, where the AIC difference was less than 2 points the simplest model was preferred. As these are estimated p-values, care was taken with marginal values in the range of 0.01 to 0.05. In this case these parameters could be removed and the model re-tested. The two models were then compared using AIC to validate the inclusion of the terms. As these are nested models, they can be compared using the "anova" function in R (test = "F") testing for a significant difference between the two models.

Once the optimum model was found it was validated by checking for homogeneity of variance, independence and outliers. Homogeneity of variance was checked by

examining scatter plots of the residuals against fitted values, whilst the dependence due to model misfit can be checked by plotting residuals against covariates; no patterns should be present. Cook's distance was calculated to look for influential outliers.

Scallop minimum landing size (MLS) is an important factor in the management of the fishery (legal landing sizes varying from 100mm length to 110mm length around the UK). In order to investigate the effect of these different sizes on the gonad weight the "predict.gam" function in the "mgcv" package was used to predict gonad weight at different shell sizes based on the optimum model. All other covariates were held at an average value. The data collected for this study and used in the model was shell height (in order to calculate the RGH index). However, fisheries landing size limits use shell length, hence the following correction factor was used to convert shell height to shell length.

$$\text{Shell Length} = 1.1382(\text{Shell Height}) - 2.263 \quad (R^2 = 0.94, n=2000)$$

2.4. RESULTS

2.4.1 Fishers Questionnaire

How many spawning events do you see each year?

The majority of fishers (seven out of eight) answered that they observed two spawning events each year. Due to the closed season from June – October the fishers generally answered that they thought that a spawning event occurred just prior to the open season on November 1ST due to the condition of the gonads in the animals caught at that time, but as they are not fishing they do not witness the spawning event directly. Several respondents considered that, in more recent years the autumn spawning occurred later, as they used to see good quality roe by the time fishing commenced on November 1ST whereas recently the gonads have been empty and poor quality indicating recent spawning.

During a spawning event, do king scallops from different fishing grounds around the island all spawn at the same time?

All the respondents considered that spawning occurred at different times at different fishing areas off the island. Most of respondents (five out of eight) answered that spawning around the island occurs over a period of three to four weeks.

Does spawning take place at the same time every year?

Five out of eight of respondents answered that the timing of spawning varied each year by two to three weeks. Two fishermen answered that spawning varied by more than a month from one year to another but they noted that this was due to spawning occurring later in more recent years.

In which months do you usually see spawning taking place?

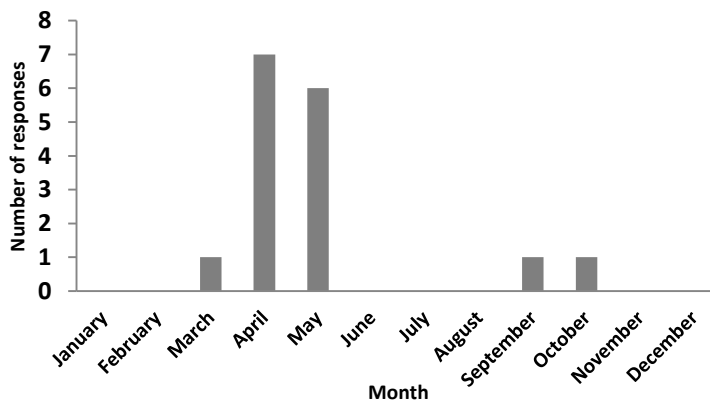


Figure 2.2. Months in which scallop fishers reported observing the occurrence of spawning in *Pecten maximus* in waters around the Isle of Man

Do the Majority of scallops at a single fishing ground spawn at approximately the same time or does it occur over a longer time period?

All of fishers felt that the majority of scallops spawned within a day or two of each other.

How long does it take a fully empty gonad to refill to the stage shown in the picture (stage IV (Mason 1958))?

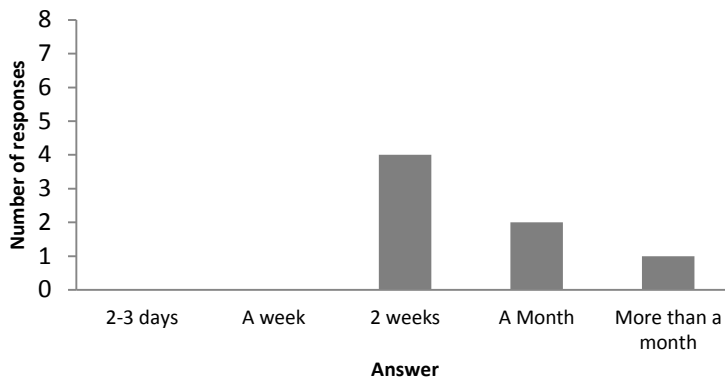
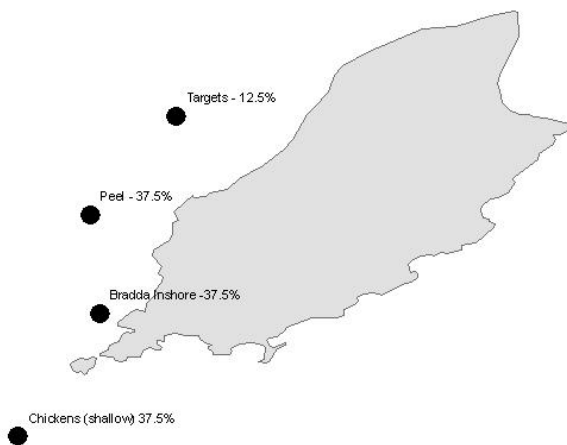


Figure 2.3. Time taken for the spent gonads of *Pecten maximus* to recover to stage IV (Mason 1958) as reported by scallop fishers in the Isle of Man

Using the map below to mark your answer, can you indicate grounds or regions which spawn the earliest and latest?

a



b

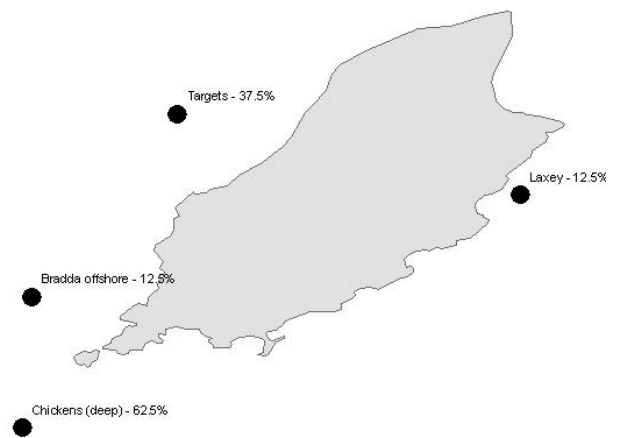


Figure 2.4. Maps of the Isle of Man showing percentage of scallop fishers who considered a site to be either a) early spawning or b) late spawning for the scallop *Pecten maximus*

Open comments/conversation

Half of the respondents commented that spawning events followed a large spring tide peak. Three quarters of respondents considered that deeper sites generally spawned later

than shallower ones. A quarter of fishers considered that there was a general pattern of spawning occurring later in both the spring and the autumn in recent years.

2.4.2 Analysis of scallop survey data

2.4.2.1 Spatial variation in gonad maturation

The two-way ANOVA of RGH by site and year indicated a significant interaction term ($F_{(8,2274)} = 19.4$, $p < 0.001$) which means that, although there are differences among sites, these are not consistent between years. One way ANOVA of RGH by Site showed significant difference between sites in both years (2009: $F_{(8,1127)} = 88.1$, $p < 0.001$, 2010: $F_{(8,1147)} = 72.4$, $p < 0.001$). Tukeys HSD test was used to investigate post hoc pairwise differences between sites (Figure 2.5). Although the interaction term was significant, indicating that patterns between years were not consistent, this seems to be driven primarily by the change in RGH at CHK and SED sites from 2009 to 2010 whilst the remaining sites showed similar patterns in both years (Figure 2.5). There was no grouping of gonad maturation by geographical or oceanographical pattern (for example; inshore v offshore, east v west) (Figure 2.5).

The proportion of scallops with gonads that fell within each class of the Gonad Observation Index (GOI) is shown in Figure 2.6. The difference between 2009 and 2010 identified using the RGH and two-way ANOVA was also observed for the GOI. In 2009 the majority of scallops were classed as stage five whereas the majority were classified as stage three in 2010.

The CV of RGH varied from 0.19 to 0.78 across different sites in June and October 2009/2010 (Table 2.1) suggesting a large variation in the synchronicity of gonad maturation state between sites and dates, but generally the synchronicity was poor (mean CV = 0.46) compared to synchronised spawning in Bay St Brieuc (CV < 0.1 all year) (Mackie and Ansell 1993).

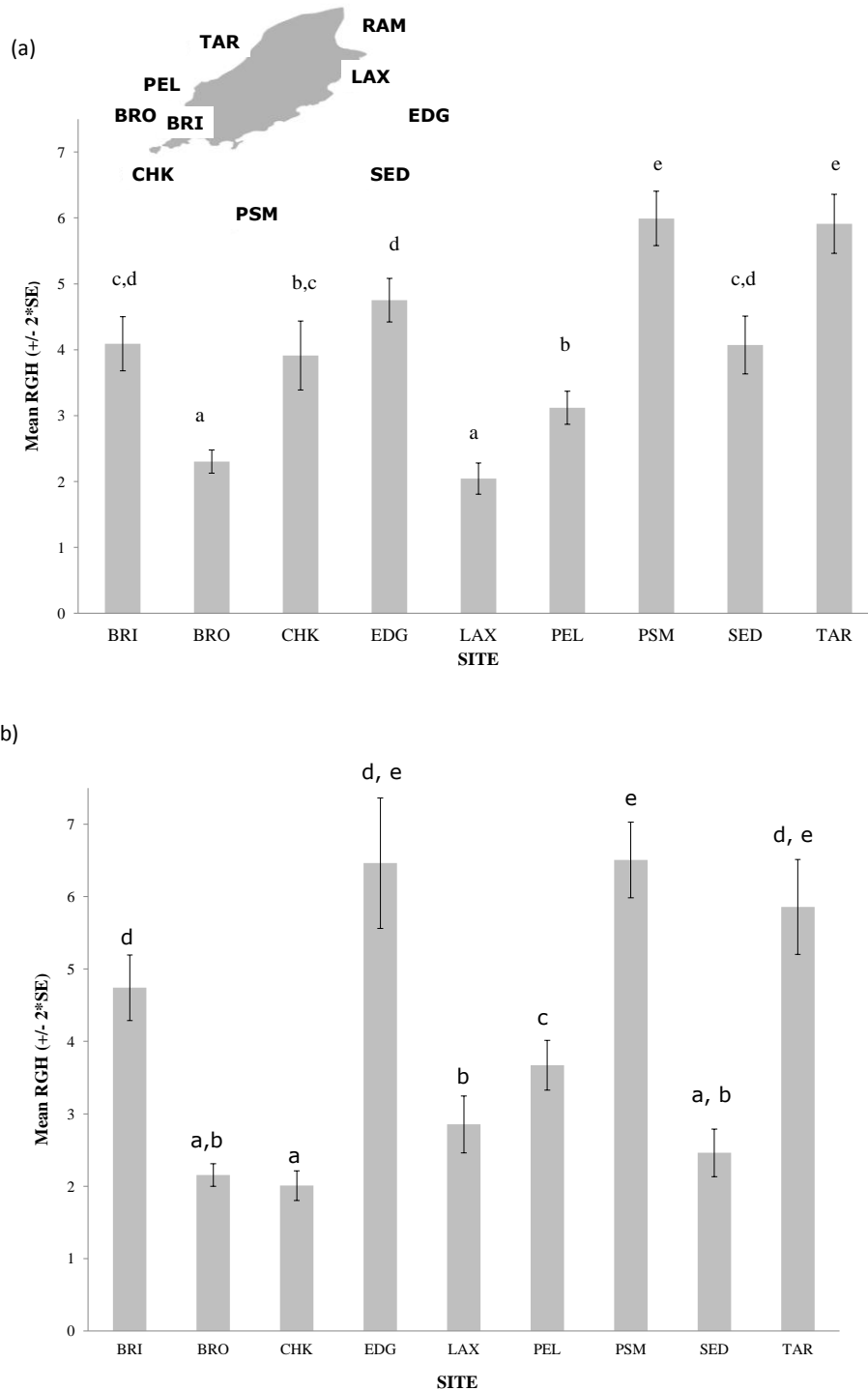


Figure 2.5. Mean relative gonad weight to height index (RGH) for scallops sampled from different beds around the Isle of Man in (a) October 2009 and (b) October 2010. Letters above chart indicate significance results of Tukeys HSD pairwise comparisons; similar letters indicate non-significant differences. Insert shows map of sampling sites around the Isle of Man

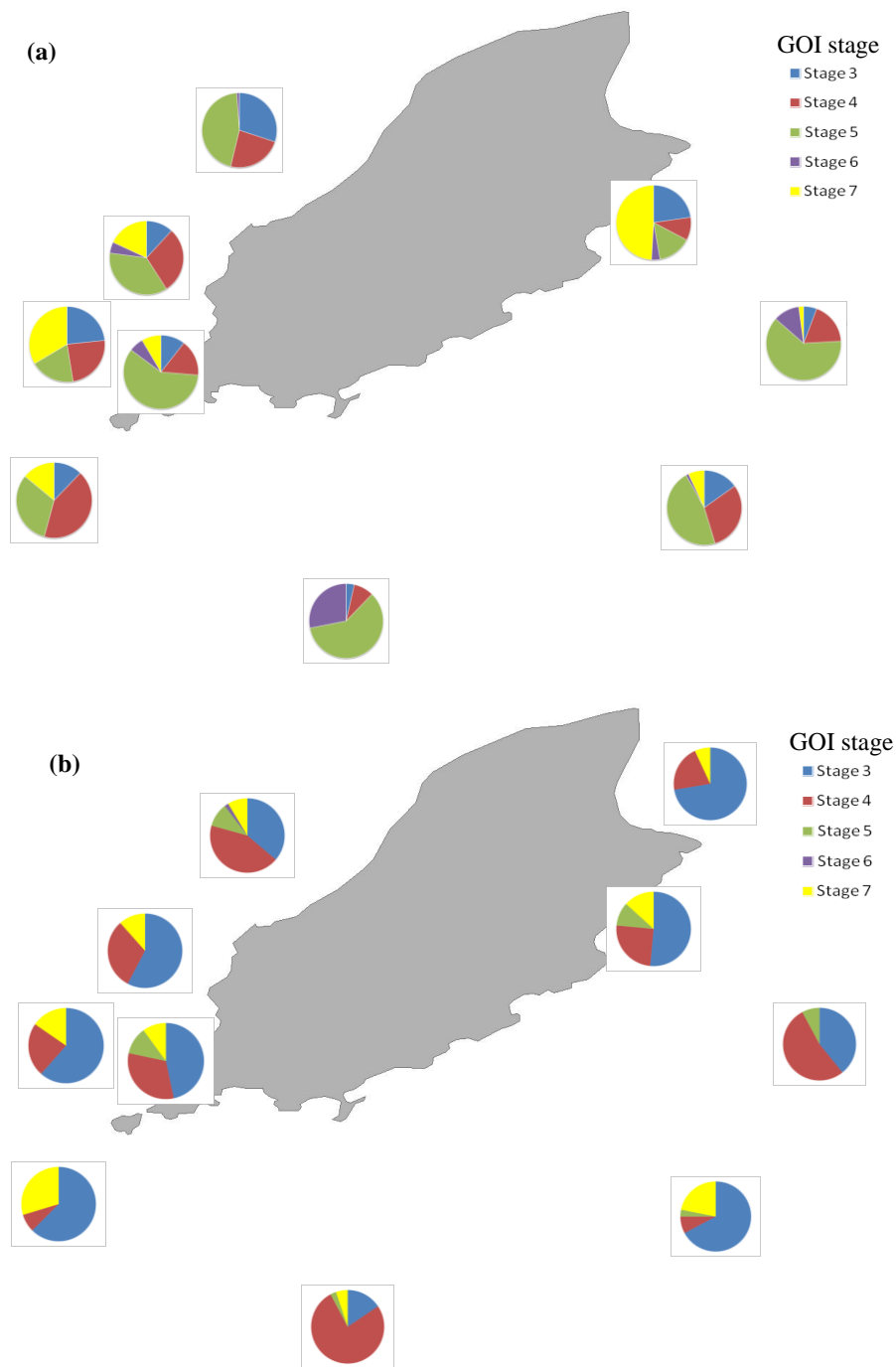


Figure 2.6. Pie charts representing the proportion of scallops from each site with gonads at each stage of the gonad observation index (GOI) (Mason 1958). (a) October 2009 (b) October 2010

Table 2.1. Coefficient of variation (CV) of the relative gonad weight to shell height (RGH) index of scallops caught off the Isle of Man in June and October 2009 and 2010. (For site codes see Figure 1)

Site	CV			
	June 2009	June 2010	October 2009	October 2010
BRI	0.41	0.41	0.55	0.53
BRO	0.49	0.32	0.43	0.42
CHK	0.47	0.38	0.54	0.77
EDG	0.54	0.26	0.41	0.62
LAX	0.40	0.35	0.67	0.78
PEL	0.43	0.39	0.48	0.55
PSM	0.40	0.22	0.37	0.45
SED	0.50	0.27	0.50	0.67
TAR	0.34	0.19	0.50	0.54

2.4.2.2 Modelling Environmental Drivers of Gonad Maturation State

No obvious outliers were identified for the gonad weight data. Plotting gonad weight against each covariate showed no obvious linear patterns. Low levels of collinearity (Pearsons $r < 0.4$) were observed between some covariates, but were not sufficient to warrant their removal from the model. There was no significant spatial autocorrelation in any of the covariates or the response variable.

The GAM was created with a total sample size of 1546. However, interactions between variables could not be included due to lack of replicates in the covariate axis.

The starting model was:

$$\text{Gonad weight} \sim s(\text{Temperature decrease}) + s(\text{Average chlorophyll}) + s(\text{Density}) + s(\text{Shell height}) + s(\text{Stratification}) + \text{factor}(\text{Sampling period}) + s(\text{Depth}) + s(\text{Fishing Pressure})$$

Sampling period, fishing pressure and depth were not significant and were dropped from the optimum model. Stratification had only 1 degree of freedom with a smoother

(indicating a straight line) so was added as a parametric parameter. This resulted in this final model:

Gamma Distribution: $E(Y) = \mu$; $\text{var}(Y) = \mu^2 / \alpha$

Alpha = 3.92 (se = 0.14)

Link: log

Gonad Weight ~ s(temperature decrease) + s(average chlorophyll) + s(density) +
s(Shell height) + Stratification

The AIC for the start model was 6083.9 compared to 6083.6 for the final model and the “anova” test in R showed no significant difference between these two models ($P = 0.21$). Therefore the simpler model was preferred. The model met the assumptions of homogeneity of variance and independence of data. All Cook’s distance values were less than 0.5 which indicated there was no effect of outliers.

The smoothers for all four remaining covariates and the parametric stratification parameter were highly significant ($P < 0.001$) (Table 2.2). The model explained 42.8% of the deviance. Figure 2.7 shows the estimated smoothers for each covariate. The graphs are plotted using the response variable scale on the y-axis and not the log link scale. Greater rates of change of temperature over the month prior to sampling initially had a larger positive effect on gonad weight up to a rate of 0.8°C decrease per month, beyond which the effect of $> 0.8^\circ\text{C}$ change in temperature per month had a reduced effect on gonad weight. Increasing shell height had a positive effect on gonad weight. Increasing scallop densities initially had a small negative effect on gonad weight but changed to a rapid increase in gonad weight at densities greater than 8 per 100m^2 . Initially increasing average annual chl_a had a positive effect on gonad weight however this levelled out and decreases slightly at chl_a levels greater than 3.5 mg m^{-3} . However, it should be noted that this part of the smoother has only a single data point driving the relationship. There was a negative linear relationship of gonad weight with increasing intensity of stratification (Table 2.2).

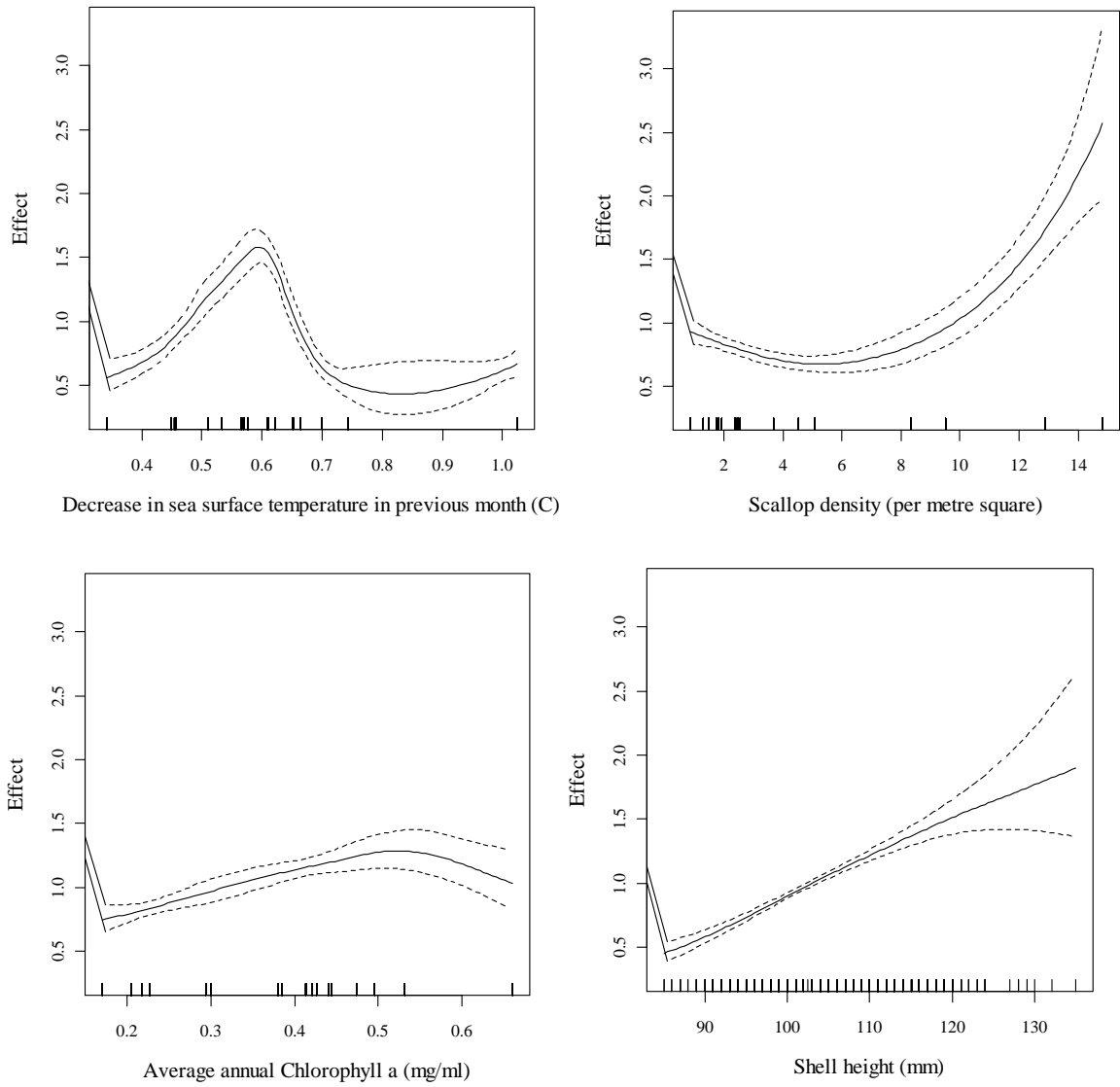


Figure 2.7. Smoothers of the effect of the 4 non-parametric terms on gonad weight (g) in the preferred GAM model. Dashed lines indicate 2 standard errors. Y axis has been back transformed from the log link scale to the response variable scale

Table 2.2. Results of preferred GAM model

<u>Parametric coefficients</u>				
Parameter	Estimate (SE)	SE	t value	p value
intercept	2.03 (0.13)	0.13	15.63	<0.0001
Stratification	-0.25 (0.04)	0.04	-5.55	<0.0001
<u>Smoothed terms</u>				
Parameter	edf	F	p value	
Temperature decrease	6.3	34.41	<0.0001	
Density	2.49	33.06	<0.0001	
Average chlorophyll	2.96	11.51	<0.0001	
Shell height	2.64	68.21	<0.0001	

Predicting the gonad weight based on different shell sizes showed that the greatest gain in gonad weight was found between a shell length of 85 and 90 mm (Figure 2.8). However it can be seen that gains in gonad weight with shell size appear to be indeterminate for the sample range used.

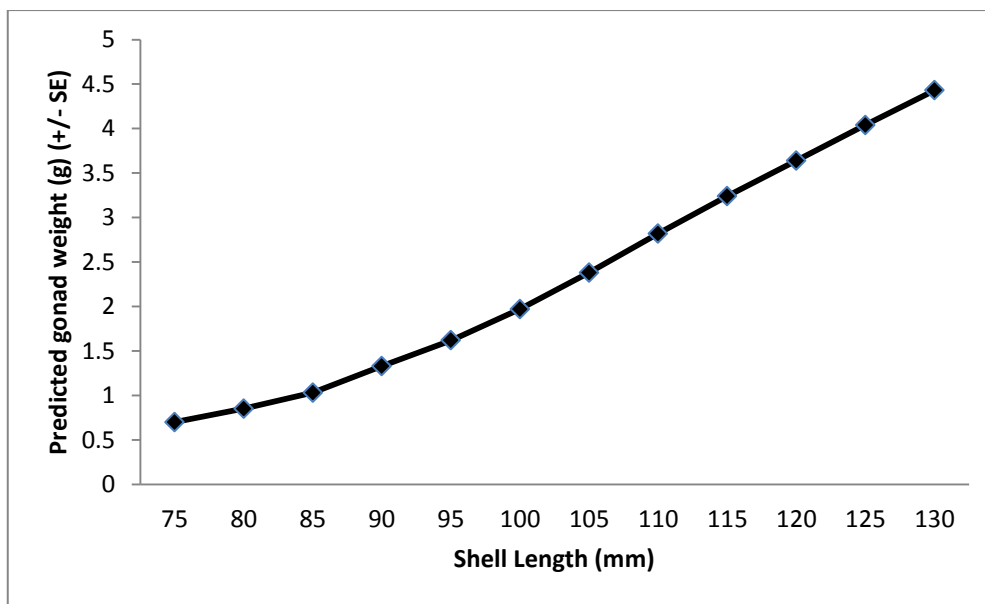


Figure 2.8. Predicted gonad weight (g) at varying shell lengths (mm) based on the preferred GAM model with all other parameters held constant at average values. Maximum measured scallop was 131mm in shell length

2.5. DISCUSSION

2.5.1 *Timing of scallop spawning.*

Mason's (1958) observations of scallops at a single site off the south west coast of the Isle of Man and these of the present study agree with the fishers' observation that there are two main spawning events per year for *P. maximus* in the waters surrounding the Isle of Man. The months in which these two spawning events take place were considered by the majority of fishers to be April/May and September/October. The timing of the spring spawning observed by the fishers matched well with Mason (1958) who found the majority of the spawning occurred in April (1952) or May (1951). However Mason reported that the autumn spawning occurred in August or September, a month earlier than that observed by the fishers in the present study. Interestingly the fishers also noted that they felt there was a trend towards later spawning, particularly in the autumn. The results of the gonad maturation survey in the second week in October 2010 suggested that spawning recently had taken place with the majority of scallop samples at all sites observed in stage three or four on the gonad observation index. This agrees with Mason's (1958) observations of gonad state in October 1952 which had a spawning event in the middle of September. Mason (1958) recorded that spawning occurred in August in 1951 and by October the majority of scallops had recovered to stage four of the GOI with few stage three gonads observed. The condition of the gonads in October 2010 therefore suggest a September spawning event rather than an earlier August event. In 2009 our survey showed a different picture, with the GOI stage with the greatest representation across all sites being stage five. However, Laxey and Bradda Offshore both had mostly spent gonads at stage seven which suggested a very recent spawning event. It was also noted that in 2009 there were significant numbers of scallops at four out of the five GOI stages at most sites. Mason's (1958) work observed that stage five did not predominate until December following a full spawning in August or September but did occur following a small, partial summer spawning event. Therefore, this suggests a partial spawning event at some sites where the scallops have recovered quickly to stage five and a full spawning at others which are seen as spent. Presence of stage seven gonads suggests that the full spawning event in 2009 occurred in October, later than the August/September found in the 1950s, supporting the observation from some fishers that recent spawning is later than in historic years.

2.5.2 *Implications of changes in the timing of spawning*

Residual flow in the Irish Sea is generally slow (0.01 ms^{-1}) and northward towards the North Channel (Bowden 1955). The mean residual flow out of the North Channel towards the Malin shelf is estimated to be $0.02 - 0.03 \text{ ms}^{-1}$ (Knight and Howarth 1999). This residual flow is primarily wind-driven hence storms and storm surges are particularly important. A large 48 hour winter storm in February 1994 displaced a volume of water equivalent to 34% of the Irish Sea through the North Channel (Knight and Howarth 1999). During low wind events some density driven currents can become important due to the temperature and salinity differences between the Irish Sea and the Malin Shelf water (Knight and Howarth 1999), but during the summer months it would be expected that residual flow is minimal due to decreased wind forcing. This low residual flow may enhance the retention of larvae within the Irish Sea. The consequence of later spawning is that scallop larvae are present in the plankton for the onset of the increased autumn wind forcing and hence increased residual flow through the northern channel. Thus a biannual spawning strategy is important for connectivity beyond the Irish Sea to the west coast of Scotland, especially if it occurs in autumn rather than late summer.

The inter-annual variation in the timing of spawning shown by our survey and others (Mason 1958) was also observed by the fishers who stated that the annual spawning events can vary by three to four weeks. This variability in the timing of spawning can mean that the scallop larvae experience different oceanographic conditions from one year to the next which may help explain the temporal variability in recruitment of *P. maximus*.

2.5.3 Synchronicity of spawning

Fishers' observations that most scallops spawn within a day or two of each other suggests a highly synchronised spawning event. However, this is not seen in our results with the coefficient of variation (CV) being, on average, high in both June and October (Table 2.2). Previous work has compared the CV of the highly synchronised population in the Bay St Brieuc, France with less synchronised populations of Scotland and Bay of Brest, France (Mackie and Ansell 1993). Bay St Brieuc showed low CV (<0.1) all year apart from during the spawning event where it rose to just greater than 0.5. Scottish scallops that generally showed a bimodal spawning pattern showed a brief period of synchronisation with low CV (<0.1) immediately following spawning in the spring and autumn (Mackie and Ansell 1993). At other times the CV rose and remained between 0.1

and 0.4. Bay of Brest scallops which show year round “trickle” spawning, never showed a period of synchronisation with CV alternating between approximately 0.1 and 0.35 (Mackie and Ansell 1993). Our scallops showed CV values of up to 0.78 following spawning in October. Even in June, outside of the spawning period, the majority of sites showed values over 0.3. This suggests that scallops around the Isle of Man are not synchronised in their gonad maturation state and spawning. The discrepancy between the fishers’ observations and the survey results is possibly due to the interpretation of the question and a matter of perspective. Fishers will seek the site with the best quality gonads in a high proportion of their catch. So if, for example, a third of the catch has spent gonads they likely will seek alternative sites, with the perception that spawning has taken place. However for a scientist a third of the population spawning whilst two thirds have not does not constitute a synchronised event. The result of this perception of synchronisation is that when the fishers move on to new sites, there will be a large proportion of scallops remaining that have ripe gonads and will be able to spawn. Therefore the fishing strategy of targeting scallops with ripe gonads will have less of an impact in the “bet hedging” reproductive strategy than on a tightly synchronised spawning strategy. The lack of synchronisation can mean that reproductive failure due to a single environmental event is less likely than in populations with a single synchronised event (Paulet *et al* 1988). However, “bet hedging” as a reproductive strategy, by having multiple spawning events, can cause difficulty in fertilisation due to Allee effects; a decreased probability of two gametes meeting compared to a large peak in spawning (Sastry 1979). This can also be compounded if sedentary species, such as *P. maximus*, occur in low densities as on commercially fished grounds. Work on sea urchins found that fertilisation success dropped significantly when the distance to the nearest neighbour increased above just a few metres (Levitan 1991; Levitan *et al.* 1992). The densities of scallops found on the commercial beds in the Isle of Man were extremely low at a maximum of 14 individuals per 100m² (Brand and Prudden 1997). However this is an average over the length of the tow and the variance between tows suggests it is likely that the distribution is patchy with aggregations at higher densities between areas of low or no scallops.

2.5.4 Spatial variation in gonad maturation state

The fishers reported that scallops found at different sites around the Isle of Man spawn at different times. Our survey showed that there was a significant difference between the RGH at different sites which supported this observation. The information regarding the

order in which sites spawn is more difficult to interpret. There was no consensus among fishers regarding which sites spawned first and this was mirrored in our results, such that most sites had at least some scallops at the same GOI stage (Table 2.1). However, five out of eight fishers reported that scallops at the deeper Chickens site spawned last. This was the case in our survey in 2010 when chickens had the highest percentage of recently spent, stage seven gonads while other sites were showing signs of recovery from spawning (Table 2.1). The 2009 results were confused by the presence of the partial spawning, making it very difficult to work out which sites had spawned first or last.

The observation of the fishers that scallops at deeper sites spawn later in the year than shallower ones could not be validated by our data.

2.5.5 Modelling drivers of gonad maturation state

The generalised additive model found that shell height, rate of decrease in temperature over the previous month, the degree of thermal stratification, scallop density and average annual chlorophyll a concentration all had a significant effect on the gonad weight. The effect of shell height is not surprising as larger the scallops would be expected to have larger gonads. Using the model to predict gonad weight at different shell sizes shows that the greatest gain in gonad weight for an increase in shell length of 5mm is found between 85mm and 90mm. Small gains are then made up to a shell size of 100mm where-after the relationship is approximately linear with around 0.4g gonad weight added per 5mm of shell. With the mean gonad weight equalling 3.99g this equates to around 20% gain in gonad size between the lower United Kingdom (UK) MLS of 100mm and the higher MLS implemented in the Isle of Man of 110mm and other parts of the UK. The relationship between large parental size and larger eggs which, in turn, increases offspring fitness is well documented in fishes (for example: Eium and Flemming 2000, Vallim and Nissling 2000, Chambers and Leggett 1996, Parker and Begon 1986). However, in marine invertebrates there appears to have been less focus on this issue in the literature. A study by Valentinsson (2002) found that the maternal size in whelks led to a greater number of eggs and hatchlings but not larger offspring. To our knowledge there have been no similar studies in Pectinids, however given larger gonads one would expect the production of greater numbers of egg and sperm. Therefore, the difference between the lower landing size limit of 100mm found in some parts of the UK and 110mm in other areas and the Isle of Man could equate to considerable increase in

reproductive output. However, growth rates vary considerably around the UK and in some areas scallops rarely reach 110mm due to food limitation (David Palmer, CEFAS, Pers. COMM.) and therefore in those areas a MLS of 110mm would be inappropriate. It is also important to note that Mason (1958) found that scallops only spawn once a year in their first two breeding seasons (age two and three). Unpublished survey data from Isle of Man shows that the average shell length at age 3 ranges from 92mm at BRO to 107mm at RAM with a mean size across all sites of 102.2mm. At age four this increases to 105mm at LAX to 121mm at RAM with a mean of 110.9mm across all sites. All sites except LAX have an average size over 110mm by age five. Scallops from the majority of sites in waters off the IOM, by virtue of the 110mm MLS, are protected until they reach an age at which they will spawn twice in a single year. Therefore a larger scallop protected from fishing mortality to 110mm is likely to produce this 20% increase in the amount amount of eggs and sperms twice in a year. So scallops protected from fishing mortality until 110mm (age 4) will produce 3.4x the amount of eggs and sperm in their lifetime compared to a scallop that suffers fishing mortality at 100mm (age 3) (Table 2.3).

Table 2.3. Calculation of increase in reproductive output of a scallop experiencing fishing mortality at 110mm (4 years) scallop compared to a scallop experiencing fishing mortality at 100mm (3 years), including a 20% increase in gonad weight between 100m and 110mm

Fishing mortality	Yr 1	Yr2	Yr 3	Yr 4(spring)	Yr 4(autumn)	Total
100mm (age 3)	Virgin	Virgin	1x	dead	dead	1x
110mm (age 4)	Virgin	Virgin	1x	1.2x	1.2x	3.4x

2.5.6 Rate of change of temperature

The decrease in temperature over the month prior to sampling suggests that the rate of change in temperature is a trigger for the onset of gonad maturation and spawning in the autumn. The sites with the smallest change in temperature had low gonad weight and a GOI suggestive of recent spawning and early stages of recovery (stages three and seven). The scallops sampled from the sites associated with the largest changes in temperature exhibited a larger effect on gonad weight and had GOIs suggestive of less recent spawning with greater evidence of recovery (stage four). The drop in effect with a higher rate of temperature change seemed to be driven primarily by LAX in 2009 which had a large proportion of spent, low weight gonads (stage 7) but also had recovered gonads

suggesting a partial spawning earlier followed by the more recent spawning event. It would be expected that this relationship of gonad maturation with rate of change in temperature would relate to a rate of increase in temperature for triggering the gonad maturation for the spring spawning event, but this still needs to be investigated.

2.5.7 Long term drivers of gonad weight

The effect of the rate of change of temperature on weight represents a short-term response associated with a biannual spawning event. The remaining parameters in the model were needed to explain the portion of the spatial variation in gonad weight that caused long-term responses to spatial heterogeneity in the environmental drivers.

The effect of scallop density on gonad weight suggested that greater densities of scallops lead to higher gonad weights. This perhaps sounds counter-intuitive considering the potential effects of increased competition but may be due to the fact that the sites with higher scallop densities may be associated with favourable conditions for scallop growth, greater investment in gonad production and higher survival. In general, higher average annual chlorophyll concentration had a positive effect on gonad weight. A single scallop sampling site had high chl_a with a lower effect on gonad weight (RAM). This site is unusual in the fact that it is very shallow and is exposed to inputs from a sewerage outflow and hence high inputs of particulate organic matter (POM) that stimulate primary production. However the potential positive effect of gonad growth associated with these high food levels is perhaps counteracted by the negative effect that high levels of turbidity have on growth. High turbidity decreases growth rates as it is energetically expensive to expel silt (Morel and Bossy 2001) and large aggregations of POM can clog the gills (Chauvaud and Paulet 1998). The only parametric term in the model was the stratification index. Increased stratification was associated with lower gonad weights in scallops compared to scallops from sites experiencing a mixed water column. This is to be expected; with such sites experiencing bottom temperatures around two degrees cooler than mixed waters (Shammon 2007) which would lower metabolism and decrease growth rates (Chauvaud and Paulet 1998). In addition sites with stratified waters will not experience the same rate of bottom temperature decrease in the autumn as those sites with a mixed water column. This could lead to later onset of gonad maturation due to lack of this environmental stimulus.

2.5.8 Conclusions

In general fishers' observations on the spatial and temporal variability in scallop gonad status and spawning events in the Isle of Man were accurate and validated by this study. When fishers' observation deviated from empirical data it might be explained by the differences in perspectives of fishers compared to scientists. This highlights that questions should be explicit and avoid language that can take on different quantitative meanings depending on an individuals' priorities. However, when appropriate questions are asked the knowledge of the fishers can provide useful insights into the ecology and biology and focus research towards specific hypotheses. This study shows that the reproductive status of king scallops in Isle of Man's waters is spatially variable over just a few kms and that rate of change in temperature acts as an environmental stimulus for the autumn spawning event. However, the within site variation is also very high, so these scallops are adopting a strategy which spreads the risk associated with environmental unpredictability by having an extended spawning season, thereby avoiding complete reproductive failure due to unfavourable conditions, which is more likely in a single synchronised event. Some years show higher within site variation in gonad maturation status than others, this poor synchronicity could cause decreased fertilisation success, especially in low density locations. Therefore areas closed to fishing which allow accumulation of high densities of adult scallops can ameliorate this risk. The fishers perceive that scallops at a single site spawn within a day or two at which time they will then fish at alternative sites. The current study suggest that the spawning event is much less synchronised than the fishers perceive and as a result, when the fishers move on to new sites, there will be a large proportion of scallops remaining that have ripe gonads and will be able to spawn. Therefore the fishing strategy of targeting scallops with ripe gonads will have less of an impact in the "bet hedging" reproductive strategy than on a tightly synchronised spawning strategy. Gonad weight increases significantly with shell size and the 110mm landing size in the Isle of Man may increase reproductive output by up to 3.4 times over a 100mm landing size.

CHAPTER 3: POPULATION GENETICS OF PECTEN MAXIMUS L.: A MICROSATELLITE STUDY OF SPATIAL DIFFERENTIATION, EFFECTIVE POPULATION SIZE AND INBREEDING

ABSTRACT

The extent of genetic structure among populations of *Pecten maximus* was investigated using twelve newly developed and four previously published microsatellite markers. The suite of microsatellites identified genetic connectivity at the regional scale of the British Isles and Ireland but with some isolated populations. There was limited gene flow between the British/Irish region and the population from Galicia. *Pecten maximus* from Norway and *Pecten jacobaeus* from the Mediterranean both showed significant genetic differentiation from all other populations. Estimation of effective population size (N_e) suggested large N_e s with the exception of Falmouth Bay and Peel which both had small estimated N_e s. The proportion of selfed individuals within the samples ranged from 6% to 25% and the level of inbreeding ranged from that which would be expected from mating between half-first cousins to that expected from mating between half siblings. The use of multiple genetic analyses provided insight on the smaller scale genetic processes that the analysis of genetic differentiation using F statistics alone may not have identified.

3.1 INTRODUCTION

Pecten maximus, is a hermaphroditic bivalve found in the eastern Atlantic from Norway to Spain. Scallop meat is considered a luxury product, supporting an important commercial fishery. Total annual global capture production for *P. maximus* has fluctuated over the last century but has steadily risen since 1999 to just under 60,000 tonnes in 2009 (FAO 2012). France and the UK land the largest annual catches and in 2009 total UK scallop landings were 34,000 tonnes which equates to a value of £47 million. Scallops are the third most valuable fishery in the UK (MMO 2012) of which *P. maximus* is the primary species.

King scallops characteristically occur with a patchy distribution at a variety of spatial scales (Brand 2006a). Geographically distant areas of scallops (100s km), at densities high enough to support a commercial fishery, may be isolated by tracts of unsuitable habitat (e.g. mud seabed). Within these ‘grounds’ there are ‘beds’ of scallops, separated by distances of 1-10s km, that are found at higher densities than occur in surrounding areas. At a smaller spatial scale, distinct patches of elevated scallop density can be separated by 10s m. This inherent patchiness at multiple spatial scales creates a metapopulation structure in which many subpopulations are connected to varying degrees via larval advection as the adults are largely sedentary. The degree of connectivity is an important factor in the management of species that have a patchy distribution. Connectivity between beds or grounds enables re-population from other areas if local abundance was to fall due to overharvesting. However, in low connectivity situations each isolated bed or ground maybe a reproductively independent unit, such that the population dynamics within it are independent of other populations. In such situations managers need to control fishing effort to maintain sufficient spawning stock biomass locally to enable repopulation without immigration through extended larval transport. Scallop populations have traditionally been managed at a spatial scale closer to the 100s km (grounds) rather than 10s km (beds). The creation of these management units typically represents political and geographical boundaries rather than biological knowledge about reproductively independent units. Scallop fisheries traditionally follow boom and bust cycles (Beukers-Stewart *et al.* 2003; Wolff *et al.* 2007; Tracey & Lyle

2011) and are reliant on the recruiting year class (Beukers-Stewart *et al.* 2003), a phenomenon that suggests effort is not managed successfully.

Connectivity and differentiation in marine biological systems has been inferred from the estimation of pelagic larval duration (PLD) (Grantham *et al.* 2003; Shanks 2009). Whilst the extended larval phases of many marine invertebrates, can serve to link populations increasing evidence suggests that larvae do not always realise this potential dispersal distance (Bohonak 1999; Jones *et al.* 2005; Bowen *et al.* 2006; Shanks 2009). Larval behaviour, together with oceanographic features such as eddies and gyres, can allow a mechanism for local retention creating a more complex picture that PLD alone cannot unravel (Knights *et al.* 2006; Cowen *et al.* 2006b; Paris *et al.* 2007; Woodson and Mcmanus 2007). Thus additional methods are needed to further our understanding of the complex nature of connectivity in marine populations.

Genetic information can assist in identifying management units. If connectivity among populations is low and geneflow is restricted, genetic drift may lead to divergence in allele frequencies among populations. This divergence can then be detected using molecular markers such as microsatellites. The above scenario of divergence due to genetic drift is only likely to occur where natural selection is not a significant contributing factor. Whilst some sections of DNA are linked to genes and are subject to natural selection other neutral sections of DNA occur that are not subject to natural selection. Such “neutral genetic markers” are desirable for population genetic studies. The major limitation of the approach is that, in large populations, homogenisation of allele frequencies can occur if only one individual every two generations migrates among study populations (Crow and Kimura 1970). Whilst this level of connectivity is significant over evolutionary timescales to connect and homogenise subpopulations, it is negligible at the timescale of fisheries management that operates over much shorter timescales. However, whilst non-significant differentiation does not necessarily equate to high connectivity the converse is true such that significant differentiation *does* represent low connectivity, which occurs at a scale that is highly significant for managers, i.e. less than one individual per two generations. Genetically distinct stocks represent the largest unit at which a species should be managed.

The first published population genetic work on *P. maximus* used allozyme loci and studied scallops from 13 sites around the UK and France, but failed to show any differentiation among sites (Beaumont *et al.* 1993). Following this work, evidence of genetic differentiation between Norway and pooled UK samples (primarily from the Irish Sea and the Western English Channel) was identified also using allozymes (Ridgway *et al.* 2000). Mulroy Bay, an enclosed sea loch in Ireland, has been shown to differ from the rest of the UK using both mitochondrial DNA and Randomly Amplified Polymorphic DNA markers (RAPD) (Wilding *et al.* 1997; Heipel *et al.* 1998, 1999), and that a site to the east of the Isle of Man was distinct from the western sites (Heipel *et al.* 1998, 1999). The same mitochondrial study also suggested that Plymouth was distinct from the Irish Sea samples but there was a strange result in that the RAPD markers showed high similarity between Plymouth and the northern most Isle of Man sites but not with the southernmost ones. Such conflicting results will occur if different loci evolve differently within a genome and thereby lead to variation between loci or repeatability issues with RAPD markers. This variance can be decreased by increasing the number of loci used.

The present study used 12 newly developed plus four published (Watts *et al.* 2005) microsatellite markers to gain information on the genetic diversity, connectivity and population structure of *P. maximus* at different geographical spatial scales. Site location was chosen to represent areas that maybe isolated or connected by oceanographic forces. The results are then discussed in the framework of fisheries management.

3.2 METHODS

3.2.1 Microsatellite Development

Microsatellite markers were identified using next generation sequencing of the scallop transcriptome. Briefly, cDNA was isolated from gill, muscle, and mantle tissue from four individual scallops and sequenced on one fifth of a plate (sequenced on a full plate with four other non-scallop libraries using library specific MID tags) on Roche's Titanium Flex 454 sequencer. 422,372 sequences (19bp-905bp in length) were generated that were cleaned of vectors and primers using Seqclean (TGIP 2012) before contig assembly using iAssembler

(<http://bioinfo.bti.cornell.edu/tool/iAssembler/>). Microsatellite containing contigs were then identified using microsatcommander (Faircloth 2008) and primers designed using

Primer3 (Rozen and Skaletsky 2000). From an original set of 82 potential microsatellite loci, 12 loci were optimised into 3 multiplex PCR reactions (Table 3.1). Full development methodology can be found in the supplementary documentation.

3.2.2 Sample collection

P. maximus tissue samples were collected from thirteen sites from across Europe (Figure 1) and tissue from the closely related species/sub-species *Pecten jacobaeus* was sampled as an out-group from the Mediterranean (figure 3.1). Scallop tissue samples from around the Isle of Man were collected by the author during an annual research survey on the RV Prince Madog. Scallop tissue samples from the Falmouth area were collected by the author during a CEFAS research survey on-board the RV Endeavour. All other samples were collected by third parties, fixed for 3 days in 100% ethanol and then shipped in a maximum of 24% ethanol before being stored in 90% ethanol once received in the laboratory. Mantle tissue was preferred over muscle tissue due to improved amplification success (unpublished data). Gill tissue also produced good quality DNA and successful amplification, however gill tissue tended to dissolve in the ethanol making it difficult to use. Therefore, wherever possible mantle tissue was used, although in some cases only muscle tissue was available (Spain and Isle of Skye).

3.2.3 DNA Extraction and microsatellite genotyping

DNA was extracted using CTAB extraction buffer (Doyle and Doyle 1987) due to the high levels of mucopolysaccharides associated with molluscs. Samples were homogenised in 400µl of CTAB buffer (40ml of 2% CTAB buffer to 20µl of mercaptoethanol) and incubated with Proteinase K (200µg) overnight at 59 °C, before phenol-chloroform-isoamyl alcohol (24:24:1) extraction followed by two chloroform-isoamyl alcohol washes (24:1). DNA was precipitated using 2x volumes ice cold absolute ethanol and 0.1x volume of sodium chloride (pH8, 5M). The DNA was resuspended in 50µl of TE buffer (pH 8) and stored long term at -20 °C or at 4 °C for short term storage.

15 microsatellites (9 dinucleotides, 3 trinucleotides and 3 tetranucleotides) were genotyped of which 12 loci were our transcriptome derived markers and 4 were taken from Watts et al. (2005) (List15-004, List15-005, List15-008, List15-012). Microsatellites were amplified using 4 multiplex polymerase chain reactions (PCRs).

Each 12µl PCR contained 1ul of DNA (~10ng), 6µl of Qiagen Type IT multiplex PCR mix, 0.5ul of BSA (10mg/ml), 2.5µl of water and 2µl of Primer mix. The primer mix contains the forward and reverse primers for all loci in the multiplex plus the appropriate fluorescent tails (PET[®], VIC[®], NED[®], FAM[®]) (Table 3.2). Fluorescent tails are more economical than fluorescently labelled primers; oligonucleotide tails are added to the 5' end of the forward primer (Schuelke 2000). Then fluorescently labelled oligonucleotides complementary to the oligonucleotide tail are incorporated during the PCR. There were 3 PCR thermocycling profiles used (Profile one for multiplex A and C, profile two for multiplex B and profile three for multiplex D) (Table 3.3). PCR products were resolved on an ABI 3130XL sequencer using the LIZ 600 size standard. Genemapper[®] software was used to size alleles.



Figure 3.1. Site location from which *Pecten maximus* and the closely related Mediterranean species *Pecten jacobaeus* were sampled. GAL = Galicja (N=28), IRE = Clew Bay (N=48), Ireland, MLB = Mulroy Bay Ireland (N=48), IOS = Isle of Skye, Scotland (N=48), NES = North east Scotland (N=48), SES = South East Scotland (N=48), NOR = Norway (N=48), CHK = Chickens rock (N=48), Isle of Man, PEL = Peel, Isle of Man (N=48), TAR = Targets ground, Isle of Man (N=47), LAX = Laxey Bay, Isle of Man (N=48), EDG = East of Douglas, Isle of Man (N=47). MED = *Pecten jacobaeus*, Castellon, Mediterranean (N=28).

Table 3.1 Characteristics of 12 transcriptome derived microsatellite markers for *Pecten maximus* tested on 48 samples from the Isle of Man. Motif = repeat sequence for the isolated clone; Ta = annealing temperature; N = number of samples amplified out of 48; Na = number of alleles; Ne = Number of effective alleles; Range = allele size range in base pairs; Ho = observed heterozygosity; He = expected heterozygosity; HWE = probability of confirmation to the Hardy-Weinberg Equilibrium.

Primer Name	Forward Primer (5' to 3')	Reverse Primer (3' to 5')	Motif	Ta	N	Na	Ne	Allele range	Ho	He	HWE
P9	CTGATTTACCCTCCGCAACG	TCAGTAATTCTACTCGTGACCC	CTGT	58	48	6	1.460	199-215	0.146	0.315	0.0002
P11	GCCATGGTCGGAAATCACC	CAAACGCGCCAAGTCTACG	ATTT	51	48	2	1.411	304-308	0.271	0.291	0.6255
P19	TCCCTGTTCCCTCATTTACTGC	ACCGAATAATACCACTAACCAG	AATT	58	48	5	1.387	353-369	0.104	0.279	0.0510
P20	TGACAGCTGGGTATAATGGTATG	AGGTGACCGACAACCTAATC	ATTC	58	48	13	5.352	187-260	0.313	0.813	<0.0001
P23	AAATGCCGTCAGCTTTCAG	ACTGTACAAATCGGCCACG	AT	51	48	3	1.314	253-257	0.188	0.239	0.0983
P59	CGAAGGTTTGTGCTGTGAATC	CCAGCAATGACATCCGATCG	AT	58	48	5	3.499	275-281	0.646	0.714	0.2690
P60	TTGTACAAATGCTGGCGTGG	TCTACTCTGGCAGATCATGGG	AT	58	48	5	1.402	186-192	0.333	0.287	1.0000
P62	GGGACCACTGTAAACAATGTG	GCGTGACAGTCGACCATTTTC	AAC	58	48	6	3.252	254-266	0.708	0.692	0.6707
P68	CCATTGGGTAGGGAGCATCG	AAACAAACACTCGCGAACTC	AT	58	48	9	4.608	118-136	0.375	0.783	<0.0001
P70	AGTTGTGCTATTGAATGGGAAC	ATGCACTGCTTGCCACTTC	AAT	58	48	3	1.043	140-146	0.042	0.041	1.0000
P73	CATAGCGATGCAGGACAAGG	ATTCCAATGTCTGCCGTCTG	ACC	58	48	6	1.300	213-231	0.208	0.231	0.1123
P75	CGCTGAAAGTGGTTACCGTG	GCACACAGGTCCAATATAACG	AC	58	48	7	2.339	116-126	0.417	0.572	0.0039

Table 3.2 Multiplex PCR primer concentrations and tails for *Pecten maximus* microsatellite markers.

Multiplex	Primer	PCR concentration	Fluorescent tail (PCR Concentration)	Thermocycle profile
A	P9 Forward	0.05	VIC (0.2)	1
	P9 Reverse	0.20		
	P19 Forward	0.08	PET (0.33)	
	P19 Reverse	0.33		
	P20 Forward	0.05	FAM (0.2)	
	P20 Reverse	0.20		
B	P11 Forward	0.05	PET (0.2)	2
	P11 Reverse	0.20		
	P23 Forward	0.05	NED (0.2)	
	P23 Reverse	0.20		
C	P59 Forward	0.05	FAM (0.2)	1
	P59 Reverse	0.20		
	P60 Forward	0.04	VIC (0.17)	
	P60 Reverse	0.17		
	P62 Forward	0.05	PET (0.2)	
	P62 Reverse	0.20		
	P68 Forward	0.04	PET (0.2)	
	P68 Reverse	0.17		
	P70 Forward	0.04	FAM (0.17)	
	P70 Reverse	0.17		
	P73 Forward	0.05	NED (0.2)	
	P73 Reverse	0.20		
P75 Forward	0.04	FAM (0.17)		
P75 Reverse	0.17			
D	W4 Forward	0.05	PET (0.22)	3
	W4 Reverse	0.20		
	W5 Forward	0.05	NED (0.2)	
	W5 Reverse	0.20		
	W8 Forward	0.05	VIC (0.2)	
	W8 Reverse	0.20		
	W12 Forward	0.05	FAM (0.2)	
	W12 Reverse	0.20		

Table 3.3 Three thermocycling profiles for *Pecten maximus* microsatellite multiplex PCR reactions.

PROFILE 1			PROFILE 2			PROFILE 3		
Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
95 °C	5 minutes	1	95 °C	5 minutes	1	95 °C	5 minutes	1
95 °C	30 s	6	95 °C	30 s	16	95 °C	30 s	10
64 °C; -1 °C per cycle	90 s		51 °C	90 s		60 °C - 1°C cycle	90 s	
72 °C	30 s		72 °C	30 s		72 °C	30 s	
95 °C	30 s	9	95 °C	30 s	19	95 °C	30 s	13
58 °C	90 s		50 °C	90 s		50 °C	90 s	
72 °C	30 s		72 °C	30 s		72 °C	30 s	
95 °C	30 s	15	72 °C	45 minutes	1	72 °C	45 minutes	1
50 °C	90 s							
72 °C	30 s							
72 °C	45 minutes	1						

3.3 Data analysis

3.3.1 Data quality

Genotyping error rates were estimated by genotyping a set of 48 samples twice on the ABI sequencer. Each allele for each sample was cross-checked allowing a percentage error rate to be calculated. Data was checked for null alleles, stuttering and large allele dropout using Microchecker (Van Oosterhout *et al.* 2004). Null allele frequencies were estimated using the software FreeNa. This program was preferred over Microchecker as the “EM algorithm” method of estimation of null allele frequencies (Dempster *et al.* 1977) used in FreeNa was shown to produce less biased estimates than those used in Microchecker (Chapuis and Estoup 2007). The statistical power of the loci to detect F_{ST} values of 0.025, 0.005 and 0.001 was determined using the software PowSim v4.0 (Ryman and Palm 2006). For an F_{ST} of 0.025 an effective population size (N_e) of 10,000 was used along with a generational time of 506 generations. N_e of 10,000 with generational time of 50 generations for F_{ST} of 0.0025 and for F_{ST} of 0.001 N_e was set to 10,000 with a generation time of 20 generations. For the Markov-chain a burn-in of 10,000 followed by 100 batches of 5000 iterations each were used for the 600 runs using the overall average allele frequencies.

3.3.2 Locus characteristics and Hardy-Weinberg Equilibrium

Genepop v4.0 (Rousset 2008) was used to test each pair of loci in each population for evidence for linkage disequilibrium and conformity to Hardy-Weinberg Equilibrium (HWE) using Fisher's exact tests. For both tests the Markov chain parameters used were a burn-in of 10,000 followed by 100 batches of 5,000 iterations each. To correct the alpha value following multiple testing the False Discovery Rate method was used (Benjamini and Hochberg 1995). The software GENALEX 6 (Peakall and Smouse 2006) was used to estimate observed and expected heterozygosity (H_o and H_e respectively), the number of alleles and the effective number of alleles. Allelic richness adjusted for variation in samples sizes by rarefaction was calculated using the software hp-rare (Kalinowski 2005).

3.3.3 Testing transcriptome microsatellites for evidence of selection.

Traditional population genetic theory is underpinned by the assumption that the loci being used are neutral and not under the influence of selection. Whilst this is assumed to be the case for microsatellite markers it is rarely tested. However, there is evidence that microsatellites can be influenced by selection due to hitchhiking and that they can also be found within protein-coding genes and their untranslated regions where they have functional importance (Li *et al.* 2004). As our newly developed loci were transcriptome derived and could therefore be subject to stronger selection pressure than microsatellites located in other regions of the genome it was decided to test them for evidence of selection. We used the Bayesian estimates implemented in the software BayeScan (Foll and Gaggiotti 2008). Although there remains a risk of detecting false positives and false negatives this method's false discovery rate (FDR) and false non-discovery rate (FNDR) were superior to other methods in several simulation studies using Single Nucleotide Polymorphisms (SNPs), Amplified Fragment Length Polymorphisms (AFLPs) and Microsatellites (Foll and Gaggiotti 2008; Pérez-Figueroa *et al.* 2010; Narum and Hess 2011). Data from all populations except Falmouth, Norway, Mediterranean and Galicia were used. Falmouth was excluded due to the problem with linkage disequilibrium at this site and a major assumption of this test is that the loci are independent. It is possible that Norway and the Mediterranean are isolated populations and including isolated populations can inflate the FDR (Foll and Gaggiotti 2008). Both the Mediterranean and Galicia had very poor amplification on one locus with more than 60% missing data,

therefore Galicia was also excluded from this analysis. This left ten populations which should give good power to detect both diversifying and balancing selection (Foll and Gaggiotti 2008). The Sample size was set to 5000 and the thinning to 100, giving a total of 500,000 iterations. 50 Pilot runs of 5000 iterations were run prior to a burn-in of 50,000. If the data are relatively uninformative, the prior will have a large influence on the posterior odds. With a low prior odds of 1:1 (i.e. you are assuming that the model with selection is equally probable as without) you have more power to detect outlying markers but with the trade-off of increased false discovery. With higher odds of neutrality over selection you have less false discovery but lower power to detect outliers. In the present study both the default odds of 10:1 and a lower prior odds of 5:1. Once BayeScan had produced an outfile the results were further analysed in R using the function supplied with the program. This identifies statistical outliers whilst controlling the false discovery rate (FDR). We used an FDR of 1% which is suggested for codominant polymorphic data such as microsatellites (Foll and Gaggiotti 2008)

3.3.4 Population differentiation analysis

The F_{ST} measure of population differentiation was devised originally for biallelic (Wright 1951) markers which ranged from 0 for no differentiation to 1 for complete differentiation. There are several multiallelic versions of F_{ST} e.g. G_{ST} (Nei 1972) and θ (Weir and Cockerham 1984). These do not specify the identity of alleles but measure the amount of variation between subpopulations in comparison to the total population. Due to this the upper limits are dependent on the amount of diversity and for highly polymorphic loci, such as microsatellites, the average within subpopulation and total population heterozygosity can approach unity, causing the upper limit not to be 1. In fact G_{ST} can be very small even if the subpopulations have no shared alleles (Hedrick 1999). Simulation studies have found that microsatellites with high genetic diversity (0.7-0.8) gave higher precision in the estimates of F-statistics but at very high diversity (>0.85) estimates became biased negatively (Leblois *et al.* 2003) and such markers should not be used for estimation of F-statistics. Pairwise F_{ST} for multiple alleles were calculated using the methods of Weir and Cockerham (1984) ($F_{ST}(W\&C)$) in the software FSTAT (Goudet 2001) both with and without markers with a genetic diversity (heterozygosity) > 0.85. Marker P9 was not used due to the poor amplification success in the Mediterranean and Galicia samples. The presence of null alleles can influence the apparent

differentiation between subpopulations; decreased F_{ST} values in the presence of null alleles (Chapuis and Estoup 2007). The software FreeNa calculates pairwise F_{ST} values after the data has been corrected for null alleles using the EM algorithm correction method (Dempster *et al.* 1977) and was used on our dataset ($F_{ST}(\text{null})$).

A spatial analysis of molecular variance was carried out using SAMOVA (Dupanloup *et al.* 2002), which uses longitudes and latitudes of each sampling site along with genetic distances to cluster sites together and aid identification of barriers to gene flow. The preferred value for K was selected using the program PORGS- MFA (Candy *et al.* 2009; Candy *et al.* 2011).

Visualisation of phylogeographic relationships was achieved using PopTree2 (Takezaki *et al.* 2010). The tree was drawn using D_A (Nei *et al.* 1983) genetic distances and the UPGMA method. D_A genetic distances seem to work well with microsatellite data for obtaining correct tree topology (Takezaki and Nei 2008). Principle Component Analysis (PCA) was carried out in GenAlEx to identify principle populations which are responsible for the differences in allele frequencies.

3.3.5 Relatedness

The presence of full and half siblings within each subpopulation was tested using the maximum likelihood method in the software programme Colony (Jones and Wang 2010). Species information was set to; monoecious, polygamous and diploid. Inbreeding was allowed as it has been shown that self-fertilisation in Pectinids is possible even in natural environments (Winkler and Estévez 2003). Each run was set to “long” with “high” likelihood accuracy. Marker genotyping error rates were set to 2%. This level was chosen to reflect the low scoring error estimated. The presence of null alleles was accounted for by setting the allele dropout error rate to those estimated by FreeNa for each locus. There were no known paternal, maternal or sibling relationships.

3.3.6 Effective population size and history

Effective population size was estimated using the linkage disequilibrium (LD) method implemented in the program LDNe (Waples and Do 2008). The mating system was set to random mating and alleles were included in the analysis if frequencies were greater than

0.02 as recommended by Waples & Do (2010). Appropriate habitat and environmental conditions in much of the northern range of *P. maximus* have only been available since the end of the last glacial maxima as recently as 10 -12,000 years ago. Therefore many of the populations could have been expanding since that time or suffered bottlenecks during the glaciation period. The presence of the genetic signature of population expansion was tested using the K and g statistics (Reich *et al.* 1999) using the Microsoft excel macro developed by Bilgin (2007).

3.4 RESULTS

3.4.1 Data Quality

Amplification success was generally high (94% over all markers and populations) with one exception; marker P9 in both the Mediterranean and Galicia had very low amplification success (25% and 43% respectively). Therefore this marker was excluded from the population differentiation analysis and from all of the analyses for those two sites.

Error rates for the genotyping process were very low with all markers having zero anomalies between the two different runs except for P9 (2.6% error) and P60 (3.2% error).

Microchecker showed no evidence of stuttering or large allele dropout. Microchecker identified one locus which had evidence of null alleles in all populations (P20), four loci which had no evidence of null alleles in any population (P59, P62, W4,W5) and the remaining 11 loci had evidence of null alleles in some populations (Table 3.4). Simulations using the software PowSim predict that our suite of markers can detect an F_{ST} of 0.025 and 0.0025 with a probability of 1.0, however for an F_{ST} of 0.001 this power drops to 0.7.

3.4.2 Loci characteristics and HWE

Observed heterozygosity was generally lower than expected (Table 3.5), most likely due to the presence of null alleles. Overall heterozygosity was similar across all populations ranging from a low of 0.33 in LAX to a high of 0.41 in NOR. The mean number of alleles ranged from 4.94 in GAL to 7.31 in EDG but the mean number of effective alleles

ranged very little; from 2.55 in MLB to 2.89 in MED. Markers P11 and P70 had the lowest numbers of alleles (ranging from 2 to 5 across the populations) whereas W5 had the highest (ranging from 13 to 20 across the populations). Mean allelic richness, corrected for sample size, ranged from 4.53 in Galicia to 5.21 in East of Douglas (Table 3.5) with marker W5 showing the highest allelic richness. Marker P9 was not included due to poor amplification in the Mediterranean and Galicia.

Following correction for multiple testing, 14 out of the 1155 pairwise, within population linkage disequilibrium tests were significant. Of these 14 tests 13 of them were within the Falmouth population whilst one was in north east Scotland. No loci showed significant linkage disequilibrium in the global test. 65 out of the 222 population/locus combinations did not conform to Hardy-Weinberg expectations. One locus (P20) was out of HWE in all populations, three loci (P23, P60 and P70) conformed to HWE in all populations and the remaining 12 loci varied between populations (Table 3.5). All significant deviations were due to heterozygote deficiency which was possibly caused by the presence of null alleles as indicated by Microchecker. Allele frequencies that conform to Hardy-Weinberg expectations is an assumption for many analyses in population genetics. Such methods (e.g. Structure) have therefore been avoided as our data strongly violates this assumption. The systematic deficit of heterozygotes, as with many other bivalve species, would have confounded tests of population bottlenecks that test for the presence of an excess of heterozygotes. For this reason it was decided not to use such programs on the current data (e.g. Bottleneck).

Table 3.4 Results for evidence for null alleles using Microchecker for 16 microsatellite markers in 14 populations for *Pecten maximus*. Red results indicate significance following correction for multiple testing using the false discovery method. Percent = percent of populations with evidence of null alleles.

Population	P9	P11	P19	P20	P23	P59	P60	P62	P68	P70	P73	P75	W4	W5	W8	W12
CHK	YES	NO	YES	YES	NO	NO	NO	NO	YES	NO	NO	YES	NO	NO	NO	NO
PEL	YES	NO	YES	YES	NO	NO	NO	NO	YES	NO	NO	YES	NO	NO	NO	NO
TAR	YES	NO	YES	YES	NO	NO	NO	NO	YES	NO	NO	YES	NO	NO	NO	NO
LAX	YES	NO	YES	YES	NO	NO	NO	NO	YES	YES	NO	YES	NO	NO	NO	NO
EDG	NO	YES	YES	YES	YES	NO	NO	NO	YES	YES	NO	NO	NO	NO	YES	NO
FAL	YES	NO	YES	YES	NO	NO	NO	NO	YES	NO	NO	YES	NO	NO	NO	NO
IRE	NO	NO	YES	YES	NO	NO	YES	NO	YES	YES	YES	YES	NO	NO	YES	NO
MLB	NO	NO	NO	YES	NO	NO	NO	NO	YES	NO	YES	YES	NO	NO	NO	NO
IOS	NO	NO	YES	YES	NO	NO	NO	NO	YES	NO	YES	YES	NO	NO	NO	YES
NES	YES	NO	YES	YES	NO	NO	NO	NO	YES	NO	NO	YES	NO	NO	NO	YES
SES	YES	NO	YES	YES	NO	NO	NO	NO	YES	NO	NO	NO	NO	NO	NO	NO
NOR	NO	NO	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	NO
GAL	NO	NO	NO	YES	NO	NO	NO	NO	YES	NO	NO	NO	NO	NO	NO	YES
MED	YES	NO	YES	YES	NO	NO	NO	NO	YES	YES	YES	YES	NO	NO	NO	NO
PERCENT	57	7	93	100	7	0	7	0	93	29	29	71	0	0	21	21

3.4.3 Microsatellites under selection

Using Prior odds of 10:1 and a FDR of 1% there was no evidence of directional selection at any of our markers. However, 9 out of 12 markers showed significant evidence of balancing selection as indicated by the negative alpha value (Table 3.6). This pattern did not change using prior odds of 5:1.

3.4.4 Population structure

The global $F_{ST}(W\&C)$ value was 0.012 (95% CI 0.008 – 0.015) suggesting population structure. This was not equal across loci but varied from -0.001 at locus W4 to 0.025 at locus P60. Pairwise $F_{ST}(W\&C)$ values were generally low (< 0.07) (Table 3.7). $F_{ST}(W\&C)$ comparisons between NOR and the remaining populations contained the largest $F_{ST}(W\&C)$ values (0.038 – 0.070) which were all significantly different from zero following correction for multiple testing. MED, FAL and MLB were lower but significantly different from zero $F_{ST}(W\&C)$ values in pairwise comparisons with the other populations. Pairwise $F_{ST}(W\&C)$ values between MED and IOS, IRE, GAL, EDG and TAR were significant at the 0.05 level but not at 0.01 following correction for multiple testing. This is probably due to the decreased sample size in MED and therefore reduced statistical power. GAL shows mixed $F_{ST}(W\&C)$ results, being significantly different from zero ($p = 0.01$) from NOR, MLB, FAL and MED and at the 0.05 level with CHK and EDG. However the remaining pairwise $F_{ST}(W\&C)$ comparisons are not significantly different from zero. They were however, generally larger than the $F_{ST}(W\&C)$ values obtained from comparisons between populations within the Irish Sea, Scotland and the west of Ireland, which were generally an order of magnitude lower and not significantly different from zero. The only exception to this was the $F_{ST}(W\&C)$ between CHK and IRE, AND PEL. Results after removing the marker with diversity > 0.85 (W5) (Table 3.7) and the FreeNA method corrected for null alleles (Table 3.8) did not change the pattern of structure but both corrections increased all the F_{ST} values slightly and the F_{ST} value between CHK and IRE became non-significant. The dendrogram (Figure 3.2) shows the same overall pattern of differentiation as the F_{ST} values. The PCA suggested that Norway, the Mediterranean and Falmouth are the most differentiated in terms of allele frequency (Figure 3.3). The SAMOVA showed similar results except that it separated PEL from the Irish/Scottish/Isle of Man region. The program was run using a K of 7 as suggested by PORGS-MFA. This grouped IRE, IOS, NES, SES, CHK, TAR, LAX, EDG together and had PEL, MLB, FAL, NOR, GAL and MED as independent populations. The percentage of variation among groups was 2.43%, between populations within groups it was 0.01% and 97.56% of the variation was within populations. All of these variance components were significant ($p < 0.001$).

Table 3.5 Microsatellite locus information for all 13 populations of *Pecten maximus* and the Mediterranean population of *Pecten jacobaeus*. He = expected heterozygosity; Ho = Observed heterozygosity; Na = Number of alleles; Ne = Number of effective alleles; Nr = Allelic richness adjusted by rarefaction. Expected heterozygosity values in bold italics represent those samples not conforming to hardy Weinberg equilibrium using the fishers exact test after adjusting for multiple tests using the false discovery rate.

Locus	Measure	CHK	EDG	FAL	GAL	IOS	IRE	LAX	MED	MLB	NES	NOR	PEL	SES	TAR
P9	He	0.31	0.19	0.38	N/A	0.24	0.13	0.23	N/A	0.09	0.21	0.25	0.29	0.29	0.16
	Ho	0.15	0.16	0.13	N/A	0.21	0.14	0.08	N/A	0.05	0.09	0.18	0.21	0.09	0.11
	Na	6.00	7.00	7.00	N/A	6.00	5.00	5.00	N/A	2.00	6.00	4.00	7.00	5.00	6.00
	Ne	1.46	1.24	1.60	N/A	1.31	1.15	1.30	N/A	1.10	1.27	1.33	1.40	1.40	1.20
	Nr														
P11	He	0.29	0.20	0.12	0.25	0.26	0.21	0.19	0.52	0.12	0.25	0.06	0.24	0.27	0.23
	Ho	0.27	0.18	0.13	0.21	0.26	0.15	0.17	0.14	0.13	0.20	0.07	0.23	0.19	0.17
	Na	2.00	2.00	2.00	2.00	4.00	2.00	2.00	5.00	4.00	2.00	2.00	2.00	2.00	4.00
	Ne	1.41	1.25	1.14	1.32	1.35	1.26	1.24	2.08	1.14	1.33	1.07	1.31	1.37	1.30
	Nr	2.00	1.99	1.92	2.00	2.68	1.99	1.99	4.39	2.48	2.00	1.73	2.00	2.00	2.89
P19	He	0.28	0.43	0.26	0.12	0.33	0.18	0.20	0.40	0.36	0.40	0.53	0.32	0.31	0.45
	Ho	0.10	0.26	0.10	0.13	0.05	0.08	0.07	0.05	0.30	0.08	0.30	0.11	0.12	0.09
	Na	5.00	7.00	6.00	2.00	5.00	2.00	5.00	4.00	3.00	4.00	7.00	4.00	5.00	7.00
	Ne	1.39	1.74	1.35	1.13	1.50	1.21	1.25	1.65	1.56	1.66	2.12	1.48	1.45	1.82
	Nr	3.67	4.87	3.54	2.00	3.92	1.99	3.30	3.84	2.85	3.54	4.74	3.32	3.79	5.24
P20	He	0.81	0.80	0.85	0.80	0.79	0.81	0.80	0.74	0.77	0.79	0.80	0.77	0.78	0.77
	Ho	0.31	0.57	0.55	0.40	0.34	0.40	0.40	0.38	0.47	0.38	0.31	0.49	0.40	0.40
	Na	13.00	13.00	13.00	11.00	13.00	11.00	15.00	9.00	10.00	14.00	8.00	11.00	11.00	8.00
	Ne	5.35	5.05	6.68	5.04	4.69	5.27	4.95	3.84	4.43	4.66	5.07	4.31	4.47	4.30
	Nr	8.97	8.74	9.81	8.71	8.12	8.29	8.94	7.62	7.62	8.76	7.05	7.02	7.45	6.43
P23	He	0.24	0.26	0.08	0.18	0.19	0.38	0.18	0.17	0.31	0.29	0.43	0.20	0.39	0.27
	Ho	0.19	0.18	0.09	0.20	0.17	0.38	0.17	0.19	0.26	0.33	0.51	0.21	0.28	0.30
	Na	3.00	4.00	2.00	2.00	4.00	3.00	4.00	4.00	4.00	4.00	3.00	3.00	4.00	4.00
	Ne	1.31	1.35	1.09	1.22	1.24	1.61	1.22	1.21	1.45	1.41	1.76	1.24	1.63	1.36
	Nr	2.55	3.21	1.82	2.00	2.65	2.92	2.85	3.27	3.15	3.09	2.97	2.76	3.17	3.27
P59	He	0.71	0.68	0.70	0.71	0.71	0.68	0.69	0.72	0.65	0.71	0.65	0.66	0.69	0.68
	Ho	0.65	0.57	0.75	0.59	0.71	0.60	0.65	0.61	0.54	0.70	0.77	0.60	0.73	0.72
	Na	5.00	7.00	5.00	8.00	7.00	6.00	6.00	5.00	5.00	5.00	5.00	6.00	6.00	8.00
	Ne	3.50	3.13	3.28	3.43	3.41	3.13	3.17	3.51	2.84	3.40	2.82	2.93	3.23	3.12
	Nr	4.84	4.77	4.54	6.55	5.17	4.99	5.14	4.82	3.92	4.62	4.60	4.60	4.97	4.93
P60	He	0.29	0.46	0.35	0.32	0.36	0.43	0.36	0.41	0.22	0.26	0.50	0.34	0.32	0.38
	Ho	0.33	0.55	0.29	0.32	0.27	0.27	0.35	0.33	0.24	0.26	0.47	0.28	0.39	0.32
	Na	5.00	5.00	5.00	2.00	5.00	5.00	4.00	4.00	3.00	4.00	3.00	5.00	4.00	5.00
	Ne	1.40	1.85	1.54	1.47	1.56	1.77	1.56	1.69	1.27	1.36	2.02	1.51	1.47	1.62
	Nr	3.00	3.63	3.67	2.00	3.07	3.30	3.27	3.43	2.56	2.69	2.34	3.25	2.82	3.50
P62	He	0.69	0.70	0.68	0.65	0.65	0.72	0.71	0.73	0.67	0.67	0.68	0.65	0.68	0.68
	Ho	0.71	0.72	0.56	0.67	0.67	0.62	0.64	0.74	0.70	0.62	0.59	0.68	0.66	0.70
	Na	6.00	6.00	7.00	3.00	5.00	6.00	6.00	7.00	6.00	6.00	5.00	6.00	6.00	6.00
	Ne	3.25	3.32	3.08	2.85	2.81	3.61	3.41	3.74	3.07	3.00	3.08	2.85	3.09	3.09
	Nr	4.70	4.90	5.01	3.00	4.09	5.47	5.25	6.12	4.04	5.16	4.24	4.40	4.51	4.95
P68	He	0.78	0.79	0.72	0.83	0.81	0.74	0.79	0.76	0.74	0.82	0.76	0.78	0.74	0.82
	Ho	0.38	0.60	0.44	0.63	0.58	0.47	0.42	0.61	0.59	0.61	0.70	0.47	0.48	0.62
	Na	9.00	11.00	7.00	8.00	9.00	8.00	9.00	7.00	8.00	11.00	9.00	9.00	9.00	11.00
	Ne	4.61	4.81	3.53	5.90	5.15	3.87	4.68	4.23	3.88	5.58	4.16	4.56	3.87	5.53
	Nr	6.64	8.09	5.32	7.18	6.94	6.58	6.30	6.51	6.59	8.03	6.78	6.96	7.14	8.17
P70	He	0.04	0.14	0.04	0.08	0.02	0.13	0.06	0.11	0.06	0.13	0.02	0.04	0.16	0.12
	Ho	0.04	0.06	0.04	0.08	0.02	0.00	0.02	0.04	0.02	0.09	0.02	0.00	0.18	0.04
	Na	3.00	2.00	2.00	3.00	2.00	2.00	3.00	3.00	2.00	4.00	2.00	2.00	3.00	4.00
	Ne	1.04	1.16	1.04	1.08	1.02	1.15	1.07	1.12	1.07	1.14	1.02	1.04	1.19	1.14
	Nr	1.67	1.95	1.56	2.23	1.38	1.94	1.89	2.43	1.73	2.73	1.34	1.57	2.36	2.52

Locus	Measure	CHK	EDG	FAL	GAL	IOS	IRE	LAX	MED	MLB	NES	NOR	PEL	SES	TAR
P73	He	0.23	0.15	0.19	0.28	0.21	0.28	0.21	0.26	0.33	0.20	0.18	0.35	0.19	0.23
	Ho	0.21	0.11	0.13	0.24	0.09	0.22	0.19	0.15	0.20	0.17	0.17	0.17	0.15	0.17
	Na	6.00	6.00	3.00	4.00	5.00	8.00	7.00	4.00	6.00	5.00	4.00	6.00	6.00	5.00
	Ne	1.30	1.17	1.24	1.39	1.26	1.39	1.27	1.36	1.48	1.25	1.22	1.54	1.23	1.31
	Nr	3.97	3.20	2.52	3.51	3.38	4.81	3.97	3.42	4.35	3.35	2.97	4.50	3.63	3.61
P75	He	0.57	0.56	0.52	0.53	0.56	0.53	0.53	0.61	0.60	0.47	0.54	0.58	0.55	0.54
	Ho	0.42	0.47	0.23	0.50	0.36	0.29	0.29	0.35	0.35	0.28	0.47	0.26	0.58	0.47
	Na	7.00	5.00	4.00	4.00	5.00	7.00	4.00	5.00	4.00	5.00	4.00	6.00	6.00	6.00
	Ne	2.34	2.25	2.07	2.12	2.26	2.12	2.14	2.56	2.53	1.88	2.15	2.37	2.21	2.15
	Nr	4.84	3.88	3.51	3.14	3.68	4.39	3.58	4.23	3.74	3.65	3.96	4.13	4.38	4.19
W4	He	0.06	0.11	0.24	0.05	0.16	0.12	0.15	0.14	0.16	0.12	0.10	0.19	0.18	0.20
	Ho	0.07	0.09	0.27	0.05	0.17	0.08	0.10	0.14	0.17	0.08	0.11	0.16	0.20	0.15
	Na	3.00	3.00	4.00	2.00	3.00	4.00	4.00	4.00	5.00	3.00	2.00	4.00	4.00	5.00
	Ne	1.07	1.12	1.32	1.05	1.18	1.14	1.17	1.16	1.20	1.13	1.12	1.24	1.22	1.25
	Nr	1.94	2.35	3.36	1.76	2.48	2.60	2.87	2.96	2.93	2.21	1.89	3.19	2.96	3.61
W5	He	0.89	0.88	0.90	0.89	0.89	0.90	0.86	0.89	0.86	0.88	0.86	0.89	0.87	0.89
	Ho	0.87	0.91	1.00	0.95	0.83	0.96	0.77	1.00	0.87	0.94	0.87	0.89	0.87	0.85
	Na	17.00	20.00	15.00	13.00	19.00	20.00	15.00	16.00	16.00	16.00	13.00	18.00	17.00	16.00
	Ne	9.38	8.58	9.80	8.82	9.11	9.58	7.12	9.01	7.03	8.47	7.32	8.92	7.75	8.76
	Nr	12.20	12.70	12.23	11.91	12.72	13.23	10.57	13.10	11.20	11.14	9.53	11.73	11.61	11.45
W8	He	0.15	0.32	0.14	0.09	0.11	0.19	0.18	0.04	0.24	0.15	0.44	0.24	0.25	0.20
	Ho	0.11	0.22	0.10	0.10	0.11	0.08	0.14	0.04	0.22	0.15	0.26	0.16	0.24	0.16
	Na	5.00	7.00	4.00	3.00	6.00	4.00	5.00	2.00	8.00	6.00	6.00	7.00	9.00	7.00
	Ne	1.18	1.48	1.16	1.10	1.12	1.24	1.22	1.04	1.32	1.17	1.77	1.31	1.33	1.24
	Nr	2.93	4.78	2.86	2.52	2.74	3.03	3.42	1.64	4.50	3.12	4.82	4.18	4.55	3.85
W12	He	0.82	0.81	0.76	0.83	0.83	0.83	0.82	0.79	0.82	0.82	0.77	0.84	0.83	0.83
	Ho	0.76	0.80	0.71	0.67	0.67	0.73	0.83	0.64	0.71	0.71	0.74	0.86	0.81	0.70
	Na	11.00	12.00	11.00	11.00	10.00	13.00	12.00	11.00	11.00	13.00	9.00	13.00	12.00	13.00
	Ne	5.41	5.28	4.23	6.03	5.75	5.82	5.40	4.74	5.42	5.65	4.35	6.07	5.91	5.98
	Nr	8.29	9.08	8.09	9.45	8.16	8.68	8.63	8.74	7.61	9.07	7.05	9.37	9.06	8.98
Mean	He	0.45	0.47	0.43	0.41	0.44	0.45	0.43	0.50	0.44	0.45	0.47	0.46	0.47	0.47
	Ho	0.35	0.40	0.34	0.36	0.34	0.34	0.33	0.36	0.36	0.36	0.41	0.36	0.40	0.37
	Na	6.63	7.31	6.06	4.94	6.75	6.63	6.63	5.88	6.06	6.75	5.38	6.81	6.81	7.19
	Ne	2.84	2.80	2.76	2.81	2.79	2.83	2.64	2.89	2.55	2.77	2.65	2.76	2.68	2.82
	Nr	4.81	5.21	4.65	4.53	4.74	4.95	4.80	5.10	4.62	4.88	4.40	4.86	4.96	5.17

Table 3.6 Results of BayeScan testing for selection in transcriptome derived microsatellite markers for *Pecten maximus*. Prior odds set to 10:1. PO = posterior odds. Strength of evidence is based on Jeffrey's Scale of evidence. Negative alpha values indicate balancing selection whilst positive indicate directional selection.

Locus	log10(PO)	alpha	fst	Strength of evidence
P9	1.5	-1.6	0.009	Strong to very strong
P11	-0.9	0.1	0.043	Not significant
P19	1000	-1.6	0.008	Decisive
P20	1.2	-1.5	0.010	Strong
P23	-0.8	-0.1	0.033	Not significant
P59	2.1	-1.8	0.008	Decisive
P60	1000	-1.8	0.007	Decisive
P62	-1.0	0.0	0.035	Not significant
P68	3.7	-2.0	0.005	Decisive
P70	2.9	-1.8	0.007	Decisive
P73	2.2	-1.3	0.011	Decisive
P75	3.7	-1.8	0.007	Decisive

Table 3.7 Pairwise FST values calculated using Weir and Cockerham (1984) in FSTAT for *Pecten maximus* from 14 populations using 15 microsatellite markers. Values above the diagonal used all markers and below the diagonal are FST values calculated after removing markers with heterozygosity >0.85. * = significant at 0.05, ** = significant at 0.01 (alpha adjusted for multiple testing using the False Discovery Rate method).

	CHK	FAL	IOS	LAX	NOR	MLB	SES	NES	IRE	MED	GAL	EDG	TAR	PEL
CHK	-	0.0100**	-0.0013	-0.0015	0.0415**	0.0123**	-0.0007	-0.0002	0.0019*	0.0228**	0.0034*	0.0050*	-0.0015	0.0027*
FAL	0.0116**	-	0.0091**	0.0199**	0.0589**	0.0275**	0.0184**	0.0093**	0.0165**	0.0289**	0.0149**	0.0184**	0.0091**	0.0178**
IOS	-0.0012	0.0102**	-	0.002	0.0488**	0.0091**	0.0024	-0.0018	0.0031	0.0095*	-0.0025	0.0041	-0.0022	-0.0027
LAX	-0.0017	0.0228**	0.0021	-	0.0477**	0.0104**	0.0012	0.0076	0.0016	0.0239**	0.0089	0.0004	0.0007	0.0012
NOR	0.0458**	0.064**	0.0536**	0.0521**	-	0.0571**	0.0395**	0.0495**	0.0374**	0.0668**	0.0351**	0.0358**	0.0369**	0.0511**
MLB	0.0119**	0.0292**	0.008**	0.01**	0.06**	-	0.0091**	0.0127**	0.0074**	0.0211**	0.0115**	0.0108**	0.0086**	0.0047**
SES	-0.0002	0.0214**	0.003	0.0021	0.0425**	0.0084**	-	0.0014	-0.0043	0.0179**	0.009	-0.0014	-0.0035	0.002
NES	-0.0002	0.0098**	-0.0022	0.0072	0.0527**	0.0132**	0.0002	-	0.0035	0.0176**	0.0074	0.0041	-0.0031	0.0038
IRE	0.0018	0.0186**	0.0036	0.0012	0.0383**	0.0082**	-0.001	0.0041	-	0.0154*	0.0072	-0.0025	-0.0005	-0.0002
MED	0.025**	0.0331**	0.0097*	0.0255**	0.0702**	0.023**	0.0199**	0.0193**	0.0179*	-	0.0152*	0.0107*	0.0125*	0.0129**
GAL	0.0035	0.0174**	-0.003	0.0094	0.0557**	0.0126**	0.0106	0.0092	0.0089	0.0177*	-	0.0125**	0.0033	0.0053
EDG	0.0052	0.0203**	0.004	0.0004	0.0382**	0.0117**	-0.0007	0.0039	-0.0032	0.0116	0.0133	-	-0.0026	0.0011
TAR	-0.002	0.0104**	-0.002	0.0003	0.0385**	0.0092**	-0.0009	-0.0029	-0.0006	0.0147*	0.0039	-0.002	-	-0.0012
PEL	0.0029**	0.0199**	-0.0022	0.0008	0.0535**	0.0044**	0.0018	0.0039	0.0006	0.015**	0.0059	0.0014	-0.0014	-

Table 3.8 Pairwise F_{ST} values for *Pecten maximus* from 14 populations. Calculated using allele frequencies corrected for null alleles (Chapuis and Estoup 2007).

	CHK	FAL	IOS	LAX	NOR	MLB	SES	NES	IRE	MED	GAL	EDG	TAR	PEL
CHK														
FAL	0.0105													
IOS	0.0015	0.0117												
LAX	-0.0028	0.0163	0.0048											
NOR	0.0413	0.0577	0.0485	0.0463										
MLB	0.0141	0.0307	0.0121	0.0112	0.0594									
SES	-0.0008	0.0188	0.0064	0.0022	0.0407	0.0136								
NES	0.0011	0.0108	0.0006	0.0078	0.0503	0.0159	0.0025							
IRE	0.0097	0.0257	0.0097	0.0049	0.0401	0.0107	0.0042	0.0108						
MED	0.0298	0.0350	0.0234	0.0314	0.0779	0.0388	0.0273	0.0257	0.0312					
GAL	0.0107	0.0221	0.0053	0.0139	0.0613	0.0169	0.0160	0.0149	0.0141	0.0372				
EDG	0.0064	0.0201	0.0080	0.0014	0.0347	0.0120	0.0015	0.0063	0.0002	0.0258	0.0183			
TAR	0.0043	0.0143	0.0016	0.0026	0.0386	0.0129	0.0011	-0.0007	0.0030	0.0225	0.0133	-0.0007		
PEL	0.0042	0.0172	0.0005	0.0017	0.0505	0.0066	0.0061	0.0066	0.0043	0.0250	0.0152	0.0032	0.0011	

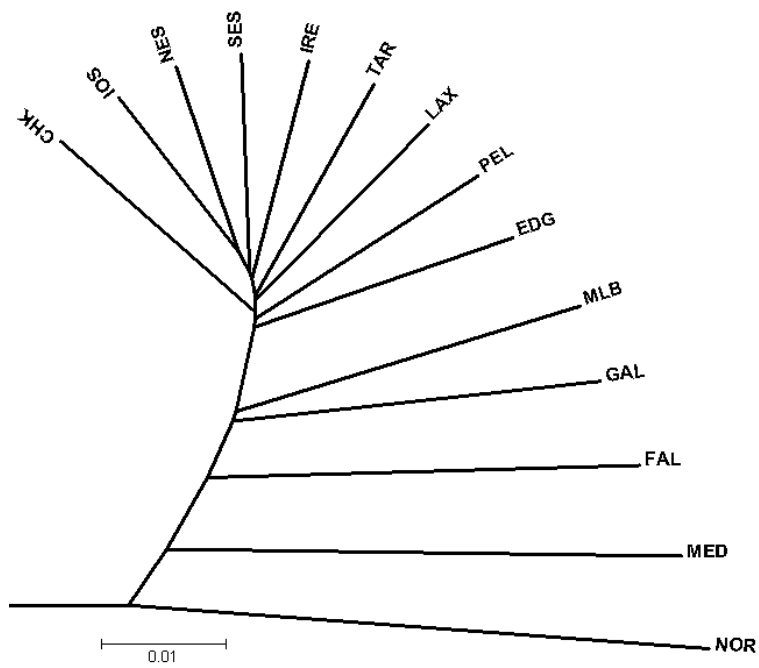


Figure 3.2. Unrooted UPGMA dendrogram showing the relationship between populations of *Pecten maximus*. Nei, Tajima, & Tatenos genetic distance D_A (1983) was used to construct the tree.

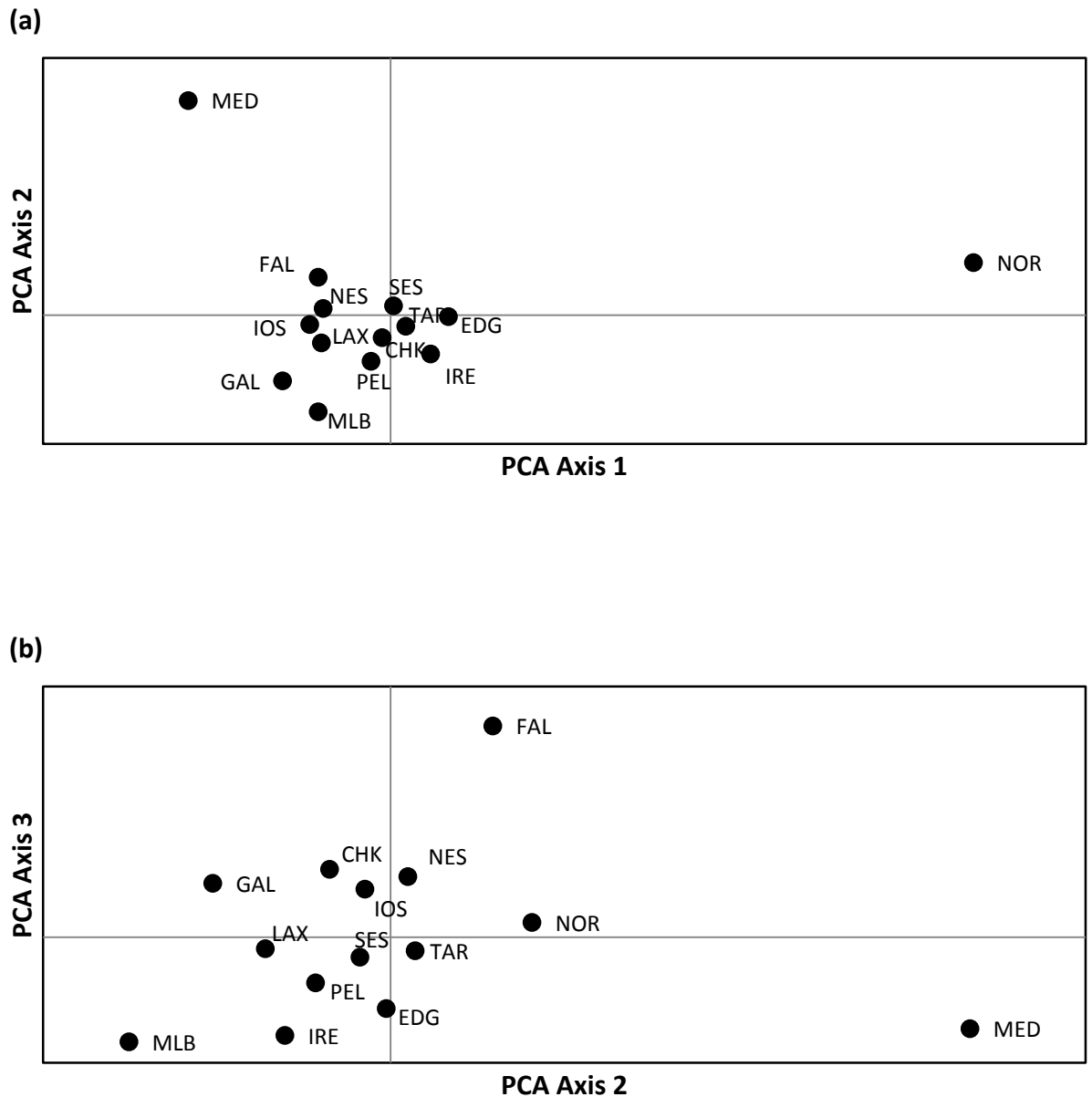


Figure 3.3. Principle component analysis of population differentiation in *Pecten maximus*. (a) PCA axis 1v2, (b) PCA axis 2v3. Axis 1 explains 40.25% of the differences, axis 2 - 21.29% and axis 3 - 17.33%

3.4.5 Relatedness

The results from the maximum likelihood estimation of sibship relationships suggests that the Falmouth population has many more full sib pairs than any other site (18 compared to the next highest of 4 in Peel) (Table 3.9). Of these 18 pairs of full sibs the maximum in a single family is 3, with the remaining only being single pairs of siblings. The number of half siblings estimated was higher than full siblings at all sites, ranging from six in the Mediterranean (N=28) to 76 in Norway (N=48). The proportion of all

pairwise comparisons at each site which had a probability (> 0.95) of being either full or half siblings ranged from 0.02 in the Mediterranean to 0.07 in Norway. The program estimates the parental allele frequencies and gives the number of inferred mothers and fathers. Although it is not possible to confer maternal versus paternal inheritance from microsatellites and therefore the mother and father assignments must be arbitrary. However, this data are still interesting as it provides an insight into the balance between successful male and female gametes. This data suggested that there was an imbalance in the success of male to female gametes in *P. maximus*. Inbreeding ranged from a low of 3% in the Falmouth population to a high of 14% in *Pecten jacobaeus* in the Mediterranean and the level of selfing estimated was generally high (6% - 25%).

Table 3.9 Results from the maximum likelihood estimation of sibship relationships and inbreeding in *Pecten maximus* using Colony. N = sample size, FS = number of full sib pairs (> 0.95 probability), HS = number of half sib pairs (> 0.95 probability), P = proportion of pairwise comparisons which were either full sibs or half sibs (>0.95 probability), Family = number of pairs in the largest full sib family, ♀ = number of inferred mother genotypes, ♂ = number of inferred father genotypes, IB = maximum likelihood estimate of inbreeding, selfing = maximum likelihood estimate of selfing.

Population	N	FS	HS	P	Family	♀	♂	IB	Selfing
CHK	48	1	64	0.06	2	37	27	0.078	0.15
EDG	45	2	50	0.05	2	40	28	0.056	0.11
FAL	48	18	26	0.04	3	32	19	0.030	0.06
GAL	28	0	19	0.05	-	28	19	0.055	0.10
IOS	48	1	46	0.04	2	39	30	0.116	0.21
IRE	48	2	42	0.04	2	39	29	0.055	0.10
LAX	48	3	62	0.06	2	36	26	0.103	0.19
MED	28	0	6	0.02	-	28	18	0.143	0.25
MLB	48	1	57	0.05	2	40	28	0.067	0.13
NES	48	2	46	0.04	2	40	29	0.055	0.10
NOR	48	1	76	0.07	-	35	23	0.032	0.06
PEL	47	4	38	0.04	2	45	29	0.081	0.15
SES	48	2	69	0.06	2	36	28	0.067	0.13
TAR	47	1	54	0.05	2	42	29	0.081	0.15

3.4.6 Effective population size and history

For most populations the confidence intervals (CIs) of the estimated effective population (N_e) included infinity (Table 3.10). This is likely to happen when the N_e is large as the methods have difficulty in differentiating between medium and large populations (Waples and Do 2010; Waples and England 2011) However 2 populations had estimates with tighter CIs which did not include infinity; Falmouth ($N_e = 24$, CI = 20-29) and Peel ($N_e = 155$, CI = 87-511).

The two tests for expansion of population size show conflicting results; the k-test showed strong evidence for population expansion in all populations whilst the g-test showed no

evidence of population expansion (Table 3.11). The g test is an inter-locus test and relates to the population's more distant past compared to the intra-locus K test. So this conflicting result could be due to the time scale of the population size change.

Table 3.10 Effective population (N_e) size estimates and 95% confidence intervals (CI) for *Pecten maximus* and *Pecten jacobaeus* samples using linkage disequilibrium.

Population	95% CIs	N_e estimate
CHK	605.8 - infinity	-438.9
EDG	152.3 - infinity	467.0
FAL	20.2 -29	24.1
GAL	151.6 - infinity	173.8
IOS	139.1 - infinity	454.5
IRE	764.4 - infinity	-352.4
LAX	102.3 - infinity	233.9
MED	-55.1 - infinity	-37.2
MLB	435.3 - infinity	-408.1
NES	520.5 - infinity	-440.4
NOR	311.3 - infinity	-504.3
PEL	87.2 - 511.1	154.9
SES	158.9 - infinity	939.4
TAR	-3496 - infinity	-259.0

Table 3.11 Population expansion tests for 14 populations of *Pecten maximus*.
 No. Negative = the number of loci with a negative k. k = p-value of k test
 for hypothesis of stable population size. G = the result of the g-test.
 Cut off value used for g-test for evidence of expansion is 0.22

Population	No. Negative	k	g
CHK	13	0.0026	4.0
FAL	15	0.0002	2.1
IOS	16	<0.0001	1.5
LAX	15	0.0002	1.9
NOR	15	0.0002	3.5
MLB	14	0.0014	4.8
SES	14	0.0014	2.0
NES	14	0.0014	1.6
IRE	14	0.0014	1.5
MED	13	0.0026	1.1
GAL	12	0.0131	3.5
TAR	15	0.0002	1.7
PEL	16	<0.0001	3.6

3.5 DISCUSSION

3.5.1 Data Quality

Hardy-Weinberg Equilibrium

Departure from Hardy-Weinberg equilibrium (HWE) was observed in 65 of 222 locus/population combinations. This deviation was not systematic across all loci in any population and it is likely that null alleles at some loci are the underlying cause of this deviation. The presence of null alleles is particularly prevalent in Bivalves (e.g. Reece *et al.* 2004; Kenchington *et al.* 2006; Yu & Li 2008; Lallias *et al.* 2010; Lemer, Rochel, & Planes 2011) and leads to an artificial excess of homozygotes which causes the departure from HWE. For this reason we avoided further analyses which require populations to be in HWE (for example STRUCTURE) and we also decided not to use the program Bottleneck for estimation of population bottlenecks as it uses the heterozygote excess method, and the artificially high homozygosity would make identification of heterozygote excess problematic. Although it would have been possible to drop those

markers with evidence of null alleles and then carry out these analyses, it was decided not to follow this route. The markers responsible for the presence of null alleles varied between populations and we would have been left with only four markers which showed no evidence of null alleles. Instead we chose to correct for null alleles for FST calculation and to focus on analyses which do not require HWE or are robust to violation of this assumption.

Markers under selection

The transcriptome derived microsatellite markers showed no evidence of directional selection but nine out of the twelve markers showed evidence of balancing selection. Balancing selection will tend to homogenise allele frequencies, decreasing FST values and masking population structure. However, balancing selection is difficult to detect, with the false discovery rate being greater than that for directional selection (Foll and Gaggiotti 2008; Pérez-Figueroa *et al.* 2010; Narum and Hess 2011). Mutation rates naturally vary across loci which leads to variance in the FST values returned for each locus for which a distribution can be identified. The theory behind tests for detection of selection is that the loci under selection will have FST values which lie outside this distribution of FST values from neutral markers. However, in this study we had only twelve markers from which to build the neutral distribution and as such any outliers should be interpreted with caution, especially as nine of these twelve reference markers are considered outliers. Therefore we did not exclude any markers from further analysis based on the evidence of balancing selection, but caution should be maintained when interpreting low FST values.

3.5.2 Population structure

The population structure of the marine, broadcast spawning bivalve, *Pecten maximus* was studied by means of variation in the allele frequencies in 15 microsatellite markers. Results suggest some heterogeneity in the genetic structure of the populations due to a significant global FST. Pairwise FST comparisons suggest some connectivity between populations within the Irish Sea, around Ireland and Scotland (hereon called Irish/Scottish region) with very low and negative FST values (negative values meaning that the variance and error in the sample produced more noise than the weak signal of differentiation from the data) and there was clustering of these populations in the PCA and on the unrooted UPGMA dendrogram. The general pattern in residual oceanographic

flow would support this connectivity; the Irish Coastal Current flows northwards connecting the north coast of Cornwall with Malin Head at the northern tip of Ireland (Fernand *et al.* 2006). The Scottish coastal current flows northwards along the west coast of Scotland and round the northern coast to the Orkney Islands and south before being guided eastwards to the Norwegian Trench with a proportion of this flow carried further southwards along the east coast of Scotland (Hill *et al.* 2008). These currents are primarily driven by thermal stratification from May to October (Fernand *et al.* 2006; Hill *et al.* 2008) providing opportunity for larval dispersal during these months. Outside these months where stratification breaks down and density driven currents are no longer the primary drivers of circulation (Hill *et al.* 2008) wind forcing becomes more important. Oceanographic connectivity between the Irish Sea and Scotland is achieved through the Northern Channel with the average annual residual flow through the channel estimated to be northwards at approximately $0.02 - 0.03 \text{ m s}^{-1}$ (Knight and Howarth 1999). This flow however, is primarily wind driven, with autumn and winter storms being responsible for the majority of the outflow (Knight and Howarth 1999). So sporadic larval dispersal associated with unusual climactic events (e.g. summer storm surges) and later autumn spawning events may provide the primary connectivity between the Irish Sea and Scottish populations. However, whilst the flow out of the Northern Channel is lower in the summer months, the presence of temperature and salinity differences between the Irish Sea and the Malin Shelf can lead to density driven currents connecting the Irish Sea with the west coast of Scotland (Knight and Howarth 1999) allowing opportunity for larval dispersal through the North Channel following the spring spawning event. There is no obvious oceanographic means of connectivity between the Irish Sea and the west coast of Ireland. The lack of differentiation between populations in these two areas is probably due to a mutual upstream source population, for example the north coast of Cornwall or even the coast of Brittany. However the principle currents travel up from the Atlantic coast of France connecting with the western English Channel and the Celtic Sea before travelling up the west coast of Ireland as the Irish Coastal Current. This forms a front in the Celtic which acts as a barrier that limits flow into the Irish Sea (Hill *et al.* 2008). Another possibility is that there is limited contemporary connectivity between the west coast of Ireland and the Irish Sea but they have shared a recent common ancestor and have not experienced enough time for divergence of allele frequencies. The current shelf-wide circulation system has most likely been in existence since deglaciation about 8000 years ago (Hill *et al.* 2008). In evolutionary terms 8000 years is quite a shallow

divergence time, representing approximately 1600 generations for *P. maximus* based on age structures observed in contemporary exploited populations. However, *P. maximus* are long lived and in historically unexploited populations the generation time could have been much higher with the populations experiencing significantly fewer generations to diverge. The spatial analysis of molecular variance showed the same Irish/Scottish grouping with the exception of Peel. The oceanographic circulation off the west coast of the Isle of Man could decrease connectivity between Peel and neighbouring populations: The Manx West Coast Front is situated close to shore in the south of the Island and then moves further offshore as it moves northwards (Lee *et al.* 2007). This creates a current which connects southern populations with offshore northern populations, which may bypass Peel. Mulroy Bay in the North of Ireland was an exception to this Irish/Scottish group with pairwise FST values an order of magnitude larger than those in the rest of this region and they were significantly different from zero. In addition it did not cluster with the Irish/Scottish region on the unrooted UPGMA dendrogram and was an isolated group on the SAMOVA. Mulroy Bay is a semi-enclosed sea loch; limited water exchange and the presence of eddies (Moreno Navas *et al.* 2011) will decrease larval dispersal outside the loch and create an isolated population. Decreased migration via larval dispersal will lead to increased allelic differentiation due to genetic drift. This result is in agreement with previous genetic studies for *P. maximus*; mitochondrial and RAPD markers both showed Mulroy Bay as a genetically diverged population (Wilding *et al.* 1997; Heipel *et al.* 1998, 1999).

The second exception to the pattern of connectivity around the British Isles is the population of scallops in Falmouth Bay which had significant FST values in all pairwise comparisons and was isolated in the PCA, SAMOVA and the UPGMA dendrogram. This population showed interesting and complex genetic characteristics and as such warrants in depth discussion and will be covered in section 4.5.

Scallops from Galicia had mixed results, FST values were generally higher than those within the Irish/Scottish region but significance from zero of pairwise FST values between Galicia and the Irish/Scottish region varied, possibly due to the smaller sample size and decreased power. Galicia did not cluster with the Irish/Scottish region on the dendrogram, was an isolated group in the SAMOVA and was slightly removed from the tight cluster of that region in the principle component analysis. These results indicate that

there is likely to be only very low levels of gene flow between northern Spain and the British Isles, insignificant on an ecological timescale but enough on an evolutionary timescale to maintain very low F_{ST} values. The presence of this barrier between Spain and the British Isles was also seen in the spiny spider crab, *Maja brachydactyla*, which had an F_{ST} value of 0.06 between Galway and the coast of Galicia using microsatellites (Sotelo *et al.* 2008). In the same study mitochondrial DNA (mtDNA) showed less differentiation and lower F_{ST} values than microsatellites. This study also showed Galway as an isolated population using spatial analysis of variance with microsatellites, whilst with the mtDNA SAMOVA, crabs from Galway clustered with those from France and the very northernmost Spanish population with the rest of the Iberian Peninsula in a separate group. A connected “Atlantic region” has been seen in studies of marine organisms whose range extends from the Iberian Peninsula to the British Isles: The European lobster, *Homarus gammarus*, showed a homogenous group including the Iberian peninsula, British Isles and the Atlantic coast of France (Triantafyllidis *et al.* 2004) as did studies of the European flat oyster *Ostrea edulis* (Launey 2002) and the shore crab *Carcinus maenus* (Roman and Palumbi 2004; Domingues *et al.* 2010). On the basis of the results from the present study it seems that *P. maximus* is intermediate between these two scenarios.

The Norwegian population showed the largest pairwise F_{ST} values and these differences in allele frequencies were supported by the principle component analysis, SAMOVA and the UPGMA dendrogram. The principle currents on the coast of Norway flow from the Skagerrak northwards along the Norwegian coast (Huthnance 1991) which provides limited opportunities for larval dispersal across the North Sea or up from the southern North Sea. There is also the presence of the Norwegian Trench; approximately 100km across and ranging from 300m deep to more than 700m deep at the Skagerrak (Huthnance 1991). This deep water would likely provide inhospitable habitat for scallops, this along with large areas of muddy sediment in the central North Sea will limit the stepping stone method of connectivity across the North Sea. The Norwegian coastline is highly heterogeneous with many deep fiords. As with the semi-enclosed sea loch of Mulroy Bay, these fiords could provide locally isolated water systems with limited circulation to the coast. However, the samples for this study were collected from the “outer part” of the Hardangerfjorden Fiord which has high levels of water circulation due to both tides and density driven currents (Skjoldal *et al.* 2011). This relative isolation of

Norwegian populations has been seen in other genetic studies of marine species; shore crab, *Carcinus maenus* (Roman and Palumbi 2004; Domingues *et al.* 2010), European lobster, *Homarus gammarus* (Triantafyllidis *et al.* 2004) and European flat oyster, *Ostrea edulis*, (Launey 2002).

Pecten jacobaeus from the Mediterranean had pairwise F_{ST} values that were significantly different from zero in all pairwise comparisons and it was isolated in the principle component analysis, SAMOVA and the UPGMA dendrogram. *P. jacobaeus* and *P. maximus* are generally considered sub-species or different “races” as opposed to separate species (Ríos *et al.* 2002; Saavedra and Peña 2005). They are thought to have diverged no earlier than 300,000 years ago during the period of variable climactic conditions that started approximately 2 Ma ago and lasted until the end of the last glacial maximum, 18,000 years ago. The 2 “races” have previously shown a stepped cline distribution using allozyme markers (Rios *et al.* 2002), which is associated with the Almeria-Oran Front (AOF). The AOF has been proposed as a boundary to gene flow which maintains the two differentiated populations (Rios *et al.* 2002; Saavedra and Peña 2005) and this stepped cline around the AOF has also been recorded in *Mytilus galloprovincialis* (Quesada *et al.* 2002). It is therefore not surprising that the Mediterranean population of *P. jacobaeus* was genetically differentiated from all other populations, what was surprising however, was that the magnitude of the F_{ST} values in all the pairwise comparisons with the Norway population were larger than those achieved with the *P. jacobaeus* Mediterranean population, suggesting greater divergence. Comparing the magnitude of the F_{ST} s when they are such small numbers may be risky as the effects of sampling and errors make the values an estimate rather than an absolute, however it does suggest that the level of divergence seen between Norway and the other populations is at least equivalent to that seen across the AOF.

Balancing selection would tend to homogenise allele frequencies, so whilst we can be confident that the significant population differentiation identified in this study is real and not the result of local diversifying selection, we need to be cautious in the interpretation of the homogenised region around the British Isles and Ireland. However, the patterns of population structure seen around the British Isles in this study correlate well with those found with mitochondrial DNA (mtDNA) and allozymes (Beaumont *et al.* 1993; Wilding *et al.* 1997; Heipel *et al.* 1999) and it not be expected that all three marker types would be

affected to the same extent by balancing selection. In a study on oysters balancing selection was proposed as an explanation for the lack population structure shown by allozymes compared to the presence of genetic structure indicated by mtDNA and nuclear DNA (Karl and Avise 1992), but no such discrepancies are obvious in *P. maximus*. It is also difficult to imagine that 75% of microsatellite markers developed would be under balancing selection. Therefore, whilst the presence of balancing selection is still a possibility, it is felt that false discovery of balancing selection is a more likely explanation. The inclusion of isolated populations can increase the false discovery rate (FDR). Although the most differentiated populations were removed from the analysis, MLB was included and this population showed weak but significant structure with the remaining populations in the analysis and is a possible explanation for an increased FDR.

3.5.3 Effective population size

For the majority of populations in this study the estimation of effective population size (N_e) was unreliable with confidence intervals that included infinity. This is likely to occur when estimating large N_e s as the noise from the sampling is larger than the weak signal from the linkage disequilibrium (Waples and Do 2010). However, the method has been shown to be more accurate for estimation of a small N_e (Waples and Do 2010) and it is unlikely that a small N_e would be mistaken for a medium or large one. This can be seen in our data set as the two populations which have low N_e estimates also have much tighter confidence intervals. In the Falmouth Bay population the low estimate of N_e is supported by the presence of multiple loci in linkage disequilibrium after correction for multiple testing. A decrease in the population size can increase genetic drift and cause linkage disequilibrium, but this equilibrates to zero within a few generations due to recombination (Hedrick 1985). A strong bottleneck can take longer to reach linkage equilibrium and the presence of non-random mating or inbreeding can also slow the rate of decay of linkage disequilibrium (Hedrick 1985). Non-random mating and inbreeding are possible in *P. maximus* so the rate of approach to equilibrium could be slow (Hedrick 1985). Scallops from Peel do not show evidence of linkage disequilibrium and this could be due to a small population size being stable for multiple generations such that the population has had time to reach equilibrium. The estimated effective population sizes will provide an estimate of the total metapopulation size if migration between subpopulations is larger than five to ten percent (Waples and England 2011), however if immigration is lower than five percent then the N_e estimated will more accurately reflect

the local population size that would be considerably smaller than the metapopulation. It is possible that Peel experiences lower migration than other Isle of Man sites due to the trajectory of oceanographic currents discussed previously and therefore the N_e estimate may be indicative of the local population rather than the overall metapopulation. Alternatively, as the signature of a small N_e can last for considerable numbers of generations, the low N_e could have been a temporary phenomenon which occurred far enough into the past that all loci have had time to return to equilibrium.

The small N_e estimated for Peel and the extremely small N_e estimate for the Falmouth population are surprising for a species so widely distributed and sufficiently abundant to support large commercial fisheries, especially in light of the levels connectivity suggested in the previous section. However, the perception that marine species will show large N_e s has been put into doubt recently with many studies suggesting an N_e many orders of magnitude lower than the census size (e.g. Turner *et al.* 2002; Hoarau *et al.* 2005; Hedgecock *et al.* 2007). This is especially significant for broadcast spawners that suffer from spatial and temporal variation in reproductive success which can lower N_e (Hedgecock 1994; Hedrick 2005).

3.5.4 Population expansion

The analysis of population expansion showed conflicting results from the two different tests. All populations showed significant evidence of population expansion using the k test whilst the converse was true for the g test. Although the k test has more power than the g test with a sample size of 50, the g test has greater power with 15 loci (Reich *et al.* 1999) so it is unlikely that a lack of power in the g test would cause this discrepancy. Deviation from the single step mutation model can cause increased probability of false rejection of a stable population size in the k test whilst this is not the case for the g test (Reich *et al.* 1999), so it is possible that there was a false detection of population expansion in the k test. This is however an unlikely explanation as the number of negative k values is at least 12 out of 16 loci and higher in most populations, giving good statistical power (Bilgin 2007). Also a previous study of *P. maximus* has shown signatures of population expansion using mitochondrial DNA (Saavedra and Peña 2005) and therefore the results of the k test support these results. The most probable

explanation is that the population expansion occurred relatively recently as the g test, which has greatest sensitivity to population expansions on a time scale that occurred approximately 3 times older than the k test, has poor sensitivity to recent population expansions (Reich *et al.* 1999). The g test has less than 50% probability of detecting population expansion if it occurred more recently than $1.02 N_0$ generations ago (N_0 is the pre-expansion population size) with its greatest sensitivity at $14.6 N_0$ generations ago (Reich *et al.* 1999). The population expansion suggested by mitochondrial data was estimated to have occurred 200,000 to 400,000 years ago, so for the g test to have high sensitivity the pre-expansion population size would have to be less than approximately 5,500 which is highly unlikely for such an abundant species.

3.5.5 Relatedness

Sibship

In a broadcast spawning species it is expected that eggs and sperm will be well mixed by diffusion and turbulence and that larvae will be dispersed during the planktonic larval phase. In theory these larvae will mix with larvae from other populations such that it should be rare for siblings to spatially recruit together in discrete patches. However, other studies have shown that larva movements in the water are not correctly modelled by diffusion, but rather 'packets' of water travel coherently with larvae being transported together within this water packet (Siegel *et al.* 2003) which decreases the number of independent larval paths (Selkoe *et al.* 2006). Also larval behaviour can act to keep siblings together throughout their larval phase (Selkoe *et al.* 2008). The numbers of siblings within a population can also be increased if there are a small number of parents per generation as in sweepstake recruitment (Hedrick 2005). The proportions of half siblings found in this study are similar to those found in a study of *Paralabrax clathratus* (kelp bass). The same study showed through simulations that these estimated proportions of siblings were higher than what would be expected by chance using seven unlinked loci (Selkoe *et al.* 2006). However, the current study had only population (Falmouth) that had full siblings in proportions which would be significant based on the simulations in the kelp bass study. In our study we used 15 (for Galicia and Mediterranean) or 16 loci and therefore it would be expected that false positives would be less likely to occur and it has been shown in simulations that the maximum likelihood method used can recover the correct family structure almost perfectly when just six to ten microsatellites are used (Wang and Santure 2009).

Inbreeding and self fertilisation

The estimated inbreeding coefficients ranged from 0.03 (half-first cousin mating) in Falmouth to 0.14 (higher than half brother/half sister or grandparent/grandchild mating) for *P. jacobaeus* in the Mediterranean. The model allows for deviation from HWE and can account for null alleles (Wang 2004; Wang and Santure 2009), therefore it was expected that these estimates were not attributed to the presence of null alleles and the associated excess of homozygotes. These high levels of inbreeding are probably due to the presence of siblings within the scallop populations and to the high levels of estimated selfing. The estimates for selfing obtained for this study agree with the estimate of ten percent for spontaneous self-fertilisation from an empirical study on the scallop *Argopecten purpuratus* (Winkler and Estévez 2003). The inbreeding depression associated with self-fertilisation has been estimated for several aquaculture scallop species; decreased hatching, growth and survival in *Argopecten irradians* (Zheng *et al.* 2008), decreased growth of larvae in *P. maximus* (Beaumont and Budd 1983) and decreased growth and survival of larvae in *Argopecten cicularis* (Ibarra *et al.* 1995). The prevalence of self-fertilisation in *P. maximus* may be confounded by the low densities generally found on commercial beds (e.g. in the Isle of Man average scallop densities at the end of the open fishing season can be as low as 3 scallops 100m⁻² Brand 2006b) because sedentary broadcast spawners rely on proximity to conspecifics to ensure fertilisation success (Levitan *et al.* 1992). In sea urchins it is estimated that the fertilisation success declines quickly when distances between conspecifics increase to just a few metres (Pennington 1985; Levitan 1991) and that greatest fertilization success is achieved with dense aggregations of large numbers of individuals (Pennington 1985; Levitan 1991; Levitan *et al.* 1992). Scallops have a naturally patchy distribution which may mean that within areas of low density there may patches of scallops at higher densities, however even if behaviour can increase the aggregation of scallops it cannot increase the overall numbers.

3.5.6 Falmouth population

The population from Falmouth was distinct from the wider Atlantic region with significant FST values in all pairwise comparisons and was isolated on the UPGMA dendrogram and the principle component analysis. Falmouth is unusual in that it had a very low effective population size estimate, high numbers of full siblings and showed significant linkage disequilibrium. The oceanography in Falmouth Bay (also known as

Eddystone Bay) is characterised by seasonally stratified waters offshore with a frontal jet travelling from east to west just outside the bay which then continues into the Celtic Sea and joins the Irish Coastal Current (Hill *et al.* 2008). Inshore from this frontal jet is a mixed water column with tidally induced eddies; anticlockwise in the west and clockwise in the east (Ferentinos and Collins 1979). It is therefore feasible that there is increased local retention of larvae within the bay within the eddy systems. This would explain the increased prevalence of siblings. The presence of siblings in a sample would have the effect of reducing the effective population size as the genetic diversity obtained from two siblings would be less than that obtained from two unrelated individuals. A low effective population size would lead to an increased rate of genetic drift even with some migration which would explain the F_{ST} values which are higher than those for other sites in the Atlantic region. The persistence of linkage disequilibrium and the fact that allelic richness and heterozygosity have not decreased in comparison to the other populations in this study would suggest that the low N_e is a recent change; however the high likelihood of non-random mating and the presence of inbreeding will slow the rate of decay of linkage disequilibrium considerably.

3.5.7 Fisheries management

The genetic structure of *P. maximus* suggests that there is some connectivity over large spatial scales, at least around the British Isles and Ireland. It is difficult to assess whether this connectivity is significant on an ecological timescale relevant to fisheries management but the presence of some populations that showed signs of low effective population size and divergence suggest that it would be unwise to manage stocks at a regional scale with the assumption that there are high levels of connectivity between all populations. The populations that showed low effective population size and lower levels of immigration could be at risk of over-exploitation with limited potential for recruitment from neighbouring populations. Low densities could also increase the level of inbreeding and self-fertilisation which has been shown to lead to inbreeding depression in scallops, it is therefore advisable to manage the fisheries at a local scale so that scallop numbers on the majority of scallop grounds, if not all, do not drop to levels with low densities that can lead to Allee effects. This potential dependence on high densities and scallop aggregation for successful fertilisation and decreased self-fertilisation means that spatial management in the form of marine protected areas (MPA) or no take zones could be particularly beneficial for scallops. It has been shown that areas protected from scallop

dredging have increased densities of scallops compared to adjacent areas and that there is increased reproductive output compared to fished areas (Beukers-Stewart *et al.* 2005, 2006; Kaiser *et al.* 2007). This would help build up a healthy spawning stock biomass and the general lack of genetic structure seen in this study means that MPAs have the potential to help seed fished grounds outside of their boundaries. However, there should be careful consideration of the oceanographic circulation in the positioning of MPAs and they should not be relied on alone to maintain good recruitment as migration between populations is not universal but some populations can be significantly more isolated than others, as was seen in this study.

CHAPTER 4: ENHANCING COMMERCIAL SCALLOP STOCKS WITH HATCHERY REARED SEED: GENETIC CONSIDERATIONS

ABSTRACT

This present study was undertaken to investigate the Genetic efficiency of enhancing the populations of wild scallops using hatchery produced seed scallops. Scallops from four sites around Isle of Man (Irish Sea) and from a scallop hatchery were genotyped using 15 microsatellite markers. Heterozygosity was Equivalent in both the IOM samples and the hatchery scallops. Allelic richness was slightly lower in the hatchery sample than for the IOM scallops (4.14 compared to 5.07 – 5.50). The effective population size (N_e) of the hatchery scallops was estimated at 32.4 (95% CI: 24.4 – 44.9). The confidence intervals for the estimates of N_e for the IOM samples included infinity. When N_e becomes large the genetic signal is weak compared to the sampling noise and so estimation of larger values of N_e is difficult. Therefore, whilst we can be confident that the N_e of IOM scallops is larger than that of the hatchery the precise difference is uncertain. Simulations showed that with a census size larger than 10^8 , enhancement with small numbers of scallop seed (100,000 surviving to reproduce) should not affect the enhanced population's effective population size however with larger numbers of surviving seed or a smaller census size it is likely that the N_e of the enhanced population could be decreased. Some benefit from enhancement of wild populations of scallops with hatchery seed is possible when the wild population has a very low effective population size and the number of seed scallops used is small. However this can rapidly change from an increase in the enhanced population's effective population size to large decreases when larger numbers of seed scallops are used. In order to avoid a possible detrimental outcome of introducing hatchery scallop seed we suggest that effort is made to estimate both the wild population's census size and effective population size to allow the prediction of the outcome of stock enhancement.

4.1 INTRODUCTION

The king scallop, *Pecten maximus*, supports a valuable European fishery with landings of approximately 60,000 tonnes in 2009 (FAO statistics). In the Isle of Man (IOM), Irish Sea, king scallops comprise over 65% of all fishery income (Beukers-Stewart *et al.* 2003). High fishing intensity since the start of the IOM fishery in 1937 has led to a decrease in the density of scallops found on commercial beds, with less than 3/100 m⁻² now typical at the beginning of the open season and 1/100 m⁻² at the end of the fishing season compared to a maximum estimate of 4/100 m⁻² to 11/100 m⁻² in the early 1980's (Brand 2006b). There also has been a reduction in the average age from greater than nine years old to less than five years old over an exploitation period of 15 years (Brand *et al.* 1991; Brand and Prudden 1997). This has meant that the king scallop fishery is now vulnerable to the variability and relative strength of each recruiting year class (Beukers-Stewart *et al.* 2003). This uncertainty could be alleviated through stock enhancement and scallop ranching projects. Juvenile scallops can be produced in hatcheries and grown out on the seabed to supplement the natural exploitable population and thereby enhancing the spawning stock biomass.

However, there are concerns about the genetic consequences of the release of aquaculture stock into the natural environment (Utter 1998; Gaffney 2006; Roodt-Wilding 2007). Gaffney (2006) identified two main genetic concerns related to shellfish restocking programs: (i) changes in the effective population size (N_e) and (ii) the genetic composition in the enhanced population relative the original native wild population. The problem of using hatchery progeny, with a low N_e , to enhance wild populations was first highlighted in the case of endangered salmonid populations. Ryman & Laikre (1991) identified that if the transferred hatchery population made up approximately 30% of the post enhancement population then there would be a decrease in the overall N_e of approximately 50%. However, even overfished shellfish populations rarely reach such low population sizes as these salmonids and Gaffney (2006) argued that an increase in N_e is the more likely scenario in hatchery enhanced shellfish populations. A population with low N_e will have a greater rate of loss of alleles and heterozygosity (Crow 1986) and such loss of genetic variability could lead reduced resilience. There is mounting evidence that the genetic diversity of hatchery progeny may be lower than their wild counterparts (Hedgecock and Sly 1990; Liu *et al.* 2010). The introduction of progeny with low genetic diversity into wild salmonid populations has led to lowered overall genetic variability

leading to concerns regarding the fitness of the resulting populations (Utter 1998). However, whereas the low population sizes in salmonid species led to a “swamping” of native populations with “hybrid swarms” (Utter 1998), the comparatively large population sizes of scallops may mean that such swamping is less likely to occur unless extremely large numbers of hatchery derived seed are used. However, low genetic variability in Scandinavian populations of the European oyster (*Ostrea edulis*) was thought to be partially due to transplantation from one location to another as well as overfishing and disease (Johannesson *et al.* 1989). It is therefore important that seed stock has as high a genetic variability as possible to avoid adverse effects on the recipient wild population. The seed should also have genetic composition that is representative of the recipient population to avoid outbreeding depression and the breakdown of local genetic adaptation.

The present study quantified the genetic composition and diversity at microsatellite loci in wild scallops from the Isle of Man, Irish Sea as compared with that of hatchery progeny were being considered for use in scallop restocking projects around the island, and thereby evaluates the efficiency of such an approach to enhance the local fishery.

4.2 METHODS AND MATERIALS

4.2.1 Native and hatchery scallop sampling

Sampling was carried out in 2009 onboard the RV Prince Madog using Newhaven dredges at each of four commercially fished sites around the Isle of Man (Figure 4.1). 50 scallops from each site were collected and approximately a 3mm² piece of mantle tissue from each scallop and stored in 90% ethanol. Each scallop was aged by counting the growth check rings on the flat valve of the shell following Mason (1957) who studied scallop growth off the south west coast of the IOM.

A sample of 50 scallops from a single spawning event was sourced from a hatchery, Iroise Peche, in Port du Tinduff, Rade de Brest, France. Wild scallops collected from Rade de Brest were spawned in March 2009 and the juveniles produced were grown on in sea cages until June 2010. From each of the scallops a sample of mantle tissue was then preserved in 90% ethanol. The Iroise Peche hatchery use new wild broodstock from the Rade de Brest for every spawning and the F1 generation is never used as broodstock,

thereby minimising the occurrence of inbreeding. The total number of individuals used for broodstock varies from year to year depending on the gamete production of the spawners and the survival of the eggs. However, the number of broodstock exceeds several hundred each year (Iroise Peche hatchery, pers comm).

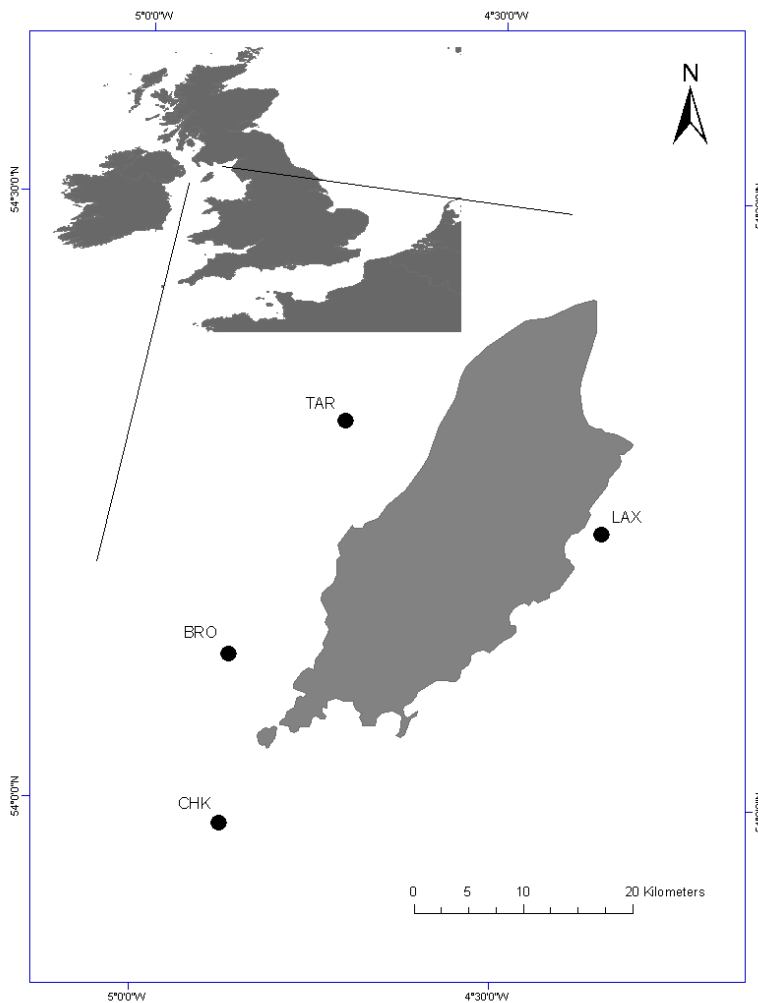


Figure 4.1. Sampling sites for wild *Pecten maximus* from the waters around the Isle of Man, Irish Sea. CHK= Chickens Rock, BRO = Bradda Offshore, TAR = Targets, LAX = Laxey

4.2.2 DNA extraction and microsatellite genotyping

DNA was extracted using CTAB extraction buffer (Doyle and Doyle 1987) due to the high levels of mucopolysaccharides associated with molluscs. Samples were homogenised in (400µl) of CTAB buffer (40ml of 2% CTAB buffer to 20µl of mercaptoethanol) and incubated with proteinase K (200µg) overnight at 59 °C, before phenol-chloroform-isoamyl alcohol (24:24:1) extraction, followed by two chloroform-

isoamylalcohol washes (24:1). DNA was precipitated using 2x volumes absolute ethanol and 0.1x volume of sodium chloride (pH8, 5M). The DNA was resuspended in 50ul of TE buffer (pH 8) and stored at -20 °C for long term or at 4 °C for short term storage.

15 microsatellites (9 dinucleotides, 3 trinucleotides and 3 tetranucleotides) were genotyped of which 12 loci were transcriptome derived (Chapter 3) and 4 (List15-004, List15-005, List15-008, List15-012) were taken from Watts et al. (2005). The transcriptome derived markers are labelled with a prefix of P and those from Watts et al (2005) with a prefix of W (W4, W5, W8 and W12 respectively). Microsatellites were amplified using 4 multiplex PCR reactions. Each 12ul reaction contained 1ul of DNA (~10ng), 6ul of Qiagen Type IT multiplex PCR mix, 0.5ul of BSA (10mg/ml), 2.5ul of water and 2ul of Primer mix. See Chapter 3 for full PCR conditions. PCR products were resolved on an ABI 3130XL sequencer using the LIZ 600 size standard (Applied Biosystems). Genemapper software (Applied Biosystems) was used to size alleles.

Wild scallop census size estimation

Scallop abundance in the Isle of Man waters up to the 12 nautical mile limit were estimated using still photography methods adapted from Lambert *et al.* (2011). Scallop abundance was analysed for 50 photographs at 145 sites (Figure 4.2). The abundance at each station was assumed to be representative of an area of 25 km² (each station was 5 km apart). The total abundance for all 145 sites (3625 km²) was then calculated.

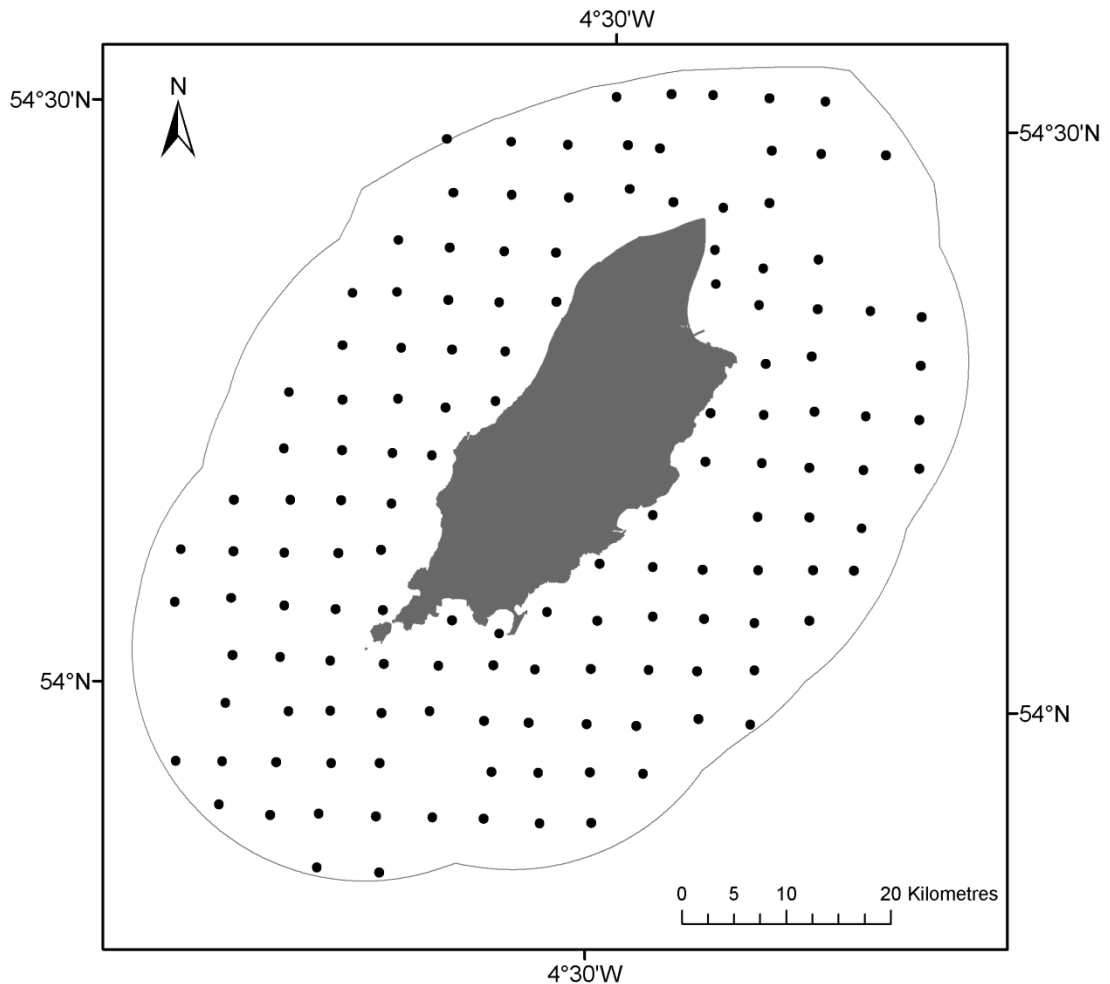


Figure 4.2. Map showing the video sampling sites for the estimation of abundance of *Pecten maximus* in the waters off the Isle of Man. Grey line indicates the 12 nautical mile limit.

4.3 Data analysis

4.3.1 Data quality

Microchecker (Van Oosterhout *et al.* 2004) was used to check for scoring errors and null alleles. Allele frequencies from each population were then tested for concordance with Hardy-Weinberg Equilibrium (HWE) using a test analogous to the Fisher's exact test but using a more computationally efficient Markov-Chain random walk (Guo and Thomson 1992) in the software package Arlequin v 3.5 (Excoffier and Lischer, 2010). Linkage disequilibrium (LD) was tested for using Genepop v 4.0 using the log likelihood ratio statistic (G-test) (10,000 iterations, 100 batches, 5000 iterations).

4.3.2 Genetic diversity

The number of alleles, number of effective alleles, observed and expected heterozygosity were calculated using GenAEx (Peakall and Smouse 2006). To test the difference in heterozygosity between IOM and hatchery scallops, individual heterozygosity was estimated by assigning a value of 0 to homozygous loci and 1 for heterozygous loci for each individual at each locus in all populations. The score for each individual was then divided by the number of loci genotyped for that individual. Analysis of variance (ANOVA) was then used to investigate any significant differences in individual heterozygosity among populations.

For the comparison of allelic richness, only the age group of scallops with the greatest number of individuals was used for each IOM site (This varied between sites). This was due to the fact that the hatchery samples were all from a single cohort and it is possible that scallops show variable reproductive success with different spawning events. This means that the diversity of a single cohort may be less than the overall population, the sweepstake effect (Hedgecock 1994). Using a single age group for each site from the IOM meant that there was less bias in the comparison of allelic diversity. However, this approach resulted in unequal sample sizes, therefore allelic richness estimates were adjusted for unequal sample sizes which we performed using adjustment by rarefaction using the software HP-RARE v1.1 (Kalinowski 2005).

4.3.3 Effective population size

The temporal method of N_e estimation in populations with overlapping generations requires at least two samples which, in the case of populations with overlapping generations, should be separated by 2-4 generations (Wang and Whitlock 2003). The IOM samples consisted of 3 to 5 year olds and the hatchery was a single cohort and was therefore not suitable for use with the temporal method. Therefore, the effective number of breeders (N_b) was estimated by using single cohorts and the linkage disequilibrium method implemented in the software package LDNe (Waples and Do 2008). As small sample size can bias the estimates of N_b , especially for larger N_b s, and as no significant population structure has been observed in IOM samples (Chapter 3) scallops of the same age group from all sites were combined into a single sample group. The four year old age group had largest representation, with 126 individuals. The effective number of breeders

was then calculated for this group and compared to that of the hatchery samples. Random mating was specified in the program settings and only alleles with a frequency of greater than 0.02 were included in the analysis as recommended by Waples & Do (2010).

To estimate the effect of hatchery seed on the effective population size of the wild stock we used the method of Ryman and Laikre (1991);

$$1/N_e = X^2 / N_h + (1-X)^2 / N_w \quad \text{Equation 1}$$

Where N_e is the effective population size following enhancement, X is the relative contribution to the offspring from hatchery progeny, N_h is the effective population size of the hatchery progeny and N_w is the effective population size of the wild population. Each individual scallop was assumed to have the same chance of breeding, therefore the relative contribution of hatchery stock to offspring is simply the number of seed (that survive) divided by the census size.

4.3.4 Population structure

Pairwise F_{st} (Reynolds et al 1983, Slatkin 1995) were estimated in Arlequin to investigate genetic structure between populations, with P values generated using 10,000 permutations. Fisher's exact test of allele frequency differentiation among populations as calculated in Genepop v 4.0 (Rousset 2008) (10,000 iterations, 100 batches, 5000 iterations). Analysis of Molecular Variance (AMOVA) was performed using Arlequin v3.5. The threshold for missing data was set to 0.1 which allowed all loci to be used. A hierarchical structure with two groups (hatchery and IOM populations) was used, with sampling site nested within group.

4.4 RESULTS

4.4.1 Data quality

No stuttering or large allele drop-out was identified by Microchecker. There was no evidence of LD for any pair of loci in any population with the exception of markers W12 and W5 in the hatchery sample after correction for multiple testing. There was no evidence of LD in any pairs of loci in all populations suggesting that loci were not closely linked and can be treated as independent variables. Twenty two out of the 75 locus/population combinations deviated from HWE, which occurred due to a deficiency

of heterozygotes and all populations were significant for the global test over all loci for heterozygote deficiency ($P < 0.0001$). Two markers deviated from HWE in all populations but none of the populations deviated from HWE at all loci. Microchecker highlighted the possible presence of null alleles in the population and loci combinations which were not in HWE, this can cause an excess of homozygotes and is the likely cause of the deviations from HWE.

4.4.2 Genetic diversity

The mean number of alleles per locus was lowest in the hatchery samples, and the mean effective number of alleles was slightly lower in the hatchery populations than the Isle of Man populations (Table 4.1). Average allelic richness varied between 4.14 in hatchery samples to 5.50 in the Laxey population after rarefaction (Table 4.1) However, single locus comparisons show that hatchery allelic richness is only lower than IOM samples at 8 out of the 15 loci. The mean heterozygosity was similar in all populations, including the hatchery, ranging from 0.35 in Laxey to 0.39 in Targets (Table 4.1), this heterozygosity was not significantly different between the IOM and hatchery samples (ANOVA: $F_{4,234} = 1.78$, $P = 0.13$).

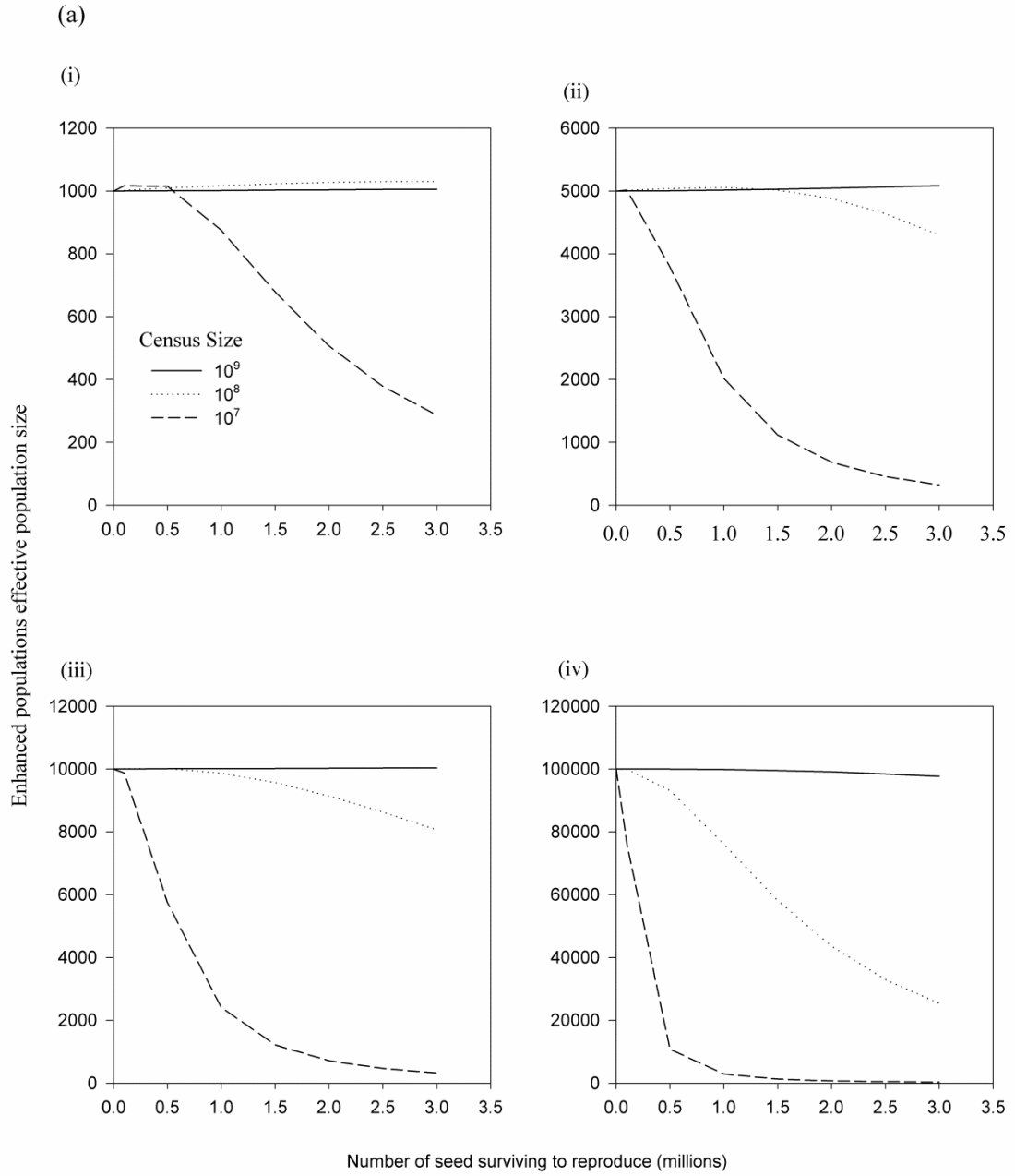
4.4.3 Census and Effective population size

Linkage disequilibrium (LD) estimates of the effective number of breeders (N_b) showed that the hatchery population had a low N_b of 32.4 (95% CI: 24.4 – 44.9). The estimate for the IOM had confidence intervals which included infinity (394.6 to infinity). The point estimate was 1141.4. The estimated census population size was 3.2×10^8 .

3.4 Effect of Hatchery Seed on Wild Populations N_e

Due to the likely error associated with the estimation of the census population size and the inherent variability in population numbers on a temporal scale, three different census sizes were used to estimate the effect of seeding on the effective population size of the enhanced population; 107, 108 and 109 (Figure 4.3). In the equation for estimating the effect of seed on enhanced the populations N_e , N_h was set to 30 (representative of our estimate of N_b from the hatchery samples), but due the inaccuracy of the estimate of N_b for the wild population, a range of values were used for the value of N_w (500, 1000, 10000, 100000). The effect of enhancing a wild population with hatchery progeny

depends on both the number of seed which survive to reproduce and both the wild N_e and census size (Figure 4.3). With a moderate to large wild N_e there is little benefit from enhancing with hatchery seed (up to 1% increase in wild N_e in some scenarios) and these increases are generally only achieved with low numbers of hatchery seed (Figure 4.3). The wild N_e can drop by as much as 96% when large numbers of seed are added to wild populations that have a moderate to large N_e . Doubling the hatchery scallops N_e only slightly improved the outcome of these seeding scenarios (Figure 4.3b). A wild population with a small N_e can potentially benefit from enhancement with hatchery seed ($N_e = 60$) in some scenarios (Figure 4.4). In these scenarios, with a hatchery effective population size of 60 and a census size that is small to moderate then an increase in the wild populations N_e is achieved with low numbers of hatchery seed, but higher numbers of seed scallops can lead to a rapid decline in the wild N_e (Figure 4.4). With larger census sizes and a small wild N_e an increase in the wild populations N_e can be achieved even with larger numbers of seed but as the census size increases further then this benefit plateaus out becoming neutral (Figure 4.4). Using a lower N_h of 30 in these low N_e scenarios leads to declines in the recipient populations N_e with lower numbers of seed even with larger census sizes compared to the $N_h = 60$ scenarios (data not shown).



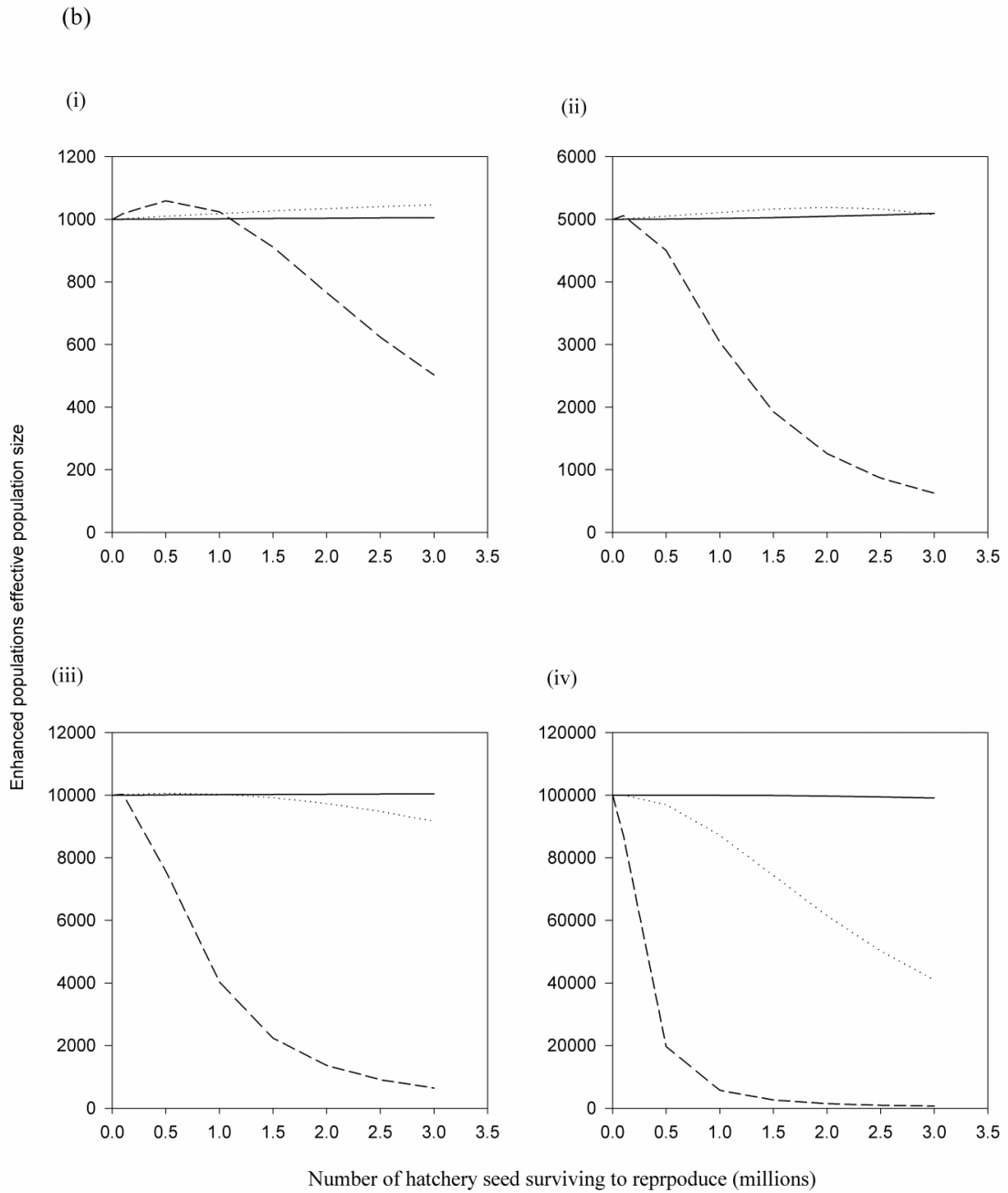


Figure 4.3. Predicted post enhancement effective population size following the introduction of hatchery seed. Enhanced with hatchery seed with an effective size of (a) 30 and (b) 60. The effect of different wild effective population size is shown in the different graphs: (i) 1000 (ii) 5000 (iii) 10,000 (iv) 100,000. The effect of variation in census size is shown by: solid line $N=10^7$, dashed line $N=10^8$ and dotted line $N=10^9$.

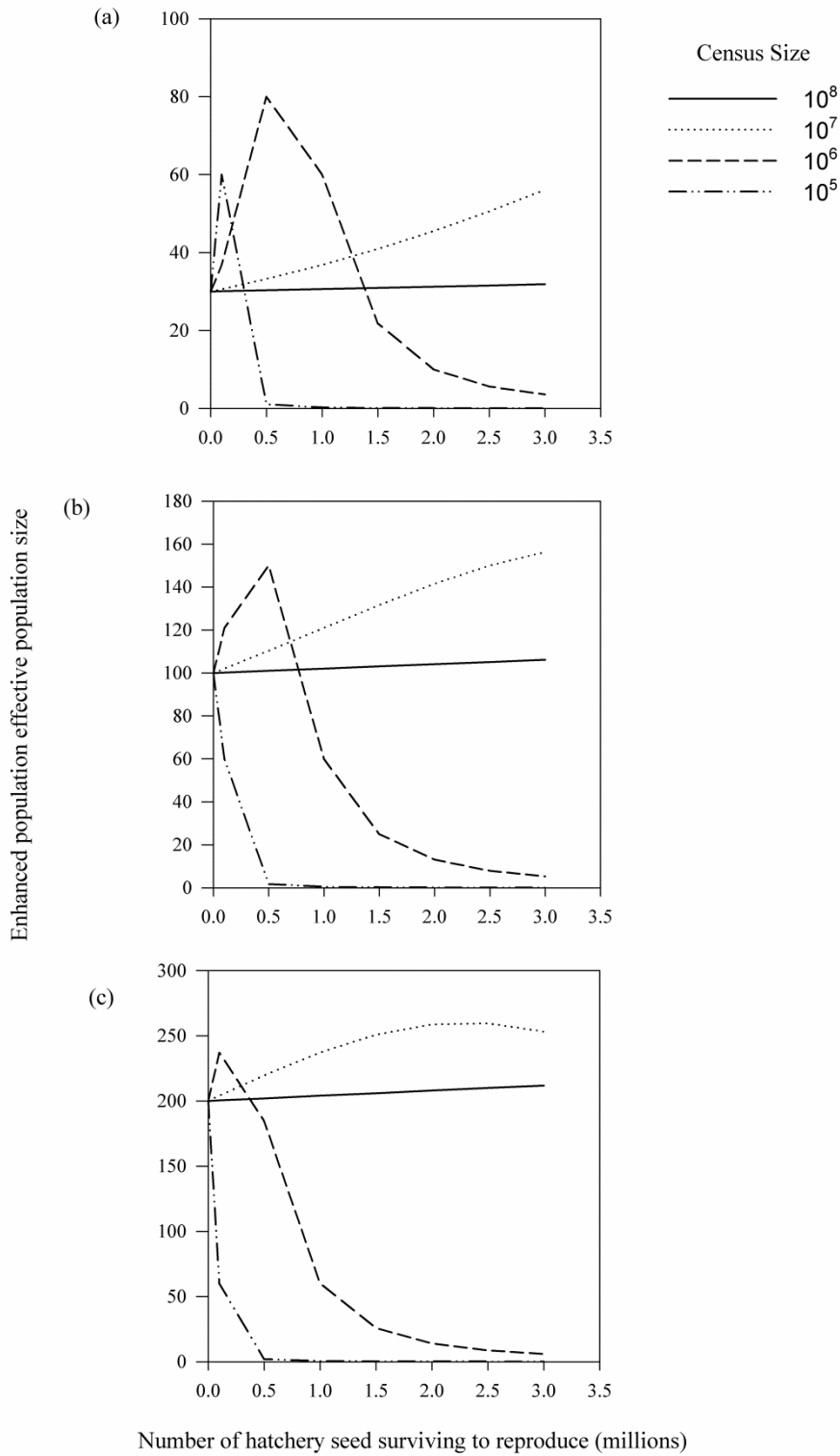


Figure 4.4. Predicted post enhancement effective population size following the introduction of hatchery seed ($N_e = 60$) to wild populations with small effective population sizes of (a) 30, (b) 100 and (c) 200. Wild population census sizes of: Solid line; 10^8 , dotted line; 10^7 , dashed line; 10^6 , dash and dots; 10^5 .

4.4.4 Population structure

F_{ST} values among the IOM sample groups were very low and were not significantly different from zero. Between the IOM and hatchery populations there was low but statistically significant genetic structure between the hatchery sample and three of the four IOM sample groups (Table 4.2). AMOVA showed that 98.3% of the variance occurred within populations, 1.5% of the variance occurred between the hatchery samples and IOM samples and 0.2% was between the populations within the IOM group. Fisher's exact test of population differentiation revealed a similar pattern: The hatchery sample was significantly different from all IOM samples, but sites within the IOM were not significantly different to each other after FDR correction of alpha (Table 4.2).

Table 4.1. Locus characteristics for *Pecten maximus* samples from the Isle of Man and hatchery progeny. Na = number of alleles, Ne = number of effective alleles, Ho = observed heterozygosity, He = expected heterozygosity, Nr = allelic richness adjusted by rarefaction.

Population	Measure	P9	P20	P23	P11	P60	P62	P70	P73	P59	P68	P75	W12	W4	W5	W8	Mean
BRO	Na	3	15	3	2	3	7	2	5	6	10	6	13	3	18	5	6.73
	Ne	1.17	5.50	1.30	1.19	1.56	3.49	1.09	1.24	3.44	4.94	2.34	6.52	1.12	8.91	1.20	3.00
	Ho	0.07	0.49	0.21	0.17	0.21	0.60	0.00	0.13	0.67	0.52	0.50	0.75	0.11	0.88	0.08	0.36
	He	0.15	0.82	0.23	0.16	0.36	0.71	0.08	0.19	0.71	0.80	0.57	0.85	0.11	0.89	0.17	0.45
	Nr	2.63	10.33	2.92	1.99	2.40	5.93	1.88	3.72	4.85	7.29	4.63	10.42	2.35	13.84	3.58	5.25
CHK	Na	6	13	3	2	5	6	3	6	5	9	7	11	3	17	5	6.73
	Ne	1.46	5.35	1.31	1.41	1.40	3.25	1.04	1.30	3.50	4.61	2.34	5.41	1.07	9.38	1.18	2.93
	Ho	0.15	0.31	0.19	0.27	0.33	0.71	0.04	0.21	0.65	0.38	0.42	0.76	0.07	0.87	0.11	0.36
	He	0.31	0.81	0.24	0.29	0.29	0.69	0.04	0.23	0.71	0.78	0.57	0.82	0.06	0.89	0.15	0.46
	Nr	5.00	9.97	2.71	2.00	2.46	5.03	1.93	4.32	4.84	6.27	5.24	7.35	2.23	13.24	3.44	5.07
TAR	Na	6	8	5	4	5	6	4	5	8	11	6	13	5	16	7	7.27
	Ne	1.20	4.30	1.36	1.30	1.62	3.09	1.14	1.31	3.12	5.53	2.15	5.98	1.25	8.76	1.24	2.89
	Ho	0.11	0.40	0.30	0.17	0.32	0.70	0.04	0.17	0.72	0.62	0.47	0.70	0.15	0.85	0.16	0.39
	He	0.16	0.77	0.27	0.23	0.38	0.68	0.12	0.23	0.68	0.82	0.54	0.83	0.20	0.89	0.20	0.47
	Nr	3.88	6.82	3.81	3.19	3.88	5.38	2.42	3.55	5.15	7.98	4.79	9.01	3.84	12.18	4.20	5.34
LAX	Na	5	15	4	2	4	6	3	7	6	9	4	12	4	15	5	6.73
	Ne	1.30	4.95	1.22	1.23	1.56	3.41	1.06	1.27	3.17	4.68	2.14	5.40	1.17	7.12	1.22	2.73
	Ho	0.08	0.40	0.17	0.17	0.35	0.64	0.02	0.19	0.65	0.42	0.29	0.83	0.10	0.77	0.14	0.35
	He	0.23	0.80	0.18	0.19	0.36	0.71	0.06	0.21	0.68	0.79	0.53	0.81	0.15	0.86	0.18	0.45
	Nr	4.92	13.10	2.82	2.00	3.82	5.80	1.97	4.45	4.95	6.65	3.00	9.00	3.00	13.00	4.00	5.50
Hatchery	Na	3	9	2	2	3	6	2	3	6	7	4	10	4	10	3	4.93
	Ne	1.17	4.88	1.21	1.11	1.38	3.30	1.02	1.25	3.30	2.86	2.02	5.79	1.25	5.13	1.11	2.45
	Ho	0.05	0.50	0.15	0.07	0.31	0.60	0.02	0.13	0.80	0.45	0.42	0.83	0.21	0.77	0.11	0.36
	He	0.15	0.79	0.18	0.10	0.27	0.70	0.02	0.20	0.70	0.65	0.50	0.83	0.20	0.80	0.10	0.41
	Nr	2.70	7.27	1.99	1.94	2.97	5.52	1.45	2.47	4.66	5.83	3.09	8.06	3.47	8.46	2.28	4.14

Table 4.2. Above diagonal: Fisher's exact test of population differentiation in the scallop *Pecten maximus*. Below diagonal: Pairwise F_{st} values comparing *P. maximus* from four Isle of Man (IOM) Sites and hatchery progeny. Bold face type face = significant following correction of 0.05 alpha value for multiple testing using the false discovery rate.

	BRO	CHK	TAR	LAX	Hatchery
BRO	-	0.034	0.250	0.441	<0.0001
CHK	0.0001	-	0.025	0.029	<0.0001
TAR	0.0000	0.0009	-	0.432	<0.0001
LAX	0.0014	0.0043	0.0018	-	<0.0001
Hatchery	0.0111	0.0120	0.0069	0.0146	-

4.5 DISCUSSION

Levels of genetic diversity and population structure were quantified for four IOM and a hatchery sample. Heterozygosity was found to be similar in both the IOM and the hatchery sample, whereas allelic richness was slightly lower in the hatchery samples when compared to all 4 of the IOM sites (when averaged over all loci), but this was not the case for all of the single locus comparisons. It is not possible, with only a single hatchery replicate, to test if this decrease is significant. To compensate for the presence of only a single cohort in the hatchery samples they were compared to individuals from a single age group at each IOM site. Nevertheless, average allelic richness was still higher in all IOM samples compared to hatchery. However it is possible that more than one cohort is present in each IOM age group as it has been shown that there are two spawning peaks in the Isle of Man (Mason 1958). Ageing the scallops to either spring or autumn spawning is very difficult due to the lack of a visible first year growth check ring. This could increase the allelic richness in the Isle of Man samples as sweepstake reproductive success is often seen in broadcast spawning marine invertebrates and causes lower allelic richness in single cohorts compared to multiple cohorts (Hedgecock 1994; Flowers *et al.* 2002; Hedrick 2005). It is unclear whether other studies that compared wild and hatchery bivalves have used single cohorts or a total population sample. Decreased allelic richness with the heterozygosity maintained has been seen in other studies (e.g. Dillon & Manzi (1987)). A decrease in allelic richness means that some rare alleles, and therefore genetic diversity, has been lost in the hatchery samples. If this decrease is real and not an artefact of sampling more than a single cohort in the IOM, it

could be detrimental to the wild recipient population. Utter (1998) described the negative effect of population enhancement using seed with compromised allelic richness as a “swamping” of the native genetic diversity. However, in the case of the scallop around the Isle of Man, which supports a large annual fishery, it is unlikely that the census number would be low enough that this could occur without extremely large numbers of seed being used and surviving thereafter to reproduce.

There was low but significant genetic structure between the IOM and hatchery sample as supported by the Fisher’s exact test. The AMOVA analysis suggested that 1.5% of the variance occurred between the IOM group and the hatchery samples. This finding could lead to concern that the genetic composition of the native scallops might be compromised with the introduction of such seed scallops. Local populations will have evolved in response to the conditions in which they reside and transferring scallops that have evolved in different environments could affect their survival to maturity and the subsequent success of their direct or “hybrid” (hatchery x wild) offspring such that transferred scallops may have poor survival and therefore may not be economically viable. Alternatively, hatchery scallops may have preferential survival over native scallops and outcompete them. An example of ecological differences that are thought to be genetically mediated in *P. maximus* is the reproductive strategy. Scallops at some locations have single peak spawning events whilst others show bimodal or even extended “trickle” spawning. This is thought to be genetically mediated as, when transplanted among sites, the transplanted scallops maintained their native spawning schedule (Cochard & Devauchelle 1993, Mackie & Ansell 1993, Magnesen & Christophersen 2008). However, there is a history of movement of *P. maximus* around its range including transfers of scallops into IOM waters (Beaumont 2000), so contamination of the native genetic composition has probably occurred already. It should also be noted that the Irish Sea is subject to considerable marine traffic. Ballast water contains large quantities of plankton and the numbers of larvae transferred in ballast water may be greater than in transfer projects (Beaumont 2000).

The N_e estimate we obtained for the hatchery sample was small with small CIs. However the estimate for IOM sample had extremely wide CIs, including infinity, so little weight should be given to the point estimates of N_e for the IOM. N_e estimation has difficulty in distinguishing between estimates of moderate and large size and has poor precision with

large N_e s because the genetic signal is weak and the sampling noise is large (Waples and Do 2009). It is likely that this was the case with the IOM sample, so although we can be confident that the N_e of the wild population is larger than that of the hatchery scallops we cannot be precise about the magnitude of this difference. This high effective population size is to be expected for such an abundant marine species however, it was found in chapter three that a wild population in Falmouth Bay, western English Channel, had an extremely small N_e of 24 (20 – 29 – 95% confidence intervals) suggesting that the protocols used by the hatchery are able to at least match the N_e s found in smaller wild populations.

By using a range of values for both wild N_e and census size we were able to simulate the effect that transferring seed would have on the wild population in these different scenarios. The effective population size used for the hatchery seed was 30 (to represent that estimated for our hatchery seed) and 60 (to represent combining seed from multiple spawning events with different broodstock). Enhancing wild populations with seed scallop can have vastly differing outcomes depending on the census and effective size of the recipient population, ranging from an increase in the enhanced populations' N_e to a decrease. Thus it is important to estimate both of these parameters prior to transferring seed to ensure a positive outcome for the wild population with the enhancement with hatchery scallop seed. The effect of doubling the hatchery effective population size from 30 to 60 has only a very small effect on the outcome in these scenarios. However, increasing the effective population size of the hatchery seed, for example by using multiple spawning events with different broodstock, can be beneficial when enhancing a wild population with a very low effective population size and using small numbers of seed. Using multiple spawning events with different broodstock should also increase the allelic richness of the seed scallops which was shown to be slightly lower in the hatchery scallops than the wild population. The importance of maintaining high N_e sizes relates to the negative relationship of N_e to rate of loss of alleles and heterozygosity (Crow 1986). Therefore populations with low N_e are at higher risk of losing genetic variability.

The simulations assume that the hatchery seed have an equal chance of contributing to the next generation compared to the wild scallops. Whilst this is a reasonable assumption it is not reasonable to assume that this occurs with random mating within the whole population. In broadcast spawning invertebrates, many studies have shown that fertilisation success decreases rapidly with distance (Levitan 1991; Levitan *et al.* 1992) and therefore scallops are much more likely to mate with their neighbours than scallops

some distance away. A concern of scallop ranching would be that if a large number of hatchery seed were released into one area of the seabed that these scallops would be more likely to mate with other hatchery seed than with the wild population, creating an F1 generation. This is also confounded by the fact that enhancement of wild populations would be more likely to occur in depleted and low density wild populations rather than an area of the seabed that has high densities of wild scallops.

This study has shown that there is no change in heterozygosity but that there is the possibility of lower allelic richness in the hatchery scallops compared to wild IOM populations. When combined with the weak, but significant genetic structure between the hatchery and IOM populations and a low hatchery N_e , these data indicate that caution should be taken when transferring scallops into different areas of the seabed. For example the scallop population in Falmouth Bay identified in Chapter three with an effective population size of 20 – 29 would be much more at risk of negative effects from the enhancement with hatchery scallops than larger populations of scallops. These data indicate that it would be wise to take a precautionary approach to the transfer of hatchery scallops to enhance wild populations.

If stocking is to take place then we recommend the following precautions:

1. Efforts should be made to estimate the census population size and, if possible, the wild N_e in order to more accurately estimate the outcome of the effect of transplanted seed on the effective population size.
2. Even with a moderate to large wild N_e populations with a census size of less than 109 could lead to a decrease in the enhanced populations N_e with the introduction of large amounts of seed.
3. Ranching should not be used as an alternative for good management of the wild populations as, if the native population numbers decline to numbers lower than 108 then there can be negative effects from enhancement even with small numbers of seed.
4. With a census size larger than 108, enhancement with small numbers of seed (100,000 surviving to reproduce) should not affect the enhanced populations' effective population size.

5. Increasing the hatchery scallops effective population size (for example by using multiple spawning events with different broodstock) should be considered in scenarios with very low wild effective population sizes.

CHAPTER 5: GENERAL DISCUSSION

Spatial Scales in the Management of *Pecten maximus*

The current study employed two distinct approaches to try and understand the spatial scales of connectivity and spatial variation in the timing of reproduction in populations of *P. maximus* in the NE Atlantic. Firstly, genetic variation and structure was investigated which provided information on integral stock units on an evolutionary timescale (Carvalho and Hauser 1994) and secondly variation in the reproductive status was studied, providing information at the opposite end of the integrity spectrum on very short timescales. Genetic structure suggested that connectivity at the regional scale covering the British Isles and Ireland, but with several isolated populations within this region. In comparison there was variation at very small spatial scales of just a few kms in the reproductive status of *P. maximus*. So although it is likely that there is connectivity between neighbouring populations via larval dispersal which homogenises allele frequencies the various populations do not act as a single random mating unit; if populations spawn at different times then there is no chance of fertilisation between individuals at different sites, even if the gametes are able to disperse from one population to another. Poor reproductive synchronisation will lead to high variance in the reproductive success of individuals within a population (Pennington 1985). This sweepstake effect (Hedgecock 1994) can also be amplified by low densities of scallops as sperm will be rapidly diluted (Pennington 1985; Levitan 1991) and fertilisation success may rapidly decrease when individuals are separated by just a few metres (Pennington 1985). This again will lead to only a few individuals being reproductively successful, but may also increase the proportion of self fertilised larvae in the total larval pool. A final factor in variation in reproductive success is the non-random survival of larvae after fertilisation. A mismatch of some individuals between reproductive activity and oceanographic conditions favourable for survival and recruitment of larvae will again increase the variance in reproductive success (Hedgecock 1994). Variance in reproductive success and environmental stochasticity can lead to spatial and temporal variation in recruitment (Botsford *et al.* 1994). In a fishery such as that of the king scallop, which relies on the year class recruiting to the fishery, such variance can lead to large variability in the landings between grounds from year to year. The variance caused

by environmental stochasticity cannot be influenced, therefore good management of a scallop stock would require strategies to maximise reproductive success and minimise reproductive variance. This could be achieved by increasing scallop densities; the closer a scallop is to a potential mate the higher the chance of fertilisation success. Also, by maximising the spatial distribution of high density populations the proportion of spawning stock which mismatch reproductive activity with favourable environmental conditions may be able to be minimised. One way of achieving this goal is to have a network of suitable habitat closed to scallop extraction, which will allow increases in scallop density (Beukers-Stewart *et al.* 2005, 2006; Kaiser *et al.* 2007).

Maximising the potential “spillover” from protected areas to commercial grounds requires careful consideration of their positioning. For example, around the Isle of Man microsatellite analysis suggests gene flow between populations, however it is likely that the area offshore from Peel experiences lower migration than other populations, probably less than five percent (with migration greater than five to ten percent the effective population size estimate should have been representative of the metapopulation not the local population as was likely with the small N_e estimated for Peel (Waples and England 2011)). Such low levels of migration are probably not substantial enough to influence population dynamics as the shift between demographic independence and dependence is estimated to occur at levels of migration in excess of 10% (Hastings 1993). Therefore scallops within the Peel population are unlikely to supply significant numbers of larvae to neighbouring populations. However, although populations with lower connectivity are not good candidates for MPAs it must also be remembered that they are more reliant on self-recruitment and are therefore much more susceptible to over-exploitation.

The sweepstake effect also decreases the overall effective population size (Hedgcock 1994; Hedrick 2005). Small effective population size increases the rate of loss of heterozygosity and increases the variance of the frequency of an allele (Crow 1986) which can decrease the fitness of a population. Inbreeding depression has been shown to cause decreased hatching, growth and survival in scallops (Beaumont and Budd 1983; Ibarra *et al.* 1995; Winkler and Estévez 2003). The high levels of inbreeding and selfing estimated in this study will add to the decreased fitness of a population. Therefore areas of high density scallops, which can increase fertilisation success and therefore decrease the proportion of selfed offspring and the level of inbreeding, may increase the effective population size and may act to maintain high fitness within population. Another

management tool for increasing scallop densities is to enhance natural scallop populations with hatchery reared juvenile scallops. The study on the genetic effects of using hatchery reared scallops to enhance wild populations suggests that caution should be used when using large numbers of seed, especially when the census and effective population sizes of the wild population are not known.

It can be seen in the above discussion that management of king scallop stocks should occur at spatial scales much smaller than that suggested by the genetic structure alone. Including genetic information on effective population sizes, inbreeding and self fertilisation as well as ecological information about reproductive variability has allowed identification of processes occurring at much smaller spatial scales than those of the genetic stock on an evolutionary timescale alone.

The last glacial maxima and migration drift equilibrium

Calculation of F_{ST} values assumes that populations are in migration drift equilibrium and therefore, with changes in connectivity, genetic differentiation measures will often reflect the past levels of movement or isolation rather than the contemporary structure (Hellberg 2009). Migration-drift equilibrium can take a long time to reach; time (in generations) required for F_{ST} to go **half way** to equilibrium values is:

$$\approx (\ln 2)/(2m + 1/2N_e) \quad (\text{Crow and Aoki 1984})$$

where m is the migration rate and N_e is the effective population size. Therefore when effective population size is large it can take many thousands of generations to reach equilibrium and any changes in migration or effective population size will cause the time to equilibrium to be reset.

This phenomenon is particularly important to consider at high latitudes where many populations have only been colonised since the last glacial maximum (LGM). The Irish Sea Glacier was grounded as far south as the Isles of Scilly (Hiemstra *et al.* 2006) as late as 22,000-21,000 years ago with rapid retreat through the southern Irish Sea to the Isle of Anglesey by 20,000-18,000 years ago (McCarroll *et al.* 2010). There was then a gradual retreat northwards after this. The extent of the ice cover over Ireland has been debated, but evidence suggests that there was total ice cover over Ireland with self edge glaciations present west and southwest of Ireland (Scourse submitted). Further south the

conditions within the southern Irish Sea during the LGM are thought to be similar to those experienced in the Fjords of eastern Greenland (76°N) today (Scourse submitted). These conditions made survival in the current northern extent of *P. maximus* range impossible during the LGM. It should also be noted that sea temperatures would have remained low even after deglaciation and current day thermohaline driven density currents probably developed about 8000 years ago (Hill *et al.* 2008). As colonisation events followed the northwards retreat of the ice sheet from glacial refugia the patterns of connectivity or isolation would be established but, until equilibrium is reached, genetic differentiation based on allele frequencies, would reflect the relationship between founding populations and a common ancestral population. Hence differentiation could remain low between newly settled populations and their source refugia for many thousands of generations even if current day connectivity is low (Hellberg 2009). So assuming that current day population structure has been in existence for 8000 years without change, a scallop population with a generation time of 6 years and a moderately large effective population size of 100,000 would have required a migration rate greater than 0.001 (proportion exchanged each generation by migration) to have reached 7/8 of the way to equilibrium today.

Patterns of genetic differentiation have been used to predict species ranges and locations of glacial refugia during the LGM (e.g. Coyer *et al.* 2003; Provan *et al.* 2005; Maggs *et al.* 2008; Kenchington *et al.* 2009). Some of these studies have suggested glacial refugia in Northern Europe at the ice margins including; Hurd Deep – a trench in the western English Channel (Coyer *et al.* 2003; Provan *et al.* 2005; Maggs *et al.* 2008), off the southwest coast of Ireland (Provan *et al.* 2005; Maggs *et al.* 2008) and the Faroe Islands (Maggs *et al.* 2008). Maggs *et al.* (2008) describe the theory behind this genetic model;

“Traditional models of glacial refugia and routes of recolonisation include these predictions: low genetic diversity in formerly glaciated areas, with a small number of alleles/haplotypes dominating disproportionately large areas, and high diversity including “private” alleles in glacial refugia. In the Northern Hemisphere, low diversity in the north and high diversity in the south are expected”.

However there has been some discordance between these findings and the majority of the geological evidence of the extent of ice cover and inhospitable conditions (Scourse submitted). For example, the Hurd Deep would have been disconnected from the open

ocean and would have been filled with melt water, not a marine lake (Scourse submitted). It is therefore unlikely that this would have provided habitat in which *P. maximus* would have been able to survive, especially as they have poor tolerance to low salinity conditions at low temperatures (Christophersen and Strand 2003). During the LGM the Faroe Islands would have reached summer temperatures of approximately 6-8°C (Kettle *et al.* 2011). Spawning takes place in northern Norway when summer temperatures reach 7-9°C (Strand and Parsons 2012) therefore it is possible that the Faroe Islands could have reached the minimum temperature required for spawning and was a possible glacial refugia. The currents in the northern Atlantic and Nordic Seas would have permitted recolonisation via larval dispersal from the Faroe Islands to Northern Norway, but much less so between the Faroe Islands and The British Isles and Ireland (Holm *et al.* 2000). However, in its' present northern range *P. maximus*, does not experience regular sea ice during the winters and it is predicted that during the LGM the Faroe Islands would have experienced sea ice for three to four months of the year, so whether the Faroe Islands could have acted as a northern glacial refugia is unclear. In comparison southern glacial refugia are supported by geological, archaeozoological and genetic data which suggest that populations of marine taxa would have been displaced from northern Europe south to the Iberian Peninsula and into the Mediterranean Sea (Kettle *et al.* 2011). However, the differentiation between Norway and the other sites is difficult to explain with recolonisation from Iberia or the Mediterranean but the location of a separate refuge for Norway is unclear. Analysing samples from the Faroe Islands and the north of Norway may be useful in this respect.

Significantly different from zero?

The use of hypothesis testing and P-values in biology and ecology has been heavily criticised for its misuse and misinterpretation, e.g. interpreting statistical significance as biological significance; regarding the P-value as the probability that the null hypothesis is true; assuming the P-value is a measure of effect size or evidence (Gerrodette 2011). In the analysis of population differentiation using F_{ST} , P-values have also been questioned. P-values depend on 1) the effect size, 2) variation in observations and 3) the sample size. Therefore for the same effect size you could obtain different P-values or conversely the same P-value may relate to vastly different effect sizes depending on the sample size and the variance. With the increasing ability and decreasing cost of obtaining large amounts

of data it is possible to obtain enough loci to attain a significant P-value for an F_{ST} even if this has no biological significance (Hellberg 2009). Conversely increasing the number of loci does not necessarily mean that you have enough power to detect meaningful levels of migration. There is large variance in the F_{ST} values associated with different markers, therefore lack of statistical significance could be due to high variance rather than high migration (Hellberg 2009). P-values in biology and ecology are usually calculated based on false null hypotheses (Gerrodette 2011); we know that F_{ST} is not going to be zero to ten or twenty decimal places in natural populations so we can reject the null hypothesis without testing it. The approach needs to be turned around; how much support does the data lend to a hypothesis? (Gerrodette 2011). Using inference methods to measure the degree of support can result in improved understanding than that provided by P-values (Gerrodette 2011). Maximum likelihood and Bayesian inference methods are increasingly being used in genetics, with programs like DIYABC (Cornuet *et al.* 2008) making these inference methods more widely available to the empirical geneticist. Following on from the work within this thesis it would be interesting to use Bayesian inference to gain a greater understanding of the genetic structure in *P. maximus*, especially in the connectivity between Galicia and the British Isles and also on the level and time of isolation of the Norway population.

Limitations and future work

Null alleles in bivalves

Null or non-amplifying alleles are common in microsatellite loci (Dakin and Avise 2004) and are present in most taxa but seem to be particularly prevalent in marine Bivalves (Reece *et al.* 2004; Hedgecock *et al.* 2007; Yu and Li 2008; Brownlow *et al.* 2008; Lallias *et al.* 2010; Lemer *et al.* 2011). *P. maximus* has shown rapid evolutionary divergence from other Ostreoida in both sequence and copy numbers (Biscotti *et al.* 2007) indicating high levels of mutation. Bivalves tend to show low numbers of microsatellites within the genome and short length of repeats relative to those found in mammals (Cruz *et al.* 2005; Biscotti *et al.* 2007) but with an increased propensity towards imperfect or interrupted repeats (Watts *et al.* 2005; Biscotti *et al.* 2007). An explanation for the lack of extremely long microsatellites suggests that there is a balance between length (slippage) and point mutations; slippage will tend to increase the length of a microsatellite whilst point mutations will act to break the long microsatellite into

smaller units (Ellegren 2004). Therefore the presence of high numbers of interrupted repeats again suggests high point mutation rates which can help explain the high levels of null alleles found in marine Bivalves. The effect of the presence of null alleles on various statistical methods has been investigated and it was generally found that the methods were robust. For example although null alleles created a significant decrease in the percentage of correctly assigned individuals in both model-based clustering and Bayesian assignment methods, this effect was small and would probably not alter the overall outcome of the assignment testing (Carlsson 2008). At the null allele frequency levels typically reported in the literature ($p < 0.2$), there was a slight under-estimation of the average exclusion probability in parentage analysis, but this was not considered sufficient enough to merit concern (Dakin and Avise 2004). However, null alleles do tend to inflate F_{ST} values and genetic distances slightly (Chapuis and Estoup 2007; Carlsson 2008) but this can be easily corrected before estimating F_{ST} values (Chapuis and Estoup 2007). Interestingly the F_{ST} values calculated with a correction for null alleles in this study actually increased the F_{ST} values.

The presence of null alleles increases the apparent proportion of homozygotes which causes a deviation from Hardy-Weinberg Equilibrium (HWE). Although an excess of homozygotes can also be caused by inbreeding or the Wahlund effect, these phenomenon are likely to cause deviations from expected frequencies systematically across all loci, whereas null alleles are likely to be locus specific. In this study the deviation from HWE due to an excess of homozygotes was not statistically significant in all loci suggesting the cause is not systematic and that null alleles are the more likely explanation, which was supported by Micro-checker. Analyses were corrected for the presence of null alleles where possible (F_{ST} estimates and null allele error rate included in the Colony analysis), but some methods were avoided completely due to the presence of null alleles, for example the software program STRUCTURE (Pritchard *et al.* 2000). Although shown to be fairly robust up to null allele frequencies of $P < 0.2$ (Carlsson 2008), this study only had three loci which had estimated null allele frequencies less than 0.2 in all populations.

Solutions to the problem of null alleles have generally focused on the development of multiple pairs of primers for each loci (Brownlow *et al.* 2008; Lemer *et al.* 2011) and it would be possible with the next generation sequencing data from the current study to develop a second set of primers for the loci that showed high levels of null alleles in this study. Eliminating null alleles will allow true levels of heterozygosity to be seen and a

greater understanding of conformance to Hardy-Weinberg Equilibrium, genetic diversity and population expansion.

Sweepstake Recruitment

Hedgecock (1994) predicted that variation in reproductive success, the sweepstake effect, would lead to temporal genetic change due to random drift and lower genetic diversity within cohorts than in the overall breeding population. It was hoped that this could be analysed with the microsatellite data from this study, however three problems were encountered; Firstly not all samples were able to be aged due to tissue being obtained from third parties and the shell was not available. Secondly, for samples which shells were available for aging, it was possible to age using the winter growth check rings (Mason 1957), but it was very difficult to place into a cohort. For example in the Isle of Man there are two peak spawning events, spring and autumn, and therefore two cohorts that would have the same number of winter growth check rings. Finally, the sample from each population that shells were available for were dominated by a single age group. For example in the Targets population forty of the forty seven samples were age four. This meant that we did not have a representative sample of the overall breeding populations to compare the cohort genetic diversity to. For future work it is hoped that we can use stable oxygen isotope analysis to accurately age a shell not only to the year but also into a spring or autumn cohort. Delta 18 oxygen is absorbed in greater quantities at lower sea temperatures. Although the degree of change in absorption rates varies slightly with growth rates in scallops it can still be used to age scallop shells by enabling identification of the winter decrease in sea temperature (Owen *et al.* 2002). Identification of spring or autumn spawned cohort may be possible by tracking the temperature change from the umbo through to the first winter growth check. Water temperatures will rise through the summer before falling in the autumn and winter with a spring spawned scallop whilst there will only be a temperature decrease in an autumn spawned scallop. In order to obtain a representative sample of the breeding population would require greater sampling effort to obtain enough scallops from older and less abundant age groups.

Sampling; Gonad condition survey

Survey sites for the collection of scallop samples for the gonad condition survey were largely dictated by the overall research cruise objectives with little prior consideration of the environmental and fishing parameters. This resulted in an uneven distribution of

samples within the range of some of the covariates, which created outliers. Outliers force the GAM to estimate a relationship based on one or two points at one end of the range. Transformations can help to pull outliers in but this does alter the relationship that is being investigated. A better approach would have been to choose survey sites based on their environmental parameters. Another problem with the sampling protocol was the low replication at each of the covariate coordinates. With poor x-axis replication it is very difficult to investigate interactions and in the current study there were not enough replicates to include interactions in the model. The GAM model without interactions explained 42.8% of the overall deviance and it would be expected that this may increase with the inclusion of interaction terms. An improved distribution of sampling effort would have been to decrease the number of scallop samples at each site but increase the number of sites studied.

Sampling; Population Genetics

The sampling for the population genetics study was designed to cover a variety of spatial scales from small scale within the Irish Sea, to a regional scale around the British Isles up to a large scale covering the extremes of *P. maximus* range. Now that the general patterns of connectivity and isolation have been identified it would be useful to add additional sites to elucidate some of the connections between populations. For example additional sites along the Atlantic coast of France, the Iberian Peninsula and round through the Strait of Gibraltar would help clarify the connectivity between northern European populations and southern populations.

The currents in Falmouth Bay are split; the west of the bay experiences anticlockwise circulation and east of the bay experiences clockwise circulation (Ferentinos and Collins 1979). The samples from the current study were collected from the west of the bay, within the area of anticlockwise circulation. To elucidate the scale of isolation of the Falmouth population further samples from the east of the bay and then from neighbouring bays and offshore would be useful.

There has been speculation that the northern and southern populations of scallops in Norway are genetically distinct based on their reproductive schedules: In the west and south (60°N) scallops have an extended spawning season from march until September with rebuilding of the gonad taking place from October until December (Strand and Parsons 2006) or even as late as the following spring (Magnesen and Christophersen

2008). In comparison populations from further north have a synchronised spawning in June when temperatures reach 7-9°C and rapidly rebuild their gonads throughout July and August whilst the water temperatures are warm (Strand and Parsons 2006). This difference in reproductive schedules is thought to be genetically mediated because the scallops retain their natal pattern of spawning upon translocation (Magnesen and Christophersen 2008). Samples for genetic analysis would assist to clarify this relationship.

The connection of the Norwegian coast from the south is via a current flowing from the central and southern North Sea into the southern Skagerrak-Kattegat (the sea between Denmark, Sweden and Norway linking the North Sea with the Baltic Sea) circulating anticlockwise around the coast of Sweden (Rodhe 1996) and the southern coast of Norway, exiting the Skagerrak-Kattegat to the north and up the Norwegian coast as the Norwegian Coastal Current (Rodhe 1996; Kershaw *et al.* 2004; Hill *et al.* 2008). Although this water circulation could serve to link Norwegian populations of scallops with those from the southern North Sea and the English Channel the salinity conditions within the Skagerrak-Kattegat (< 28 ‰ in places) may create a barrier to larval dispersal as hatching of eggs and survival and growth of larvae are significantly diminished at low salinity levels (Gruffydd and Beaumont 1972). A barrier in the Skagerrak-Kattegat may allow differentiation to develop between Norwegian scallops and scallops from the southern North Sea and the English Channel. Further sampling across this potential barrier would be interesting.

Summary of the thesis

The current study shows that integrating different types of data allows a clearer picture to be formed of the spatial scale over which populations interact, react and function than when a single method is used in isolation. Genetic estimates of population differentiation based on F_{ST} look at the largest scale at which populations relate but may not provide a clear picture of migration at the levels required for demographic independence. However, the addition of ecological data and different genetic analyses refined the large scale patterns identified by F_{ST} values. Using genetic markers to provide additional information by means of spatial analysis of molecular variance, effective population size and relatedness helped to identify scallop populations which were an exception to the

overall pattern of differentiation, and the inclusion of ecological and biological data showed fine scale differences in the reproductive patterns among populations.

In addition to the above general conclusions, several key findings were found:

1. Scallop populations in the Irish Sea show among population variability in the timing of the conditioning of gonads over small (< 5 km) spatial scales.
2. The within population variation in the timing of gonad conditioning is high, leading to a decreased possibility of fertilisation with a conspecific and a possible increased proportion of selfed offspring in the population. This would be exacerbated by the low densities of scallops typical of commercial grounds.
3. Estimates of the proportion of self fertilised scallops in the current study ranged from six percent to twenty five percent. Inbreeding ranged from that expected with mating between grandparent/grandchild up to that expected with mating between half siblings.
4. Falmouth Bay had a very small estimated effective population size (20 – 29).
5. Enhancing wild scallop populations with large numbers of hatchery seed (> 100,000) may have detrimental effects on the enhanced population's effective population size, especially if the census size is less than 10^8 .

APPENDIX 1: PREPARATION FOR NEXT GENERATION SEQUENCING AND MICROSATELLITE DEVELOPMENT METHODOLOGY

A1.1 RNA STABILISATION AND EXTRACTION

A1.1.1 Tissue harvesting and RNA stabilisation

Ribonucleases (RNases) are very stable, active, difficult to inactivate and are present not only in the tissue that the RNA is being extracted from, but also in bacteria and moulds commonly found in hand and dust particles, therefore RNA can very easily and quickly degrade (Fritsch *et al.* 1989) For quality RNA extraction the tissue first has to be stabilised to stop any RNA degradation. Secondly all subsequent steps must use aseptic techniques to avoid any contamination with RNases from dust particles. Finally all solutions should be RNase-free.

Four live *P. maximus* samples were collected and kept alive in aquariums until tissue harvesting. To limit the degradation of RNA, tissue was harvested from live individuals on ice and immediately stabilised in RNA-later (Sigma catalogue number R0901) following the manufacturers protocol. Three 50-100mg tissue samples, a mixture of muscle, gill and mantle, were harvested from each of the four individuals (S1, S2, S3 and S4), to give a total of 12 samples.

A1.1.2 RNA extraction

RNA extraction was performed using TRIZOL™ single-step methodology (Simms et al. 1993). TRIZOL™ is a monophasic solution of phenol and guanidine isothiocyanate and maintains the integrity of the RNA while disrupting the cells and homogenising cell components (Simms et al. 1993). TRIzol™ manufacturers' protocol was adapted and consists of the following steps;

1. Tissue disruption and homogenisation.

1.5ml of TRIZOL™ was added to each microcentrifuge tube containing 50-100mg of tissue. Sterile pestles attached to a rotary drill were used to disrupt and homogenise using six to nine short bursts (~ten seconds). Short bursts avoid heating the sample. The cleared homogenate was separated from the insoluble material by centrifugation (12,000g for ten minutes at 4°C) and pipetted to a new 1.5ml microcentrifuge tube. This additional step was carried out due to the high content of proteins, lipids and polysaccharides that are likely to be present in bivalve muscles tissues.

2. Phase separation

Incubation of the homogenate at room temperature for 10 minutes allows complete dissociation of nucleoprotein complexes (Simms et al. 1993). For phase separation, 0.2ml of chloroform was added to the homogenate per 1ml of TRIZOL used in homogenisation and centrifugation. Lids were secured firmly before shaking vigorously for 15 seconds. This was incubated at room temperature for three minutes and then centrifuged at 12,000g for 15 minutes at 4°C. RNA is retained in the upper aqueous phase and DNA and proteins are retained in the lower organic phase. This clear aqueous phase was pipetted off into a new 1.5ml microcentrifuge tube.

3. RNA precipitation

0.5ml of cold isopropanol per 1ml of TRIZOL used in homogenisation was added and mixed by inversion. Following incubation on ice for 20 minutes tubes were centrifuged at 12,000g for ten minutes at 4°C to create a gel-like pellet of RNA. The supernatant was removed and discarded.

4. RNA wash

1ml of cold 75% ethanol per 1ml of TRIZOL used during homogenisation was added to the pellet and vortexed to mix. This was centrifuged at 7500g for five minutes at 4°C. The supernatant was removed and discarded.

5. Eluting RNA

Residual ethanol was removed by air-drying the pellet for five minutes. RNA was eluted in 50µl of RNase-free water. The six extractions from Scallops 1 and 2 were combined to make one sample (S1/2) of 300µl and the same for scallops three and four (S3/4).

6. NanoDrop 1000 spectrophotometer analysis

Samples were quantified and checked for contamination using the NanoDrop 1000 (Thermo Scientific). Blank readings used 1.5 µl of RNase-free water and measurements were taken on 1.5 µl of each RNA sample. Quantity of nucleotides (ng/µl), absorbance profile, 260:280 and 260:230 nm absorbance ratios were recorded. For pure DNA samples the 260:280 ratio should be around 1.8 and for pure RNA samples it should be around 2.0. The 260:230 ratios should be above 1.5 and ideally above 2, with lower values indicating contaminants with absorption around 230 nm, for example phenol and polysaccharides.

7. Sodium acetate precipitation

Initial results showed contamination at the 230 nm wave length so the samples were precipitated and washed again. 1/10TH volume of NaAc was added to RNA extract (30µl to 300µl of extract). Then three times the volume (900µl) of ice cold 100% ethanol was added. This was incubated at -20°C for 20 minutes then centrifuged at full speed for 15 minutes at 4°C. The supernatant was discarded. 500µl of 70% ethanol was added and centrifuged at full speed for five minutes at 4°C. The supernatant was discarded leaving behind the RNA pellet. This was air dried for five minutes and then eluted in 250µl of RNase-free water. 250µl is the volume needed for the next step in the protocol. The results of the Nanodrop analysis of these final samples are shown in Table A1.1. Samples were stored at -20 °C.

Table A1.1. Nanodrop quantification and quality check results following initial extraction (Total RNA)

Sample	Concentration ng/µl	260:280 nm ratio	260:230 nm ratio	Total RNA (µg)
S1/2	77.35	2.17	1.87	30.94
S3/4	69.23	2.17	1.97	27.69

A1.2 MESSENGER RNA PURIFICATION

On initial testing it was found that, although the cDNA synthesis methodology used targeted mRNA, it yielded 90% ribosomal sequences. So for the final methodology an mRNA purification step was included. mRNA purification was carried out using Oligotex[®] mRNA Kit (Qiagen catalogue number 70022) which contains Oligotex suspension that binds to the Poly-A tail of messenger RNA. Spin columns are then used to retain the Oligotex and mRNA hybrid whilst other nucleotides are washed away using a wash buffer. The hybridisation requires a high salt content and so the mRNA is easily released by using an elution buffer that lowers the ionic strength. The manufacturers' protocol was adapted to increase yield by increasing the hybridisation time and increasing the elution volume.

1. Oligotex mRNA purification

Total RNA was defrosted on ice. The oligotex suspension was heated to 37°C, mixed by vortexing and then left at room temperature. 250µl of buffer OBB and 15µl of warm oligotex suspension was added to the 250µl of total RNA in an RNase-free 1.5ml microcentrifuge tube and mixed gently by pipetting. This was incubated at 70°C for three minutes then placed at room temperature for 15 minutes. The mixture was centrifuged for two minutes at maximum speed at room temperature to create a pellet. The supernatant was pipetted off the pellet but leaving about 1µl to avoid loss of resin and resuspended in 400µl of buffer OW2 by pipetting. This was pipetted into a small spin column which is placed in a 1.5ml microcentrifuge tube and centrifuged at max speed for one minute at room temperature. The spin column was transferred to a new 1.5ml microcentrifuge tube, 400µl of buffer OW2 was added then centrifuged again for one minute and the flow through discarded. The spin column was transferred to a new 1.5ml microcentrifuge tube, 40µl of 70°C buffer OBE was added, pipetted up and down 3-4 times and centrifuged at full speed for one minute to elute the mRNA (Pipetting was carried out in 70°C heat block to avoid cooling of the buffer). This buffer OBE stage was repeated to give a total volume of 80µl per sample.

2. Quantification

Following mRNA purification, samples were quantified again using the nanodrop 1000. Results are shown in Table A1.2. The minimum starting amount of mRNA for cDNA synthesis is 0.1 µg. Total mRNA from both samples is 1.21 µg.

3. A 16S universal primer pair was optimised for use with *P. maximus* DNA (Table A1.3). *P. maximus* mRNA samples (S1/2, S3/4) with a positive DNA control (Samples C) and a negative control were amplified with the 16S primers (Table A1.3). PCR product was run on a 2% agarose gel with TBE buffer at 100V for 30 minutes. Contamination was found in both sample S1/2 and S3/4 (Figure A1.1).

Table A1.2. Nanodrop quantification and quality check results following mRNA purification

Sample	Concentration ng/µl	260:280 nm ratio	260:230 nm ratio	mRNA (ng)
S1/2	10.25	2.07	1.18	804.6
S3/4	5.14	2.51	1.22	403.5

Table A1.3. PCR conditions for 16S universal primers with *Pecten maximus*
 16S UniF 5'- CGC CTG TTT AWC AAA AAC AT – 3'
 16S UniR 5' - CCG GTY TGA ACT CAG ATC AYG T – 3'

Reagent	<u>PCR reaction</u>		<u>Thermocycling</u>		
	10 µl Reaction		temp	time	cycles
10x ABgene Reddymix buffer	1 µl		94°C	3 min	x1
dNTP (1mM)	3.2 µl				
16S uni F primer (10 µM)	0.3 µl		94°C	1 min	
16S uni R primer (10 µM)	0.3 µl		53°C	1 min	x 30
<i>Taq</i> Polymerase	0.1 µl		72°C	1 min	
RNase-free water	4.6 µl				
DNA/RNA	0.5 µl		72°C	10 min	x1

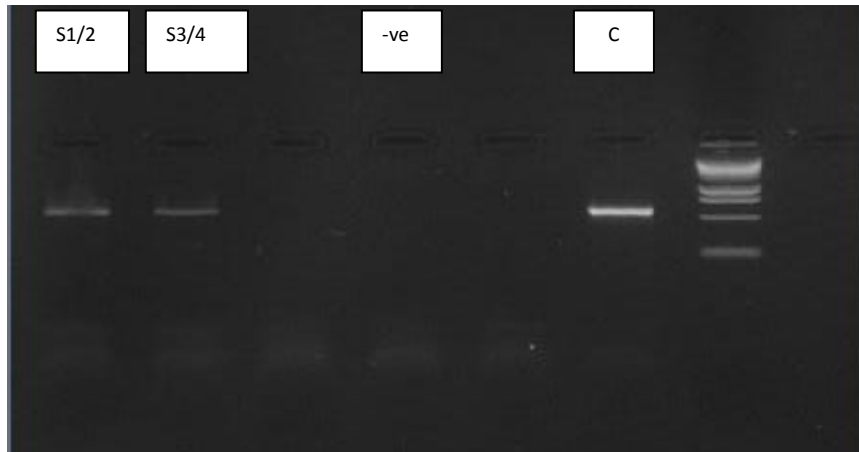


Figure A1.1. Agarose gel result for positive DNA PCR check. *Pecten maximus* mRNA samples S1/2, S3/S4, a negative control and DNA control sample C

4. RNA clean up and DNase treatment

The contaminated samples were treated with DNase using RNase-free DNase kit (Qiagen). Each sample was made up to 87.5µl using RNase-free water in 1.5ml microcentrifuge tubes. 10µl of buffer RDD was added and 2.5µl DNase I. This was incubated at 20-25°C for ten minutes. Qiagen's RNeasy mini kit was used for RNA clean up. To the 100µl of DNase treated samples, 350µl of buffer RLT was added and mixed by pipetting. Next 250µl of 100% ethanol was added and mixed by pipetting and immediately the whole 700µl sample was transferred to an RNeasy mini spin column with a 2ml collection tube. This was centrifuged for 15 seconds at 10,000g and 22°C. The flow through was discarded. 500µl of buffer RPE was added to the spin column, centrifuged at 10,000g, 22°C for 15 seconds and the flow through discarded. This stage was repeated. The spin column was placed in a new 2ml collection tube and centrifuged for one minute at full speed. The spin column was placed in a new 1.5ml microcentrifuge tube and 30µl of RNase-free water was added to the spin column, centrifuged at 10,000g for one minute at 22°C. This elution step was repeated to give final elution volume of 60µl for each sample. Following clean-up a second positive DNA PCR check was carried out. No DNA contamination was seen on agarose gel electrophoresis.

5. Sodium acetate ethanol precipitation and concentration of samples.

The cDNA synthesis requires a minimum of 0.1 µg of mRNA in a maximum of 3 µl. However eluting the precipitate in 3 µl is very difficult so a slightly larger volume was used. In order to concentrate the samples sufficiently, both samples were combined and a sodium acetate ethanol precipitation was carried out, eluting in 8µl of RNase-

free water. The final sample was quantified using the Nanodrop 1000 which estimated the concentration to be 19.8 ng/μl

6. Agilent RNA 6000 Nano Assay RNA quality check

The Agilent RNA 6000 was used for a final accurate quantification and quality check before cDNA synthesis. The protocols in the manufacturers' manual were followed. No ribosomal peaks at 18s or 28s could be seen, there was a small peak between these two ribosomal sequences showing presence of mRNA. The Agilent suggests very low contamination with rRNA (5.6%) with a final concentration of 34 ng/μl. This gives a total of 0.102 μg of RNA in 3 μl, just sufficient for cDNA synthesis. The total yield in the 8 μl was 272 ng mRNA from a starting amount of 2g of tissue.

A1.3 cDNA SYNTHESIS

Evrogen MINT cDNA synthesis kit was used and the manufacturers' protocol was followed. MINT cDNA synthesizes the first strand cDNA from 3' end of the RNA using reverse transcriptase. The 3' end of mRNA has a poly A+ stretch of RNA and the synthesis uses a 3' – Primer which is a dT oligo to anneal to the poly A+ stretch. When the reverse transcriptase reaches the 5' end of the mRNA it adds several nucleotides (primarily dC) to the 3' end of the newly synthesised first strand cDNA. A special 30-mer deoxyribo-oligonucleotide, called PlugOligo has a complementary dG sequence. The efficiency of reverse transcriptase using the PlugOligo as a template and incorporating it into the first strand cDNA, is increased by a solution in the MINT kit called IP-solution. This essentially targets mRNA and filters out rRNA. The end product is a single strand of cDNA with a tail from the 3' – Primer. The second strand synthesis then uses a Primer (M1 Primer) that compliments the tail on the 3' – Primer in a PCR reaction.

A1.3.1 First strand cDNA synthesis

1. For each RNA sample the following was added in a 0.2ml microcentrifuge tube; 3 μl RNA sample, 1 μl 3' Primer from MINT kit, 1 μl PlugOligo adapter from MINT kit. For our samples we use 3 μl of RNA and no water as we had low concentrations of RNA. The starting amount of RNA needs to be 0.1 to 1 μg per tube.
2. This was gently mixed by pipetting and spun down.
3. Tubes were placed in a thermocycler with the following profile; 2 minutes at 70 °C, hold at 42 °C, 42 °C for 30 minutes, hold at 42 °C, 42 °C for 90 minutes.

4. During the first two steps on the thermocycler the following master mix was made for each reaction (adjusted for multiple samples); 2 μ l 5x first strand buffer, 1 μ l DTT (20 mM), 1 μ l dNTP mix (10 mM each), 1 μ l MINT reverse transcriptase.
5. This was gently mixed by pipetting and spun down.
6. The thermocycler should now have reached 42 °C. 5 μ l of master mix were added to each tube in the thermocycler and pipette gently to mix. The tubes were only removed from the thermocycler for the master mix to be added and to be spun down if necessary. The hold stage was skipped to continue with the cycle.
7. After 30 minutes at 42 °C 5 μ l of IP solution was immediately added to each reaction, pipetted gently to mix and the thermocycler skipped on to the next stage.
8. After 90 minutes at 42 °C the reactions were immediately placed on ice to stop the reaction. They can be stored at -20 °C for up to 3 months.

A.1.3.2 Second strand cDNA synthesis PCR optimisation

The PCR reaction needed the number of cycles optimised using three reactions with differing number of annealing temperature cycles (18, 21 and 24 cycles). The reagents were added to a 0.2ml microcentrifuge tube in the order shown in table A1.4. The reactions were mixed by flicking the tube gently and spinning down. 16 μ l was added to each of the three PCR tubes and thermocycled as shown in table 4. The PCR product was visualised on 1.2% agarose gel with TAE buffer and run at 98V for 45 minutes. PCR products were run alongside 1kb DNA ladder (Promega) and control samples supplied with the kit (5 μ l + 1 μ l 6x loading buffer for both products and ladder). The PCR plateaued at 18 cycles (bright smear and bands with 18 cycles but the loss of some intensity with 21 cycles). The optimum number of cycles is lower than the plateau, so around 17 cycles.

A1.3.3 Full size cDNA second strand synthesis

Nanodrop quantification was carried out on one of the test PCR products to calculate approximately how many reactions would be required to achieve the desired quantity of cDNA (10 μ g). The nanodrop showed that one reaction (16 μ l) produced approximately 50 ng/ μ l or 0.8 μ g of cDNA. In order to achieve a final quantity of at least 10 μ g it was decided to carry out 13 second strand PCR reactions using 17 cycles as determined during optimisation. 5 μ l from one of the PCR reactions was visualised

on 2% agarose gel with TAE buffer, 98v for 45 mins, to check that the reaction had worked (Figure A1.3).

PCR Clean up and final quantification

For PCR clean up the samples were pooled together to create two cDNA samples which were then cleaned using QIAquick spin PCR clean-up kit (Qiagen, USA) following manufacturers protocol. Samples were eluted in 48 µl of buffer EB from the kit

Following clean-up the nano drop was used for a final quantification and quality check (Table A1.5). The final cDNA yield was 11.5 µg of cDNA.

Table A1.4. PCR conditions for second strand cDNA synthesis PCR optimisation. X is the parameter were are determining and tested at 18, 21 and 24 cycles during testing and was optimal at 17 cycles

<u>PCR reaction</u>		<u>Thermocycling</u>		
Reagent	50 µl Reaction	temp	time	cycles
Sterile water	40 µl	95°C	1 min	x1
10x Encyclo PCR Buffer	5 µl			
dNTP (10mM each)	1 µl	95°C	15 s	
PCR Primer M1	2 µl	66°C	20 s	x X
50x Encyclo Polymerase Mix	1 µl	72°C	3 min	
First Strand cDNA	1 µl			
		66°C	15 s	
		72°C	3 min	x1

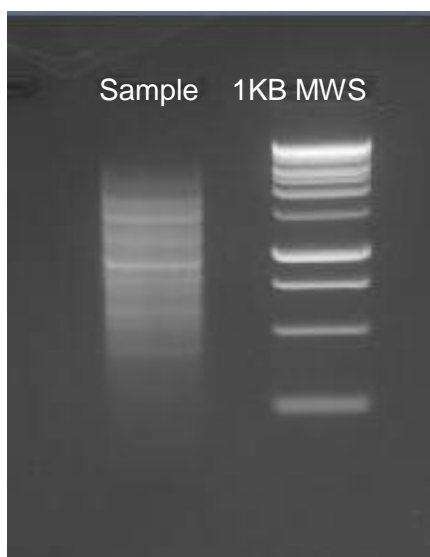


Figure A1.3. Evaluative agarose gel for second strand cDNA synthesis. *Pecten maximus* sample shows moderately bright smear indicating successful synthesis

Table A1.5. Final quantification of second strand cDNA synthesis using Nanodrop 1000

Sample	Concentration (ng/ μ l)	260:280 ratio	Total cDNA (μ g)
cDNA A	103.8	1.80	4.827
cDNA B	145.6	1.81	6.770

A1.4 SCREENING SEQUENCES

To check for sample quality and contamination with rRNA some fragments of the synthesised double stranded cDNA were sequenced. This was achieved by using plasmid vectors and cloning to amplify fragments.

A1.4.1 Preparation of Luria-Bertani (LB) plates

LB plates were prepared using the following recipe:

2.5 g Tryptone

1.25 g Yeast

2.5 g NaCl

3.75 g Agar

Made up to 250ml with distilled water

The LB mixture was autoclaved and then cooled to approximately 55 °C in a water bath before adding ampicillin (50 mg/ml), and pouring into 6 plates. Once set the plates were inverted and stored at 4 °C until needed.

A1.4.2 Cloning reaction

Topo TA Cloning® Kit (Invitrogen) with pCR 2.1 TOPO vectors was used for the cloning reaction. *Taq* polymerase PCR products have a single dA at their 3' end. A complimentary single overhanging dT on the linearised plasmid vector allows the PCR products to ligate efficiently with the vector. The following reaction, using the kit contents, was mixed gently and incubated at room temperature for 20 minutes and then placed on ice; fresh PCR product (2 µl), salt solution (1 µl), water (2 µl), TOPO vector (1 µl) (Total volume 6 µl).

A1.4.3 Transformation into competent E coli cells

One Shot® Competent Cells Kit (Invitrogen) was used with TOP10 cells for transformation. 2 µl of the vector reaction were added to the Top 10 cells and mixed very gently (do not pipette up and down). This was incubated on ice for 20 minutes then heat shocked at 42 °C for 30 seconds and immediately returned to ice. 250 µl of room temperature S.O.C was added to the cells which were then shaken horizontally for 1 hour. 15 µl of transformant were spread onto one LB plate and 20 µ on another. Due to the use of ampicillin resistant E coli and ampicillin incorporated into the LB plates successful colonies appeared white and non-transformed colonies appeared blue. After incubating the colonies overnight good numbers of white plaques were seen on the LB plates.

A1.4.4 PCR of transformants

50 white plaques were harvested and amplified using the PCR conditions shown in table A1.6. One plaque was added to each PCR reaction. Once complete, PCR products were run out on a 2% agarose gel with TBE buffer at 90V for 40 minutes (Figure A1.4). 31 samples with bright single bands of PCR products were chosen for sequencing.

Table A1.6. PCR conditions for amplification of transformants

Reagent	PCR Reaction		Thermocycling Profile		
		20 μ l reaction	Temp	Time	Cycles
10x ABgene ready mix buffer		2.0 μ l	94°C	10 mins	x1
Magnesium Chloride (25 mM)		1.6 μ l			
dNTPs (1mM)		4.0 μ l	94°C	45 s	
M13 (-20) F (20 μ M)		0.2 μ l	57°C	45 s	x30
M13 R (20 μ M)		0.2 μ l	72°C	3 mins	
<i>Taq</i> polymerase		0.2 μ l			
Water		11.8 μ l	72°C	10 mins	x1

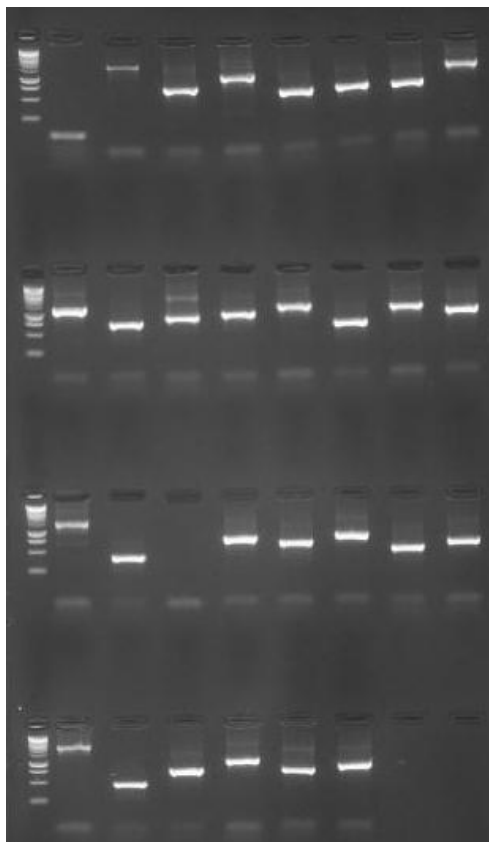


Figure A1.4. Agarose gel images of transformants from pCR 2.1 TOPO vector cloning. Colony samples 1 – 50 with 1000 kb ladders

A1.4.5 Exosap clean-up

Before sending off for sequencing, PCR product was cleaned using Exosap. For each PCR reaction 1 µl of Sap (1 U/µl) and 0.1 µl Exo I (20 U/µl) was added and incubated at 37 °C for 30 minutes and 80 °C for 20 minutes. Products were then sent to Macrogen for sequencing.

A1.4.6 Quality checking sequences

Sequences received from Macrogen were loaded into the software Chromas Lite (http://www.technelysium.com.au/chromas_lite.html) and the vector sequences identified and removed leaving only the sequence from the PCR product from the cDNA template. Many sequences failed to read due the presence of Poly-T stretches at the start of the sequence. These were assumed to be cDNA from mRNA sequences (complimentary from the Poly-A tail of the mRNA). Other readable sequences were “BLAST’ed” in GENBANK to check for the presence of ribosomal sequences or other hits. Only 3 out 31 sequences appeared to be ribosomal and it was felt that this was satisfactory for next generation sequencing.

A1.4.7 Next generation sequencing

Roche’s 454 titanium flex sequencing was carried out by Liverpool University. cDNA had to be at concentrations greater than 10 µg/200µl. Therefore before sending the sample for sequencing a sodium acetate precipitation was carried out and the cDNA eluted in 200 µl. This gave us the required concentration (10 µg/200µl).

APPENDIX 2: REPORT TO THE ISLE OF MAN GOVERNMENT

A2.1 INTRODUCTION

Marine Protected Areas (MPAs) can be implemented to achieve one of two goals. Firstly to conserve biodiversity and secondly as a fisheries management tool (Hastings and Botsford 2003). In the role of fisheries management, MPAs have the potential to enhance the sustainability of stocks, especially of sessile or sedentary species by protecting habitats where adults can achieve higher densities (Beukers-Stewart *et al.* 2006; Kaiser *et al.* 2007) with larger gonad sizes (Kaiser *et al.* 2007) leading to increased reproductive output. High densities of adults can also lead to an increase in the reproductive success of sedentary broadcast spawners as close proximity to potential mates is essential for fertilisation (Leviton *et al.* 1992). The planktonic larval phase in many marine species can lead to eggs and larvae being able to “spill-over” to areas outside the reserve, thereby allowing the reserve to act as a source of recruits for the fishing grounds (Gell and Roberts 2003).

The success of a closed area for fisheries management will depend on the level and patterns of connectivity between it and the exploited grounds. Genetic data can provide information on connectivity as it relies on two contrasting factors; firstly, larval dispersal and adult migration will serve to connect populations (gene flow) and this will be shown by a homogenisation of genetic structure between populations; conversely lack of larval dispersal and adult migration will lead to random genetic drift and local adaptations causing genetic heterogeneity between populations and genetic structure will be evident. King scallops (*Pecten maximus*) are sedentary during their adult lives with their pelagic larvae spending approximately 25 days at sea (Le Pennec *et al.* 2003) therefore, there is potential for dispersal of larvae from an MPA to neighbouring exploited areas. There have been several studies identifying low levels of genetic structure in king scallop populations (Beaumont 1982; Heipel *et al.* 1998; Heipel *et al.* 2000; Kenchington *et al.* 2006). However these are on a large geographic scale and looking at connectivity on a finer scale, between an MPA and neighbouring fishing grounds, it is less likely that any

genetic differences will be found, thus making the use of genetic tools for studying connectivity in such situations less feasible.

Port Erin is a small (2 km²) closed marine area in the Isle of Man (IOM). It has been closed to fishing since 1989 and densities of *P. maximus* are higher inside the closed area than outside (Beukers-Stewart *et al.* 2006), reproductive output is also higher inside the closed area (Beukers-Stewart *et al.* 2005). However, the question of whether the closed area contributes to recruitment in local fishing grounds has yet to be answered. In 2003 approximately 40,000 *P. maximus* were transferred into the Port Erin closed area from the Isle of Skye (IOS) as part of a stock enhancement program. If differentiation between the two populations exists then an opportunity to track this IOS genetic signature outside the boundaries of the closed area has been created which could allow identification of the contribution of Port Erin to the local fishing grounds.

This paper analyses the genetic structure of the IOS and IOM populations and reports on the feasibility of using genetic techniques to trace transplanted IOS progeny outside of the MPA.

A2.2 METHODS

A2.2.1 Sample Collection

P. Maximus samples were collected from the same grow out facility in the IOS that the transferred juveniles came from. *P. maximus* samples from the IOM of an age that prohibits them being the progeny of the transferred scallops (ie: 6 years or older at the beginning of 2009) were collected from the scallop processing plant in the Isle of Man and from the annual scallop survey onboard the research vessel Prince Madog. The left shell of each individual was kept to allow aging using annual growth rings (Mason 1957). Tissue was preserved in 90% ethanol.

A2.2.2 DNA Extraction

Molluscs have high levels of mucopolysachharides which can contaminate DNA extractions therefore DNA was extracted using CTAB extraction buffer (Doyle and Doyle 1987). Samples were homogenised in 400ul of CTAB buffer (40ml of 2% CTAB buffer to 20ul of mercaptoethanol) and incubated overnight with Proteinase K (200ug) at 59 °C. Phenol-chloroform-isoamyl alcohol (24:24:1) extraction was followed by two Chloroform-Isoamyl alcohol washes (24:1). The DNA was precipitated using 2x the

volume of ice cold absolute ethanol as the volume of DNA extract and 0.1x volume of sodium chloride (pH8, 5M). After precipitation at -20 °C for 1 hour and centrifugation at max speed for 10 minutes a pellet was formed and the remaining ethanol drained off. The pellet was then washed in 70% ethanol and re-centrifuged for 5 minutes. The Ethanol was drained and the pellet allowed to air dry for 10 minutes to ensure all remaining ethanol was removed. The DNA was resuspended in 50ul of TE buffer (pH 8) and stored long term at -20 °C or at 4 °C for short term storage.

A2.2.3 Microsatellite amplification and genotyping

Previous testing of published microsatellite markers for *P. maximus* (Watts *et al.* 2005) yielded usable PCR products in seven out of the nine markers(List15-004, List15-005, List15-008b, List15-011, List15-012, List15-013, List15-15). Three of these markers (List15-011, List15-013, List15-015) were too polymorphic for use at the population level (for example in 68 individuals 63 alleles were identified) which left only 4 markers (which we will call W4, W5, W8 and W12 to indicate that they are markers from Watts *et al* 2005). To increase power 12 new transcriptome derived microsatellite markers were developed (See Hold *et al* 2012), which we will label with a prefix of “P”, giving a total of 16 microsatellite markers which were amplified using 4 multiplex PCR reactions. Each 12µl reaction contained 1µl of DNA (~10ng), 6µl of Qiagen Type IT multiplex PCR mix, 0.5µl of BSA (10mg/ml), 2.5µl of water and 2µl of Primer mix. The final primer concentrations and fluorescent tails of each primer are shown in Table 1. Three thermocycling profiles were used (Table 2). PCR products were resolved on an ABI 3030XL sequencer using the LIZ 600 size standard and Genemapper software was used to size alleles.

Table 1. Multiplex PCR primer concentrations and tails for *Pecten maximus* microsatellite markers.

Multiplex	Primer	PCR concentration	Fluorescent tail (PCR Concentration)	Thermocycle profile
A	P9 Forward	0.05	VIC (0.2)	1
	P9 Reverse	0.20		
	P19 Forward	0.08	PET (0.33)	
	P19 Reverse	0.33		
	P20 Forward	0.05	FAM (0.2)	
	P20 Reverse	0.20		
B	P11 Forward	0.05	PET (0.2)	2
	P11 Reverse	0.20		
	P23 Forward	0.05	NED (0.2)	
	P23 Reverse	0.20		
C	P59 Forward	0.05	FAM (0.2)	1
	P59 Reverse	0.20		
	P60 Forward	0.04	VIC (0.17)	
	P60 Reverse	0.17		
	P62 Forward	0.05	PET (0.2)	
	P62 Reverse	0.20		
	P68 Forward	0.04	PET (0.2)	
	P68 Reverse	0.17		
	P70 Forward	0.04	FAM (0.17)	
	P70 Reverse	0.17		
	P73 Forward	0.05	NED (0.2)	
	P73 Reverse	0.20		
P75 Forward	0.04	FAM (0.17)		
P75 Reverse	0.17			
D	W4 Forward	0.05	PET (0.22)	3
	W4 Reverse	0.20		
	W5 Forward	0.05	NED (0.2)	
	W5 Reverse	0.20		
	W8 Forward	0.05	VIC (0.2)	
	W8 Reverse	0.20		
	W12 Forward	0.05	FAM (0.2)	
	W12 Reverse	0.20		

Table 2. Three thermocycling profiles for *Pecten maximus* microsatellite multiplex PCR reactions.

PROFILE 1			PROFILE 2			PROFILE 3		
Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
95 °C	5 minutes	1	95 °C	5 minutes	1	95 °C	5 minutes	1
95 °C	30 s	6	95 °C	30 s	16	95 °C	30 s	10
64 °C; -1 °C per cycle	90 s		51 °C	90 s		60 °C - 1°C cycle	90 s	
72 °C	30 s		72 °C	30 s		72 °C	30 s	
95 °C	30 s	9	95 °C	30 s	19	95 °C	30 s	13
58 °C	90 s		50 °C	90 s		50 °C	90 s	
72 °C	30 s		72 °C	30 s		72 °C	30 s	
95 °C	30 s	15	72 °C	45 minutes	1	72 °C	45 minutes	1
50 °C	90 s							
72 °C	30 s							
72 °C	45 minutes	1						

A2.3 DATA ANALYSIS

A2.3.1 Data Quality and loci characteristics

Microchecker (Van Oosterhout *et al.* 2004) was used to check for stuttering, large allele dropout and null alleles. Pairwise genotypic linkage disequilibrium (LD) was tested in Genepop v 4.0 using the log likelihood ratio statistic (G-test). Concordance with Hardy Weinberg Equilibrium (HWE) was tested using the exact test in Genepop v4.0. For all Genepop analyses burn-in for the Markov Chain Monte Carlo algorithm was set at 10,000 iterations and there were 100 batches each with 5000 iterations. Alpha values for multiple testing were corrected using the False Discovery Rate (FDR) of Benjamini & Hochberg (1995). Observed and expected heterozygosity was calculated using GenAIEx (Peakall and Smouse 2006).

A2.3.2 Population differentiation

Population structure was tested using Weir & Cockerham's (1984) multiallelic and multilocus adaptation of F_{ST} as implemented in Genepop v 4.0. F_{ST} corrected for null alleles was also calculated using the software FreeNA (Chapuis and Estoup 2007).

A2.3.3 Assignment testing

The ability of the suite of microsatellite markers to assign individuals to either IOM or IOS populations was tested by self assigning the samples using GeneClass2 (Piry *et al.* 2004). Each individual was assigned to either IOS or IOM based on their genotypes and the percentage of individuals correctly assigned to their population of origin was calculated.

A2.4 RESULTS

A2.4.1 Data quality and loci characteristics

There was no evidence of stuttering or large allele dropout. Four markers showed evidence of null alleles in both populations (P9, P19, P20 and P68) There was no evidence of significant linkage disequilibrium between any loci pairs in either population. Four markers (P9,P19,P20 and P68) deviated significantly from HWE in both populations and a further four (P70, P75, W4, W8) deviated only in the IOM population. Observed and expected heterozygosity (Table 3) show an excess of homozygotes causing the deviation from HWE, possibly due to null alleles as suggested by Microchecker.

A2.4.2 Population differentiation

Both calculated F_{ST} Values, FSTAT (-0.00011) and FreeNA (0.00002), were very low and not significantly different from zero suggesting no evidence differentiation between the IOM and IOS populations.

A2.4.3 Assignment testing

Percentage correct assignment was low, only achieving 47.7% of individuals correctly assigned back to their source population (more were assigned to the wrong population than the correct one).

Table 3. Observed (Obs.Het.) and expected (Exp.Het.) heterozygosities and tests for Hardy-Weinberg Equilibrium for *Pecten maximus* microsatellites from the Isle of Man and Isle of Skye. P-values estimated using the exact test in Genepop v4.0. P-values in bold italics were significant after correction for multiple testing using the False Discovery Rate of Benjamini and Hotchberg (1995).

Locus	Obs.Het.	Exp.Het.	P-value	Obs.Het.	Exp.Het.	P-value
	ISLE OF MAN			ISLE OF SKYE		
P9	0.11765	0.22125	<i>0.01227</i>	0.25581	0.35267	<i>0.00448</i>
P11	0.29787	0.25623	0.57026	0.34694	0.40269	0.58243
P19	0.13333	0.24802	<i>0.00839</i>	0.02500	0.33196	<i><0.00001</i>
P20	0.43750	0.85913	<i><0.00001</i>	0.40476	0.79231	<i><0.00001</i>
P23	0.28571	0.30952	0.39302	0.19048	0.21715	0.45344
P59	0.61765	0.67559	0.94868	0.64865	0.69900	0.20637
P60	0.35556	0.42172	0.48223	0.39623	0.45103	0.39209
P62	0.68182	0.65517	0.40772	0.63462	0.69044	0.07002
P68	0.50000	0.74363	<i>0.00096</i>	0.48649	0.83710	<i>0.00001</i>
P70	0.06977	0.15650	<i>0.00090</i>	0.09804	0.09416	1.00000
P73	0.08889	0.12884	0.17162	0.11538	0.18148	0.03323
P75	0.26471	0.59394	<i>0.00007</i>	0.38889	0.49257	0.10021
W4	0.02632	0.07754	<i>0.01326</i>	0.23913	0.21906	1.00000
W5	0.95000	0.88797	0.80008	0.89744	0.89677	0.80101
W8	0.15556	0.24694	<i>0.00217</i>	0.19298	0.24034	0.05718
W12	0.71429	0.85944	0.03286	0.67347	0.83589	0.06794

A2.5 DISCUSSION

Microsatellite data suggests that there is no genetic population differentiation between the Isle of Man and Isle of Skye populations of *P. maximus*. This lack of structure will make tracing the offspring of the transplanted IOS seed unfeasible as demonstrated by the very low success in correctly assigning the parent samples back to their population of origin.

Homogenisation of the allele frequencies could be a consequence of current day connectivity between the two populations. Connectivity during the larval phase would be possible through the North Channel. The mean residual flow out of this passage towards the Malin shelf is estimated to be $0.02 - 0.03 \text{ m s}^{-1}$ (Knight and Howarth 1999). This residual flow is primarily wind-driven with large winter storms accounting for much of this flow (Knight and Howarth 1999). During the summer months it would be expected that residual flow would be minimal due to decreased wind forcing. However, Mason (1958) felt that the primary spawning event in the IOM was in the autumn, which he observed in August and September. With the planktonic larval phase being approximately 3-4 weeks long this could mean that the larvae are in the water column when the outflow increases due to the onset of the autumnal storms. It has also been suggested that spawning may be happening approximately a month later than this in recent years (Chapter three) increasing the likelihood of the larvae encountering increased northerly residual flow. The connectivity required to homogenise allele frequencies only needs to equate to one or two individuals per generation (Crow and Kimura 1970) and therefore chance spawning events coinciding with an autumnal storm allowing flow through the North Channel would suffice.

Another possibility is that the 2 populations have limited contemporary gene flow but share a common ancestor in relatively recent history and have not yet had time to diverge. With parts of the British Isles being glaciated as recently as 10,000 years ago it is likely that the IOM and IOS population expanded from a common refugia population within this timescale. However, the lack of genetic structure taken together with predicted larval dispersal from particle tracking models (Neil and Kaiser 2008) suggests that gene flow is occurring through the North Channel, connecting scallop grounds

within the Irish Sea and with the west coast of Scotland. The presence of connectivity between scallop grounds will allow scallop larvae spawned inside an MPA to potentially settle a distance away from their natal grounds. MPAs allow increases in adult scallop density, size and reproductive output (Beukers-Stewart *et al.* 2005; Kaiser *et al.* 2007) and can therefore create a protected source of new scallop recruits for neighbouring and possibly more distant commercial fishing grounds. However, from this genetic data it is not possible to estimate the magnitude of this migration between populations of *P. maximus* or to estimate if this level of connectivity is on a scale which would influence population dynamics over an ecological timescale.

Appendix 3: FISHERS QUESTIONNAIRE

I am conducting research into the spawning patterns of the King scallop around the Isle of Man. Your answers to this short questionnaire will add valuable information to the research. Thank you. Natalie Hold, Bangor University

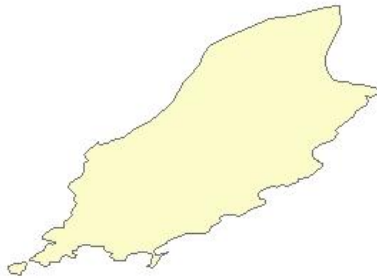
Q1. During the spring spawning event, do king scallops from different sites around the island all spawn at the same time?

- Yes, scallops across the island all spawn within a few days of each other
- No, some sites can spawn before others
- By a week or two
- By three or four weeks
- By more than a month

Q2. If no is there a pattern in which sites spawn first or does it vary from year to year?

Yes

(please label on the map the order (1,2,3 etc) in which areas tend to spawn)



No, it varies from year to year

Q3. Does the spring spawning event take place at the same time every year?

Yes it varies by less than a week

No, it can vary by 2 or 3 weeks

No, it can vary by a month or more

Q4. Do the majority of scallops at a single fishing ground all spawn at approximately the same time or does it occur over a longer time period?

Yes, most scallops spawn within a day or two of each other

Most scallops spawn within a week of each other

No the spawning carries on for longer than a week

Q5. How long does it take for a fully empty gonad to refill to the stage shown in the photo?

2-3 days

A week

2 weeks

A month

More than a month



Q6. Was this spring a typical year for scallop spawning or was it unusual?

Typical

Unusual

(please explain)

Q7. How many times a year do you usually see spawning taking place?

Once

Twice

More than twice

Varies from site to site

Please can you use the calander shown to indicate when spawning approximately takes place (place cross in months which you would expect to see spawning).

January	February	March
April	May	June
July	August	September
October	November	December

Please feel free to add any comments below. Thank you for your time.

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