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Faculty of Engineering, Science and Mathematics

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The Response of a Protandrous Species to Exploitation, and the Implications for Management: a Case Study with Patellid Limpets.

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ABSTRACT

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A zygote production model for the patellid limpet *Patella vulgata* has been developed to examine the effect of exploitation on the reproductive output of a protandrous (male to female sequential) hermaphrodite. Patellid limpets are broadcast spawners which can have specific implications for the effect of exploitation on reproductive output, due to sperm limitation. The combined zygote production model was made of three component sub-models; a population fecundity model, a gamete dispersal model, and a fertilisation model. The model makes explicit account of sperm limitation, and is based upon data collected through field and laboratory investigations conducted as part of this thesis.

The model was used to examine the relationship between egg and zygote production, and spawning stock biomass (SSB) and fishery yield for a range of *P. vulgata* populations across a wave exposure gradient. The effect of different management strategies, minimum landing size or marine protected areas, on the relationship between reproductive output and yield was also examined.

Protandry lead to a decoupling between SSB and zygote production as the populations were exposed to the simulated fishery. There was a five-fold variation in zygote production per unit area across a wave exposure gradient. Comparison of different management strategies indicates that the fishery yield could vary by up to three-fold depending on the management strategy used, whilst still protecting the same level of population reproductive output.

The genetic population structure of the Azorean *Patella candei* population was also examined to determine the scale of larval dispersal to allow the management recommendations of the zygote production model to be examined in a wider ecological context. Due to evidence of a recent population bottleneck in the Azorean *P. candei* population no firm conclusions could be drawn from this study as to the scale of larval dispersal.

Declaration of Authorship

I, **William Le Quesne**, declare that the thesis entitled **'The effect of protandry on the effect of exploitation: a case study with patellid limpets'** and the work presented in it are my own. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed: .....

Date: .....

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Contents

List of Figures

List of Tables

Chapter 1

Introduction

Although it was not explicitly stated, early work on the population dynamics of marine organisms did not seriously consider that reproductive output could be a limiting factor on population size. Indeed, impressed by the fecundity of marine organisms, in his opening address to the International Fisheries Exhibition in London in 1883 T.H. Huxley famously said;

> *All the great sea fisheries are inexhaustible....nothing we do seriously effects the number of fish.*

Since then it has become apparent that human exploitation can lead to rapid reductions, and local, regional and even complete loss of marine populations (Jackson et al., 2001; Pauly et al., 1998; Russell, 1934). This is undesirable on social (Dalzell et al., 1996), economic (FAO, 1997) and environmental (Hall, 1999; Jackson et al., 2001) grounds. As a result a great deal of effort has been exerted over the last century to understand how exploitation impacts upon target populations (see Beverton and Holt, 1957 for an early, and Hilborn and Walters, 1992 for a recent account) to try and ensure sustainable management.

The developments made in population and fisheries biology have lead to considerable understanding of population dynamics (e.g. Beverton and Holt, 1957), responses to exploitation (Hilborn and Walters, 1992), and the effects of a changing environment (Murawski et al., 2001). Differences in the life history and biology of species cause differences in their responses and resilience to exploitation (Dulvy and Reynolds,

2002; Jennings et al., 1999; Levitan and Petersen, 1995; Roberts and Hawkins, 1999). Therefore the original general formulations which were developed for demersal finfish fisheries, making such assumptions as constant sex ratio, relative fecundity, and catchability (Beverton and Holt, 1957) cannot necessarily be applied to all species with confidence. Indeed, attempting to do so may lead to flawed scientific advice and the failure of management schemes.

This thesis examines the affect of protandry (male to female sequential hermaphroditism) on the response of a population to exploitation, using patellid limpets as a model organism. I have also examined the spatial scale at which patellid limpet populations operate, as this is critical to informing management strategies that aim to allow for the effects of protandry. The use of patellid limpets as the model organism was prompted by the widespread exploitation of patellid limpets in north Atlantic islands that has lead to dramatic crashes of stocks (Ferraz et al., 2001; Hawkins et al., 2000; Martins et al., 1987; Santos et al., 1989). Patellid limpets are also a good model organism as they are sedentary intertidal species, allowing detailed population studies to be conducted. Furthermore, patellid limpets have been widely studied and their general biology and ecology is well understood; this previous work is reviewed below in section 1.7.

A population model has been developed to examine how population fecundity and population zygote production by the protandrous limpet *Patella vulgata* are affected by a simulated fishery, and how well traditional fisheries reference measures (e.g. spawning stock biomass, SSB) indicate the reproductive potential of an exploited protandrous population. The model was also used to examine the effect of different fishery management schemes on the relationship between exploitation, larval production and fishery yield.

Patellid limpets are broadcast spawners (Fretter and Graham, 1962). Population zygote production by a broadcast spawner depends on the number of male and female gametes released; how these gametes are subsequently advected and dispersed; and, depending on sperm concentrations that are achieved, the proportion of eggs fertilised. Modelling these processes requires an understanding of population biology, population fecundity, hydrodynamics and fertilisation kinetics. These topics are reviewed below along with the scale of larval dispersal, and patellid limpet biology

and ecology. In the final section of the introduction I outline the specific aims and objectives of this thesis.

1.1 Fisheries Management and Population Biology of Exploited Species

The primary goal of a fisheries manager is to ensure continuity of a population to safeguard future potential yield. Apart from inter-population movements, the only source of additions to a population is recruitment. Therefore ensuring sufficient continual recruitment is one of the main tasks of fishery management. A fundamental assumption underlying much of modern fishery science is that recruitment is related to the level of population reproductive output (Myers, 2002). Therefore considerable efforts have been made to predict future recruitment on the basis of the size and state of a stock to predict the effects of exploitation (Beverton and Holt, 1957; Cushing, 1973; Ricker, 1954; Shepherd, 1982). There has, however, only been limited success in finding a transparent reliable stock recruitment relationship, although a meta-analysis of 364 spawner-recruit time series concluded that recruitment was positively related to spawning stock size (Myers and Barrowman, 1996).

Due to the difficulties in determining stock-recruitment (S-R) relationships (Myers and Barrowman, 1996) many fisheries managers have taken the alternative approach of managing population reproductive output rather than trying to directly manage recruitment (Mace and Sissenwine, 1993). This is based on the assumption that sufficient continual recruitment will be ensured in the long term by conserving adequate reproductive output. As the required level of reproductive output cannot be quantitatively determined without a S-R relationship there has been a tendency to select arbitrary levels of spawning stock biomass (SSB), or reproductive output, to be conserved of around 20-35% of the unexploited level on the basis of "reasonable" assumptions (Mace and Sissenwine, 1993; Thompson, 1993).

Beverton and Holt (1957) originally proposed that SSB could be used as a potential predictor of recruitment as it can act as a predictor of reproductive output on the

basis of the relationship:

$$E = \Omega f SSB \tag{1.1}$$

where E is the population egg production, Ω the relative fecundity (eggs kg^{-1}), and f the proportion of females. Assuming Ω and f are known then E can be estimated from SSB. Furthermore, even if Ω and f are unknown but are assumed to be constant, then SSB can be used as a relative measure of E. However, egg production itself is not a direct measure of the realised reproductive output of a population. What ultimately matters is zygote production and larval output. Larval production (L) can be related to E according to the relationship:

$$L = \rho E \tag{1.2}$$

where ρ is the proportion of eggs fertilised. Beverton and Holt (1957) took the approach that egg production could be considered synonymous with larval production as:

> *"within a wide range of population abundance there is likely to be, at any time, an excess of spermatozoa. For our present purposes we shall therefore disregard the contribution of males to reproduction, and assume the latter is limited by egg production only." (Beverton and Holt, 1957, p.61)*

Therefore, if ρ is taken to equal 1, then;

$$L = E \tag{1.3}$$

which leads to;

$$L \; \alpha \; SSB \tag{1.4}$$

The use of SSB as a predictor of population reproductive output is reliant upon the assumptions that Ω, f and ρ are constant. However, each of these parameters has been shown, at times, to be variable due to long term systematic and short term stochastic effects (e.g. Gundersen et al., 2000; Marconato and Shapiro, 1996; Punt et al., 1993) indicating that SSB can be an inaccurate predictor of population reproductive output. Although population reproductive output is only part of the equation that relates stock size to recruitment, recent studies have shown that direct

measures of E and reproductive output can provide a better predictor of recruitment than SSB (Gundersen et al., 2000; Kjesbu et al., 1998; Kraus et al., 2002; Marshall et al., 1998).

1.2 Protandry, Sex Allocation and Population Biology

Hermaphroditism in all its forms, simultaneous, protandric (male to female), or protogynic (female to male) is widely reported in marine animals, for example at least 18 families of teleost fish (Warner, 1975) and 40% of mollusc genera (Heller, 1993) contain hermaphroditic species. Furthermore a number of protandrous and protogynous fin-fish and invertebrate species are targeted by commercial fisheries (Bannerot et al., 1987; Blaber et al., 1999; Buxton, 1992; Ferraz et al., 2001; Hesp et al., 2004; Milton et al., 1998; Punt et al., 1993). Sequential hermaphroditism is of particular concern to biological resource managers as it breaks the assumption of a constant sex ratio across all size classes, thus the population sex ratio can vary as a population is exploited (Blaber et al., 1999; Hawkins and Roberts, 2003).

The adaptive significance of sequential hermaphroditism has become to be understood in terms of Ghiselin's (1969) size advantage model (Charnov, 1982; Warner, 1975) which states that if the product of the probability of survival to a given age and the realised fecundity at that age increases faster for one sex rather than the other, then there is an adaptive advantage to individuals which change sex. The direction of sex change will depend on the reproductive strategy of the species (Charnov, 1982). In general protogyny will occur in species with mate selection, and protandry occurs when reproduction is unselective (Charnov, 1982), such as for broadcast spawners.

At this point it must be noted that in this thesis I follow Anger and Moreira's (1988) definitions of fecundity which are: potential fecundity, the number of gametes in the gonad; actual fecundity, the number of gametes released during a spawning event; realised fecundity, the number of gametes producing viable zygotes.

According to the size advantage model, an individual will change sex if the sex

that enables the individual to achieve greatest reproductive success for a given size changes as size increases. This implies that an individual that changes sex at the optimum size will achieve greater lifetime reproductive success than an equivalent gonochronist, be it male or female. However, should an individual change sex at a non-optimum size it will be losing out on the greater reproductive success that would have been achieved at that size by being the alternative sex. Furthermore, if an individual is particularly non-optimal in its size at sex change, greater reproductive success may be achieved as a gonochronist. Thus, under certain circumstances, there will be a selection pressure favouring optimum sized sex changers, and under other circumstances gonochronisim may be selected for.

The original size advantage model has been developed to allow for the metabolic and evolutionary limitations, and costs of sex change (Iwasa, 1991). The size advantage model has also been developed to include social control of reproduction (Kuwamura and Nakashima, 1998; Munoz and Warner, 2004) as the original size advantage model did not account for social traits such as partner allocation and the effects of post-reproduction parental care for offspring (St Mary, 1994). Furthermore, Iwasa (1991) demonstrated that sex change could be favored due to sexual differentiation in growth or mortality rates even in the absence of a size advantage. It should be noted that the modern developments on sex change theory in sequential hermaphrodites have been dominated by work on protogynic fish species with complex social control of mate selection (Kuwamura and Nakashima, 1998; Munday, 2002; Munoz and Warner, 2003, 2004; Shinomiya et al., 2003) which may have little direct application to randomly mating broadcast spawning species where the determination of sex change is dominated by non-socially mediated environmental factors (Wright, 1989).

1.2.1 Sequential hermaphroditism and fishery exploitation

In the case of sequential hermaphrodites, sperm and egg production can become concentrated in separate size ranges of the population (Bannerot et al., 1987; Milton et al., 1998). Since fishery mortality is often size related one sex can be predominately exploited, which it has been claimed leaves sequential hermaphrodites particularly vulnerable to recruitment overfishing (Blaber et al., 1999; Hesp et al.,

2004; Milton et al., 1998). In support of this claim several studies have noted changes in sex ratios as a result of exploitation of sequential hermaphrodites (e.g. Hawkins and Roberts, 2003; Milton et al., 1998; Punt et al., 1993). Furthermore, traditional fisheries management measures such as minimum landing size (Hesp et al., 2004) or net mesh size (Hobday et al., 2001) may only marginally, or totally fail to protect a specific sex within a population.

To quantify the affects of sequential hermaphroditism on population dynamics and the yield and resilience of an exploited stock, models have been developed which specifically account for sex change (Bannerot et al., 1987; Buxton, 1992; Punt et al., 1993). Unfortunately, the paucity of observational data has not allowed the model predictions to be robustly validated and the modelling efforts have been hampered by the inability to accurately fit relevant model parameters.

Buxton (1992) developed a simple per recruit model for two protogynous reef fish which indicated that even at low levels of fishing mortality (F) the sex ratio becomes markedly skewed towards the smaller females. The prediction of Buxton's (1992) model showed good agreement with observed sex ratios in both exploited and unexploited populations. Punt et al. (1993) developed a similar per recruit model for a different protogynous reef fish which predicted that a low level of F could skew the female:male sex ratio to 4:1 and under high F to 19:1 which was in agreement with observational data. Both models were able to accurately predict observed sex ratios without recourse to density dependent changes in the age at sex change. No compensatory effect on age at sex change leaves the population highly vulnerable to recruitment overfishing unless changes in the reproductive social interactions compensate for the change in sex ratio (e.g. each male mating with more females), apart from cases where there was an excess of gamete production by the preferentially exploited sex prior to exploitation. This is unlikely for females due to the energetic costs associated with oogenesis and the assumption that eggs rarely compete with each other for fertilisations (Levitan, 1998). Contrastingly sperm are often produced in excess due to the evolutionary pressure of sperm competition (Levitan, 1998; Munoz and Warner, 2003). In such circumstances reducing population sperm production may have little or no effect on population embryo production.

To predict the effect of altered sex ratios on recruitment and long term yield Punt

et al. (1993) developed a theoretical model which related recruitment to either female numbers, a product of male and female numbers, or just male numbers. The different recruitment schemes represent different levels of social compensation for altered sex ratios. For a given level of F the three different stock-recruitment schemes gave very different long term yields. This result highlighted the significant effect of sequential hermaphroditism on the population dynamics of exploited species. Punt et al.'s (1993) model also indicates that in the case of certain assumptions about compensatory adjustments, sequential hermaphroditism renders a population more resilient to exploitation than an equivalent gonochronistic species. Similarly, Bannerot et al. (1987), working on protogynous, groupers developed a static reproductive model that demonstrated that for certain parameter combinations protogynous species were more resilient to exploitation than analogous gonochronistic species.

The above models were developed for protogynous species with mate selection. Hesp et al. (2004) noted that protandrous species would be less able to adjust to skewed sex ratios by simple behavioral changes as egg production would be reduced rather than sperm production. In most cases it is assumed that excess sperm is produced, therefore more efficient use of the remaining sperm would still lead to the same level of larval production; however a drop in egg production would most likely lead to a direct proportionate drop in larval production. Therefore, as non-selective breeders, protandrous patellid limpets would not be able to make simple social adjustments to offset the effects of increased mortality.

Although broadcast spawners cannot make social adaptations to variations in mortality rates, they can respond by changing their size at sex change to maximise individual reproductive success (Hoagland, 1978). Therefore a sex changing individual may still retain a selective advantage over a gonochronist, even in the face of exploitation, if the individual can change sex at the new optimum size for sex change. Environmentally mediated density dependent sex change is predicted by theory to occur as at any given time the optimum size at sex change is not only determined by the relative number of male or female gametes producible by the individual, but also by the population sperm to egg ratio (Charnov and Bull, 1977; Charnov et al., 1978). Thus size at sex change should be labile and able to respond to environmental or demographic conditions; environmentally mediated sex change has been demon-

strated in the lottid limpet *Lottia gigantea* (Wright, 1989), the coralliophilid snail *Coralliophila violacea* (Soong and Chen, 1991), calyptraeid limpets (Collin et al., 2005), pandalid shrimp (Charnov et al., 1978), fish (Kuwamura and Nakashima, 1998) and terrestrial plants (see Charnov and Bull, 1977). Furthermore, a trend in change of size at sex change has been observed in relation to an increase in fishery pressure for *Pandalus borealis* (Charnov, 1981).

Therefore, despite the assertion by various authors that sequential hermaphrodites are more prone to recruitment overfishing than gonochronists (Blaber et al., 1999; Hesp et al., 2004; Milton et al., 1998), sequential hermaphrodites can alter size at sex change, and may thus be more resilient to exploitation than gonochronists. However sequential hermaphrodites will only be resilient to recruitment overfishing if they are able to make sufficiently large and rapid response in size at sex change to altered demographic parameters. If the sufficient response is not possible, or does not occur, sequential hermaphrodites are left more prone to recruitment overfishing than gonochronists.

Sex change within four weeks of a change in mate has been demonstrated for socially mediated sex changing fin-fish (Nakashima et al., 1995). However, of more direct relevance in the case of protandrous patellid limpet fisheries is the observation of Wright (1989) that the lottid limpet *L. gigantea* displayed a 2-3 year lag in response of size at sex change to density manipulations leaving a population possibly prone to over exploitation whilst individuals adjust to the new conditions. Charnov (1981) followed a trend in declining size at sex change by *P. borealis* in response to increasing fishery pressure over an eight year period, but it is not known how optimal the size at sex change was during this period. However large changes in population sex ratio have been observed in relation to fishery exploitation (Branch and Odendaal, 2003; Hawkins and Roberts, 2003; Milton et al., 1998; Punt et al., 1993) indicating that size at sex change does not always alter rapidly enough to keep up with the demographic changes caused by exploitation.

1.3 Protandry and Population Fecundity

To develop a larval production model it is necessary to model population fecundity and how it will be affected by a simulated fishery. Therefore I will examine the factors that affect population fecundity. Furthermore I shall examine the relationship between population fecundity and SSB, as an alternative explanation for the failure to find reliable S-R relationships may be because SSB is not a sensitive measure of population reproductive output. Indeed, as noted above, recent studies have shown that population egg production, or population fecundity are better predictors of recruitment than SSB (Gundersen et al., 2000; Kjesbu et al., 1998; Kraus et al., 2002; Marshall et al., 1998), and E is expected to become a better proxy for reproductive output than SSB when the assumptions of constant Ω or f fail. This is of particular concern to sequential hermaphrodites as the assumption of a constant f is expected to fail when a sequential hermaphrodite is exploited.

Population potential fecundity is the summation of the gametic output of all the individuals across a population. Therefore population potential fecundity can be calculated by combining a length-fecundity relationship with the population length-frequency structure.

I will first discuss size-fecundity relationships before I go on to discuss the effects of length-frequency structure on population fecundity, and the effect of exploitation on length-frequency structure.

1.3.1 Fecundity-length relationships

In the simplest case, under the assumptions of constant egg size and allometric growth, fecundity is expected to be linearly related to weight or length^3. These conditions lead to constant relative fecundity, whereby a female produces the same number of eggs per kg body weight regardless of age or size. Beverton and Holt (1957) based their relationship between SSB and egg production on this assumption and cited Raitt (1933) and Simpson (1951) as examples of this relationship holding true for haddock and plaice respectively. Linear weight-potential fecundity relationships have also been found in gastropod molluscs (Clavier, 1992).

In contrast, energetic theory would generally suggest that relative fecundity should be related to age, due to the reallocation of energy from somatic growth to reproduction with increasing age (Ramirez Llodra, 2002). For example, a number of bivalve species, including scallops (Claereboudt and Himmelman, 1996; MacDonald and Thompson, 1985), mussels (Kautsky, 1982) and oysters (Rodhouse, 1978) show rapid early growth which declines with age; however fecundity continues to increase with increasing age as energy is diverted from somatic growth to reproduction.

In addition to size and age, environmental factors such as food availability (Kautsky, 1982; Nakaoka, 1994) and temperature (Claereboudt and Himmelman, 1996; Kjesbu et al., 1998) modulate underlying age or weight fecundity relationships in both fin fish (Kjesbu et al., 1998; Marshall et al., 1998; Tereshchenko, 2002) and invertebrates (see Ramirez Llodra, 2002, for review). Kjesbu et al. (1998) found relative fecundity varied between years by up to 40% for Arcto-Norwegian cod, *Gadus morhua*, and suspected that inter-annual variation may be as much as 90%. Relative fecundity in the Barents Sea capelin, *Mallotus villosus*, was found to vary by over 30% over a 20 year period (Tereshchenko, 2002). In both the cases variations in food supply and temperature were cited as the cause of variation.

Similar variations in relative fecundity over space and time have been found in invertebrates. *Mytilus edulis* populations show variation in relative fecundity between and within populations over space and time (Bayne and Worrall, 1980; Kautsky, 1982) due to variable food availaibilty. Potential fecundity in the scallop *Placopecten magellanicus* shows a three-fold difference between shallow and deep-water populations, due to variations in food supply and temperature between the two depths (Barber et al., 1988).

Inter-specific community interactions can also modify size- or age-fecundity relationships. Relative fecundity in the limpet *Patella granularis* varied by an order of magnitude depending on the extent of barnacle cover (Branch, 1976), with fecundity declining as barnacle cover increased. Although this is ultimately a result of reduced food availability in barnacle dominated areas, it highlights the extent to which variation in intraspecific relative fecundity occurs between habitats.

The assumption of constant Ω breaks down when there is a non-linear relationship between individual weight and fecundity, and when there is spatial or temporal

variation in relative fecundity. Spatial and temporal variations in relative fecundity are common and non-linear relationships between weight and fecundity have been widely reported for fin fish (Kjesbu et al., 1998; Lasiak, 1991; Tereshchenko, 2002)) and invertebrates (Garwood, 1987; Kautsky, 1982; Kido and Murray, 2003; Ramirez Llodra, 2002). Thus in many cases the linear relationship between SSB and reproductive output is broken due to a failure in the assumption of constant Ω.

The fact that the E/SSB ratio varies within a population suggests there may be opportunities to selectively exploit areas or sections of the population with a lower E/SSB ratio, and protect sections with a higher E/SSB ratio. However efforts to opportunistically exploit a stock in such a manner must consider the loss of future reproductive potential as much as the loss of present reproductive potential. Opportunistic targeting of temporal variation in E/SSB ratios may also be achieved, but is less desirable due to the desire to minimise temporal variation in yield. Nevertheless temporally variable relative fecundity needs to be accounted for by fishery managers.

1.3.2 Population structure

Population fecundity is the summation of individual fecundities across the population. Therefore the number and size of individuals in a population will have major implications for population fecundity and larval production. Under conditions where E α SSB the relationship between population structure and population fecundity is clear: as large individuals tend to produce more gametes than small individuals, for a given population density the larger the individuals the greater the population fecundity.

Exploitation will directly reduce population fecundity as individuals are removed from the population. The effects of exploitation on target species are expected to be a reduction in mean length and a reduction in the relative abundance of larger size classes (Jennings and Kaiser, 1998). This has been shown to hold for fin fish (Jennings et al., 1999; Jennings and Kaiser, 1998) as well as invertebrates (Roy et al., 2003), including patellid limpets (Pombo and Escofet, 1996). Comparing re-

productive output by protected and unprotected populations of the limpet *Cymbula occulus* Branch and Odendaal (2003) found that limpets were on average 30-50% smaller and 75% less abundant in unprotected compared to protected areas. The variation in population structure between the two sites led to an 80-fold difference in reproductive output per unit area between the sites, indicating how changes in length-frequency structure can lead to important differences in population fecundity.

The length-frequency structure of a population is determined by recruitment, growth, mortality, immigration and emigration. For sessile, sedentary and territorial species heterogeneity in habitat quality can cause consistent differences in population structure between areas (Dias, 1996; Wing et al., 2003), and lead to large differences in population fecundity of sub-stocks (Branch and Odendaal, 2003). Extreme variation in habitat quality can lead to a mosaic of sub-populations covering a gradient from strong larval sources to strong larval sinks (Pulliam, 1988; Wing et al., 2003).

The composition and structure of intertidal rocky shore communities are strongly influenced by the extent of wave action (Lewis, 1964; Stephenson and Stephenson, 1972). The important effect of exposure gradient on population structure and dynamics has been shown for a range of limpet species (Branch, 1974; Spencer Davis, 1969; Thompson, 1979) including *P. vulgata* (Baxter, 1983; Lewis and Bowman, 1975; Southward and Orton, 1954; Thompson, 1980). For example the average size of the patellid limpet *Cymbula oculus* is 65% greater in sheltered areas than exposed areas and biomass per unit area is 80% greater in sheltered areas (Branch and Odendaal, 2003). This implies a large variation in reproductive output between sheltered and exposed sites. Therefore the population fecundity of limpet populations, and the response to exploitation will vary along an exposure gradient (Branch and Odendaal, 2003).

Beyond the simple case where the assumption E α SSB holds true, the implications of the influence of population structure on population fecundity in relation to SSB and yield become more subtle. To examine this, consider the simplified case of a population where there is no spatial or temporal variation in fecundity, but the length-fecundity relationship is exponential rather than cubic. In such a population the E/SSB of an individual will increase with increasing size. Thus to achieve

a given yield more eggs would be removed from the population if the yield was made up of large older individuals than small younger individuals. Furthermore a population of given SSB made up predominantly of old and large individuals would have greater egg production than a population with the same SSB made up of small young individuals. The decoupling of SSB and E under such circumstances has been demonstrated for Greenland halibut, *Reinharditus hippoglossoides*, where over 18 years population egg production dropped compared to SSB as the average age of spawners declined due to the selective removal of larger individuals by the fishery (Gundersen et al., 2000).

The discussion of population fecundity has so far generally assumed a constant sex ratio. In the case of protandrous hermaphrodites, females are disproportionately concentrated in the larger size classes. Most fisheries tend to be size selective, especially hand collected fisheries such as patellid limpet fisheries (Kido and Murray, 2003), preferentially removing larger individuals. Thus females will be disproportionately removed as fishery pressure is applied to a previously unharvested limpet stock and hence E will drop faster than SSB. This will be the case even if all the other assumptions hold as female SSB will drop faster than the overall population SSB. The exact long-term effect of exploitation on the population fecundity of a protandrous hermaphrodite is harder to gauge. Although exploitation will lead to a reduction in individual body size (Roy et al., 2003) leading to an overall reduction in fecundity the population may respond by sex change occurring at an earlier size/age and hence offsetting some of the effects of the reduced egg production (Wright, 1989). This, however, would lead to a greater decline in sperm production as males are promoted to females at a smaller size leading to a potential decline in fertilisation success.

In this section I have only been considering how exploitation may effect gamete production, and have not considered how varying levels of sperm production may effect fertilisation success. The total number of eggs produced will define the upper limit of zygote production, however the number of zygotes actually produced will depend on the proportion of eggs fertilised. In the next two sections I will consider fertilisation success and how it effects zygote production.

1.4 Fertilisation Kinetics

In the early 20^{th} Century the first suggestions that fertilisation may be a limiting step in the population dynamics of natural populations came to light. Belding (1910) suggested that fertilisation success may be a limiting factor in the Massachusetts scallop fishery, and Gross and Smyth (1946) proposed reduced fertilisation success as a reason for the failure of *Ostrea edulis* populations to recover around Britain after the introduction of management strategies. Furthermore, during this period, work by Lillie (1915) and Sparck (1927) showed that under laboratory conditions reduced sperm concentrations lead to a reduced proportion of eggs fertilised.

Subsequently it has been accepted that fertilisation success can under some circumstances be limiting in reproduction, and hence recruitment, and several recent reviews discuss sperm limitation and the implications for natural resource managers (Levitan, 1995; Levitan and Petersen, 1995; Levitan and Sewell, 1998; Roberts and Hawkins, 1999; Yund, 2000). Per capita reduction in reproductive output due to a lack of fertilisation at reduced densities is not confined to marine benthic invertebrates (Courchamp et al., 1999) and has also been demonstrated in marine algae (Berndt et al., 2002; Serrao et al., 1996) and terrestrial plants (Groom, 1998; Roll et al., 1997).

There are three main aspects of concern for fisheries managers in relation to sperm limitation. Firstly, fertilisation success can be negatively density dependent (Denny and Shibata, 1989; Roberts and Hawkins, 1999). This is an example of the Allee effect (Allee, 1931), a decline in per capita reproductive output with declining population numbers or density. This is in contrast to standard fisheries theory which assumes that positive compensation occurs with decreasing population numbers or density due to a reduction in intraspecific competition (Beverton and Holt, 1957). Secondly, empirical evidence demonstrates that fertilisation success shows a non-linear response to sperm concentration (Babcock et al., 1994; Levitan et al., 1991; Pennington, 1985). This means that per capita rates of larval output may show a non-linear response to reduced population numbers or densities, and that threshold levels may be reached after which fertilisation success drops rapidly and population collapse may occur (Levitan and Petersen, 1995; Quinn et al., 1993; Yund, 2000).

Thirdly, variable fertilisation rates mean that the relationship between population egg production and larval production in equation 1.2 is not constant; thus egg production and zygote production can become decoupled. Population larval production can thus decline faster than predicted by demographic indicators such as SSB, with the consequence that management efforts will be ill judged, rendering broadcast spawners potentially more susceptible to recruitment overfishing.

The effect of sperm limitation on fertilisation success means that in order to develop an integrated model of the effect of exploitation on zygote production, population fecundity and dispersal models need to be coupled with a fertilisation kinetics model to simulate how the reduction in population numbers will effect population larval production.

1.5 Egg Production, Sperm Limitation and Larval Production

To understand the relationship between E and L it is necessary to understand how fertilisation success decreases as egg production and sperm production are reduced due to exploitation. Despite being an example of an Allee effect which can lead to precipitous population collapses below critical threshold levels (Levitan and Sewell, 1998; Roberts and Hawkins, 1999; Yund, 2000), a majority of theoretical and empirical studies have either been concerned with individual reproductive output (Wahle and Peckham, 1999), or have been conducted on spawning pairs or small groups (e.g. Babcock and Keesing, 1999; Encena et al., 1998; Levitan et al., 1991). Few studies have attempted to directly examine the effect of exploitation on egg production, fertilisation success and larval production at the population level (e.g. Claereboudt, 1999), thus predictions as to the impact of Allee effects on fishery yields and harvest strategies remain largely untested.

Quinn et al. (1993) developed a dynamic pool model of the effects of harvesting on a marine invertebrate with density dependent fertilisation and post-settlement mortality to examine the management implications of marine reserves. Even with a simplified 2-patch model which did not include post-settlement density dependence

there were threshold levels beyond which sudden population collapse occurred. This was dependent upon the yield taken and the distribution of effort between patches. A notable result from the model is that the system is more stable with fishery reserves and complete population collapse was not possible when reserves were included. Similarly, for high levels of fishery pressure, the highest equilibrium yield is achieved with a scenario including reserves rather than the no reserve scenario. However, the maximum possible yield is achieved when low harvest intensity is applied across all populations, although in this case only a small increase in harvest intensity lead to complete stock collapse.

A study of the effect of population size and density on fertilisation success of *Clypeaster rosaceus* (Levitan and Young, 1995) found that for observed values of current speed and sperm release rate predicted fertilisation success varied between 0% and 100% depending on the patch size, density, and degree of aggregation. For any specific combination of population size and overall density, fertilisation increased as the degree of aggregation increased. For a given level of aggregation and population size, fertilisation success increased as density increased. Population size had little effect on fertilisation success at high densities, but at low densities fertilisation success increased with increasing population number. However the study only examined the effect of varying population parameters on fertilisation success rather than absolute larval production.

Claereboudt (1999) exposed a theoretical scallop population to a simulated fishery. The initial population was composed of identical 150mm scallops, the result of the fishery was to reduce the population density by half and reduce the scallops to 100mm, equivalent to a 65% reduction in per capita reproductive output. Also the simulated fishery could either disrupt the initial distribution of the scallops or not. The model predicts that in the un-fished case fertilisation success is 62%. In the case where the fishery disturbs the scallop distribution, fertilisation success dropped to 16% and larval production dropped to less than 5% of the unexploited value. In the case where the fishery does not disturb the distribution, fertilisation success dropped to 29% and larval production is approximately 10% of the unexploited level. Claereboudt (1999) conclude from this that sperm limitation is an important concern and that moderate fishing pressure can lead to a greater than 90% decrease

in larval production.

The dynamics of Allee effects suggests the existence of threshold levels below which precipitous population crashes may occur. Lundquist and Botsford (2004) questioned whether threshold levels really do occur. To test this they developed a population zygote production model based upon the urchin *Stronglyocentrotus droebachiensis* to examine the occurrence of threshold levels and the subsequent fishery implications. It was found that, except for under unrealistic conditions relating to population distribution and sperm dispersal, threshold levels did not occur. Furthermore, under many conditions the relationship between population density and fertilisation success was functionally linear. The authors noted that some previous studies that have demonstrated threshold dynamics (e.g. Pfister and Bradbury, 1996; Quinn et al., 1993) have included threshold effects in the assumed density-fertilisation success relationship rather than using combined fertilisation kinetic and gamete dispersal models to examine the existence of threshold levels.

Wahle and Peckham (1999), and Meidel and Scheibling (2001) both developed zygote production models for the urchin *Stronlyocentrotus droebachiensis* to examine how fertilisation success interacts with population dynamics. Wahle and Peckham (1999) specifically examined the trade off between reduced per capita fecundity at high densities with increased fertilisation success at high densities. Over the range of densities examined they found that gonad production halved with increased density but that fertilisation success increased 2 fold over the same increase in density. Thus although per capita egg production decreased, zygote production increased with increasing density. The authors did not quantify the fishery implications, but noted that density effects on fertilisation success need to be accounted for in management strategies. Meidel and Scheibling (2001) examined the interaction between population density, gonad production, fertilisation success and zygote production of *Stronglyocentrotus droebachiensis* under different community states and found that population zygote production could be concentrated into a small part of the population's range. Grazing fronts occupied less than 1% of the area, but accounted for 7-22% of the population egg production and 11-44% of the population zygote production. This highlights the effect that variable fertilisation success has in exaggerating the importance of reproductive output from high density sections of the population

and reducing the importance of reproductive output from spawners at low density, even though there may be greater numbers of individuals at low densities.

The above examples demonstrate the importance of modelling the effect of exploitation on L rather than just E if the effect of exploitation on reproductive output is to be understood. Once a functioning larval production model has been developed different types of fishery management strategy can be compared by altering the extent of fishery pressure applied to various portions of the population in the model. Marine protected areas (MPAs) have recently received increasing attention as to their use instead of, or in conjunction with, traditional fishery management measures such as minimum landing size (Palumbi, 2001; Polunin, 2002). Although any form of hypothesised fishery management scheme can be applied to a theoretical model it must be considered whether the theoretical calculations retain any biological or practical management coherence. Fundamental to the application of MPAs to species with sessile and sedentary adult forms, is that reproductive output from within reserves spreads beyond the reserves during larval dispersal (Crowder et al., 2000; Rowley, 1994). Therefore it is important to try and determine the spatial distance over which larvae disperse before management strategies relying on MPAs can be reasonably promoted.

1.6 The Spatial Scale of Populations

The spatial scale over which populations act as a coherent self-sustaining unit has significant implications for defining the scale of management units (Begg et al., 1999; MacLean and Evans, 1981), and the siting and spacing of marine reserves (Lockwood et al., 2002; Roberts, 1997). For sessile, sedentary, and many territorial marine species the larval phase is the main dispersive phase, and larval dispersal acts as the major determinant of the spatial scale of population structure.

I will begin by discussing the stock concept in fisheries management, before discussing the implications of the scale of populations on the appropriateness and siting of marine reserves.

1.6.1 Stock identification and fisheries management

The fundamental basis of fisheries assessment and management assumes knowledge of a population's dynamics in terms of additions and losses, coupled with the ability to control the level of fishery mortality (Beverton and Holt, 1957). In the absence of any understanding of stock structure the ability to assess and manage a fishery can be fundamentally flawed; this can be due to a failure to either i) correctly assess the population dynamics of the fishery, or ii) control the fishery mortality at the desired level.

Not only is identification of stock structure necessary to tailor management measures to the appropriate spatial scale to optimise yield (Begg et al., 1999), but it is also critical for the effective protection of fisheries. Kutkuhn (1981) noted that the inability to correctly define stock units

> *could unknowingly prejudice otherwise well-designed protection efforts.*

The understanding and study of stock structures has been hampered by the consistent failure to come to a fixed definition of a 'stock'. This is partly because the idea of a stock represents a man-made concept required by practical management necessity as much as a stock being a naturally occurring phenomenon. Ihssen et al.'s (1981) definition of a stock as

> *an intraspecific group of randomly mating individuals with temporal and spatial integrity*

covers the biological nature of a stock, but fails to account for the blurred edges imposed upon the definition by the more flexible management requirements for the definition of a stock. The earlier definition by Larkin (1972) of a stock as;

> *a population of organisms which sharing a common gene pool, is sufficiently discrete to warrant consideration as a self-perpetuating system which can be managed*

allows for more flexibility in the identification of a stock, more in line with common practice. Larkin's (1972) definition of a stock also covers the key aspect of a stock as being a viable management unit, in that the effect of births and deaths within the stock greatly outweigh the effect of immigration and emigration. Thus local births

and deaths are the main drivers of the local population dynamics. Due to the semantic complexities that have arisen several authors have taken the Wittgensteinian approach that what is more important is an appreciation of the stock concept rather than a strict definition of the term stock itself (e.g. MacLean and Evans, 1981).

The drivers behind the development of the stock concept are three-fold. Firstly there is the management requirement for stock identification, as for example with transboundary stocks there is no point in one legislative region enforcing stringent fishery control measures to rebuild a stock if fishers from another legislative area continue to heavily exploit the same stock (Kutkuhn, 1981). Secondly, the theoretical development of fish population dynamics as epitomised by Beverton and Holt (1957) is based upon the idea of stock management; managing reproductively isolated groups of individuals. Thirdly, developing alongside the development of fisheries theory, observations of differing meristic characters between populations in different areas (e.g. Schmidt, 1930; Tanaka et al., 1969) lead to the obvious conclusion that not all conspecifics reproduce randomly throughout the species' range (Templeman, 1983), indicating that the concept of a stock has biological coherence.

Stock identification has gone hand in hand with the development of methods to identify stocks (Pawson and Jennings, 1996; Templeman, 1983). Tagging studies are widely used for following the movements of finfish and post-metamorphosis shellfish (Pawson and Jennings, 1996), however the small size of larvae means that traditional tagging studies can not be applied and genetic techniques are one of the only available means of stock discrimination. Increasingly powerful genetic techniques have allowed for more detailed analysis of the fine scale of stock structure (Paetkau et al., 2004; Parker et al., 1998; Shaklee et al., 1999). As a result of this it has been recognised that some stocks traditionally managed as a single homogenous stock are in fact composed of several spawning components (Stephenson, 1999) and failure to account for the separate spawning units can lead to inefficient management. The tendency to treat stocks as discrete homogenous units with uniform life history parameters has come in for criticism as an unrealistic over-simplification driven by management needs (Gauldie, 1991; Stephenson, 1999). Regardless of this a complete disregard for stock structure would lead to an even less accurate depiction of the true population dynamics as required by management measures.

Beyond the straightforward demographic significance of stock identification, interest in stock structure has increased to take into account preservation of genetic diversity (MacLean and Evans, 1981). The concept of separate stocks inherently suggests reproductive separation between stocks. Reproductive isolation restricts gene flow between local populations, increasing genetic diversity across the full species range and allowing local adaptation of local populations (MacLean and Evans, 1981). Conserving genetic diversity should be considered an important aim of fisheries management to preserve the ability of a population to adapt to a variable environment (Koljonen, 2001). Furthermore stock identification and the conservation of separate stocks across the full geographic range of the species is important to provide potential refuges and sources of range expansion in the face of future environmental change (Wolff and Mendo, 2000).

Where fisheries scientists talk about stock identification and stock integrity, benthic ecologists talk about open and closed populations. Fisheries scientists and benthic ecologists are addressing the same fundamental questions of population regulation; however the studies tend to operate at different spatial scales (Caley et al., 1996).

Benthic biologists have noted that for sedentary and sessile species larval dispersal can occur over a far greater range than adult movements (Todd, 1998). This leads to the two observations that i) local reproductive output can become decoupled from local larval supply, and ii) larval dispersal is the link between populations and determines the scale of population processes. However, much work on benthic species has concentrated on processes of recruitment and population regulation operating on very small spatial scales (Caley et al., 1996). For example Hughes (1990) concluded that a population of the bryozoan *Cellepora pumicosa* was an open population on the basis of eight 25 x 40cm quadrats. Fishery managers are concerned with processes operating on the scale of management areas which tend to be of the order of 10^1 - 10^3 km, hence very small scale studies of population regulation have limited implications to stock management considerations (Shepherd and Brown, 1993).

Stock identification for sessile benthic invertebrates has been attempted for abalone in Australia (Shepherd and Brown, 1993) and California (Tegner, 1993) as these high value gastropod molluscs have suffered from widespread population decline as

a result of extensive harvesting (Hobday et al., 2001; McShane, 1992). Shepherd and Brown (1993) concluded that the abalone stock structure in south Australia was best understood in accordance with metapopulation dynamics with a majority of larval dispersal occurring within a few 10s of kms. However genetic homogeneity predominantly occurred over scales of about 500km, although in specific cases significant genetic differentiation occurred between populations only separated by 1.5 km of suitable habitat (Brown, 1991). Studies of Californian abalone similarly concluded that a majority of settlement occurs within 10km (Tegner, 1993), although occasional dispersal will occur over larger scales and depends upon prevailing currents at the time of spawning.

1.6.2 Larval dispersal and marine protected areas (MPAs)

The use of MPAs as a fishery management tool was originally based on the idea that not only will stocks increase within MPAs, but that yield will also increase outside MPAs (Polunin, 2002). For this to occur there must either be 'spill over' of mobile adult stocks (Rowley, 1994), or increased recruitment to recruit limited populations outside the MPA due to larval export from within the MPA (Crowder et al., 2000). Increasingly MPAs are being used in a greater variety of management roles. Temporary or rotational opening of areas to fishing allows the stock to build up within the reserve and does not rely on beneficial effects spreading outside reserve boundaries (e.g. Murawski et al., 2000). The proposal to manage migratory stocks with large scale MPAs (Polunin, 2002) no longer uses MPAs as a tool to totally protect a subsection of the stock throughout its life cycle, but instead uses MPAs as a more traditional tool to reduce the fishery pressure applied to the population as a whole.

The spatial extent of larval dispersal is not only relevant to how far the benefits of the reserve will spread, but also determines whether the protected population itself receives sufficient recruitment (Crowder et al., 2000; Sala et al., 2002). This larval supply can be self-recruiting from within the reserve, from the remaining fished stocks outside the reserve, from other reserves set up as part of a network, or from a combination of these sources. Understanding larval dispersal is critical to making decisions on the siting, spacing and size of a protected area or network of areas

(Crowder et al., 2000; Palumbi, 2003; Roberts, 1997; Warner et al., 2000).

The ability for widespread teleplanic dispersal by benthic invertebrate larvae has been demonstrated (Scheltema, 1986) and modelling has shown that recruits may come from source areas 10s or 100s of km 'upstream' of a local population (Roberts, 1997), leading to the concept of open populations where recruitment is decoupled from local reproductive output (Gaines and Lafferty, 1995). However, contrary to this, there is increasing evidence that in many cases local populations are dominated by local recruitment (Heipel et al., 1998; Knowlton and Keller, 1986; Sponaugle et al., 2002; Todd, 1998; Warner et al., 2000) operating over scales of a few kms (McQuaid and Phillips, 2000) to a few 10s of kms (Palumbi, 2003). The increase in acceptance that recruitment may be predominantly local coincides with increases in understanding that there are a range of structures and processes occurring in the water column over the relevant scales to physically constrain and reduce larval dispersal (Archambault and Bourget, 1999; Denny et al., 1992; McCulloch and Shanks, 2003; Shanks, 1983; Shanks and McCulloch, 2003; Shanks et al., 2003; Talbot and Bate, 1987b; Wolanski et al., 1989; Wolanski and Hamner, 1988). Hydrodynamic modelling also indicates that in areas of topographic complexity particles can become trapped and remain near the source point for several weeks (Black et al., 1990).

If populations are dominated by recruitment over small spatial scales this could undermine the utility of MPAs to increase fishery yield outside protected areas as the benefits of increased spawning stock would not translate to increased recruitment outside of reserves. There is scant information to date as to whether MPAs do actually increase recruitment in the surrounding unprotected areas (see Halpern, 2003; Polunin, 2002, for reviews) and few studies have examined the effect of reserves on recruitment of sessile and sedentary species outside of reserves. Hockey and Branch (1994) examined recruitment of *Patella aspera* adjacent to refuges in the Canary Islands and found that recruitment was an order of magnitude higher close to the refuges than distant, however there was little observable difference in recruitment beyond 5 km from the refuge.

Further evidence of the scale of larval dispersal has come from a number of sources. Following the collapse of the bay scallop *Argopecten irradians* population in Bogue Sound, North Carolina, Petersen et al. (1996) proposed that the population in the

Sound was recruit limited as the population did not naturally regenerate. Petersen et al. (1996) tested this hypothesis by transplanting spawners into the Sound: over the following three years recruitment increased by 568% and adult biomass increased by 258% whilst adjacent control areas showed no significant change. This indicated that the population was recruit limited, and that larval dispersal was limiting over Sound-basin scale. Similar low levels of larval dispersal have been inferred from a genetic study of blacklip abalone, *Haliotis rubra*, around south Australia. Although there was large scale genetic homogeneity following a general trend of isolation by distance there was also evidence of differentiation between populations $<$ 3km apart (Brown, 1991).

Small scale larval dispersal has also been demonstrated from observations of the range expansion of invasive species. The barnacle *Elminus modestus* spread by about 20-30km a year following its introduction to north west Europe (Crisp, 1958). The mussel *Mytilus galloprovinicalis* had a single point introduction to the south eastern South African coast and the subsequent spread was followed by McQuaid and Phillips (2000). Four years after introduction the full range was asymmetrically distributed around the introduction site, the furthest spread recruits were 166km downcurrent to the north east and 29 km upcurrent to the south west, however 90% of recruits were within 5km of the initial site of introduction. These observations coincide with Palumbi's (2003) conclusions based on genetic data sets from five different species indicating that pelagic larvae of shallow water benthic invertebrates frequently disperse by 20-50 km, although the demographically significant recruitment may occur within a few kms.

The above examples demonstrate the importance of understanding the scale a which at population operates when evaluating the implications of theoretical population models on the effects of different types of fishery management strategy.

Before discussing the more specific aims and objectives of the thesis I will provide a brief review of patellid limpet biology, ecology and fisheries. I will particulary focus on the biology and ecology *P. vulgata*.

1.7 Patellid Limpet Biology, Ecology, and Fisheries

The term limpet is widely used to cover a range of conical molluscs (Fretter and Graham, 1962), however in this thesis I am solely concerned with the "true" limpets of the order Patellogastropoda. In this thesis I use the term limpet to refer to members of the order Patellogastropoda. A recently published molecular phylogeny divides the Patellogastropoda into 9 genera that form two distinct superfamilies (Nakano and Ozawa, 2004). One superfamily, the Patelloidea, contains the genera *Patelloida*, *Lottia* and *Nipponacmea*, and the other superfamily, the Acmaeoidea, contains the genera *Patella*, *Nacella*, *Cellana*, *Scutellastra*, *Helcion* and *Cymbula*. In general the Acmaeoidea are confined to the Atlantic and Indian oceans, and the Patelloida are confined to the Pacific, although some Acmaeoidea occur in the western Pacific, and some Patelloida occur in the north-eastern Atlantic (Nakano and Ozawa, 2004).

Limpets are conical prosobranch gastropod molluscs, and their internal body plan has undergone torsion (Fretter and Graham, 1962). They are broadcast spawners with larvae that undergo pelagic development, and although they have developed many strategies for withstanding desiccation high in the intertidal they are constrained to marine habitats in order for external fertilisation and subsequent larval development to occur (Fretter and Graham, 1962).

Limpets lack an operculum as they generally spend their complete post-larval life attached by their foot to solid substratum. Limpets are renowned for the tenacity with which they can cling onto surfaces. The tenacity with which they can cling on varies between individuals and species depending on the size and shape of the foot and the composition and quantity of mucus (Branch, 1981). It is their tenacity that has allowed limpets to expand into high energy wave swept shores.

Limpets are widely distributed, stretching from the tropics to polar latitudes and are the dominant grazers in many littoral and sublittoral rocky environments and they can play a critical role in determining community structure (Branch, 1985), as a result of which their biology and ecology has been widely studied (see Branch, 1981,

1985; Fretter and Graham, 1962, for reviews). Limpets are important grazers not only because they are widespread and numerically common, but also because they are able to graze tougher material than many other grazing gastropods (Branch, 1985; Hawkins and Hartnoll, 1983; Hawkins et al., 1989). Limpets and chitons are the only herbivorous grazing gastropod molluscs that are able to graze crustose algae (Hawkins et al., 1989; Steneck and Watling, 1982). Most grazing gastropods using a sweeping action, however limpets and chitons have robust buccal muscles surrounding their radulae, reduced numbers of points in contact with the substrate at any time increasing the pressure exerted per point, and have minerally hardened teeth, allowing them to graze with an excavating action (Hawkins et al., 1989; Steneck and Watling, 1982). Thus limpets can eat crustose algae, and tough leathery algae and sporelings that would be unaffected by other herbivorous grazing molluscs.

The regional, longshore and vertical distribution of limpet species is governed by physical and biological factors (Boaventura et al., 2002a; Branch, 1985). Physical factors define the absolute limits of a species' range, and biological interactions then frequently determine where the boundaries to the range actually occur (Branch, 1985). The dominant physical factor controlling the vertical distribution of intertidal rocky shore species is tidal level, and apart from regional climatic variations, the dominant physical factor effecting alongshore distribution is the degree of wave exposure (Jenkins and Hartnoll, 2001; Lewis, 1964; Moore and Seed, 1985; Yonge, 1949).

Limpets play a complex role in rocky shore communities, with limpet populations effecting the community structure of the shore, and the community structure of the shore effecting limpet populations (Arrontes et al., 2004; Branch, 1985; Jenkins et al., 1999b; Southward and Southward, 1978). The benthic community can effect the population structure and dynamics of limpet populations (Choat, 1977; Hawkins and Hartnoll, 1982; Lewis and Bowman, 1975). Conversely limpets can not only effect the presence or absence of fauna (Branch, 1975a; Underwood et al., 1983) and flora (Jenkins et al., 1999a) on rocky shores, but can also effect the phenotypic expression of individuals in the system (Jara and Moreno, 1984). The nature of limpet-algal relationships on the rocky shore have become so developed that some algae are dependent upon grazers for their persistence (Branch, 1985). The na-

ture of limpet-algal relationships can be further influenced by other members of the rocky shore community (e.g. barnacles, Hawkins, 1981), the level of physical stress (Branch, 1981), and regional scale differences in community structure and functioning (Jenkins et al., 2005). In addition to community interaction, limpet population dynamics have been shown to be effected by intraspecific (Boaventura et al., 2003; Branch, 1975a; Creese, 1980a; Fletcher, 1984a,b), and intra-order (Boaventura et al., 2002b; Haven, 1973) competition.

Collection of limpets for human consumption has been known since prehistoric times (Cunliffe and Hawkins, 1988); patellid limpet shells have been found in middens from up to 60 000 - 70 000 years ago (Volman, 1978). Prehistoric shell middens containing limpets are widely reported, for example limpets have been found in Celtic middens in Ireland (Gibbons, 1991), Maori middens in the south Pacific (Hayward and Brook, 1981), Aleut middens in Alaska (Siegel-Causey et al., 1991), and Southern African middens (Thackeray, 1988). Limpets are still widely collected for human consumption along the European Atlantic coast, the North and South American Pacific coast, in the Mediterranean, on Atlantic and Pacific islands, and around the South African coast. In many areas reduction in population numbers and even population collapse has been attributed to human collection (Branch, 1975b; Branch and Odendaal, 2003; Ferraz et al., 2001; Hawkins et al., 2000; Pombo and Escofet, 1996). Thus understanding the effects of exploitation on protandrous limpets has tangible practical benefits in addition to purely developing a better theoretical understanding of the population dynamics of exploited protandrous populations.

1.8 Aims and Objectives

The overall aims of this thesis were to examine the effect of protandry on the response of a species to exploitation with patellid limpets as a case study. I have only examined how exploitation affects reproductive output, and have not examined how this in turn effects recruitment. More specifically, I examined how protandry effects the relationships between SSB, fishery yield, population egg production, and population larval production. I did this by developing a simple model of how population larval production is affected by exploitation.

The motivation for working with patellid limpets is that recent population crashes have occurred, and that they are a convenient model species to work with. The main species that are commercially exploited in Europe are *P. aspera*, *p. caerulea*, *P. candei*, *P. feruginea* and *P. ulysponensis*. In this thesis I predominantly worked on U.K. populations of *P. vulgata*, but where possible I applied aspects of this work to exploited *P. aspera* and *P. candei* populations in the Azores. There is no commercial exploitation of limpets in the U.K., and only very limited recreational collection occurs. Also, although not one of the main commercially exploited species, there is a limited commercial fishery for *P. vulgata* in France (Baker, 2001). *P. vulgata* was chosen as the species to work on for three main reasons: firstly *P. vulgata* is protandrous, therefore it is an appropriate species to use when examining the effects of protandry. Secondly, it is possible to examine unexploited populations; and thirdly, due to the extent of the population decline in Azorean populations, it is difficult to collect large sample sizes, and it was not considered appropriate to sacrifice large numbers of limpets from heavily exploited populations. Furthermore, as all members of the genus *Patella* share very similar biology and ecology, conclusions drawn on the basis of examining one species can be broadly applied across the genus.

In order to develop a model of how larval production by a broadcast spawner is affected by exploitation it is necessary to develop three separate sub-models: a population fecundity model that specifies the number of male and female gametes released; a physical dispersal model that simulates the dispersal and advection of the gametes once released; and a fertilisation model that determines the number of eggs fertilised dependent on the predicted gamete concentrations and the length of time for which these concentrations last.

In addition to developing the zygote production model I conducted a genetic stock segregation study of the Azorean *P. candei* populations to examine the spatial scale at which these populations segregate. This was to investigate the appropriateness of possible management strategies that I examined with the model.

The specific objectives and content of each chapter are listed below:

- Chapter 2: I confirmed the occurrence of protandry in *P. vulgata*
- Chapter 3: I examined the spawning behaviour of *P. vulgata* to determine the

hydrodynamic conditions into which *P. vulgata* spawns to develop a physical gamete dispersal model.

- Chapter 4: I conducted a laboratory based examination of the fertilisation kinetics of *P. vulgata* to develop a model of the proportion of eggs fertilised when exposed to sperm at given concentrations for a given length of time.
- Chapter 5: I examined the sex-specific population structure of example *P. vulgata* populations. Sex-specific length-fecundity relationships were measured, and a model developed of how a simulated fishery would effect population gamete production, SSB and yield under different management scenarios.
- Chapter 6: I combined the component sub-models developed in the previous chapters to create a model of how population zygote production is affected by exploitation under different management scenarios.
- Chapter 7: I examined the genetic population structure of Azorean *P. candei* populations to determine the spatial scale over which they operate to determine if management strategies based on no-take zones can be effectively applied to populations in the real world.
- Chapter 8: Provides an overview and synthesis of the thesis to examine the implications of protandry on the response of a species to exploitation. The limitations of the work, and possibilities of future developments are considered.

Chapter 2

Direct observation of protandry in *Patella vulgata*

2.1 Introduction

Protandry in patellid limpets was first suggested by Orton (1919) for *P. vulgata* on the basis of sex specific length frequency analysis. Since then further work has reinforced the evidence for protandry in *P. vulgata* (Choquet, 1971; Dodd, 1956; Orton, 1928), and suggested the occurrence of protandry more widely in the genus *Patella* (Bacci, 1947; Martins et al., 1987; Thompson, 1979) and the order Patellogastropoda (Branch, 1974; Creese et al., 1990; Wright and Lindberg, 1982) (Table 2.1). However most workers have relied upon fatal methods of sex determination, therefore the assumption of protandry remains unconfirmed. The only direct observations of protandry in the order Patellogastropoda are of the lottid *Lottia gigantea* (Wright and Lindberg, 1982).

P.vulgata follows an annual reproductive cycle with complete regression of the gonad following spawning (Orton, 1928). Orton (1919, 1928) first suggested the occurrence of protandry in *P. vulgata* as the proportion of females in a length class increases with increasing size class, suggesting that sex change occurred whilst the testis was regressed with an ovary forming the following year. Orton (1919) noted that skewed size frequencies can also be explained by variable size at maturation, or differential

growth or mortality rates, but further observations suggested that this was unlikely to be the case (Orton, 1928).

Table 2.1: Previous observations of protandry in Patellogastropods. See text for explanation of the basis of observations in describing a certain species as protandrous

Species	Family	Basis of observation	References
Patella vulgata	Patellidae	Length frequency, hormonal	Orton (1919), Orton (1928), Dodd (1956), Choquet (1971)
Patella ulysponensis	Patellidae	Length frequency	Thompson (1979), Guerra and Gaudencio (1986) (but not Dodd, 1956)
Patella aspera	Patellidae	Length frequency	Martins et al. (1987)
Patella caerulea	Patellidae	Transitional hermaphrodites, length frequency	Bacci (1947)
Scutellastra longicosta	Patellidae	Length frequency	Branch (1974)
Scutellastra cochlear	Patellidae	Length frequency	Branch (1974)
Scutellastra kermadecensis	Patellidae	Transitional hermaphrodites, length frequency	Creese et al. (1990)
Cymbula oculus	Patellidae	Transitional hermaphrodites, length frequency	Branch (1974)
Helcion pectunculus	Patellidae	Length frequency	Gray and Hodgson (2003)
Lottia gigantea	Lottidae	Direct observation, length frequency	Wright and Lindberg (1982)

Sequential hermaphroditism has been noted in a number of Patellogastropods (Branch, 1974; Orton, 1928) and has been categorised as being of two types, 'mosaic' and 'transitional', by Branch (1974). Mosaic hermaphroditism is a very rare form and is assumed to be an abboration, whereas transitional hermaphroditism has been found regularly in a number of species and is assumed to be a gonad in 'transition' from one sex to the other Branch (1974). Occasional 'mosaic' simultaneous hermaphroditism ($\ll 1\%$) has been noted in *P. vulgata* (Dodd, 1956; Orton, 1928), other members of the genus *Patella* (Bacci, 1947; Dodd, 1956) and more widely in the order Patellogastropoda (Branch, 1974). However mosaic sequential hermaphroditism is considered an aberrant form (Dodd, 1956) and not indicative of the sexuality of limpets. Furthermore Dodd (1956) notes that aberrant hermaphroditism occurs in a vast range of gonochronistic species, and can therefore be regarded as being of no relevance to this discussion.

The 'transitional' form of sequential hermaphroditism has been noted in *P. caerulea* (Bacci, 1947) and *Cymbula oculus* (Branch, 1974) in conjunction with skewed sex ratios. In the transitional form of hermaphroditism oocytes start developing in a spawned testis and spermatogenesis ceases as gonad maturation proceeds (Branch, 1974). This occurs most frequently in limpets of the size range where the population sex ratio changes (Bacci, 1947; Branch, 1974). As neither *P. caerulea* (Bacci, 1947) nor *Cymbula oculus* (Branch, 1974) fully regress their gonads following spawning Branch (1974) took this to be the sex transition from male to female. *P. vulgata* fully regresses its gonad each year following spawning prior to the start of gametogenesis for the following season, therefore the transitional stage would not be expected to be observed. The histological work required for the observations of transitional gonads is fatal, thus no single individual could be followed from the male to female condition to confirm this hypothesis.

Choquet (1971) examined the role of the endocrine system on gametogenesis and sex change in *P. vulgata* with organotypic cultures. Notably Choquet (1971) histologically established the presence of oogonia in male limpets that remained quiescent during spermatogenesis. Choquet (1971) concluded that young limpet gonads had an inherent tendency to undergo spermatogenesis which was enhanced by a cerebral gonadostimulant. During post-spawning gonad regression and rest period spermatogenesis was inhibited by a tentacular endocrinal secretion. During the female phase cerebral secretion stimulated oogenesis and spermatogenesis continued to be inhibited by the tentacular endocrinal secretion. From this Choquet (1971) concluded that *P. vulgata* underwent sex inversion rather than having gonads that started with both oogonia and spermatogonia but were conditioned to become functional gonochronists.

In order to confirm or deny the occurrence of protandry in the Patellidae I made direct observations of the sex of marked limpets over two breeding seasons.

2.2 Method

As part of a separate study 4515 *P. vulgata* were collected from Lynmouth beach, Devon, in October 2002, measured to the nearest mm and sexed by the standard

dissection method (Orton, 1928)(Figure 2.1). These observations were used to determine the likely range over which sex change might be observed for the direct observations of protandry.

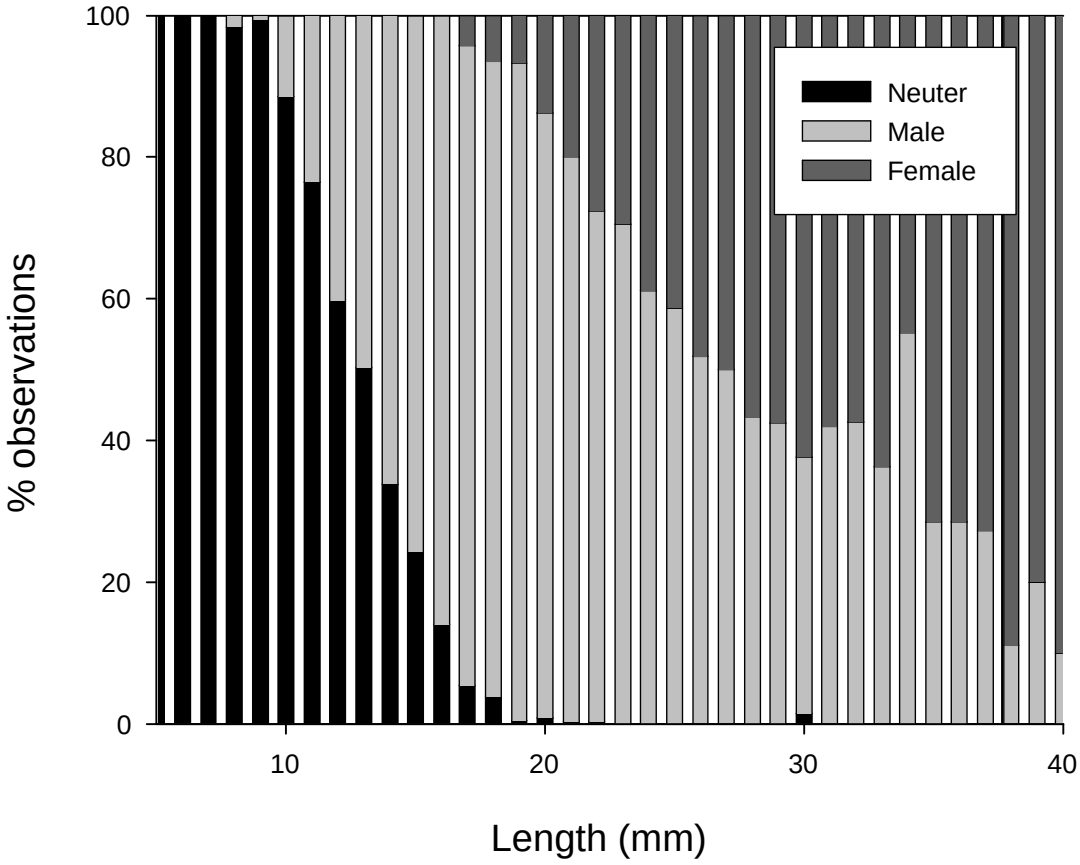

Figure 2.1: Sex ratio of *P. vulgata* per 1 mm length class for 4515 collected from Lynmouth beach, Devon. Limpets over 40 mm (max 43 mm) were pooled into a single length class due to low numbers in the largest size classes.

Non-fatal observations of the sex of selected *P. vulgata* were made at Lynmouth beach, Devon, in October 2003. 200 limpets covering the size range over which sex change was thought likely to occur (15 - 25 mm) were measured to the nearest mm and marked with small plastic numbered disks attached by epoxy resin. The sex of the limpets was determined by taking a biopsy of gonad with a hypodermic needle. The method used follows that of Wright and Lindberg (1979) with the modification that prior to inserting the needle into the limpet the syringe was pre-filled will 1ml filtered seawater to act as a carrier medium for the small amount of material

collected from the limpet. Once the biopsy had been taken the sample was squirted into a vial containing ≈ 10ml filtered seawater. In most cases it was possible to make an immediate visual determination of sex; sperm produced a pinky cream coloured cloud in the vial, and the small green spherical eggs are directly visible with the naked eye. In the cases where sex determination was uncertain no records were kept. In October 2004 the surviving marked limpets that could be found were collected and sexed by dissection.

2.3 Results and Discussion

Thirty seven of the original 200 marked limpets were found and collected in October 2004. 9 of the 31 limpets (29%) that had initially been male were female at the time of the second observation (Figure 2.2). One of the 8 limpets that had initially been female was male at the time of the second observation.

This indicates that *P. vulgata* is a protandrous hermaphrodite. The observation of a single female becoming a male is the first observation of two way sex change in a protandrous hermaphrodite. Two way sex change has been demonstrated by protogynous hermaphrodite fishes that undergo socially mediate sex change (Kuwamura and Nakashima, 1998, and refs within) however it has not been previously demonstrated in a protandrous hermaphrodite.

Choquet's 1971 work on endocrine gender control demonstrated that in recently changed female *P. vulgata* spermatogonia occurred within the ovary and spermatogenesis was suppressed by tentacular endocrine secretions, although in older females the endocrine secretions had stopped and there was no evidence of the previous male lineage in the ovary. This indicates that recently changed females may well be capable of returning to their previous male state. The results of this work demonstrate that *P.vulgata* may be more capable of labile sex change in response to altering environmental conditions than had previously been considered.

As to the key question of whether this leaves protandrous patellid limpets more or less susceptible to exploitation than an analogous gonochronist, this depends on the speed and extent to which limpets are able to alter the age at sex change in relation

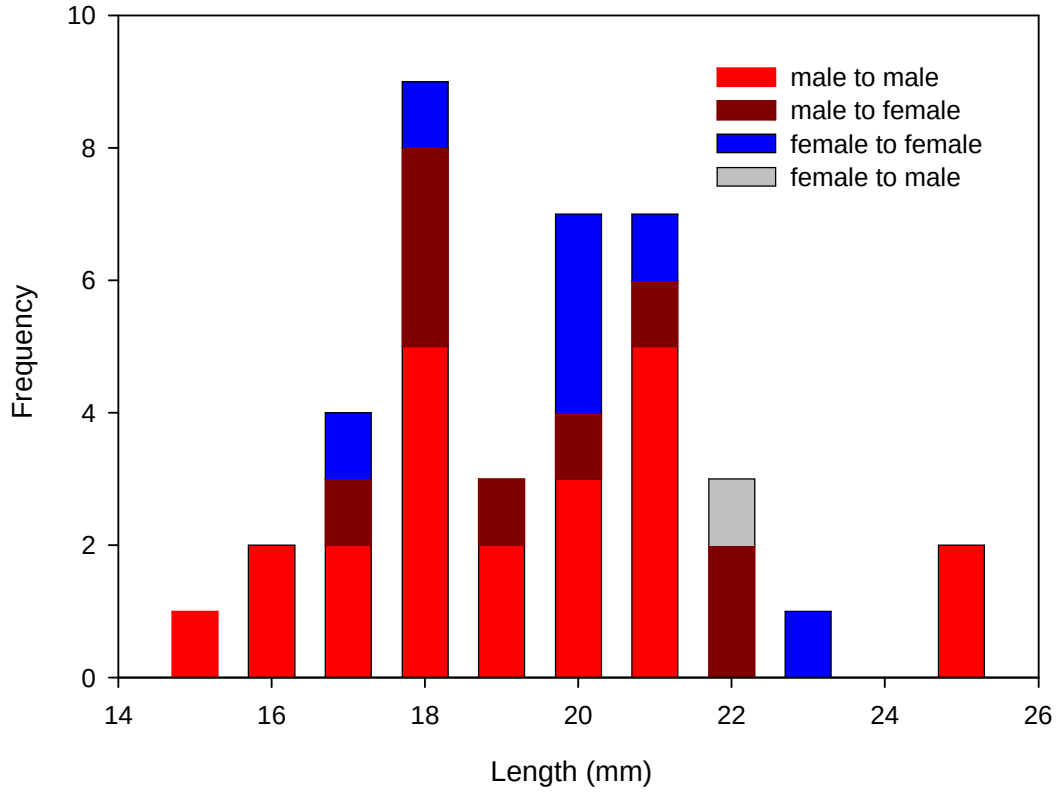

Figure 2.2: Sex transformations between observations in October 2003 and October 2004 of *P. vulgata* from Lynmouth beach, Devon.

to the rate of change in population parameters.

Chapter 3

Spawning and Gamete Dispersal

3.1 Introduction

In order for successful reproduction by broadcast spawners to occur there must be the union of gametes shed into the open water by spatially separated individuals. The hydrodynamic conditions into which a broadcast spawning organism spawns determines the dispersal and mixing of spawned gametes which has been empirically (Denny et al., 2002; Marshall et al., 2004; Mead and Denny, 1995; Serrao et al., 1996) and theoretically (Denny et al., 1992; Levitan and Young, 1995; Metaxas et al., 2002) shown to effect the proportion of eggs successfully fertilised. To develop a coupled fecundity-dispersal-fertilisation model of population larval production it is necessary to understand how gametes are dispersed. To do this I will look at the spawning stimuli controlling the spawning of *P. vulgata* and determine the associated hydrodynamic conditions. On the basis of this a simple dispersal model will be developed to simulate the dispersal of *P. vulgata* gametes during a spawning event.

The initial prerequisite for successful fertilisation is the co-occurrence in space and time of gametes of the opposite sex. One of the simplest strategies evolved to enhance fertilisation is temporal synchrony of spawning, and organisms use a number of endogenous (Holt, 1985; Soong et al., 2005), exogenous (Babcock et al., 1986), biotic (Glorioso and Davies, 1995; Starr et al., 1990) and abiotic cues (Giese and Kanatani, 1987) to achieve temporal synchrony in spawning.

For many organisms there are broad limits imposed upon the timing of spawning by seasonal or annual gametogenic cycles with spawning physiologically constrained to a certain part of the year (Giese and Kanatani, 1987). To this extent spawning triggers can be multi-factorial and organisms can undergo gated maturation and spawning; the specific proximal spawning cue will only trigger spawning once various other states have been achieved. For example, the proximal spawning clue for the abalone *Halitois discus* is rough weather; but rough weather only initiates spawning once the temperature has dropped below 20-21 °c and an effective accumulated temperature of 1200 degree days has been reached (Sasaki and Shepherd, 1995).

The conditions at the time of spawning must also be appropriate for embryonic and larvae development as well as being appropriate for the initial fertilisation. Spawning has been shown to be triggered by the onset of favourable conditions for larval development such as the presence of ample algal food (Himmelman, 1975; Starr et al., 1990) or a reduction in predation pressure (Frank and Leggett, 1982; Shanks, 1998).

Temporal synchrony on the small scale can be best achieved by sensitivity to spawning of nearby conspecifics. Spawning has been shown to be triggered by the presence of gametes, or pheromones released synchronously with gametes (Beach et al., 1975; Galtsoff, 1938, 1940; Soong et al., 2005). It has been suggested that external factors can initiate spawning processes that are then locally coordinated by the release of spawning hormones (Giese and Kanatani, 1987; Soong et al., 2005).

Irrespective of the proximal cue that initiates spawning the hydrodynamic conditions into which the gametes are spawned will determine their rate and extent of dispersal and mixing. For gametes to meet from organisms that are not immediately adjacent to each other some mixing is required for the gametes to be brought into sufficiently close contact for fertilisation to occur; however too much mixing and the gametes will be dispersed to almost infinite dilution and no fertilisations will occur. As examples of the balance that must be found between too much and too little dispersal, Serrao et al. (1996) found that *Fucus vesiculosus* living in high energy intertidal conditions spawned in periods of slack water. Conversely McEuen (1988), working on the subtidal holothurian *Pentamera populifera* in a low energy environment, found that spawning occurred during periods of flow rather than periods of non-flow.

Spawning in *P. vulgata*

The seasonal spawning cycle of *P. vulgata* was first noted by Russell (1909). Further work on *P.vulgata* (Bowman and Lewis, 1986; Orton, 1928; Orton et al., 1956) confirmed that it has a distinct seasonal spawning cycle, spawning in late autumn followed by a period of gonad regression, with the exact timing of spawning varying between location and year (Baxter, 1982; Bowman and Lewis, 1986; Lewis and Bowman, 1975). Similar seasonal patterns have been shown for other Patellogastropod limpets in the UK (Thompson, 1979), continental Europe (Guerra and Gaudencio, 1986), Australia (Creese, 1980b), and South Africa (Branch, 1974). Although some species do not have a distinct rest period following spawning (e.g. Bacci, 1947; Branch, 1974), and other species display extended spawning periods lasting for prolonged periods throughout the year (e.g. Branch, 1974; Creese, 1980b).

P. vulgata undergoes mass spawnings, but there is not a single complete spawning event each year (Lewis and Bowman, 1975; Martins et al., 1987; Orton et al., 1956). Complete synchrony in gametogenesis does not occur (Lewis and Bowman, 1975) and the first partial spawnings may occur before the whole population has reached maturity (Bowman and Lewis, 1986). The early spawnings are followed by regeneration of the gonad prior to the main spawnings. Although there are partial early and late spawnings Lewis and Bowman (1975) considered them to be demographically insignificant due to the small numbers of gametes released compared to the main spawnings.

Early work on spawning stimuli of *Patella* spp. in the UK (Orton and Southward, 1961; Orton et al., 1956; Thompson, 1979) indicated that the main spawning events were associated with the onset of autumn storms and high wave action. More detailed analysis of spawning stimuli of *P. vulgata* has been conducted by Bowman and co-workers (Bowman, 1985; Bowman and Lewis, 1977, 1986). Bowman (1985) found that the first autumn storms did not always initiate spawning, and that storms did not initiate spawning until the ambient seawater temperature had dropped below 12 °c. Furthermore regional analysis of *P. vulgata* spawning around the UK showed that in different regions the time that sea level dropped below 12 °c corresponded with the time of first spawning (Bowman and Lewis, 1986). Bowman (1985) sug-

gested that the 12 ° threshold may not be universal across the range of *P.vulgata* and the first main spawning may be triggered by the first autumn storm associated with a drop in water temperature. There is no evidence that spawning is related to tidal or lunar cycles (Bowman, 1985; Bowman and Lewis, 1986). However it should be noted that much of the work by Bowman relied up samples taken at fortnightly intervals and a direct correspondence between rough sea and spawning events could not be pinpointed.

The aim of this study is to assess with high frequency observations whether *P. vulgata* spawning corresponds with periods of high wave action.

3.2 Methods

The gonad condition index (GCI) of a population of *P. vulgata* and wave action and water temperature were monitored during the spawning season of 2002 from 4^{th} November to 9^{th} December at Warren beach, South Devon.

The GCI of limpets was assessed following the method of Orton et al. (1956) whereby a stage 0 limpet is in its neuter resting state, and a stage V limpet has a fully mature gonad. A minimum of 60 limpets were examined for GCI each day to give a measure of the average population GCI, Orton et al. (1956) found that samples of 50 individuals gave a good representation of the population's gonad developmental status. Spawning events are characterised by a drop in the population's average GCI and a drop in the proportion of individuals with stage IV and V gonads (Bowman and Lewis, 1986; Lewis and Bowman, 1975; Orton et al., 1956). The results of the GCI observations from Warren beach are analysed in terms of the population average GCI and percent stage IV and V gonads. The standard deviation is calculated for the population average GCI, and 95% binomial confidence limits (Spiegel, 1961) are calculated for the percent stage IV and V gonads.

Wave action and temperature were monitored with a Seamon temperature and pressure meter bolted onto the rock just above spring low tide level. The pressure readings were used as a measure of wave action by comparing the maximum and minimum pressure readings over a 15 minute period. The meter could only be easily

accessed at spring low tides, therefore a 22 s sampling rate was chosen as a higher frequency sampling rate would have overloaded the onboard memory before the meter could be re-accessed and the data downloaded.

Analysis of pressure/temperature data

The data were divided into 15 minute sections. The data collected at low tide were removed by removing all the 15 minute blocks of data that had pressure readings of less than 0.05 bar. The wave action during each 15 minute block was determined by subtracting the lowest pressure measurement from the highest pressure measurement during each 15 minute period. The maximum temperature measurement from each 15 minute block was ascribed as the temperature for that time period.

3.3 Results

The complete data set of pressure readings and temperature per 15 minute block and daily GCI data are in Appendix 1.

The daily average GCI and measures of environmental parameters are presented in Figure 3.1. The daily percent stage IV and V gonads and measures of environmental parameters are plotted in Figure 3.2. The results are presented alongside the maximum daily tide height to see if there is a relationship between spawning and spring-neap cycle.

The largest spawning event did coincide with the period of greatest wave action; however the relationship between wave action and spawning seems somewhat equivocal. Both indices of gonad state (average GCI and % stage IV and V) indicate four separate spawning events. The largest, which resulted in the spawning of all stage IV and V gonads (Figure 3.2), occurred between sampling on 22^{nd} and 23^{rd} November and coincided with the period of greatest wave action and a drop in sea temperature. There were two previous spawning events (8^{th} - 10^{th} Nov and 15^{th} - 16^{th} Nov), but in neither case did all stage IV and V limpets spawn, during these events wave action was moderate and there was no associated drop in temperature. The fourth spawning (5^{th} - 6^{th} Nov) also resulted in the complete spawning of all

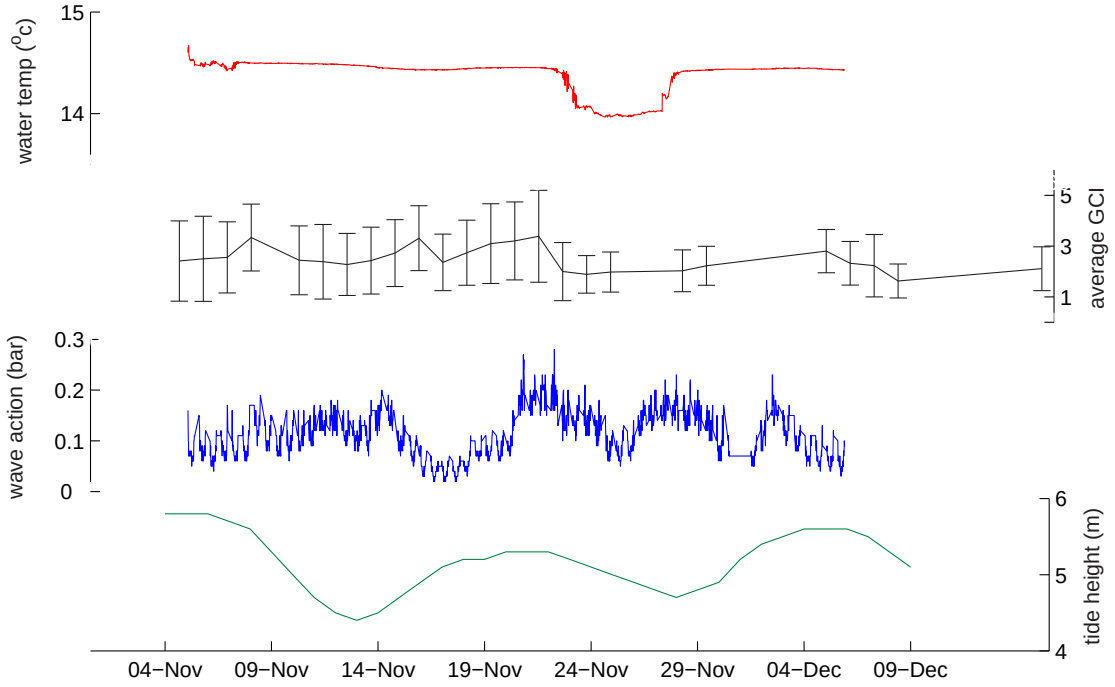

Figure 3.1: The average daily GCI of *P. vulgata* from Warren beach, Devon plotted along side environmental parameters. The error bars are ± 1 standard deviation. Tide height is the maximum tide height each day. See text for explanation of wave action.

stage IV and V gonads, however there had been little regeneration following the large spawning on the 22^{nd} November.

If the first two spawning events are considered as what Bowman and Lewis (1986) referred to as 'partial early spawnings', then the larger spawning of 22^{nd} November can be considered to be the main spawning event. This is consistent with Bowman's 1985 prediction that the main spawning occurs during the first main autumnal storm that is associated with a drop in water temperature.

3.4 Discussion

It initially seems counter intuitive for an intertidal or shallow water broadcast spawning species to spawn during periods of increased wave action as this would seemingly increase the rate of gamete dispersal and reduce the proportion of eggs fertilised. Especially as it was demonstrated by Serrao et al. (1996) that several macroalgal species spawned during slack water, and spawning was repressed during rough

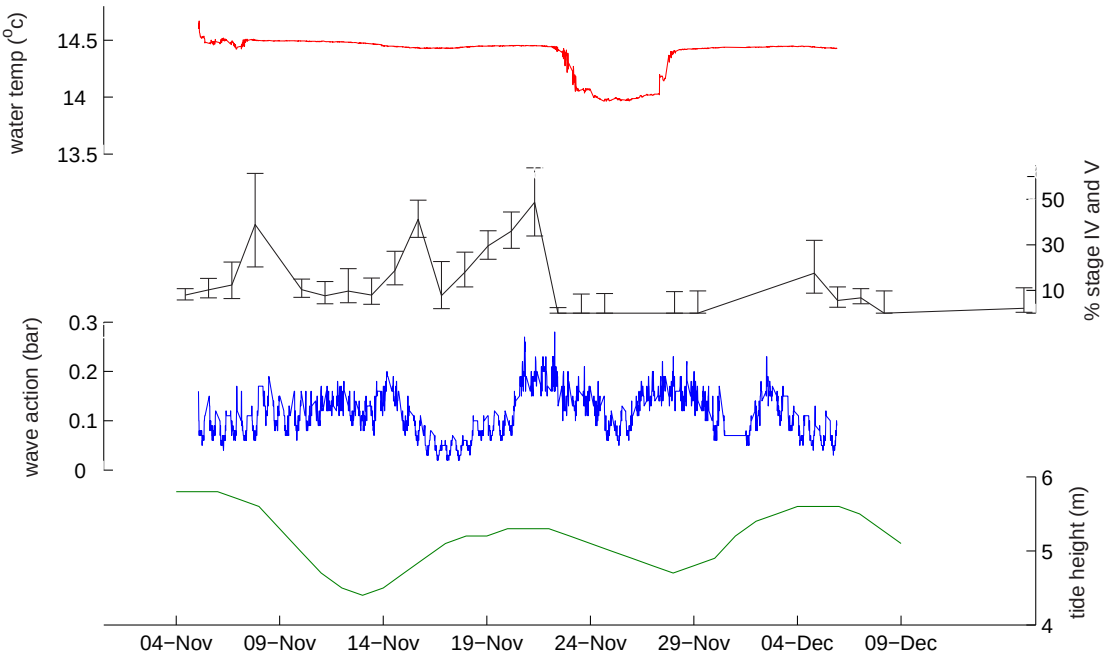

Figure 3.2: The daily percent of stage IV and V gonads of *P. vulgata* from Warren beach, Devon plotted along side environmental parameters. The error bars are 95% confidence limits. Tide height is the maximum tide height each day. See text for explanation of wave action.

conditions, presumably to reduce gamete dispersal. Contrary to this a number of intertidal and shallow water species have been reported as spawning in storms or periods of rough conditions (Table 3.1), however the practicability of working in shallow waters during storm conditions has hampered efforts to directly confirm the relationship between spawning and rough conditions.

The hydrodynamic conditions into which an individual spawns will not only affect the dispersal and advection of gametes, but will also control the initial larval dispersal trajectory. The hydrodynamic conditions optimal for one process may not be optimal for the other, therefore it may not be surprising that animals are seen to spawn into conditions that are non-optimal for fertilisation. Sasaki and Shepherd (1995) noted that the abalone *Haliotis discus* and the trochid *Tegula* spp. spawned during typhoons and minor storms. After typhoons larvae were advected further offshore and mixed deeper than larvae produced during minor storms. Sasaki and Shepherd (1995) suggested that the abalone spawned in variable conditions to enable

Table 3.1: Intertidal and shallow water species known or suspected of spawning in storms or rough conditions.

Taxanomic Group	Species	References
Gastropoda	*Patella vulgata*	Bowman (1985); Orton et al. (1956)
	Patella ulysponensis	Orton and Southward (1961)
	Patella aspera	Thompson (1979)
	Patella aspera	Martins et al. (1987)
	Cellana radians	Wright and Lindberg (1982)
	Haliotis discus	Sasaki and Shepherd (1995)
	Lottia digitalis	Shanks (1998)
	Tegula rustica	Sasaki and Shepherd (1995)
	Tegula argyostoma	Sasaki and Shepherd (1995)
	Melagraphia aethiops	Grange (1976)
	Zediloma atrovirens	Grange (1976)
	Lunella smaragda	Grange (1976)
Bivalvia	*Septifer virgatus*	Sasaki (1984)
Echinodermata	*Stronglyocentrotus nudus*	Sasaki and Shepherd (1995)
Polychaeta	*Phragmatopoma lapidosa*	Ramirez Llodra (2002)

variable dispersal to gain the benefits of short and long distance dispersal.

3.5 Surf Zone and Very Nearshore Oceanography: Modelling Gamete Dispersal

3.5.1 Surf zone hydrodynamics

Intertidal or shallow tidal organisms spawning on the wave swept rocky shore release their gametes into a highly turbulent environment with high levels of initial mixing and dispersal which would seemingly rapidly dilute spawned gametes. However the surf zone (Inman et al., 1971; Svendsen, 1992; Winter, 1983) and nearshore waters (Itosu and Miki, 1983; Shanks et al., 2003; Wolanski and Hamner, 1988) show far greater structure over a variety of scales (Shanks et al., 2003) than initially assumed. The structure in the water column as a result of the interaction of wind and wave driven currents may act to control or constrain the dispersal of gametes, even in

rough conditions.

Unfortunately the oceanography of surf zone and very nearshore waters[1] falls between the reach of offshore research vessels and terrestrial workers and for a long time was overlooked and hence little understood. Increasing amounts of work is being done on the biological oceanography of very nearshore waters, predominantly with reference to larval dispersal and supply (e.g. Archambault and Bourget, 1999; McCulloch and Shanks, 2003; Prince et al., 1988; Shanks et al., 2003) and the supply of food and nutrients to benthic macroalgae and filter feeders (e.g. Archambault et al., 1999; Shanks and McCulloch, 2003). In addition to this, work has been done on the influence of reefs on nearshore currents (e.g. Black et al., 1990; Symonds et al., 1995; Wolanski et al., 1989). However very little work has been done directly on the hydrodynamics of the surf zone of rocky shores (Denny et al., 1992, 2003; Denny and Shibata, 1989); a majority of work on surf-zone hydrodynamics has been done on sandy shores (e.g. Inman et al., 1971; Short, 1985; Svendsen, 1992; Talbot and Bate, 1987a,b; Winter, 1983).

As intertidal dwellers, limpets will spawn into the surf zone in stormy conditions, so the hydrodynamics of surf zones are the area of interest in developing a gamete dispersal model. The surf zone can be visualised as a well mixed self-contained water body with limited exchange with nearshore waters (McLachlan, 1980; Svendsen, 1992), as a topographically controlled boundary mixing front is established along the back of the outer breaker line (Wolanski and Hamner, 1988). The hydrodynamics of surf zones are dominated by two main mixing processes (Inman et al., 1971); firstly turbulent eddy diffusion within the surf zone, and secondarily advective rip currents which transfer water across the outer breaker line between the surf zone and very nearshore waters.

The turbulent eddy diffusion within the surf zone is primarily caused by the injection of turbulent energy into the water column during the collapse of a wave crest and is spread throughout the surf zone by the subsequent passage of the wave bore (Inman et al., 1971). Turbulent eddies do not penetrate the mixing front into the non-turbulent waters beyond the outer breaker line, so the rapid turbulent mixing

[1]Following Shanks et al. (2003) very near shore waters are defined as being within 1 km of the coastline.

is contained within the surf zone (Denny, 1988).

A dynamic pressure gradient, known as the radiation stress (Longuet-Higgins and Stewart, 1964), is generated by the progression of waves against the shore. Sea level rises along the shore to create a seaward directed pressure gradient to offset the shoreward directed flux of wave momentum (Komar, 1998). This wave generated super elevation of the mean water level above the mean still water level is known as wave set-up. The level of wave set-up is affected by beach angle and wave height; as a result of this there can be long shore variation in wave set-up (Komar, 1998).

Once a wave has broken it no longer acts as an oscillatory wave and transforms into a travelling wave similar to a bore (Denny, 1988), and there is a net shoreward transport of water. However water does not pile up along a coast line so there must be a return flow to offset the shoreward directed flow. This return flow can either be in the form of undertow running beneath the surface shoreward flow, or as rip currents where the water runs alongshore before turning seaward.

Longshore variation in wave set-up can occur, which can lead to longshore pressure gradients, which in turn generates longshore currents that are the basic force behind the generation of rip currents. The longshore currents run along the shore until they encounter other longshore currents directed towards them, an area of low radiation stress, or the topography forces them to turn seawards (Figure 3.3).

A series of dye releases in the surf zone were conducted by Inman et al. (1971) to examine surf zone currents on sandy beaches. A general pattern of dispersal was noted; the dye patch was rapidly dispersed across the surf zone over 0.1-2 minutes, after which the patch was advected alongshore (Inman et al., 1971). For example on a shallow sloping sandy beach the dye patch took 106 s to disperse across the surf zone, and had a residence time of 21 minutes within the surf zone. Talbot and Bate (1987b) undertook a similar series of dye releases within the surf zone of a sandy beach. They found that there were a variety of rip types ranging from rip gyres that remined totally within the surf zone and did not lead to mixing with the nearshore to exchange rips that carried the tracer up to 2 km offshore (Figure 3.4). The flux estimates for exchange of water between the surf zone and very nearshore ranged from 0 to 0.032 $m^3 s^{-1} m^{-1}$, the exchange flux increased with wave height for a given section of beach. The resident half-life of water within the surf zone ranged

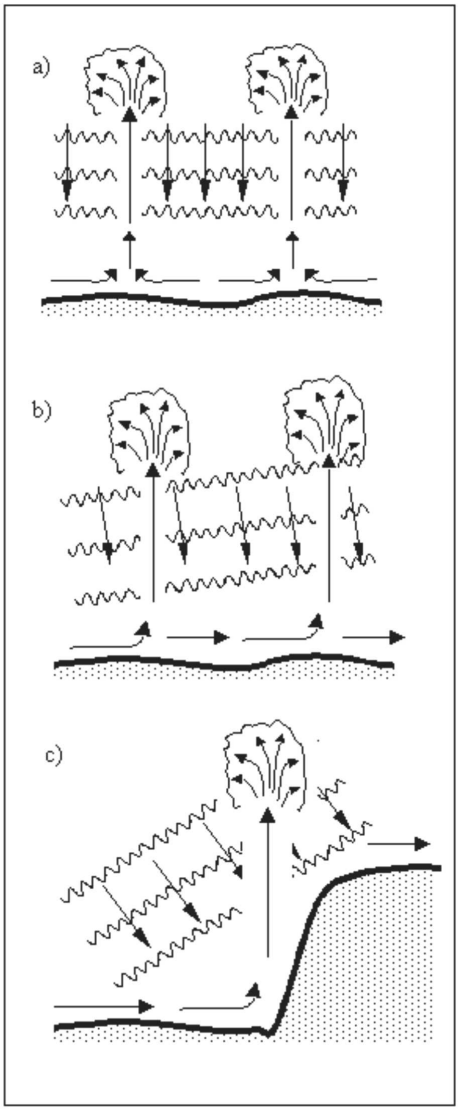

Figure 3.3: Different types of rip currents caused by a) counter flowing rip currents; b) an area of low radiation stress and; c) topography. Figure modified from (Komar, 1998).

from 22 minutes to 5 days with an average of 3.6 hours (Talbot and Bate, 1987b). In reality there is mixing between the surf zone and the very nearshore waters all along the back of the surf zone, and rips are not the only cause of exchange (Inman et al., 1971; Svendsen, 1992); however rips are the dominant exchange mechanism and the empirical dye measures of surf zone half life will have accounted for this extra mixing.

The dimensions of the surf zone are determined by the location of the outer breaker line. As a wave propagates across across shoaling water towards the coast the wave

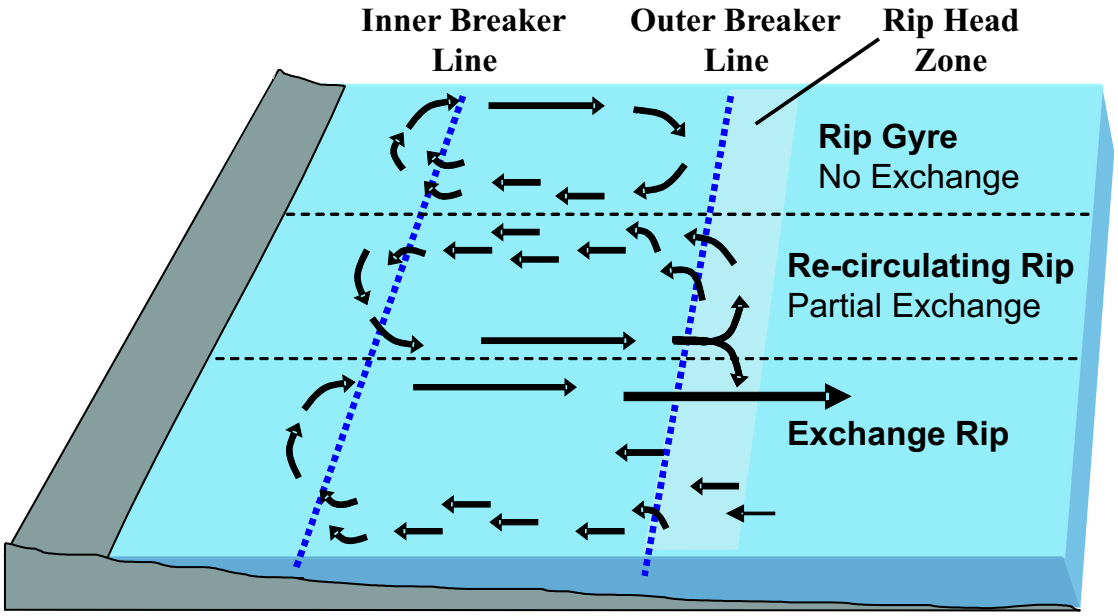

Figure 3.4: Rip currents causing different levels of exchange between surf zone and very nearshore waters.

celerity and wave length reduces, and the wave height and steepness increases. At some point the wave reaches a point of instability and breaks. The break point occurs when the velocity of water molecules in the wave crest reach the wave celerity; this commonly occurs when γ, the ratio of wave height to water depth, reaches ≈ 0.75 (Denny, 1988; Komar, 1998), although on steeper beaches γ is often between 0.78 - 1.03 (Komar, 1998).

The above discussion of dye tracer studies in the surf zone was all in relation to sandy beaches. Rocky shores differ from sandy beaches in that they tend to be steeper and have far more complex topography. Denny et al. (1992) conducted a series of dye releases in the surf zone of a rocky shore; however rather than studying the dye dispersal throughout the whole surf zone they concentrated on dispersal in wave surge channels and exchange with the main body of the surf zone. Four blind end surge channels with volumes ranging from 3.5 to 45 m^3 were examined, and it was found that the exchange parameter, λ [2], varied between 0.001 to 0.04 s^{-1}, implying average residence times of 25 to 1000 s. Partial and temporary containment of dispersal by small scale topographic features such as wave surge channels could have a significant impact on the proportion of eggs fertilised as it would allow

[2]the proportion of the water in the channel that was exchanged with water from outside the channel per second

for temporary pockets of high concentrations of gametes to build up compared to the gamete concentrations that would occur if gametes were immediately dispersed throughout the surf zone.

3.5.2 Modelling gamete dispersal

The above discussion bears out two main aspects of surf zone hydrodynamics:

> i) On the gross scale, surf zones are self-contained well mixed water masses. The mixing is characterised by rapid cross shore mixing followed by gradual longshore mixing.
>
> ii) On the fine scale, the exact topography of a section of beach will control the hydrodynamics and mixing and will determine the number, type and size of semi-self-contained water masses (e.g. wave surge channels) within the surf zone.

It is possible to develop a simple model that accounts for the gross mixing and dispersal within the surf zone, but it is not possible to develop a simple model that will account for all the fine scale structure within the surf zone.

Previous theoretical work on gamete dispersal has been based on diffusion within the benthic boundary layer using advection-diffusion models (Babcock et al., 1994; Claereboudt, 1999; Denny and Shibata, 1989; Levitan and Young, 1995), apart from the work discussed above by Denny et al. (1992) which used simple box models to model gamete diffusion in blind end surge channels. Due to the dominance of diffusion over advection within the surf zone a simple one box model is used in agreement with Wolanski and Hamner's (1988) observation that nearshore "mixing and diffusion processes ... are quite different from these processes in the open sea, and classical advection-diffusion models that were developed for the open sea are not valid near shore".

In the model presented the surf zone is represented as a single box of unit width running cross shore (Figure 3.5). If the simplification of longshore uniformity is assumed then downstream loss and upstream addition will cancel each other out, in which case the input to the box is gametes released from the benthic boundary,

and the only loss is exchange across the outer breaker line. Water within the box is assumed to be fully mixed, any gametes advected out of the box are considered to be permanently lost from the system.

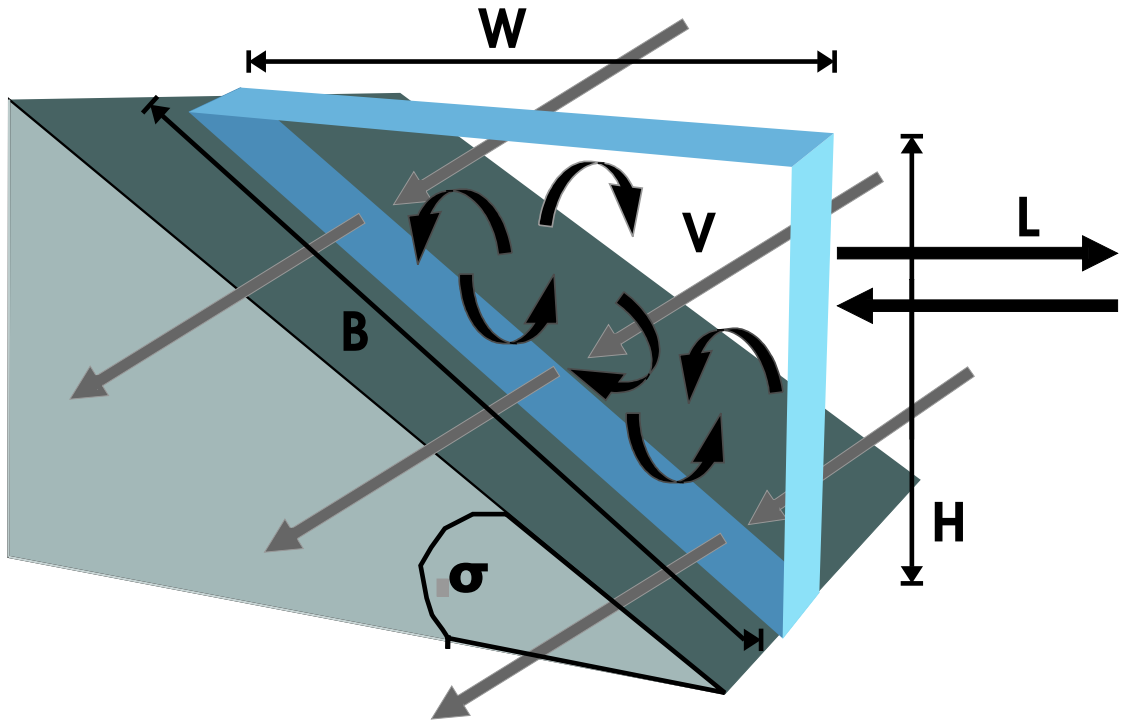

Figure 3.5: A schematic representation of the surf zone one box model showing main flows. Assuming upstream inputs are cancelled by downstream loss the only exchange flow is cross shore exchange across the outer breaker line. See text for list of variables.

The dimensions of the box are determined by the location of the outer breaker line and angle of beach slope. The location of the outer breaker line is determined by the wave height. Taking the ratio of wave height to wave depth at time of wave breaking to be a standard value for steep rocky shores of,

$$\gamma = 0.90$$

the dimensions of the box can be calculated (Komar, 1998). The variables used are as follows:

h = wave height (m)
H = depth of surf zone at outer edge (m)
W = width of surf zone (m)
B = length of bed in the surf zone (m)
V = volume of surf zone (m^3)

σ = beach angle (°)
L = leakage rate out of surf zone ($m^3 s^{-1}$)
λ = proportion of volume exchanged per unit time (s^{-1})
τ = residence time (s)

Given h and σ the remaining dimensions of the surf zone can be determined using the following relationships:

$$H = h/\gamma \tag{3.1}$$

$$W = \frac{H sin(90 - \sigma)}{sin\sigma} \tag{3.2}$$

$$B = \sqrt{W^2 + H^2} \tag{3.3}$$

$$V = \frac{W * H}{2} \tag{3.4}$$

Given a value for λ or τ the following relationships can be calculated:

$$L = V * \lambda \tag{3.5}$$

$$\tau = \frac{1}{\lambda} \tag{3.6}$$

In order to apply the surf zone dispersal model to Warren Beach the wave height during a spawning event and beach angle are needed. The maximum pressure differences recoded by the pressure meter installed at Warren beach during the main spawning event on 22^{nd} November are about 0.3 bar, which equates to a wave height (h) of 3 m (Figure 3.1). The beach angle taken by measurements of the beach (σ) is 14°.

There is no direct empirical data from Warren Beach on which to base an estimate of τ. However on the basis of the observations of Denny et al. (1992) on the mixing of

wave surge channels, and Inman et al. (1971) and Talbot and Bate (1987b) working on sandy beach surf zones an initial value for the half-life in the surf zone of 20 minutes was assumed. Sensitivity testing to the value of κ will be conducted with the full fecundity-dispersal-fertilisation model (see Chapter 6). Also a more structurally complex dispersal model building in wave surge channels similar to those examined by Denny et al. (1992) will be examined to see how the topographic complexity of the coastline affects fertilisation success.

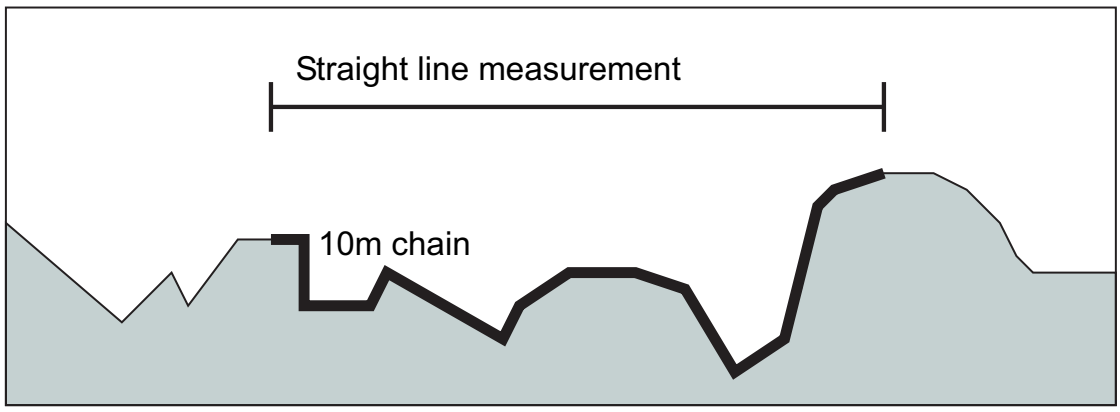

Figure 3.6: A schematic representation of the chain method for measuring large scale surface roughness. The technique is discussed in the text.

The number of gametes released into the water during a spawning event is determined by the number of spawning limpets. In this model the number of gametes released into the surf zone will depend on the number of limpets in a meter wide strip of shore. The density of limpets per m^2 on Warren Beach is examined in chapter 5. However as a result of large scale roughness the surface area of available habitat is greater than just the planar surface area of an inclined slope. To allow for surface roughness, the planar surface area of the benthos in the surf zone was multiplied by a roughness factor. The roughness factor was calculated by draping a 10m chain in a line across the rock surface 20 times starting from 20 different haphazardly chosen locations, and measuring in haphazardly selected directions (Figure 3.6). On each occasion the straight line distance covered by the chain was measured with a tape measure and the roughness factor was calculated according to Luckhurst and Luckhurst (1978) using the relationship;

$$roughness\,factor = chain\,length / straight\,line\,length \tag{3.7}$$

The average roughness factor from Warren Beach was 1.6. The surface area of rock available to limpets was calculated by multiplying the planar surface area of the benthos by the roughness factor^2. The roughness factor was squared to allow for the combined effect of roughness in the x and y planes.

The physical model of the surf zone and dispersal within the surf zone was used in conjunction with the fertilisation and population fecundity sub-models to construct a zygote production model (Chapter 6). The full model paramatisation and model runs are presented in chapter 6.

Chapter 4

Fertilisation Kinetics

4.1 Introduction

In the last 20 years an increasing amount of work has been done on fertilisation kinetics, and the proportion of eggs fertilised has been shown to be effected by the following:

- sperm concentration (Andre and Lindegarth, 1995; Babcock and Keesing, 1999; Baker and Tyler, 2001; Encena et al., 1998; Levitan, 1991; Levitan and Young, 1995; Marshall et al., 2000; Pennington, 1985; Powell et al., 2001; Styan and Butler, 2000; Vogel et al., 1982; Williams et al., 1997),
- gamete age (Andre and Lindegarth, 1995; Babcock and Keesing, 1999; Denny and Shibata, 1989; Encena et al., 1998; Levitan et al., 1991; Powell et al., 2001; Styan and Butler, 2000; Vogel et al., 1982),
- gamete ratios (Baker and Tyler, 2001; Vogel et al., 1982),
- contact time between gametes (Babcock and Keesing, 1999; Baker and Tyler, 2001; Levitan et al., 1991),
- distance between spawning individuals (Babcock and Keesing, 1999; Babcock et al., 1994; Levitan, 1991; Levitan et al., 1991; Levitan and Young, 1995; Metaxas et al., 2002; Pennington, 1985),
- spawning behaviour (Levitan et al., 1992; Marshall et al., 2004; Pennington,

1985),

- gamete characteristics (Levitan et al., 1992; Marshall et al., 2000; Styan and Butler, 2000), and
- hydrographic effects (Denny et al., 1992, 2002; Levitan, 1991; Levitan et al., 1992; Marshall et al., 2004; Mead and Denny, 1995; Metaxas et al., 2002; Pennington, 1985; Serrao et al., 1996).

Levitan et al. (1991) concluded that sperm concentration had the greatest effect on fertilisation success followed by contact time and gamete age respectively. Sperm-egg ratio has no effect on fertilisation success apart from the extreme case where sperm and eggs occur in similar concentrations (Levitan et al., 1991; Vogel et al., 1982).

Vogel et al. (1982) developed a fertilisation model (hereafter called the VCCW model) based upon bimolecular reaction rates with the assumptions that sperm follow a random search pattern and that the fertilisation rate is directly proportional to sperm concentration. The proportion of eggs fertilised after infinite exposure time is given by:

$$\Phi = 1 - e^{-\beta S_0 \tau} \tag{4.1}$$

where β is the bimolecular rate constant (see below), S_0 is the starting sperm concentration, and τ is the active half life of sperm. For cases where the exposure time is much less than the sperm active half life τ can be replaced by the exposure time t, to give the relationship:

$$\Phi = 1 - e^{-\beta S_0 t} \tag{4.2}$$

Vogel et al. (1982) initially proposed that the bimolecular rate constant β was a factor of sperm swimming velocity and the fertilisable cross sectional area of an egg. Denny and Shibata (1989) noted that in turbulent flows the sperm swimming velocity would be small compared to the eddy shear velocity and proposed that for turbulent flows the sperm swimming velocity should be replaced by the shear velocity. In reality the parameters for the bimolecular rate constant are hard to empirically determine. The rate constant β is either fitted to empirical data (Babcock and Keesing, 1999; Levitan et al., 1991; Levitan and Young, 1995), or the fitted ratio of egg cross sectional area to egg fertilisable cross sectional area has been used in

conjunction with empirical measures of egg diameter and sperm swimming speed or shear velocity(Babcock et al., 1994; Claereboudt, 1999; Metaxas et al., 2002; Styan and Butler, 2000).

4.1.1 Aims

The aims of this chapter are to assess the effects of sperm concentration, sperm age, egg age, and contact time on the fertilisation success of *P. vulgata* in order to fit the VCCW model for incorporation into a coupled population larval production model. Although the coupled population larval production model is being developed for *P. vulgata*, in order to keep the work applicable to the Azorean *P. aspera* populations, the sperm concentration experiments were repeated with *P. aspera* in the Azores.

4.2 Materials and Methods

Patella vulgata

The limpets were collected from Lynmouth, north Devon, in November 2003. Only limpets displaying stage IV or V gonads (Orton et al., 1956) were used. For all the fertilisation work the seawater used had been collected from adjacent to the site where the limpets were collected and filtered through a 0.2 μm mesh prior to use. After filtration the water was stored, for a maximum period of a week, at 5°c in a darkened container prior to use. All fertilisation work and subsequent larval incubations were conducted at 12°c ($\pm$ 1°c), the ambient temperature at which *P. vulgata* spawns (Bowman, 1985, Chapter 3).

For all the fertilisation experiments the gametes were prepared in a similar manner to make standard sperm and egg stock suspensions prior to use. The method of gamete preparation is described below, subsequent references to sperm or egg stock suspensions refer to gametes prepared as described. For each of the experiments three independent replicate runs were conducted using stock suspensions prepared

from different limpets for each run.

4.2.1 Preparation of sperm stock suspensions

Unless stated otherwise three limpets were used on each occasion to make up the stock suspension. Three mature male gonads were dissected out whole and placed in an evaporating dish with 100 ml of seawater, the gonads cut up into small chunks and the gonad material agitated in the water to release the sperm. The sperm suspension was passed through a 250 μm mesh to remove the large chunks of gonad material. The suspension was then allowed to stand for approximately 20 minutes, as previous work indicated that strip-spawned sperm needed to be exposed to seawater for a brief period to become activated (A. Hodgson pers comm.). The sperm stock suspension was then ready for use, and was always used within an hour of preparation.

4.2.2 Preparation of egg stock suspensions

Unless stated otherwise three limpets were used on each occasion to make up the stock suspension. Three mature female ovaries were dissected out whole and placed in a evaporating dish with 100 ml of seawater. The gonad was gently pulled apart and gently agitated in the water to release the eggs. The suspension was then passed through a 250 μm mesh to remove the large chunks of gonad material. Previous work (Aquino-Souza pers comm.) indicated that strip-spawned *Patella* eggs need to be pre-matured in alkalinised seawater in order to achieve high rates of fertilisations. Therefore after passing through the mesh filter the eggs were transferred to 100ml of seawater made up to pH 9 using NH_4OH for 20 minutes. Following alkalinisation the eggs were rinsed, and then ready for use. All eggs were used within 1 hour of alkalinisation.

4.2.3 Experiment 1: sperm concentration

Sperm and egg stock suspensions were prepared. Using the sperm stock suspension as the most concentrated suspension a series of six 10-fold dilutions were prepared using 90 ml of sperm suspension in a glass evaporating dish. As a control, a final

evaporating dish was prepared containing 90 ml of seawater to check for sperm contamination or parthenogenesis. The sperm concentration of the suspensions was calculated by making three replicate counts of the first 10-fold dilution using a haemocytometer and multiplying according to calculate the concentration of the other dilutions.

Once the sperm suspensions were prepared, approximately 2000 eggs were gently pipetted into each evaporating dish and the bowls were continuously gently stirred to keep the eggs in suspension for three hours whilst fertilisations were allowed to occur. After 3 hours the eggs were gently rinsed and transferred to clean seawater for 24 hours to allow development to occur before counts of fertilisations were made. During the 24 hour incubation period the dishes were continuously gently stirred to keep the eggs, and developing larvae, in suspension. After the 24 hour incubation a few drops of formalin were added to preserve the sample. Once the eggs and larvae had settled the supernatant was poured off and the sample stored prior to counting.

After 24 hours fertilised eggs had reached the trochophore stage; eggs that had been fertilised could be easily visually distinguished from those that had not. Visual counts of the numbers of fertilised and unfertilised eggs were made using a dissecting microscope. The sample was agitated to haphazardly distribute the eggs and larvae and a 1ml aliquot was pipetted into a Sedgewick-Rafter cell for the counts. The number of unfertilised eggs and larvae were noted until a combined total of at least 500 had been counted. To avoid subconscious bias the counts were made blind. From this the fertilisation percentage of each sample could be calculated.

4.2.4 Experiment 2: gamete contact time

Sperm and egg stock suspensions were prepared. The concentration of the sperm stock suspension was determined by three replicate counts with a haemocytometer, then using the appropriate dilution 100ml of 10^6 sperm ml^{-1} sperm suspension was made up. About 20 000 eggs were added to the prepared sperm suspension and quickly stirred, then the gamete mixture was left being continuously gently stirred. After set time periods (1, 5, 30, 90, 180, and 360 min) about 2000 eggs were removed

from the gamete mixture, rinsed twice, and transferred to a evaporating dish of 100ml of clean seawater, then left to incubate for 24 hours to allow the fertilised eggs to develop.

After the 24 hour incubation period the samples were treated, and counts made, as described above for the sperm concentration experiment.

4.2.5 Experiment 3: sperm age

Sperm and egg stock suspensions were prepared. The concentration of the sperm stock suspension was determined by three replicate counts with a haemocytometer. Seven evaporating dishes were then prepared containing 100ml of 10^6 sperm ml^{-1} sperm suspension. The evaporating dishes were continuously gently stirred. Approximately 2000 eggs were added to the first sperm suspension, then at set time intervals (0, 3, 6, 12, 18, 24, 48, and 72 hours) a fresh egg stock solution was prepared and 2000 eggs added to one of the aged sperm suspensions.

Three hours after the fresh eggs had been added to the aged sperm suspension the eggs were removed, rinsed, and placed in fresh clean seawater for the larvae to develop for 24 hours. After 24 hours the samples were treated, and counts made, as described above.

4.2.6 Experiment 4: egg age

A pooled egg stock suspension was made using five female limpets and continuously gently stirred to keep the eggs in suspension. A pooled stock sperm solution was made using 3 limpets and the sperm concentration determined as described above. An evaporating dish was then prepared containing 100ml of 10^6 sperm ml^{-1} sperm suspension; to this approximately 2000 eggs from the egg stock were added. At set intervals (0, 3, 6, 12, 18, 24, 48, and 72 hours) fresh sperm stock suspensions were made up in an evaporating dish containing 100ml of 10^6 sperm ml^{-1} sperm suspension to which 2000 of the aged eggs were added.

Three hours after the aged eggs had been added to the fresh sperm suspension the eggs were removed, rinsed, and placed in fresh clean seawater for the larvae to

develop for 24 hours. After 24 hours the samples were treated, and counts made, as described above.

Patella aspera

The fertilisation work on *P. aspera* in the Azores was conducted at the DOP, Horta, Faial during March 2004. Although previous workers (Martins et al., 1987) have found *P. aspera* with fully mature gonads in March, no individuals with fully mature gonads were found during the two weeks available for the fertilisation work in 2004. Despite the lack of limpets with mature gonads sperm concentration experiments were attempted.

The limpets used had stage III/IV gonads. The seawater was held at 17°c, the ambient temperature at the time of collection. Otherwise the method was identical to that used for *P. vulgata*. The sperm concentration experiment was repeated 7 times, but as only low levels of fertilisations were achieved further contact time or gamete age experiments were not attempted.

4.3 Results

The complete raw data for the results of the *P. vulgata* and *P. aspera* fertilisation experiments are given in Appendix 2.

Patella vulgata

4.3.1 Sperm concentration

Great variability in fertilisation success was shown over the range of sperm concentrations tested (Figure 4.1). At sperm concentrations of 10^3 ml^{-1} fertilisation success was less than 15%. Above 10^5 ml^{-1} fertilisation success had risen to over 90% of the maximum that was achieved for each replicate. There was little increase in fertilisations over sperm concentrations of 10^6 sperm ml^{-1}. Low levels of

fertilisation ($\approx$ 1%) were found at low sperm concentrations of $\approx 10^2$ sperm ml^{-1}. Maximum fertilisation success under optimal sperm concentration varied between sets of gametes.

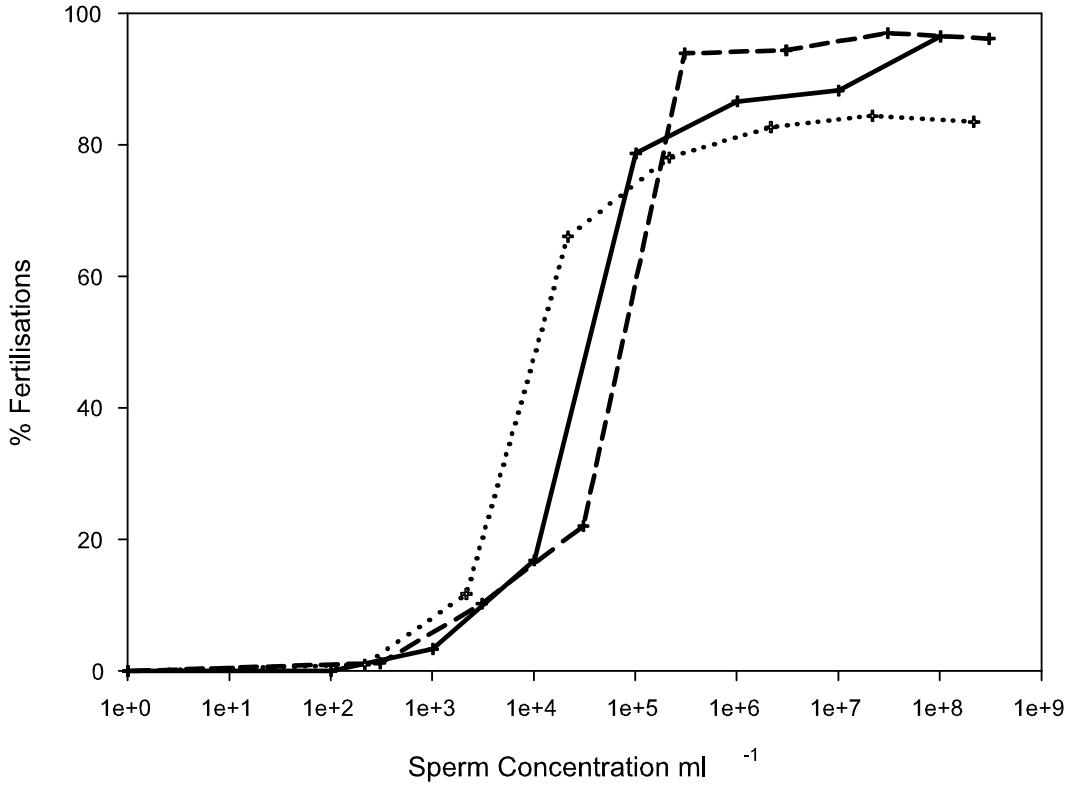

Figure 4.1: Percent fertilisations as a function of sperm concentration for *P. vulgata*. The lines indicate the three independent replicates of serial ten-fold dilutions.

4.3.2 Gamete contact time

The proportion of fertilisations increased with increasing gamete contact time until the gametes had been in contact for 90 minutes (Figure 4.2). Large variation in percentage fertilisations (16%, 58% and 72% fertilisations) in the first minute was shown between the three runs. It took over 10 minutes for the majority of fertilisations to occur.

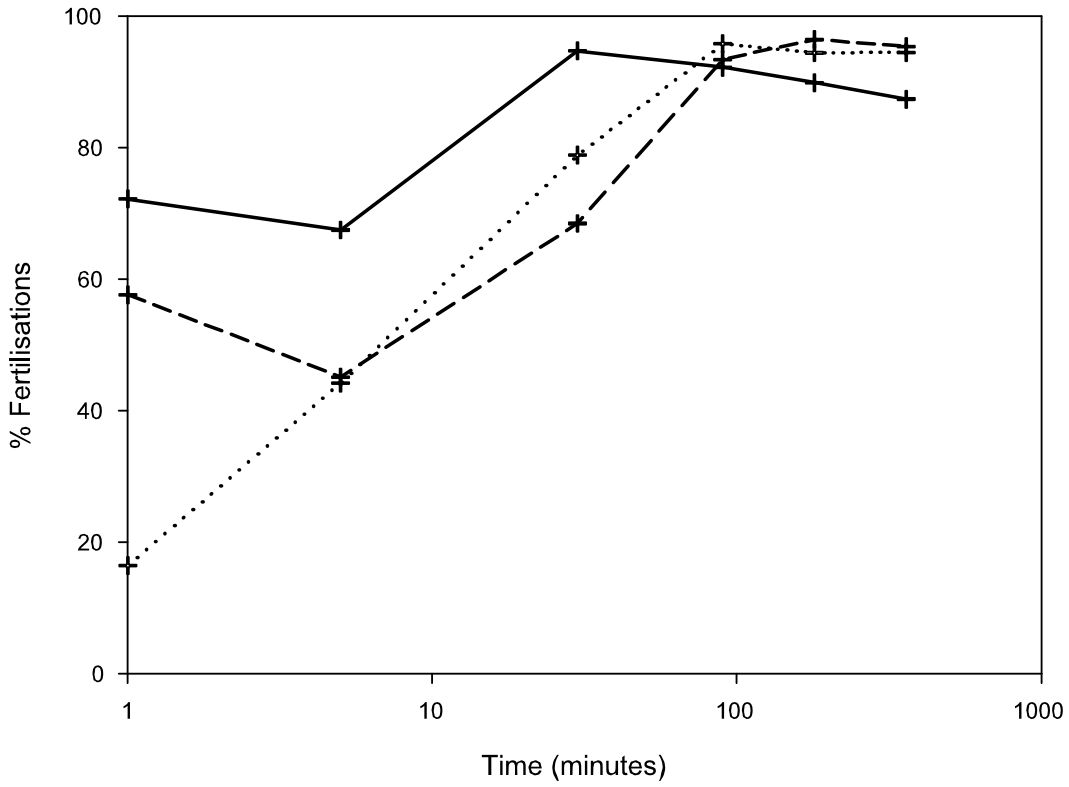

Figure 4.2: Percent fertilisations as a function of gamete contact time for *P. vulgata*. The lines indicate the three independent replicates.

4.3.3 Gamete age

The sperm age experiment showed that high levels of fertilisation still occurred after six hours (Figure 4.3). Fertilisations had dropped to low levels ($< 10\%$) by 12 hrs, but very low levels of fertilisations still occurred after 24 hrs. There were no fertilisations by the time sperm was 48 hrs old.

Unfortunately there were no data collected for the egg age experiment after 12 hrs as the later samples were not correctly preserved; however it was noted during the running of the experiment that by 72 hrs the eggs had disintegrated thus no fertilisations would be expected. By 12 hrs egg viability had started decreasing although it was still over 50% (Figure 4.3).

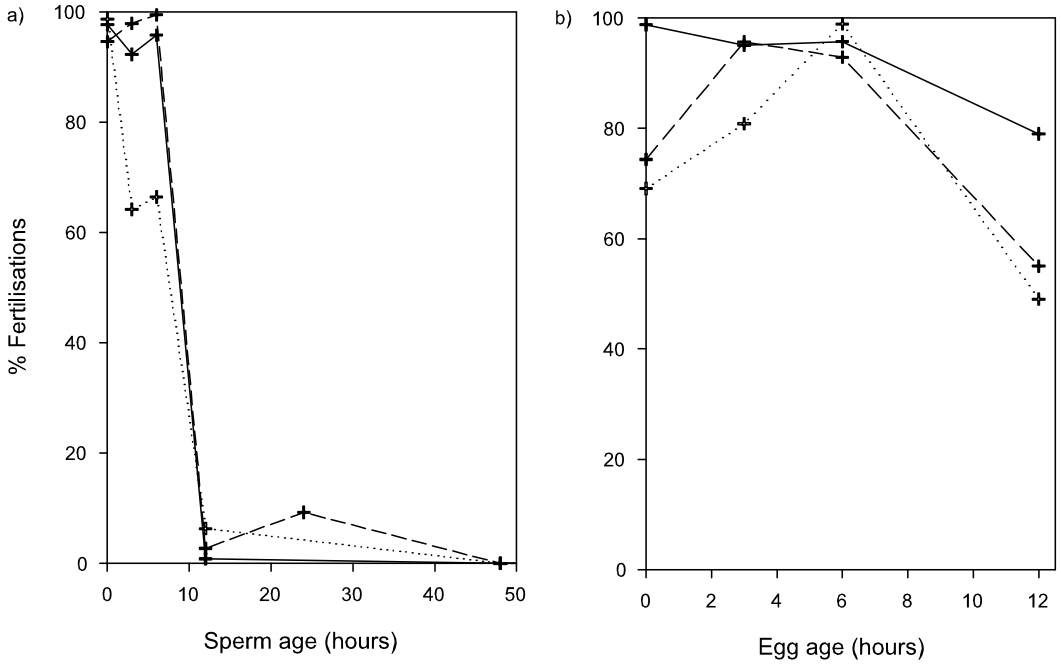

Figure 4.3: Percent fertilisations as a function of a) sperm age, and b) egg age for *P. vulgata*. The lines indicate the three independent replicates.

Patella aspera

None of the sperm concentration experiments achieved a maximum fertilisation success of over 40% (Figure 4.4) and the replicates show great variability between the maximum rates of fertilisations achieved. The low levels of fertilisations achieved is considered to be due to the immature state of the gonads used for the experiments, whilst the variation in maximum level of fertilisation is indicative of variation in the proportion of eggs viable in each run. If the results are examined as the fertilisation success proportional to the maximum fertilisation success achieved in each replicate, it can be seen that the sperm concentration at which maximum fertilisation is achieved varies over almost three orders of magnitude (Figure 4.5). This indicates variation in the sperm viability as well as the egg viability.

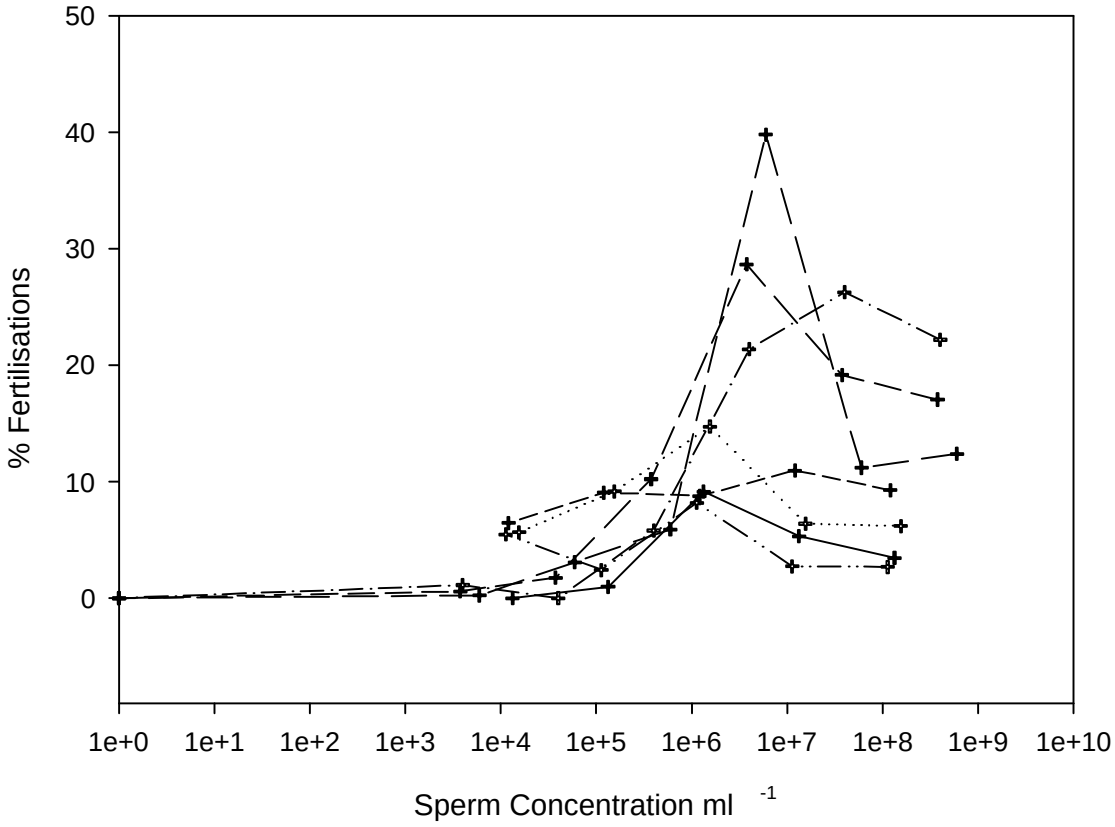

Figure 4.4: Percent fertilisations as a function of sperm concentration for *P. aspera*. The lines indicate the independent replicates of serial ten-fold dilutions.

4.4 Fitting the VCCW Model

The VCCW model (Equation 4.3) can be used to predict the proportion of eggs fertilised if eggs are exposed to a known concentration of sperm for a known time. One of the implications of equation 4.2 is that under high sperm concentrations all the eggs will be fertilised; however no empirical studies of fertilisation kinetics have found 100% fertilisations occurring on a consistent basis even under optimum conditions (e.g. Babcock and Keesing, 1999; Baker and Tyler, 2001; Claereboudt, 1999; Encena et al., 1998; Levitan et al., 1991, 1992; Levitan and Young, 1995; Metaxas et al., 2002; Styan and Butler, 2000). Therefore a modification of the original model is proposed to account for less than 100% maximum fertilisation success:

$$\Phi = \alpha(1 - e^{-\beta S_0 t}) \tag{4.3}$$

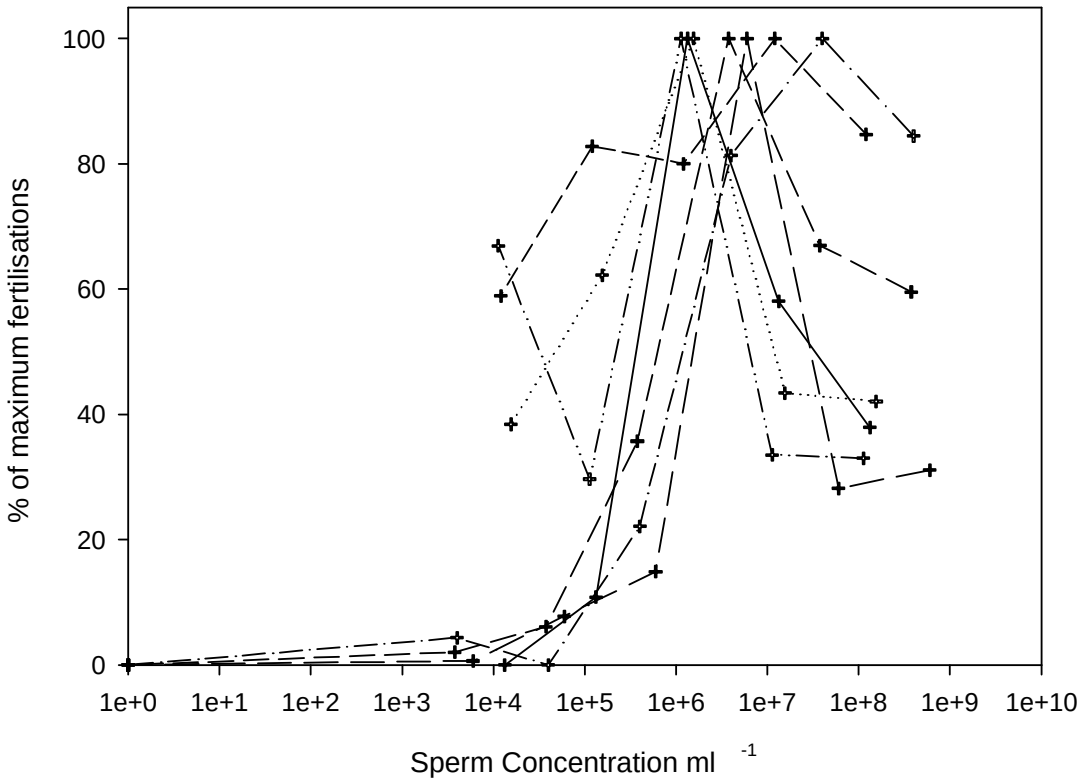

Figure 4.5: Percent fertilisations as a proportion of the maximum number of fertilisations achieved per replicate, as a function of sperm concentration for *P. aspera*. The lines indicate the independent replicates of serial ten-fold dilutions.

where the parameter α is introduced to control the level at which the fertilisation success plateaus due to egg viability.

The α and β parameters of the modified VCCW model (equation 4.3) were fitted to the empirical data using a Matlab routine to iteratively minimise the sum of squares of the residuals (Figure 4.6). Previous authors have fitted β by minimising the sum of squares against the sperm concentration relationship (Babcock and Keesing, 1999; Levitan et al., 1991; Levitan and Young, 1995); this approach overestimates fertilisations at short contact times. Therefore β and α were simultaneously fitted to the sperm concentration and contact time relationships (Figure 4.6). The Matlab routine used to find the optimal values of α and β is given in Appendix 3. The optimal values determined by the fitting routine are $\alpha = 0.880$ and $\beta = 2.0\ \mathrm{e}^{-9}$.

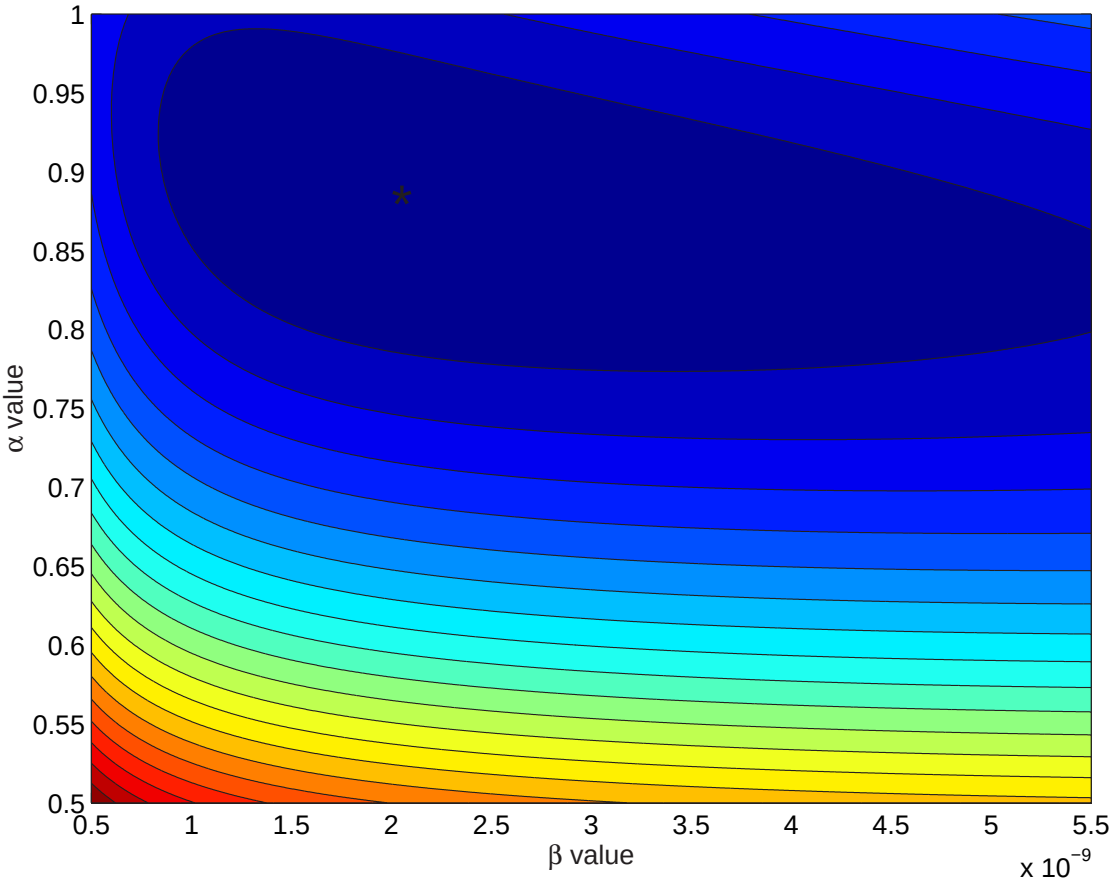

Figure 4.6: Sum of squares difference for a covarying range of parameter values. The star marks the combination of parameter values that minimises the sum of squares. Sum of squares minimised when α=0.88 and β=2 x 10^{-9}.

4.5 Discussion

Whilst evaluating the level of fertilisation success no attempt was made to differentiate between normal fertilisations and abnormal fertilisations that can occur as a result of polyspermy, although this has been shown to occur at high sperm concentrations in previous sperm concentration experiments (Baker and Tyler, 2001; Powell et al., 2001). Results from the combined fecundity-dispersal model presented in later chapters indicates that sufficiently high levels of sperm concentration to cause polyspermy does not commonly occur in the field. Therefore it is assumed that failure to account for polyspermy will not compromise the predictive ability of the fertilisation model presented above.

4.5.1 Sperm concentration

The relationship between fertilisation success and sperm concentration for *P. vulgata* corresponds with the general trends shown by many other marine benthic broadcast spawners (e.g. Babcock and Keesing, 1999; Baker and Tyler, 2001; Levitan et al., 1991; Pennington, 1985). There is only one other reported study of the fertilisation success of a patellid limpet, *Nacella concinna*, under variable sperm concentrations which found that 80% fertilisation occurred under sperm concentrations of 10^5-10^6 sperm ml^{-1} (Powell et al., 2001), an order of magnitude higher that the results presented for *P. vulgata*. *N. concinna* is an Antarctic species, and Powell et al. (2001) examining the fertilisation kinetics of *N. concinna*, and an Antarctic clam found that in both cases the optimal sperm concentration required for maximum fertilisation was one or two orders of magnitude higher than required by many temperate or tropical species (Powell et al., 2001, and refs within).

Variability in fertilisation success under optimal sperm concentration between different sets of gametes has been shown in previous studies (Baker and Tyler, 2001; Pennington, 1985; Powell et al., 2001) and has been attributed to variable egg viability (Pennington, 1985). As with this study, it has been previously noted that, although maximum fertilisation success varies, the response of fertilisation success - as a proportion of maximum fertilisations- to sperm concentration is similar between sets of gametes (Powell et al., 2001).

4.5.2 Gamete contact time

As with this study, previous work on abalone has also shown large variability for short contact times with the variability in fertilisation success decreasing as contact time increases (Babcock and Keesing, 1999; Baker and Tyler, 2001). This may be an experimental artifact as it is very hard to control the exact contact time for short contact times, although it may also be a correct observation reflecting the random and haphazard distribution of turbulence during the initial mixing of the gametes as the gametes may not become truly fully mixed in the space of a minute.

Work on abalone (Babcock and Keesing, 1999; Baker and Tyler, 2001) and sea

urchins (Levitan et al., 1991) found the majority of fertilisations occur within the first 5 minutes, or even quicker. In contrast this study found it took over 10 minutes for the majority of fertilisations to occur for *P. vulgata*. The importance of rapid fertilisation of gametes is related to the rate at which sperm are dispersed after release. Observations of *P. vulgata* spawning (Bowman, 1985; Orton et al., 1956, pers obs) showed that spawning occurred in periods of rough weather, this suggests that sperm would be rapidly dispersed due to the effects of wave action in the very nearshore. Therefore it is initially surprising that *P. vulgata* requires a longer contact time to achieve high levels of fertilisation than animals that spawn into less turbulent conditions.

4.5.3 Gamete age

Although the samples for fertilisation success of aged eggs for over 12 hours were lost the results that were obtained indicate that egg longevity is far greater than sperm longevity; this is in agreement with the results of previous studies (Andre and Lindegarth, 1995; Pennington, 1985). Assuming that eggs and sperm are simultaneously released and equally dispersed sperm aging will be the limiting factor rather than egg age.

Previous studies have found that sperm longevity decreases with decreasing sperm concentration (Babcock and Keesing, 1999; Baker, 2001; Baker and Tyler, 2001; Levitan et al., 1991; Styan and Butler, 2000), which the authors have attributed to the respiratory dilution effect (see review by Chia and Bickell, 1983). It seems unlikely that naturally spawned sperm in the field would remain for long at the concentrations used in this study (10^6 sperm ml^{-1}). However the rate at which sperm half life drops with reduced sperm concentrations suggests that whilst sperm is still at high enough concentrations to cause significant fertilisations the sperm half life will still be of the order of 10s of minutes (Levitan et al., 1991; Styan and Butler, 2000). In addition, the dispersal model (Chapter 3) predicts that gametes will have been dispersed beyond the point of achieving fertilisations within 10s of minutes.

On the basis of the observations it is concluded that gamete ageing is unlikely to

effect fertilisation success in the natural environment as gametes will be dispersed beyond the levels required to achieve fertilisations before gamete age becomes a limiting factor.

4.5.4 Applying the modified VCCW model

The sperm concentration and gamete contact time experiments show that the proportion of eggs fertilised is dependent on the sperm concentration and gamete exposure time. From this it can be concluded that if the population sperm output is reduced due to exploitation there may be a decline in the proportion of eggs fertilised; however the study of fertilisation kinetics in itself, without reference to gamete release and dispersal, does not allow for quantitative predictions and provides little information of direct ecological significance.

This can be overcome by incorporating the VCCW fertilisation model into a combined fecundity-dispersal-fertilisation model. Before the VCCW model is incorporated into a combined model it should be considered to what extent fertilisation kinetics observed in the laboratory can be assumed to represent fertilisation kinetics in the field. The experimental set up was controlled for water quality and temperature, but the main aspect of the experimental conditions that failed to mimic field conditions is the extent of turbulence, especially as *P. vulgata* spawns under highly turbulent conditions (see Chapter 3).

In a theoretical study of the effects of turbulence on plankton feeding rates Rothschild and Osborn (1988) concluded that turbulence increases encounter rates as the relative velocity of small particles in the water increases with the effect of increasing the effective searched volume. More recent modelling of plankton feeding has shown that variation in the levels of turbulence over the range of values typically found in the oceans can increase plankton prey encounter rates by over an order of magnitude (Lewis and Pedley, 2001). These studies indicate that the laboratory study of fertilisation kinetics may significantly underestimate the extent of fertilisations at low sperm concentrations and at short contact times. Contrary to the theoretical observations above Denny et al. (1992) suggested that turbulence and the associated shear stress might hinder or disrupt sperm egg contacts and subsequent successful

fertilisation.

Mead and Denny (1995) [but see Denny et al. (2002) for corrected calculations] conducted the only published empirical study of the effects of turbulence on fertilisation success with the urchin *Strongylocentrotus purpuratus*. The energy dissipation rate ϵ acts as a measure of turbulence and typically ranges from 10 Wm^{-3} in the open ocean to 3000 Wm^{-3} on the wave swept rocky shore (Mead and Denny, 1995, and refs within). Mead and Denny (1995) carried out fertilisations over the full range of natural turbulent intensities and found that the proportion of eggs fertilised increased until ϵ >1000 Wm^{-3} and the level of fertilisations remained above the 'still' water level until ϵ exceeded $\approx 2000 Wm^{-3}$. Mead and Denny (1995) used sperm concentrations that would have caused over 80% in 'still' water incubations therefore it is not known how much turbulence would increase fertilisations in sperm limited conditions.

The theoretical and empirical work on the effects of turbulence on fertilisation kinetics (Denny et al., 2002; Mead and Denny, 1995) and plankton encounter rates (Lewis and Pedley, 2001; Rothschild and Osborn, 1988) suggests that laboratory based fertilisation kinetic studies may underestimate the level of fertilisations by failing to fully account for the effects of turbulence. The role of turbulence on fertilisation success is one of the main areas in need of research in developing predictive models of how exploitation will effect larval production of broadcast spawners.

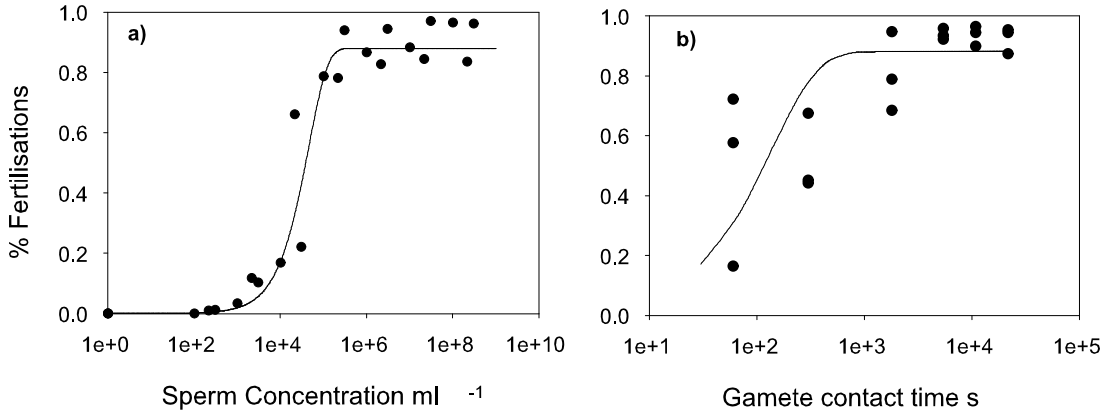

Figure 4.7: The modified VCCW model, showing the fit against a) sperm concentration and b) contact time. The points on the graphs are the data from the fertilisation experiments.

Chapter 5

Protandry, Population Fecundity and Spawning Stock Biomass

5.1 Introduction

The sex specific gamete production of a population can be calculated if the sex specific population length frequency and sex specific fecundity-length relationships are known. Similarly the population biomass, spawning stock biomass and sex specific spawning stock biomass can be calculated by combining the sex specific population length frequencies and a length-weight relationship. These simple population models can then be exposed to a simple simulated fishery to see the relationship between population fecundity, yield and SSB.

I shall begin by reviewing what is know of patellid limpet size fecundity relationships before developing a simple model of how population fecundity is affected by a simulated fishery for example populations of *P. vulgata*, and how the relationships between E, Y and SSB are influenced by protandry.

5.1.1 Limpet size-fecundity relationships

The only published length-fecundity relationship for female *P. vulgata* is from Cullercoats, Tyne and Wear, N.E. England. Garwood (1987) stated that it was an expo-

nential relationship calculated as;

$$Log\ fecundity\ =\ 0.006\ \times\ shell\,length\,(mm)\ +\ 2.93 \tag{5.1}$$

but re-analysis suggests that the stated relationship was misprinted as the data presented shows a far closer relationship with the formula;

$$Log\ fecundity\ =\ 0.06\ \times\ shell\,length\,(mm)\ +\ 2.93 \tag{5.2}$$

This indicates that there is not a direct allometric relationship between size and potential fecundity for *P. vulgata*. Baxter (1983) found that gametes accounted for a greater proportion of soft body weight with increasing size, similarly Wright and Hartnoll (1981) found that the mature gonad of a 3 yr old male made up a maximum of 19% of soft body dry mass compared to 40% for 'older males' (sic). Wright and Hartnoll (1981) give no size specific data for females. Workman (1983) found that reproductive effort as a proportion of net production increased from 11-15% at first maturity to 65-95% for individuals of 10 years or older. This supports the assertion of Garwood (1987) that fecundity increases more rapidly than a proportionate increase in weight.

Studies of other limpet species have found a variety of relationships that best explain the relationship between size and fecundity. Kido and Murray (2003) found that an exponential relationship best explained the relationship between gonad mass and shell length for *Lottia gigantea*. A study of four Australian limpet species concluded that there was a linear relationship between gonad mass and body mass (Parry, 1982). Similarly Creese (1980b) studying one of these species and three different limpet species also found that a linear relationship provided the best fit with the data. However the author noted that relative fecundity increased with shell length suggesting a non-linear relationship. A study of five South African species concluded that there was a linear relationship between gonad mass and whole organism mass, but that the relationship did not pass through the origin (Branch, 1974).

5.1.2 Aims

In this chapter I examine the population fecundity of example limpet populations and apply a simple fishery model to the populations. This model was used to

calculate the effects of simulated fishery pressure on population sperm and egg production. The output of this model will be combined with the gamete dispersal and fertilisation models developed in chapters 3 and 4 to create a combined fecundity-dispersal-fertilisation model of the effect of exploitation on larval production. The combined model will be developed and discussed in chapter 6.

The population fecundity model developed in this chapter allows analysis of the effect of exploitation on egg production, and allows for comparison of reproductive output and yield between habitats at different levels of wave exposure. The model was used to discuss the following questions;

i) What is the effect of protandry on the relationship between gametic output, SSB and fishery yield?

ii) Is SSB a good proxy for population egg production for protandrous hermaphrodites?

iii) In order to conserve egg production whilst maximising yield, is the population best managed by minimum landing size or marine protected areas?

iv) What is the effect of wave exposure on reproductive output and yield per unit area, and how are the effects of exposure best accounted for in management decisions?

5.2 Methods

5.2.1 Population structure

The population structure of *P. vulgata* at three different levels of wave exposure was examined at Warren Beach, south Devon in October 2003. A cross-shore transect was haphazardly sited for each area of different wave exposure. For each transect 10 haphazardly placed 0.25m^2 quadrats were cleared of all limpets for each of the upper shore, middle shore and lower shore across the intertidal. Each limpet was identified to species level and the maximum shell length was measured to the nearest mm. In addition each *P. vulgata* was dissected and sexed (Orton et al., 1956).

5.2.2 Fecundity

The limpets used for the determination of potential fecundity were collected from Lynmouth, north Devon during November 2003. Unfortunately due to logistical limitations it was not possible to collect ripe individuals from the same site as the population structure. Once returned to the laboratory the limpets were dissected to determine sex and gonad condition. All individuals used were weighed (whole organism wet weight), and shell length measured, prior to dissection. Only limpets with stage V gonads (Orton et al., 1956) were used for the assessment of potential fecundity. All seawater used during the preparation of gonads for fecundity counts was prefiltered to 0.2 μm.

Males

The testis of each ripe male was dissected out whole and placed in an evaporating dish with 100 ml of seawater. The testis was cut into small chunks and agitated vigorously to release the sperm. The bowl was then left for approximately 12 hours at 12°c to allow sperm to swim out of the testis material. After 12 hours the material was agitated again to release sperm still within the chunks of gonad material, and the suspension was filtered through a 250 μm mesh to separate the testis material from the sperm suspension. 20 ml of seawater was used to rinse the evaporating dish and wash through sperm retained on the mesh filter. The sperm suspension was set aside. The gonad material was washed off the mesh filter back into the evaporating dish with 100 ml of seawater and the process was repeated to try and remove any sperm remaining in the testis material. The second wash was again left for 12 hours before being agitated and passed through a 250 μm mesh. Again 20 ml of seawater was used to rinse the evaporating bowl and mesh, and this was added to the sperm suspension collected from the first wash. This left 240 ml of sperm suspension to which 10 ml of 40% formalin was added to make the suspension up to 250 ml and the samples were stored prior to counting.

The sperm concentration in the sperm suspensions collected from each of the individuals was determined using a flow cytometer (Becton Dickinson FAC Sort). The total individual potential fecundity was then calculated by multiplying the concentration

by the total volume of sperm suspension.

Females

A 3 gl^{-1} solution of protease type XIV, pronase E from *Streptomyces griseus* (Sigma-Aldrich Co. Ltd.), was prepared with filtered seawater. The ovary of each ripe female was dissected out whole and placed in a 100 ml sample tube half filled with the pronase solution and left to stand. The protease solution breaks down the connective tissue in the gonad which holds the eggs together. After 12 hours the tubes were agitated and the gonad was visually examined to see if all the eggs had been disaggregated. If the gonad had not fully disaggregated the tube was left and subsequently examined every 2 hours until the gonad was fully disaggregated. Once the gonad was fully disaggregated, 40% formalin was added to make the egg suspension up to approximately 2% formalin to stop the protease reaction. The egg suspension was passed through a 250 μm mesh to remove the larger remaining residue of gonad material whilst allowing the eggs to pass through. After filtering the egg suspension was left to settle, the supernatant poured off and the eggs suspended in 2% formalin solution until counts could take place.

Prior to counting the egg suspensions were diluted in water to reduce the egg concentration to roughly 40 eggs ml^{-1} to facilitate counting. The suspension was stirred to randomly distribute the eggs throughout the suspension and 3 independent replicate 10 ml samples were taken and counts of the eggs made using a Bogoroff tray. The mean of these counts was calculated and the result multiplied up according to the dilution used, to give the total individual potential fecundity.

Data Analysis

To convert the length frequency data into biomass and fecundity, length-weight and length-fecundity relationships were fitted to the data. A standard power law relationship was fitted to the length-frequency data using the Sigmaplot non-linear regression package. As patellid limpets do not display strict allometric growth the relationship was allowed to deviate from an exact cubic relationship.

Two possible length-fecundity relationships, a power law relationship and exponential relationship, were considered on the basis of their biological plausibility. If the limpets followed a linear weight-fecundity relationship, the length-fecundity relationship would be expected to be a simple power law relationship of the same order as the power law of the length-weight relationship. The alternative biological explanation for length-weight relationship is that of an exponential relationship, or a power law relationship of higher order than a cubic relationship. This would model a length-fecundity relationship where not only did fecundity increase with weight, but there was a further increase in fecundity due to ontogenetic reallocation of energy resources. Both relationships were fitted to the data. It transpired that both relationships had similar R^2 values (c.f. section 5.3) therefore to test significance of the fits pairwise variance ratio F tests were conducted to compare the fitted relationships with the observed data and each other for the sperm and egg data separately. The nature of the length-fecundity relationships was further examined by calculating the gradient of the line fitted to a log-log length- female fecundity plot.

The population sex-specific length frequency data from all the quadrats for each transect was compiled and normalised per m^2 to give an average population structure per m^2 for each exposure level. The sex specific population frequency data was multiplied (on a length class by length class basis) by the length-weight relationship and the fecundity-length relationship, to calculate total biomass, SSB, sex-specific SSB and sex-specific potential fecundity per length class. The contribution of each length class was summed for each transect to give average biomass, sex-specific biomass and sex specific fecundity per m^2 for each wave exposure level.

Fishery Simulation

The effects of high levels of short-term size selective exploitation on the population were simulated by sequentially removing the largest size class from each population. The smaller size classes were removed as each one in turn becomes the largest size class once the larger individuals have been removed. Once each length class had been removed the remaining SSB, sex-specific spawning stock biomass, and egg and sperm production were calculated as absolute values and as a fraction of their original unexploited value. The fishery yield was calculated as the reduction in

biomass. Thus this model explores the once-off fishery yield that is generated when a previously unexploited stock is first exposed to fishery exploitation, rather than being a per annum steady state yield calculation. This is a significant limitation which is discussed further in chapter 8.

To compare the effect of different management schemes the fishery yield and reduction in egg production were calculated for the complete range of combinations of proportional coverage of marine protected areas (MPAs) and minimum landing sizes (MLSs). For the simulation it was assumed that for a given combination of % MPA and MLS no exploitation occurs in the fraction of the habitat protected by the MPAs and that all individuals above the MLS were removed from the fraction of the habitat not covered by MPAs.

The above analysis of management options was conducted on a habitat by habitat basis for each exposure level separately. To examine the effect of varying habitat quality on management strategies three different management regimes were considered. Firstly, treating the different exposure levels identically by pooling the populations and exploiting purely on the basis of size, by sequentially removing the largest size class. The two alternative strategies involve serially exploiting each exposure level one after the other. For the first simulation the sheltered site was exploited first, then the intermediate site and finally the exposed site. In the second simulation the order in which the sites were exploited was reversed. In each case each population was exploited by sequentially removing the largest size class. Once the first population has been completely fished out the fishery then moves on to exploit the population in the next habitat. For each of the three scenarios described above, as each length class was removed, the fishery yield and reduction in population egg production was calculated. The results were normalised across the whole population to calculate yield and egg production per m^2 across the whole population. In each case it was assumed that each exposure level makes up an equal fraction of the population's range.

5.3 Results

A total of 58 male and female limpets with stage V gonads were examined for fecundity. The length weight data (Figure 5.1) fitted the relationship:

$$weight(g) = 3.45 \times 10^{-5} * length(mm)^{3.44} - 0.05 \tag{5.3}$$

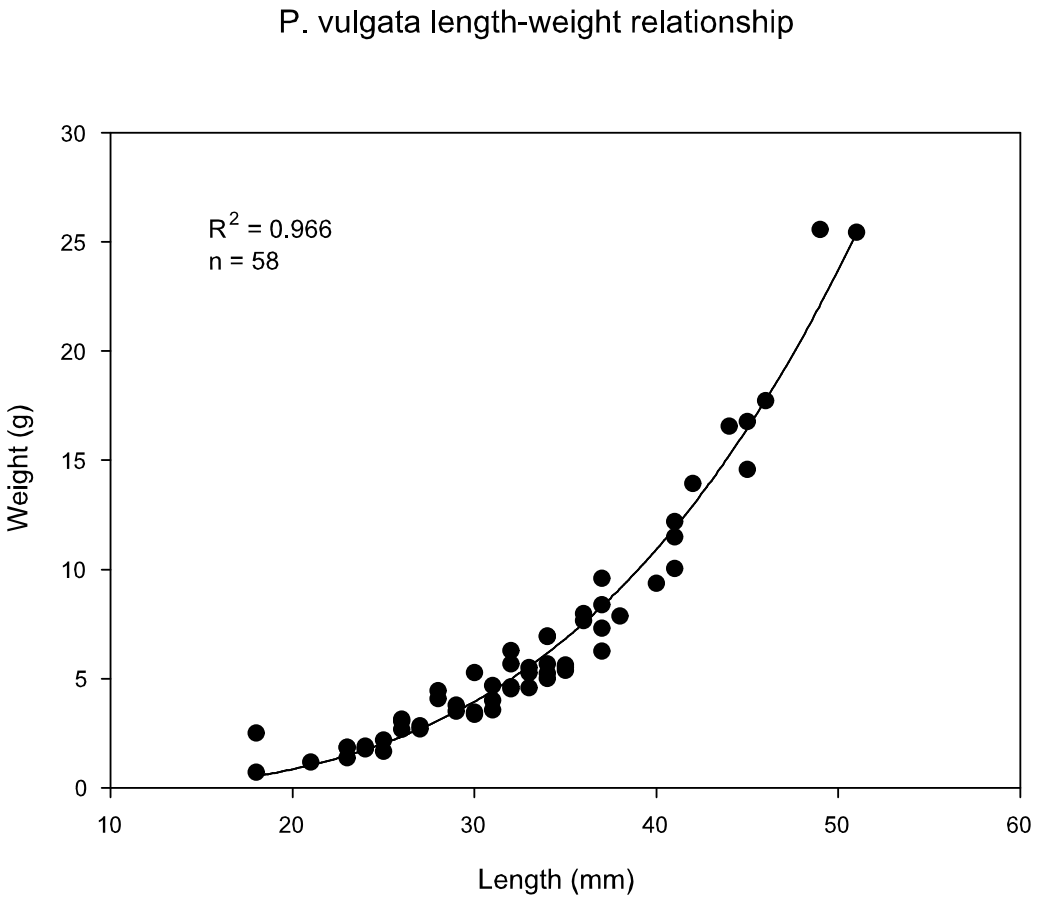

Figure 5.1: Length weight relationship for mature stage V *P. vulgata*. The formula of the regression line is given in the text.

Power law and exponential relationships were fitted to the length-egg production data (Figure 5.2a and Table 5.1). Neither fit could be rejected on the basis of variance ratio F tests ($p < 0.001$ for all pairwise tests of observed and fitted data). The relationship was further examined by calculating the gradient of the best fit straight line fitted to a log-log plot of length against fecundity (Figure 5.2b). The gradient of the line, 5.02, is larger than the power of the length weight relationship, 3.44. Although the fits can not be discriminated against on a purely statistical basis

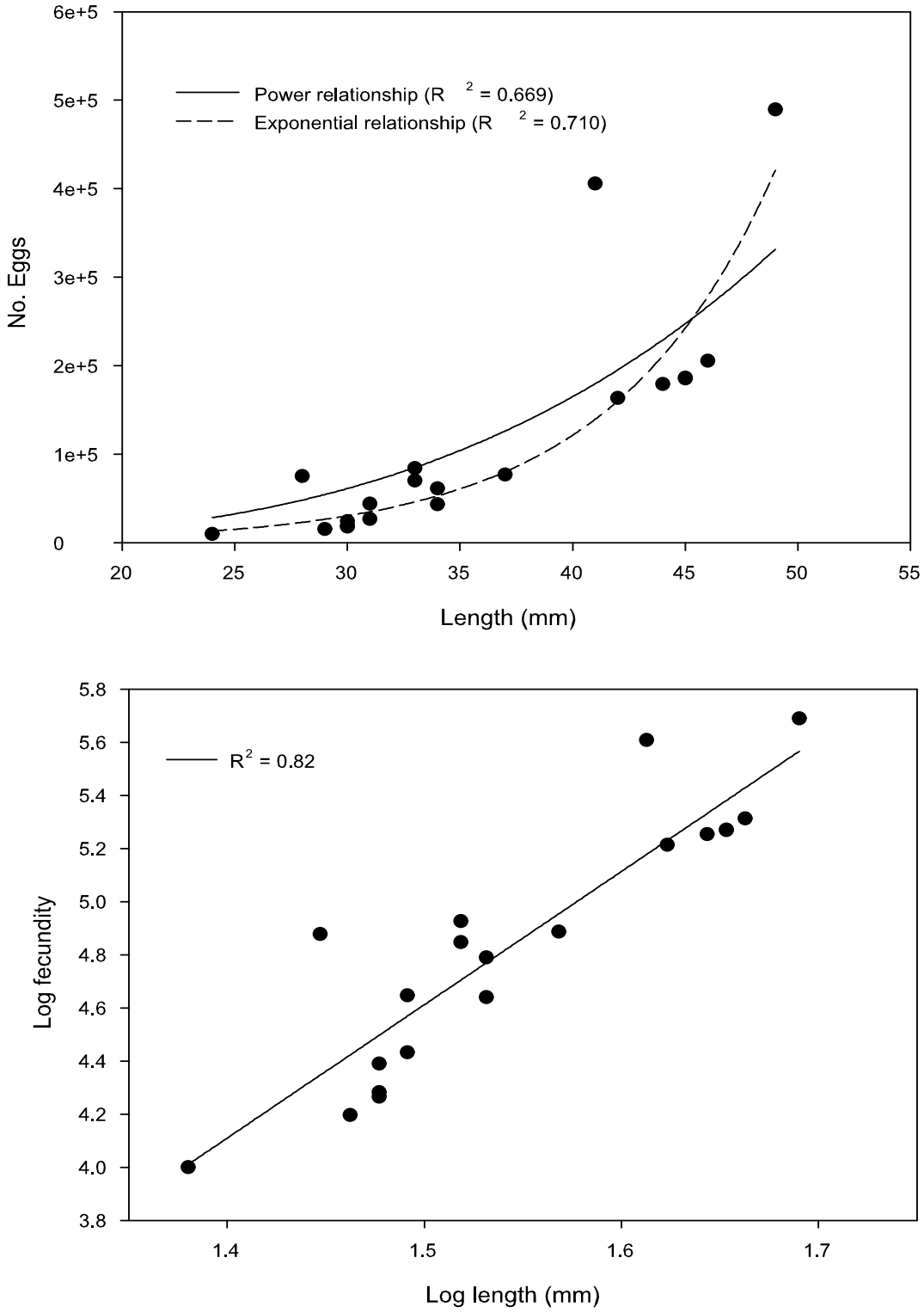

Figure 5.2: Length egg production relationship for mature stage V female *P. vulgata*. a) The formulae of the regression lines is given in the text, n= 18. b) log-log plot of length against egg production; log fecundity = 5.02*log length - 2.92. $R^2 = 0.82$

the exponential fit was considered to be more biologically appropriate and used because;

i) it has the higher R^2 value,

ii) the gradient of the log-log plot indicates a relationship of a higher power than a cubic relationship, and

iii) previous work indicates that there is an ontogenetic switch in allocation of energy reserves from somatic growth to reproduction (Baxter, 1983; Garwood, 1987; Wright and Hartnoll, 1981).

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Table 5.1: Results of fitting a power law and exponential relationships to the length-female fecundity data.

Relationship	Formula	R^2	F-test p value
Females			
Exponential	egg production = 487 * exp $^{(0.138 * length)}$	0.710	<0.001
Power	egg production = 0.511 * length $^{3.44}$	0.669	<0.001
Males			
Exponential	sperm production = 2.03 $\times 10^8$ * exp $^{(0.1117 * length)}$	0.784	<0.001

The length-sperm production data (Figure 5.3 and Table 5.1) was fitted to an exponential function as it was considered appropriate that the male and female length fecundity relationships should employ the same underlying relationship. The length-sperm production relationship is influence by a single sample of a highly fecund large male. The sample size needs to be increased to generate a more robust length-male fecundity relationship.

The population length frequency data shows a trend with respect to the exposure gradient, with more smaller individuals occurring in the more exposed sites (Figure 5.4). The full population frequency data and table of subsequent biomass and fecundity calculations is presented in Appendix 4. The most sheltered site had the longest tail to the population structure, with more larger individuals than the other sites. Transect C, the most exposed, site has the greatest proportion of the population concentrated in the smallest size classes. 87% of the individuals in transect

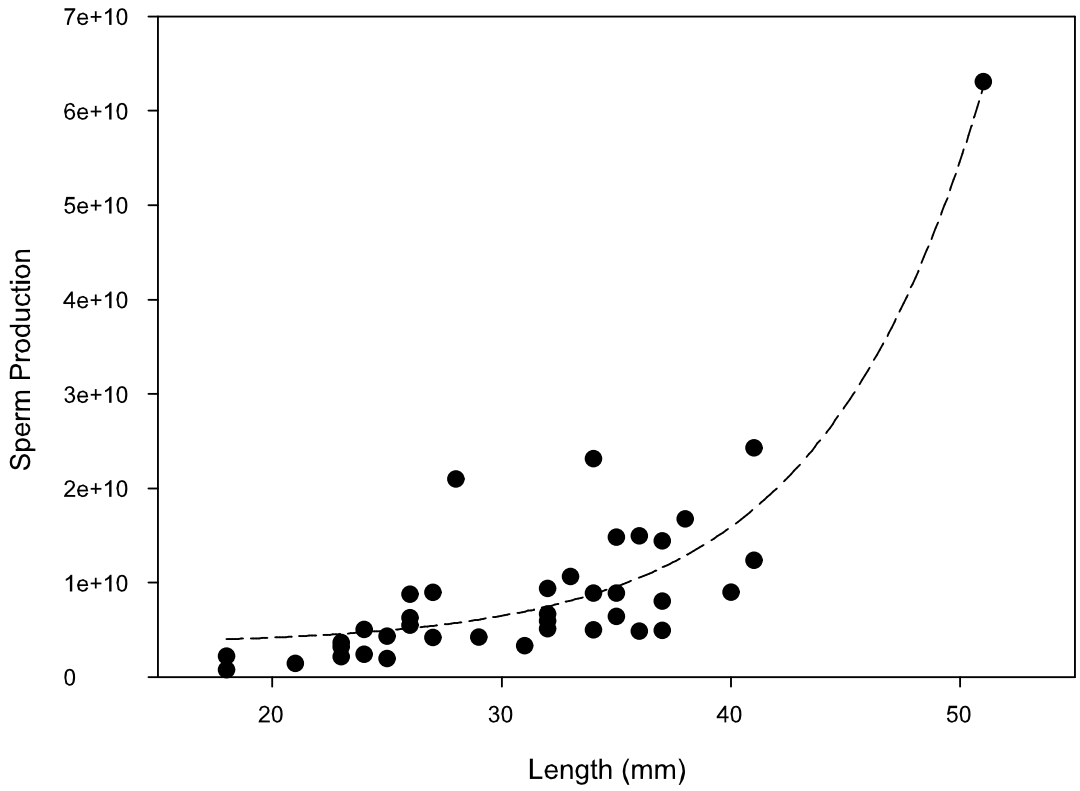

Figure 5.3: Length sperm production relationship for mature stage V male *P. vulgata*. The formula of the regression lines is given in the text, n= 39, $R^2 = 0.784$.

C (exposed) occur in the 10-20mm size range, whereas the only 81% and 77% of transect B (intermediate) and A (sheltered) populations respectively occur in the 10-20mm size range.

Summary information on the comparative population structures and reproductive outputs (Table 6.2) of the populations from across the wave exposure gradient indicate that the exposed site C had the highest population density, but the smallest average shell length, lowest biomass per unit area and least egg production per unit area. The sheltered site (Transect A) had the largest average length, and greatest biomass and egg production per unit area. Although egg production per unit area varies by 350% between populations across the exposure gradient, sperm production per unit area only varies by 14% between the different populations.

The length-frequency and length-fecundity data were combined to calculate the contribution of each length class to the population reproductive output (Figure 5.5),

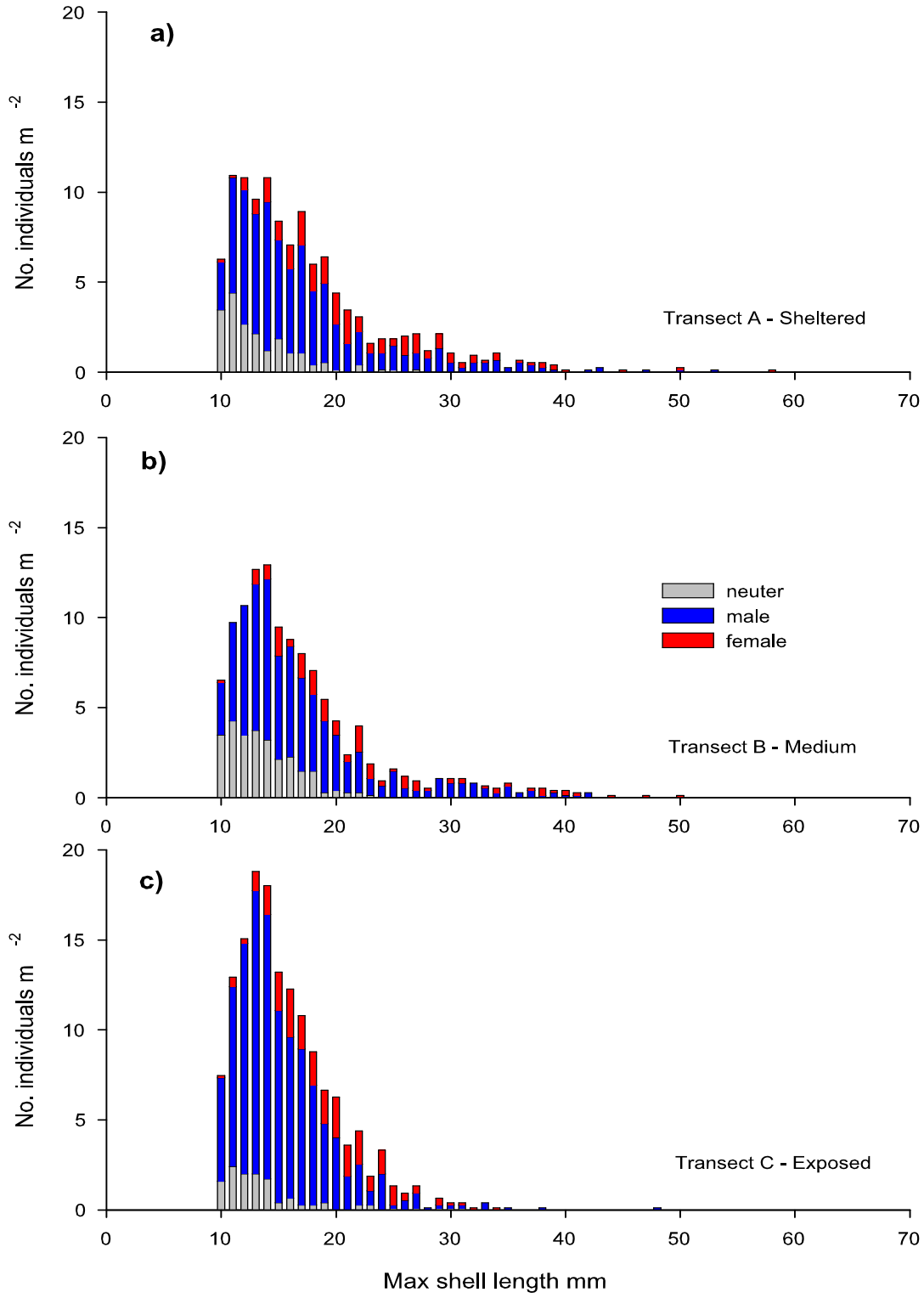

Figure 5.4: Sex-specific population structure per m^2 for *P. vulgata* at three different levels of wave exposure from Warren Beach, Devon.

Table 5.2: Results from transect population structure and fecundity assessment. All data normalised per m^2.

Transect	Wave Exposure	Mean Density # m^{-2} (± 1 s.d.)	Biomass g	Female:Male ratio	Mean Length mm (± 1 s.d.)
A	Low	123 (± 72.5)	147.8	1:3.24	17.5 (± 7.03)
B	Medium	120 (± 65.8)	120.4	1:4.96	16.9 (± 6.44)
C	High	154 (± 93.9)	87.2	1:4.54	15.7 (± 4.41)

Transect	Mean Male Length mm (± 1 s.d.)	Mean Female Length mm (± 1 s.d.)	Sperm Production sperm m^{-2}	Egg Production eggs m^{-2}	Egg:Sperm ratio
A	17.4 (± 6.83)	22.1 (± 8.15)	1.70×10^{11}	9.22×10^5	$1{:}1.84 \times 10^5$
B	17.2 (± 6.28)	22.0 (± 8.12)	1.50×10^{11}	3.95×10^5	$1{:}2.56 \times 10^5$
C	15.3 (± 4.19)	19.0 (± 4.45)	1.49×10^{11}	2.06×10^5	$1{:}7.23 \times 10^5$

and the total population fecundity per unit area (Figure 5.6 and Table **??**) according to exposure level. The few large individuals, in populations A (sheltered) and B (intermediate) especially, provided a disproportionately large input to the population fecundity. The presence of these large females which contributed a large part of the total egg production in the more sheltered populations (A and B) indicate why egg production was effected more than sperm production by the loss of the few larger individuals in the exposed population (transect C). The input of the large females in Transect A to egg production can be seen in the cumulative gamete production plots (Figure 5.6), which highlights that the over 50mm size class in Transect A contribute over 50% of the egg production.

Fishery Simulation

The impact of the simulated fishery exploitation was to rapidly reduce egg production; sperm production is less immediately effected by the removal of the largest individuals from the population (Figure 5.7). In each case a 20% reduction in population numbers concentrated on the largest individuals led to a 93%, 89% and 75% reduction in egg production and a 60%, 55% and 38% reduction in sperm production by the sheltered, intermediate and exposed populations respectively.

Sperm and egg production are less sensitive to reductions in SSB than to population numbers (Figure 5.8).Population egg production declined faster than SSB for all the exposure levels as individuals were removed from the population by exploitation, apart from when the first few individuals are removed from the exposed population

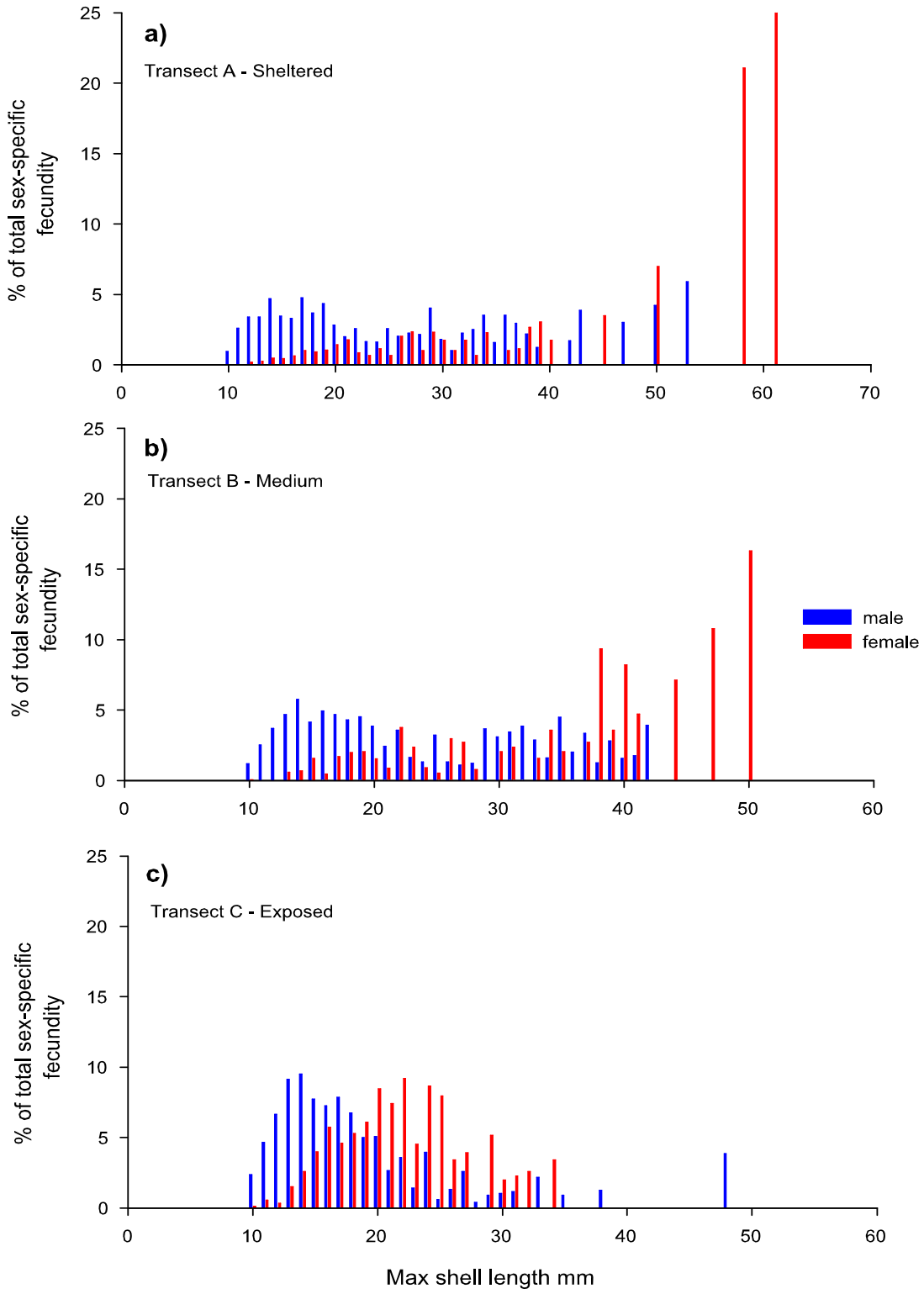

Figure 5.5: The proportion of the population sex-specific fecundity contributed by each length class. Plots a, b and c are for the sheltered, intermediate and exposed sites respectively.

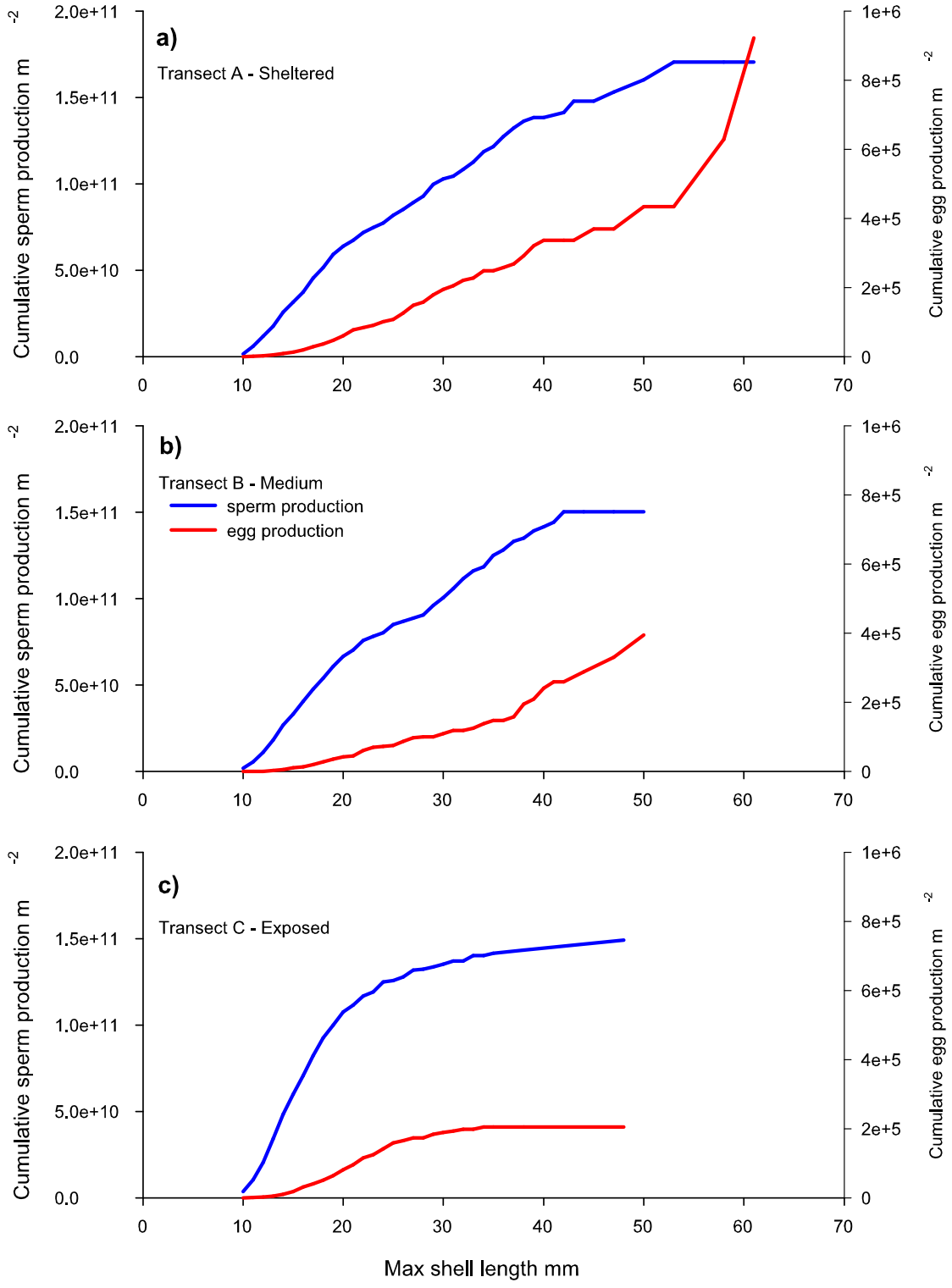

Figure 5.6: Cumulative population sex-specific fecundity Plots a, b and c are for the sheltered, intermediate and exposed sites respectively.

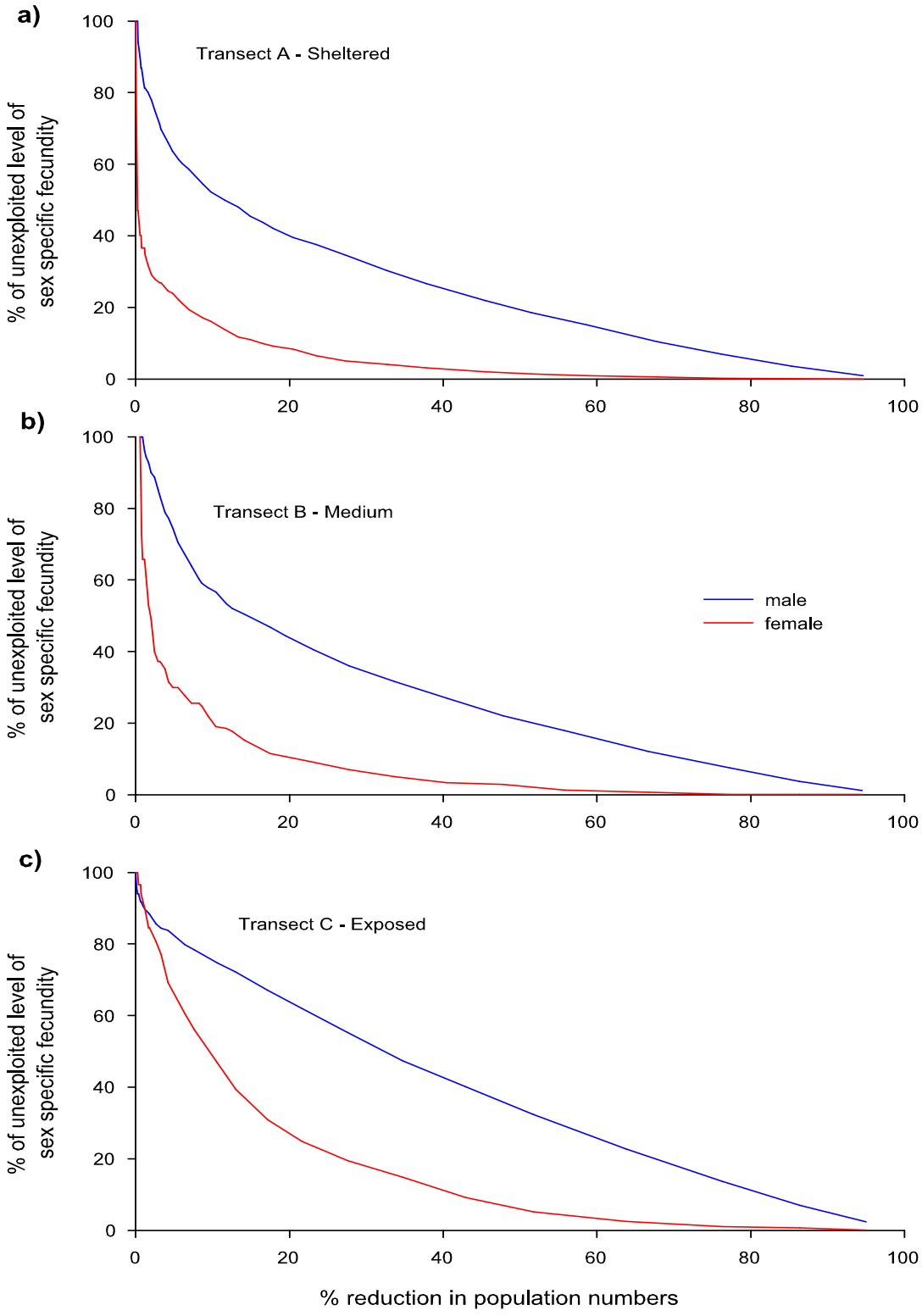

Figure 5.7: Fishery exploitation model, the effect of reduced population numbers on population sex-specific fecundity. The fishery model sequentially removes the largest individuals from the population. Plots a, b and c are for the sheltered, intermediate and exposed sites respectively.

(Transect C). In contrast the reduction in population sperm production was less than the reduction in SSB. The sheltered population, Transect A, showed the greatest departure from a proportional relationship between SSB and egg production. The more rapid decline in population egg production than SSB is consistent with the fact that both of the assumptions of constant relative fecundity, and constant sex ratio, from equation 1.1 are broken for these data.

Populations at different exposure levels showed different responses in terms of fraction of egg production remaining and fraction of biomass removed as yield for a given combination of management regulations (Figure 5.9). The different sites showed similar proportionate responses to variation in the proportion of territory covered by MPAs, but very different responses to changes in MLS. This was because the individuals in the more exposed sites were concentrated into the smaller size classes. Thus assuming there was no protection from MPAs, for the exposed site, Transect C, decreasing the MLS from 61 mm to 35 mm would have no effect on the proportion of egg production conserved or proportion of biomass taken as yield as all the individuals are below the 35mm size limit. However, if the MLS for the sheltered population, Transect A, was decreased from 61 mm to 35 mm, egg production would drop by over 70% and more than 35% of the total biomass would be available as yield. A similar change in MLS for the intermediate population, Transect B, would lead to a 60% decrease in egg production and more than 25% of the biomass would become available as yield.

The exposure gradient leads to a corresponding gradient in yield and egg production per m^2 between populations (Figure 5.10). The sheltered site (transect A) had the greatest maximum yield and egg production per unit area. However the exposed site, transect C, showed the lowest proportional drop in egg production for the greatest proportional removal of fishery yield. The extent of the variation in population length frequency, biomass and egg production per unit area means that over 70 gm^{-2} could be removed from the sheltered population and the sheltered population would still be capable of greater egg production per unit area than the exposed population. The intermediate population, transect B, showed a response to exploitation that fell between the exposed and sheltered populations.

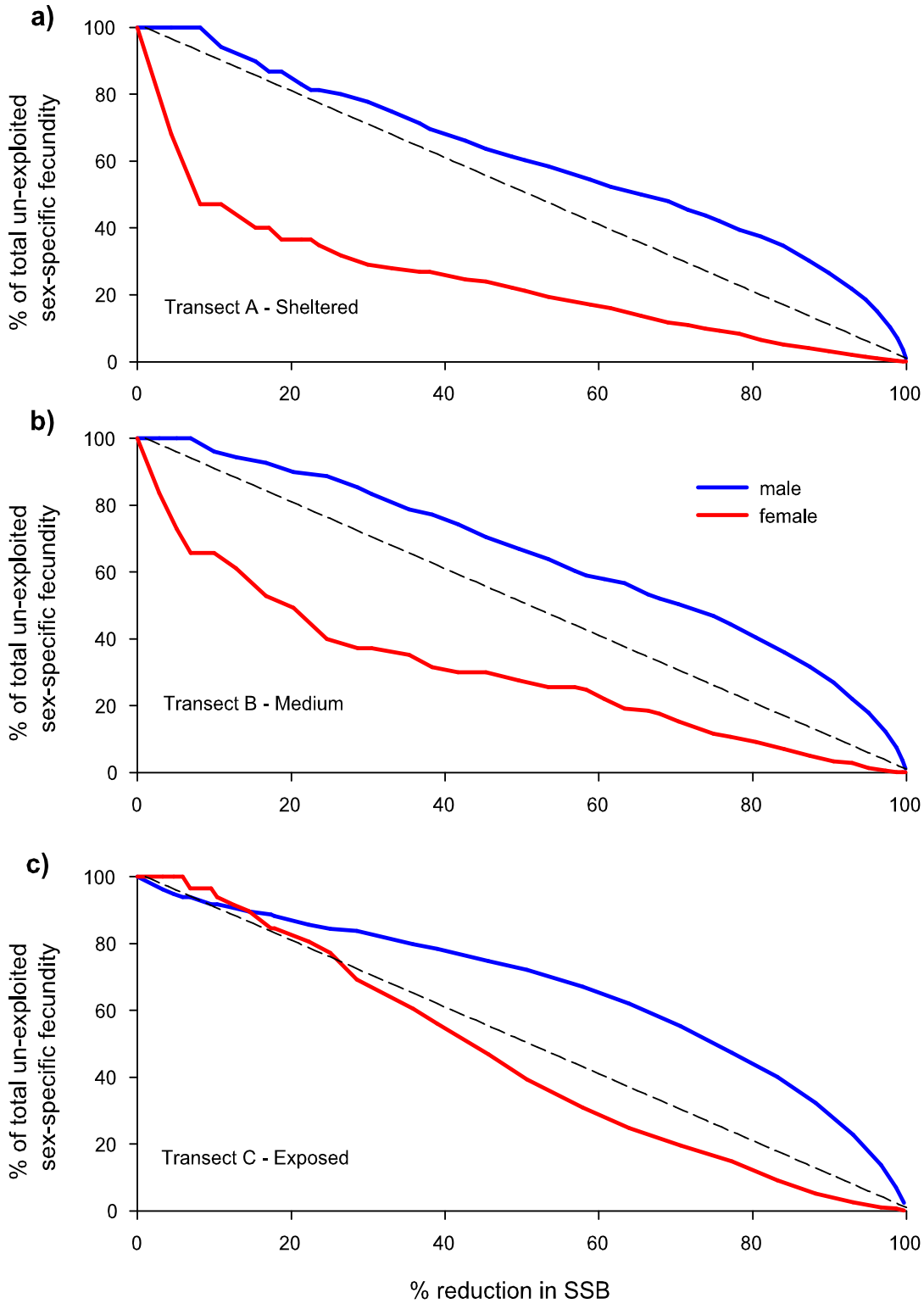

Figure 5.8: Reduction in SSB on population sex specific SSB. The dotted line indicates the expected 1:1 relationship. Plots a, b and c are for the sheltered, intermediate and exposed sites respectively.

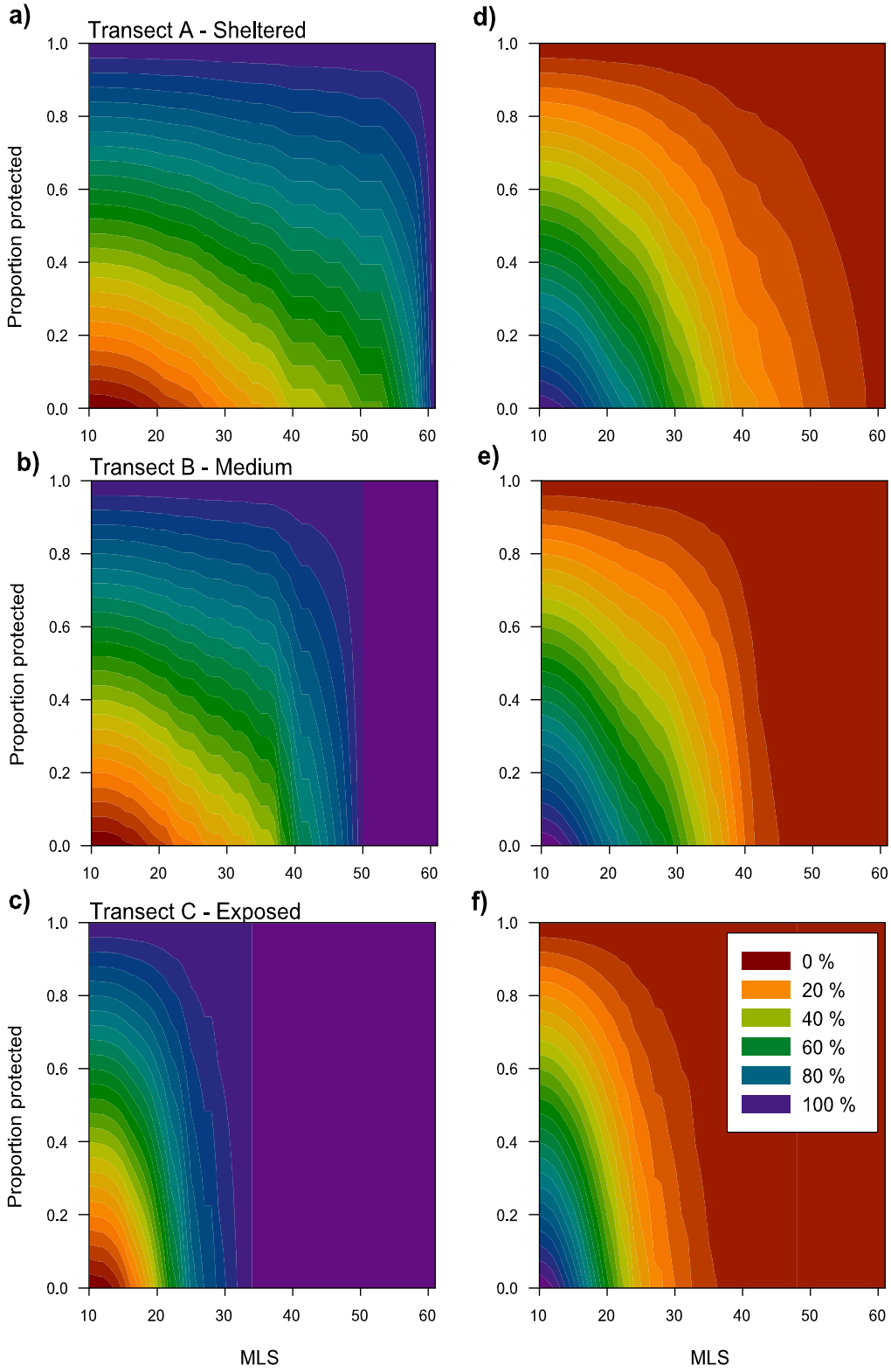

Figure 5.9: Effect of differing management regimes on the proportional yield and egg production by three different limpet populations from across an exposure gradient. For co-management by MPA and MLS, the MLS applies to the section of the population not protected by MPAs. The effect is calculated per m^2 across the full range of the fishery. a)- c) Effect of fishery on population egg production, d)- f) effect of fishery on yield.

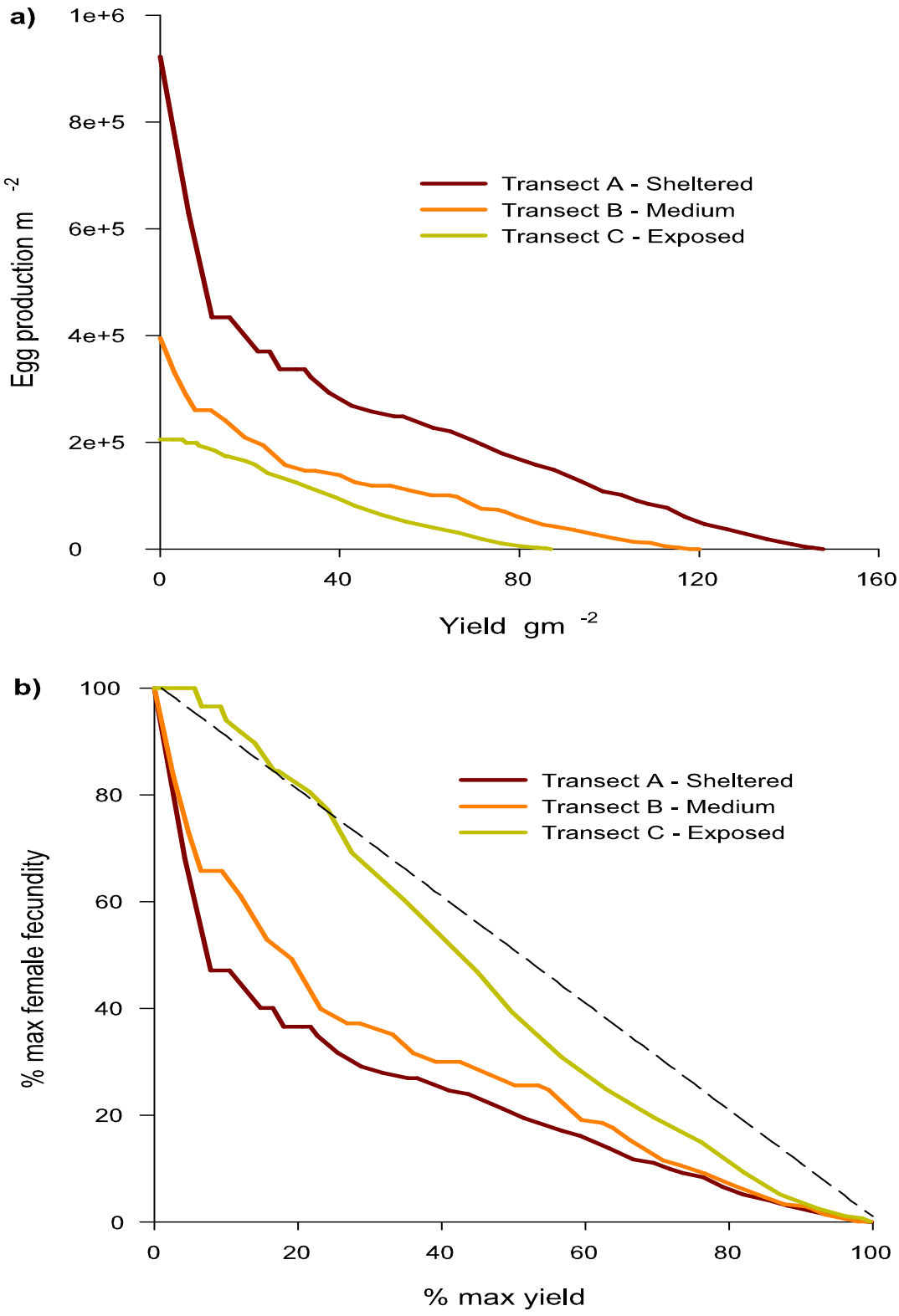

Figure 5.10: Drop in egg production per m^2 as fishery yield is taken. a) Absolute values, b) proportional values. The dotted line indicates the expected 1:1 relationship.

5.4 Discussion

5.4.1 What is the effect of protandry on the relationship between gametic output, SSB and yield?

Beverton and Holt's (1957) assertion that SSB could be used as a proxy for larval production was based on the assumption that SSB is directly proportional to egg production. It is immediately apparent from figure 5.11 that for the population studied here the assumption of direct proportionality does not hold. The failure of this relationship is due to a breakdown in the assumptions of constant sex ratio and constant relative fecundity. If the breakdown in proportionality between E and SSB was solely due to a failure in the assumption of constant sex ratio then the sex-specific SSB would be expected to be directly proportional to population egg production. As can be seen from figure 5.12 there was a non-linear relationship between female sex-specific SSB and population egg production. Therefore the failure for SSB to accurately predict E is not solely due to a failure in the assumption of a constant sex ratio, and must be at least partially due to non-linear relationship between fecundity and weight.

Does protandry have any effect on the relationship between E, SSB and yield? Or is the breakdown in direct proportionality simply due to variable constant relative fecundity? If protandry had no effect, then female SSB would be expected to be directly proportional to the population SSB. The plot of proportional reduction in SSB against proportional reduction reduction in sex-specific SSB and egg production (Figure 5.11) indicates that this was not the case. Therefore the disparity between the reduction in egg production against reduction in SSB was due to a failure in both the assumption of constant sex ratio and a failure in the assumption of constant relative fecundity. The nature of this disparity can be further examined. The distance between the 1:1 relationship and egg production (labelled x in Figure 5.11 b) indicates the full disparity between SSB and egg production. This can be separated into the disparity due to failure in the assumption of constant relative fecundity (labelled z in Figure 5.11 b) and the failure of the assumption of constant sex ratio (labelled y in Figure 5.11 b). The discrepancy between the 1:1 line and the female SSB line

(y in Figure 5.11 b) clearly illustrates the effects of protandry.

Returning to the initial selection of the length-fecundity relationship: had the exponential relationship been rejected and the linear weight-fecundity relationship described by a cubic power law length-fecundity relationship been accepted, the assumption of constant relative fecundity would still hold. In this case there would be no discrepancy between egg production and sex-specific SSB. Therefore the proportional drop in egg production would be wholly described by the proportional drop in sex-specific SSB, and thus the female sex-specific SSB line in figure 5.11 would describe the response of egg production to a decline in population SSB.

Protandry causes a decoupling of sex-specific SSB and whole population SSB; this is because females are preferentially concentrated in the larger size classes and males in the smaller size classes. Thus when a fishery selectively exploits larger size classes females will be preferentially exploited and female SSB will decline faster than whole population SSB. It can be seen that the effect of protandry on the relationship between SSB and sex-specific SSB varies between the different populations at different exposure levels. The main point to be drawn from this is that the effect of protandry on the response of a stock to exploitation will vary depending on the exact local sex-specific population structure. Thus the effects of protandry cannot be corrected for with a single simple conversion factor.

The failure of the assumptions of constant sex ratio and constant relative fecundity breaks the direct proportionality between E and SSB. Therefore some parts of the population will have a different E/SSB ratio than other parts of the population. Once this variation has been established the fishery manager could try and determine which parts of the population have the lowest E/SSB ratio as exploiting this part of the population will generate the maximum yield for the minimum drop in egg production. Unfortunately, and in complete contrast to the aims of a fishery manager, with protandry and a size selective fishery the fishery selectively exploits the section of the population with the highest E/SSB ratio first. Thus the reduction in egg production for a given yield is maximised.

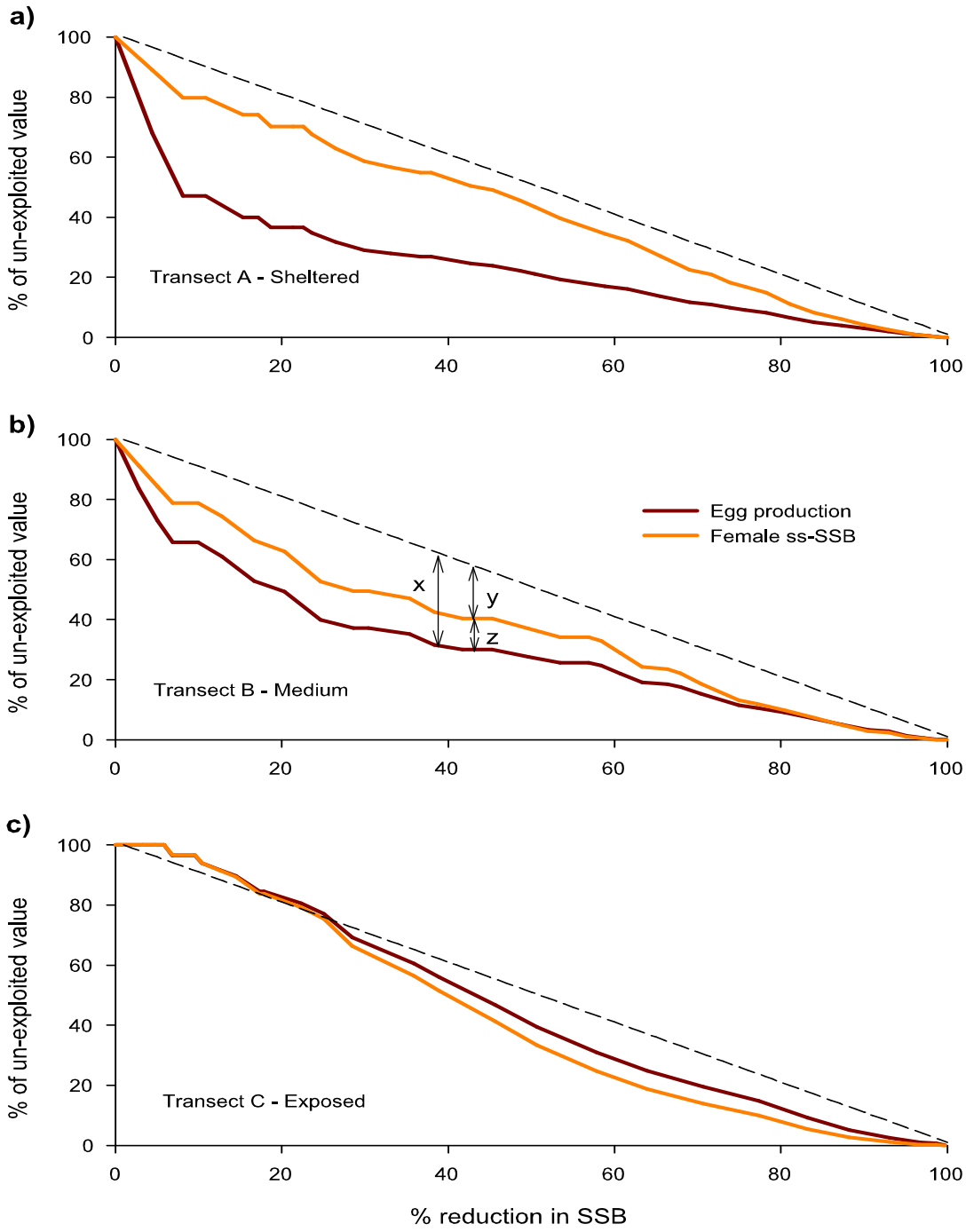

Figure 5.11: Proportional reduction in E and sex-specific SSB against proportional reduction in whole population SSB as a result of fishery pressure. For: a)transect A, sheltered; b) transect B, intermediate; and c) transect C, exposed. The arrows in part b) indicate; x- the full discrepancy between E and SSB, y- the discrepancy due to failure of the assumption of constant sex ration, and z- the discrepancy due to failure of the assumption of constant relative fecundity. The dotted line indicates the theoretical 1:1 relationship.

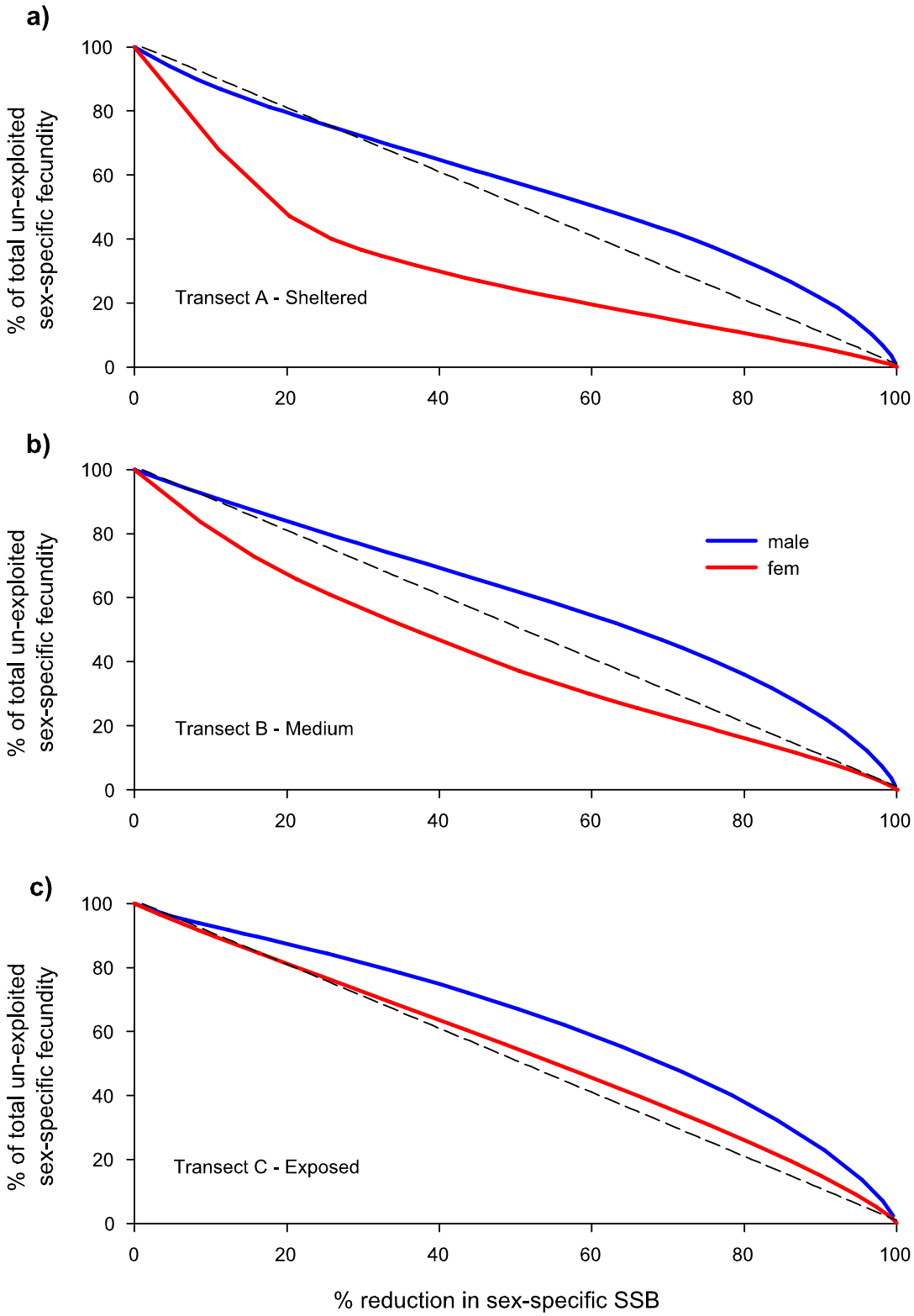

Figure 5.12: Decline in sex-specific potential fecundity plotted against decline in sex-specific SSB due to fishery pressure for: a)transect A, sheltered; b) transect B, intermediate; and c) transect C, exposed. The dotted line indicates the expected 1:1 relationship.

5.4.2 Is SSB a good proxy for population egg production for protandrous hermaphrodites?

The effects of protandry on the relationship between SSB and E vary with habitat. As can be seen from figure 5.11 there is a far greater disparity between E and SSB for the sheltered population than for the exposed population. Attempts to protect E at an arbitrary level of 30% of the unexploited level (Mace and Sissenwine, 1993; Thompson, 1993) on the basis of protecting the same level of SSB would only preserve 12% - 25% of E (Table 6.3). Thus even for the exposed population, which shows the greatest similarity between E and SSB, there would be a 17% shortfall in the proportion of E protected. For the sheltered population there would be a 60% shortfall in egg production if it were managed by SSB.

Even if the effects of the exponential length-fecundity relationship are discounted, SSB still fails to act as a direct proxy for E. It should therefore be noted that using SSB as a management tool for protandrous species should only be done with considerable caution, and with an understanding of the significance of the invalid assumptions. This is in agreement with previous studies of sequentially hermaphroditic fin fish, that have indicated that sequential hermaphrodites are susceptible to over exploitation of sex-specific reproductive potential (Buxton, 1992; Punt et al., 1993). The wider implications of the effects of protandry on fisheries management will be considered in chapter 8.

Table 5.3: The different MLS limits that would need to be applied to conserve various proxies for reproductive effort at 30% of their un-exploited level. The actual proportion of egg production that would be conserved by the MLS limit is in brackets.

	SSB	Sex-specific SSB	Egg production
Transect A - Sheltered	2530 mm (12%)	27 mm (16%)	38 mm (32%)
Transect B - Medium	23 mm (18%)	27 mm (25%)	31 mm (30%)
Transect C - Exposed	18 mm (25%)	20 mm (39%)	19 mm (%)

5.4.3 Are yield and fecundity best managed by MPAs or MLS?

Are yield and fecundity best managed by MPAs or MLS? For the time being I shall discuss this from the single species population dynamics point of view; the wider ecosystem implications of the differing management schemes shall be discussed in further detail in chapter 8.

The effects of managing by MPAs or MLS on egg production and yield are illustrated in figure 5.9. For this simulation it is assumed that the MLS is applied to any areas not covered by MPAs. The question that needs to be answered by a fishery manger is, for a given yield how can egg production be maximised, or for a given level of egg production to be conserved, how can yield be maximised? This can be resolved by overlaying the plot of yield management scenarios (Figure 5.9) with the egg production plot (see Figure 5.13). For example if it is decided to try and manage a population such that 30% of the unexploited egg production is protected; then the management strategy that maximises the yield can be found by following the black line (Figure 5.13) that represents the combination of management measures that would conserve 30% of the unexploited egg production to see which combination of management measures provides the highest yield.

From figure 5.13 it can be seen that for a given level of egg production, yield is maximised by managing on the basis of MPAs rather than with an MLS. For example if it is chosen to preserve 30% of the egg production of the sheltered population about 25% of the yield is accessible to the fishery if a MLS of 38 mm is chosen. If however 30% of the area is covered by MPAs, with no MLS outside MPAs, over 70% of the yield is accessible to the fishery whilst still conserving 30% of the unexploited egg production. The same trend holds for the more exposed populations in transects B and C although the difference in management strategies is less pronounced.

The reason for this result is that the large females have the highest E/SSB ratio. Thus once the large females have been removed from a stretch of shore the rest of the population remaining on the shore will have a lower than average E/SSB ratio. Once the large females have been removed from an area it is preferable to remove the rest of the individuals from that area, exploiting a section of the population with a

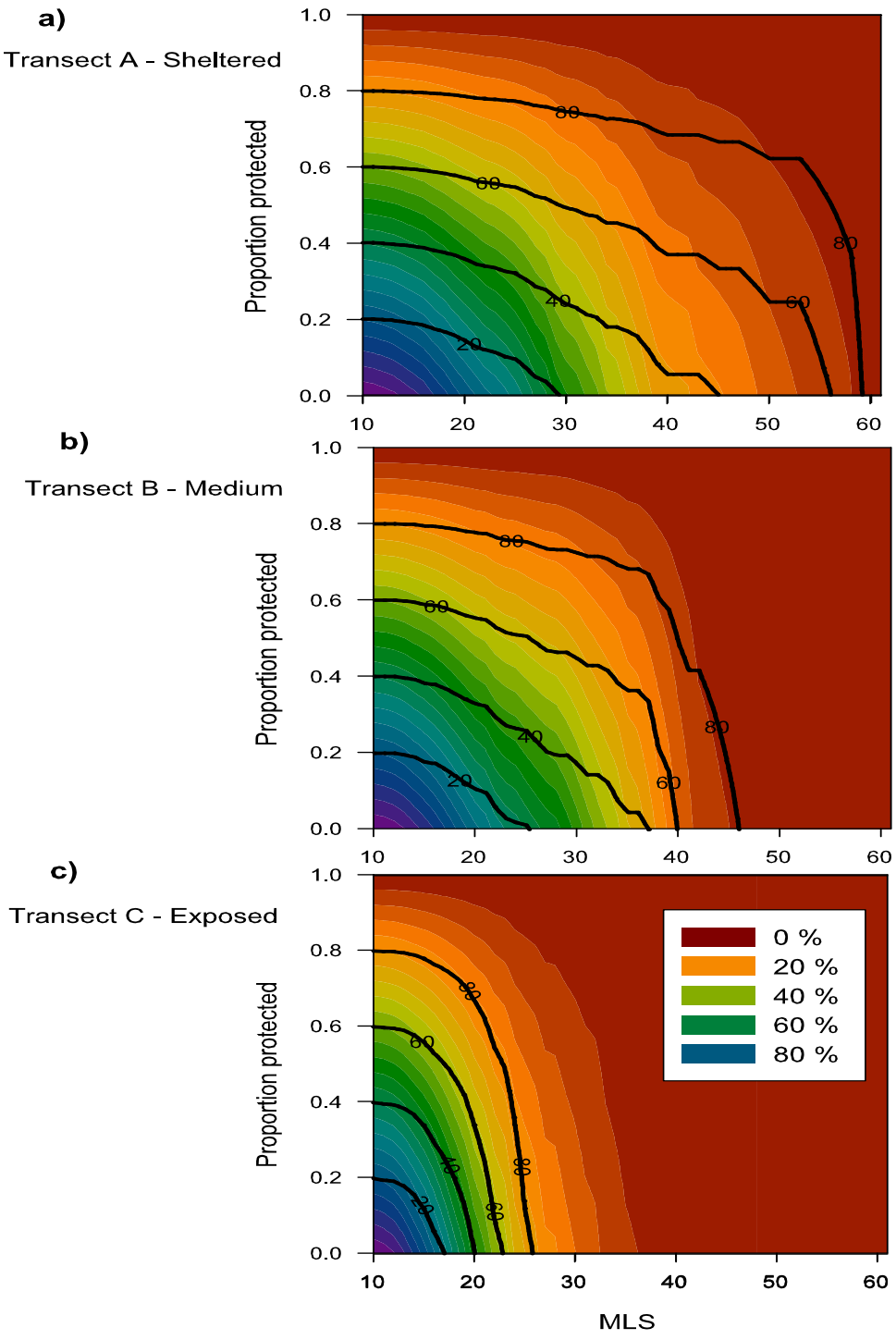

Figure 5.13: The proportion of egg production conserved and yield taken for all combinations of management scenarios. The black contour lines represent the percentage of egg production protected, whilst the colour bar represents the percentage of yield accessible to the fishery under the given management scenario

low E/SSB ratio rather than harvesting the large limpets from an unexploited area and again exploiting the section of the population with the highest E/SSB ratio. Having only MPAs with no MLS operating outside them leads to this approach to exploitation as some areas are fully protected and other areas can be fully exploited. Managing by MLS leads to the opposite approach to exploitation as it encourages the large individuals to be taken from across the whole population before then moving down to the smaller size classes. The result that the species is best managed on the basis of MPAs rather than a MLS will hold for any situation where older larger females have a higher E/SSB ratio than smaller younger females, or if there is a greater proportion of females in the larger size classes.

It should be noted that as this is a static model it under emphasises the importance of loss of younger individuals from the population, as no account is taken of the loss of future reproductive potential. The limitations of the population model and fishery simulation will be discussed further in chapter 6. The role of MPAs in fisheries management will be more widely discussed in chapter 8.

5.4.4 What is the effect of wave exposure and how should habitat variability be accounted for in management decisions?

The variation in population structure across the exposure gradient was in accordance with previous studies of *P. vulgata* population dynamics that have found that in more exposed sites population densities are higher (Jenkins and Hartnoll, 2001; Lewis, 1964; Southward, 1953), growth rate is higher (Jenkins and Hartnoll, 2001; Lewis and Bowman, 1975), mortality rates are increased (Lewis and Bowman, 1975) and average size is reduced (Jenkins and Hartnoll, 2001; Lewis and Bowman, 1975). The effect of exposure on population structure has also been shown for other patellid limpets. Branch and Odendaal (2003) found that the South African limpet *Cymbula oculus* had 80% greater biomass at sheltered than exposed shores.

In the present study at the sheltered site biomass was 70% greater, egg production 350% larger and sperm production 14% greater whilst population density was 20% reduced compared to the exposed site (Table **??**).

McShane and Naylor (1995) found small scale (hundreds of meters) habitat heterogeneity between bays and headlands led to small scale variations in life history parameters of the commercially exploited abalone *Haliotis iris*. They concluded that the MLS of 125 mm could be reduced to 110 mm within bays, as very few individuals reached 125 mm in bays, and reducing the MLS to 110 mm in bays would still meet the goal of conserving 40% of the unexploited egg production. However the practical enforcement of MLSs varying over the scale of hundreds of meters would be logistically impossible. This does not mean that small scale habitat heterogeneity can not be accounted for by fishery managers. An alternative approach would be to allow for the different life history traits whilst setting an average MLS. Thus the overall population egg production would be conserved at the desired level, although sub-populations made up by larger individuals would be over-exploited and under contribute to egg production, and sub-populations of smaller individuals would be under-exploited and over contribute to egg production.

An alternative strategy would be to manage the stock with a combination of MPAs and a MLS. To examine how such a management strategy would be effected by habitat heterogeneity three different management scenarios are examined (Figure 5.14). For these scenarios it is assumed that the total range of the population is made up of equal areas of sheltered, exposed and intermediate shoreline. For the first scenario considered the whole range of the species is fished simultaneously and the largest individuals from the whole population are sequentially removed (Even MLS - Figure 5.14). For the following two scenarios the areas of different exposure level are fished sequentially. Whilst each of the habitats is being fished the largest individuals from that area are sequentially removed until that area has been fully exploited before the next habitat area is fished. For the two scenarios the order in which the habitats were exploited are exposed - intermediate - sheltered (C-B-A - Figure 5.14), and sheltered-intermediate-exposed (A-B-C - Figure 5.14).

Again for the sake of discussion the arbitrary level of 30% of unexploited egg production is taken as the level of egg production to be conserve (Mace and Sissenwine, 1993; Thompson, 1993). The model output indicates that there is a more than 40% variation in the amount of yield per m^2 that can be taken from across the range depending on the management scenario followed. The even MLS management sce-

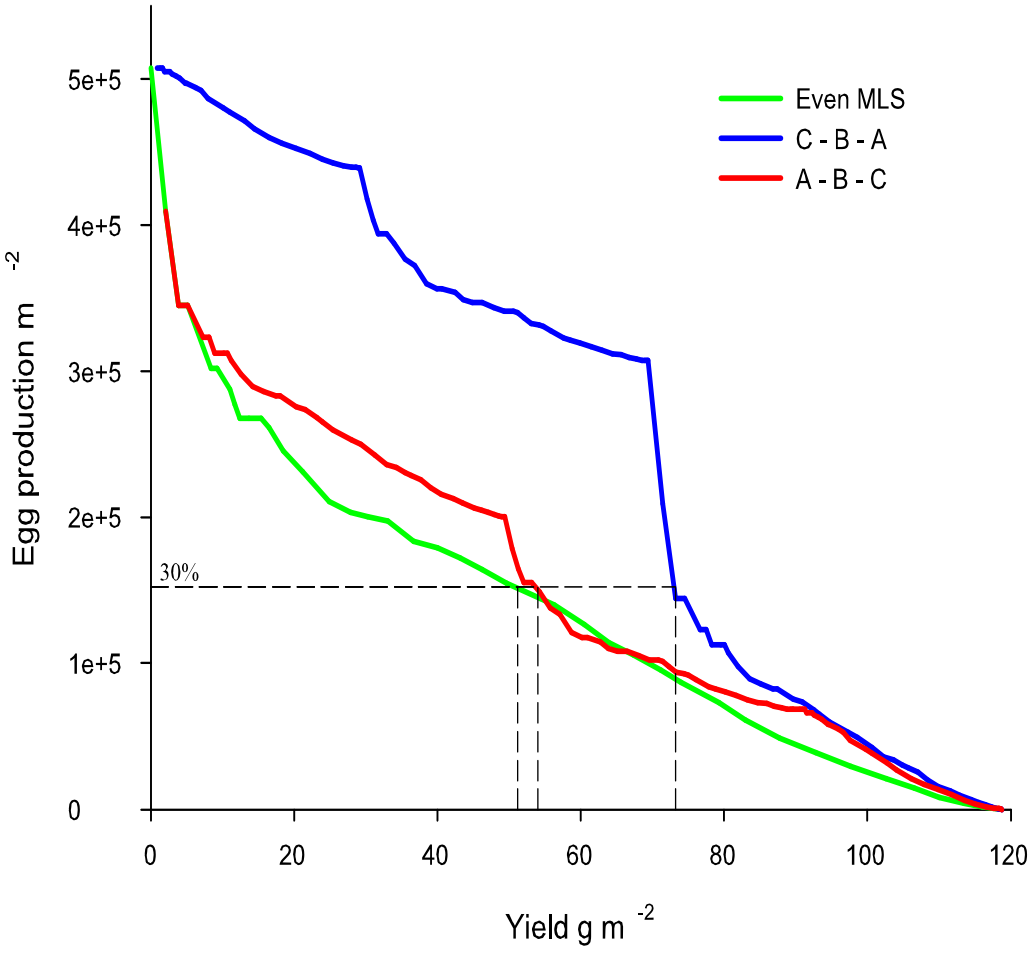

Figure 5.14: The effect of different management strategies on the relationship between yield and egg production assuming the total population range is made up of equal area of sheltered (A), exposed (B) and intermediate (C) shoreline. The different management strategies are, firstly to simultaneously fish all three areas sequentially removing the largest individuals from the population, for the other two regimes the different areas are fished sequentially with the largest individuals being sequentially removed from the area being fished. The dotted line indicates 30% unexploited egg production.

nario generated the lowest yield and the scenario sequentially exploiting first the exposed, then intermediate, then sheltered populations generated the highest yield. The sequential exploitation strategy is preferable to the even MLS strategy for two reasons. Firstly by concentrating exploitation on exposed areas rather than sheltered areas the section of the population with the lowest E/SSB ratio is exploited first. And secondly, as discussed above, the largest individuals in a population have the highest E/SSB ratio. Thus once the large individuals have been removed from a sub-population it is preferable to remove the rest of the smaller individuals from the sub-population before removing the large individuals from a pristine unexploited sub-population. However this model does not take into account loss of future reproductive potential.

The ability to tailor management strategies to heterogeneity in habitat quality will depend on the extent of knowledge about habitat quality across the harvested range of the species and the effect the variation in habitat quality had on life history traits. Even in the case of perfect knowledge on the life history characteristics of the stock across its range, the idea of a fishery management scheme to maximise yield whilst minimising the impact on egg production may not be feasible, especially if it were to require complex small scale variations in management controls. However the significant finding of this study is that the E/SSB ratio varies between different sections of a population, and a targeted fishing strategy can more than double the available yield whilst still conserving reproductive output compared to traditional MLS-only based approaches.

Chapter 6

Larval Production Modelling

6.1 Introduction

Managing stocks on the basis of egg production, whether through direct measures of egg production or through proxies, relies on the assumption that egg production provides an accurate measure of reproductive output. However zygote production is related to egg production by the relationship

$$Z = \alpha\gamma\rho E \tag{6.1}$$

where Z is population zygote production, E is population egg production, ρ is the proportion of eggs fertilised, α is the proportion of eggs viable and γ is the proportion of eggs spawned. The assumption that E = Z has become established due to Beverton and Holt (1957)'s seminal work in which they treated E as a surrogate for larval production. However in order to develop an accurate model of how zygote production is effected by exploitation it is necessary to account for $\alpha\,\gamma$ and ρ if they are variable.

It has been shown for both fin fish (Kiflawi et al., 1998; Marconato and Shapiro, 1996; Petersen, 1991) and invertebrates (Babcock et al., 1994; Levitan and Young, 1995; Pennington, 1985) that not all spawned eggs are fertilised, and that fertilisation rates are variable. Thus, as discussed in chapter 1, ρ must be accounted for by an accurate larval production model.

No empirical work on fertilisation has achieved 100% spawning success, regardless of sperm concentration or exposure time (e.g. Levitan et al., 1991; Pennington, 1985; Vogel et al., 1982) suggesting that it is common for some eggs to be inviable, although fertilisation percentages in the high 90s have been regularly observed (Babcock and Keesing, 1999; Levitan and Young, 1995; Pennington, 1985; Vogel et al., 1982). However, the reasons for loss of viability have recieved little attention. The effect of incomplete egg viability can be allowed for the purposes of a larval production model in the development of a fertilisation model. This approach has been taken in this study (see chapter 4), and the α in equation 6.1 is the same as α in equation 4.3. When egg viability, α, is accounted for in ρ, the proportion of eggs fertilised, α does not need to be explicitly included in the relationship between egg production and zygote production, and equation 6.1 can be restated;

$$Z = \gamma \rho E \tag{6.2}$$

There have been no comprehensive studies of the proportion of eggs spawned by invertebrates during spawning events, although a number of studies across a range of invertebrate taxa have noted that not all eggs are spawned during a single spawning event (e.g. Clavier, 1992; Garwood, 1987; Meidel and Scheibling, 1998). Attempts to calculate zygote production on the basis of potential fecundity need to take into account the proportion of eggs spawned. Where models have attempted to account for the proportion of eggs spawned average values from observations have been used (Meidel and Scheibling, 2001; Wahle and Peckham, 1999).

6.1.1 Aims

In this chapter I develop a combined population fecundity-gamete dispersal-fertilisation model to examine how zygote production and fertilisation success of example limpet populations is affected by a simulated fishery.

The zygote production model developed in this chapter is a development of the egg production model presented in chapter 5. The model will be used to examine the same questions as the egg production model, and the results will be compared with the egg production model to examine how well egg production functions as a proxy

for larval production. More specifically the zygote production model will be used to discuss the following questions:

i) Is sperm abundance ever limiting to zygote production?

ii) Does sperm limitation affect the relationship between zygote production, SSB and fishery yield for broadcast spawning protandrous hermaphrodites?

iii) Are SSB, or egg production good proxies for population zygote production for broadcast spawning protandrous hermaphrodites?

iv) In order to conserve zygote production whilst maximising yield, is the population best managed by minimum landing size or marine protected areas?

Chapter 5 looked at how egg production was affected by exploitation; this chapter is looking at how zygote production is affected by exploitation. Zygote production is dependent upon egg production and the proportion of eggs fertilised. Thus to avoid too much repetition of the last chapter I mainly discuss how fertilisation success affects zygote production and only discuss egg production where it is pertinent. A synthesis of the conclusions of these two chapters (5 and 6) will be presented in the final discussion chapter (Chapter 8).

6.2 Zygote Production Model

To develop a combined population-zygote production model it is necessary to know how many gametes are spawned, where and when these gametes are released, how these gametes are subsequently dispersed, and what proportion of the eggs are fertilised on the basis of predicted gamete concentrations.

Where parameter values are uncertain, a reasonable value was chosen on the basis of available data and sensitivity analysis was conducted to assess the affect of incorporating incorrect parameter values.

In this combined model the number of gametes spawned according to the chosen level of fishing intensity or management options is derived from the population fecundity

model developed in chapter 5. The only data on the proportion of gametes spawned by *P. vulgata* during a spawning event is from comparing counts of eggs remaining in six limpets examined after spawning with the number of eggs predicted from a length fecundity relationship (Garwood, 1987). The limpets had spawned an average of 83% of their eggs, ranging from 60% to 98%. Thus in the model it will be assumed that females spawn 83% of their potential fecundity during a spawning event. There is no data on the proportion of sperm released during a spawning event, therefore in the model I use the simplifying assumption that all sperm are released during a spawning event.

There were no data available on the duration of gamete release or the exact spatial location of limpets at the time of spawning. Due to the assumptions made in the hydrodynamic dispersal model the exact location at spawning is not required. Knowledge of the population structure of a one meter wide crossshore transect provides sufficient resolution for the spatial distribution of individuals for this model. As there are no data on gamete release times for limpets an appropriate value is selected on the basis of literature values for other species. The duration of spawning events varies between taxa and between hydrodynamic environments (Andre and Lindegarth, 1995; Galtsoff, 1940; Naidenko, 1989; Thomas, 1994) and the length of gamete release can vary between 15 seconds (Thomas, 1994) and 2 hours (Naidenko, 1989). As *P. vulgata* spawns into a highly turbulent environment it seems likely that their gamete release time would be towards the shorter end of the spectrum of observed values. Therefore 15 minutes was chosen as the initial starting value for gamete release time. It was assumed that gametes are shed at a constant rate during spawning.

Previous combined gamete dispersal-fertilisation models have used advection-diffusion models to determine the post-spawning movements of gametes (Claereboudt, 1999; Denny and Gaines, 1990; Denny and Shibata, 1989; Levitan and Young, 1995). Due to the nature of the highly turbulent topographically controlled mixing and dispersal in the surf zone a standard advection-diffusion model is considered inappropriate (Wolanski and Hamner, 1988) and the one box model for surf-zone dispersal developed in chapter 3 is used to model gamete dispersal. The values presented in chapter 3 for the physical dimensions and exchange parameters of the surf zone at

Warren Beach at the time of spawning were used as the initial parameter values for the model runs.

The model was run using 1 minute time steps, each model run covering a four hour period. At the start of a run the gamete concentration in the water column was set to zero, with spawning starting at the start of the model run. Gametes were added to the surf zone at the start of each time step until gamete release ceased. For the predicted gamete concentrations the proportion of eggs fertilised during each time step were calculated according to the fertilisation model developed in chapter 4. Fertilised eggs were removed from the pool of unfertilised eggs in the surf zone. Sperm were not removed from the pool of available sperm in the surf zone as a result of fertilisations as this would have negligible impact on the number of sperm available. Following the removal of fertilised eggs mixing with off-shore waters occurs and the gamete concentrations in the surf zone were recalculated to allow for gametes lost from the system.

The calculations for each time step are made in the following order:

- If spawning was still occurring, new gametes were added to the system
- Fertilisations occurred
- Fertilised eggs removed from the system
- Sperm and eggs lost from the system due to mixing.

The number of zygotes produced and the proportion of eggs fertilised per time step were summed over the full run of the model to give the total number of zygotes produced and the overall proportion of eggs fertilised for the given level of fishing intensity. The Matlab routine developed to run the model is given in Appendix 5.

6.3 Model Runs

6.3.1 Sensitivity analysis

An essential part of model development is sensitivity analysis to understand the effect of error in parameter estimates on the model output. Therefore an initial series of model runs were conducted to test the sensitivity of the model to variations in the input parameters. The default values used are listed in table 6.1. The range of values over which the input parameters were varied depending on the estimated error in the input parameters, each parameter was varied over the full range of plausible values for that parameter. As the model contains non-linear relationships parameter values were co-varied, since basing the sensitivity analysis on individually varied parameters may hide effects of the non-linear relationships within the model (Kremer, 1983). A quantitative analysis of the sensitivity of the model output to individually varied parameters was conducted (Table 6.1). However, in order to avoid over complication, the results of co-varying parameters were examined graphically (Figure 6.1), and the pertinent features of the graphical analysis are discussed.

For the quantitative analysis the sensitivity parameter, S, was calculated following (Fasham et al., 1990) with the slight modification that the absolute value was taken to simplify interpretation. Thus;

$$S = \left| \frac{\left(\frac{O_P - O_D}{O_D}\right)}{\left(\frac{P - P_D}{P_D}\right)} \right| \tag{6.3}$$

where O_D is the model output with parameters set to default values, O_P is the model output with the given parameter changed to the new value P, and P_D is the default parameter value. The sensitivity of the model prediction of fertilisation success was examined. Where there is uncertainty in the choice of upper and lower parameter values, following (Fasham et al., 1990), the limit values were taken to be half and twice the standard value respectively, apart from the surfzone residence time where a wider range of values was considered to reflect the lack of information available regarding surfzone hydrodynamics (see section 3.5.2).

In relation to the physical parameters that define the volume of the surf zone, the model displayed far greater sensitivity to the height of breaking waves than beach an-

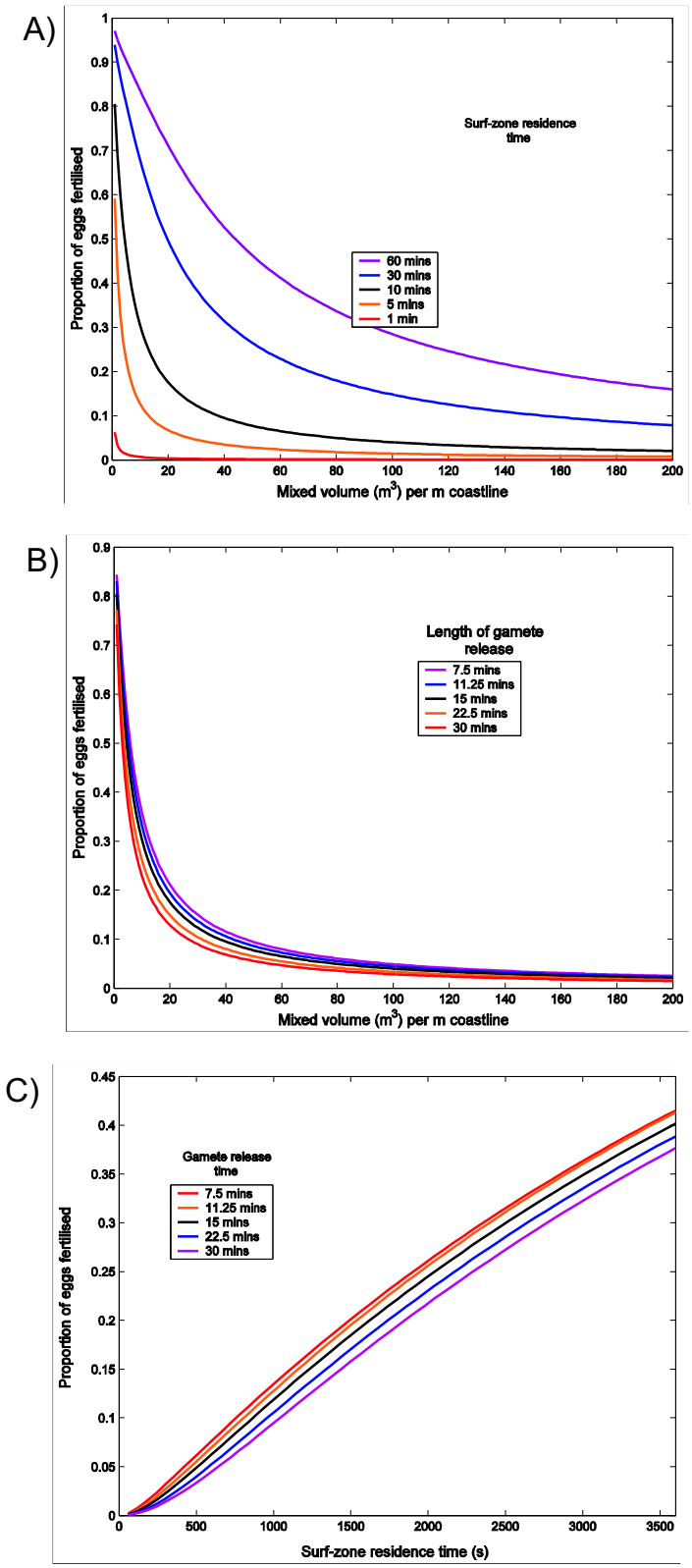

Figure 6.1: Effect of covarying parameters on the proportion of eggs fertilised. The mixed volume was directly manipulated rather than individually manipulating parameters that control the mixed volume. A) varying mixed volume of surf zone for given gamete release time values; B) varying mixed volume of surf-zone for given values of surf-zone residence time; C) varying residence time for given values of gamete release time.

Table 6.1: The default, and range of parameter values used in the model. The default values were used for each model run unless indicated otherwise. The basis for the parameter values refers to the section in the thesis where the selection of that parameter value is discussed. The sensitivity values S are the sensitivity of fertilisation success compared to the maximum/minimum parameter values. The β values used in the model is 10^6 larger than the value given in chapter 4 as the model works in m^3 whereas the original calculation measured sperm per ml.

Module	Parameter	Symbol	Default Value	Basis	Min/ Max	S
Physical	Beach angle	σ	14°	section 3.5.2	7/28	0.04/0.10
dimensions	Height of breaking waves	h	4m	section 3.5.2	2/8	1.80/0.47
	Volume	V	–	–	±50%	1.80/0.64
	Surface roughness	ϵ	1.6	section 3.5.2	1.3/1.9	1.74/2.03
	Surfzone residence time	τ	600s	section 3.5.2	60/3600	1.09/1.07
Spawning	Length of gamete release	R	900s	section 6.2	450/1800	0.44/0.28
behavior	Proportion of eggs released	γ	0.83	section 6.2	0.60/0.98	1.00/1.00
	Minimum landing size	MLS	70mm	chapter 5	–	–
	Population egg production	E	On the basis of exposure and MLS	chapter 5	–	–
	Population sperm production	S	On the basis of exposure and MLS	chapter 5	–	–
Fertilisation	Bimolecular rate constant	β	2.0×10^{-15}	section 4.4	$1/4 \times 10^{15}$	0.97/0.89
model	Proportion eggs viable	α	0.88	section 4.4	0.83/0.96	0.93/0.96
Model	Run time	T	18000s	section 6.2	–	–
conditions	Time step	t	10s	section 6.2	–	–

gle. To ease interpretation of the sensitivity analysis the volume of the surf zone was also itself directly manipulated. For the graphical analysis of covaried parameters the surf-zone volume was directly manipulated rather than varying the parameters that define the surf zone volume (Figure 6.1).

The model is sensitive to variation in all parameters. The degree of sensitivity varied between parameters (Table 6.1). The model shows a non-linear response to variations in some parameter values: this is particularly evident for the mixed volume of the surf-zone (Figure 6.1.B) and highlights the importance of conducting sensitivity testing across the full range of possible parameter values.

The model shows greater sensitivity to variation in the physical parameters defining the size and mixing in the model than the biological parameters defining rate and proportion of gamete release and the fertilisation model parameters. The single parameter analysis and covaried parameter analysis both indicate that the model is

insensitive to the duration of gamete release. Figure 6.1.B shows that the model is, however, particularly sensitive to combinations of surf-zone residence time and mixed volume. For the analysis these parameters were covaried independently, however in reality there is likely to be a positive relationship between mixed volume and residence time (Denny et al., 1992).

The sensitivity analysis indicates that the model is particularly sensitive to the physical parameters defining the model, and that considerable uncertainty exists in the selection of appropriate parameter values for the physical parameters. This indicates that the main priority for further research is to improve knowledge of the hydrodynamics of the surf-zone rather than the biology of limpet spawning. However this does not deny that further research into the biology of limpet spawning is necessary to improve the accuracy of the model.

6.3.2 Simulation runs

To address the aims of this study a variety of model runs were conducted. The model was run for the populations at each level of wave exposure to see the total proportion of eggs fertilised. The simulated fishery was applied to the populations and in each case the model was re-run and the proportion of eggs fertilised and total zygote production calculated. As in chapter 5 the reduction in population SSB and the available fishery yield was calculated for each simulation run, this enabled a comparison of the relative variation in population zygote production, population egg production, SSB and yield as simulated fishery pressure was increasingly applied. The model was also run to simulate the effects of managing on the basis of minimum landing size and\or MPAs.

6.4 Model Predictions

The model predicts that zygote production per meter of coastline, and fertilisation success vary according to exposure level (Table 6.2). The sheltered site had the highest level of zygote production. The intermediate and exposed sites only produce 39% and 20% of the sheltered site's zygote production respectively, although

maximum fertilisation success only varied by 1% between sites.

Table 6.2: Results from the zygote production model. All data normalised per m coastline.

Transect	Wave Exposure	Egg Production m^{-1}	Sperm Production m^{-1}	Fertilisation Success	Zygote Production m^{-1}
A	Low	4.34×10^7	8.00×10^{12}	6.38%	2.29×10^6
B	Medium	1.86×10^7	7.05×10^{12}	5.66%	8.83×10^5
C	High	9.69×10^6	7.01×10^{12}	5.63%	4.52×10^5

Zygote production declines with declining MLS when the populations are exposed to the simulated fishery (Figure 6.2). The MLS at which zygote production begins to decline varies between populations at different levels of wave exposure. This is due to the different maximum size of individuals between populations.

The model predictions for the effect of exploitation on fertilisation success is presented both in terms of MLS (Figure 6.3) and in terms of the proportional reduction in SSB (Figure 6.4). As the MLS is reduced the fertilisation success declines first for the sheltered site, then the medium site and finally the exposed site. The three different populations show a far more uniform response in terms of the decline in fertilisation success as SSB is reduced.

The model predictions of proportional decline in larval production per meter coastline under varying management conditions are shown in Figure 6.5. For the special case where the fished areas are completely fished out (i.e. MLS = 10mm), the proportion of the zygote production protected is identical to the proportion of the area protected. Zygote production declines across the full range of MLSs considered for the sheltered population. For the medium and exposed populations zygote production only declines across a subset of the MLSs, as the whole population is protected by MLSs greater than 50 and 47 mm for the medium and exposed populations respectively.

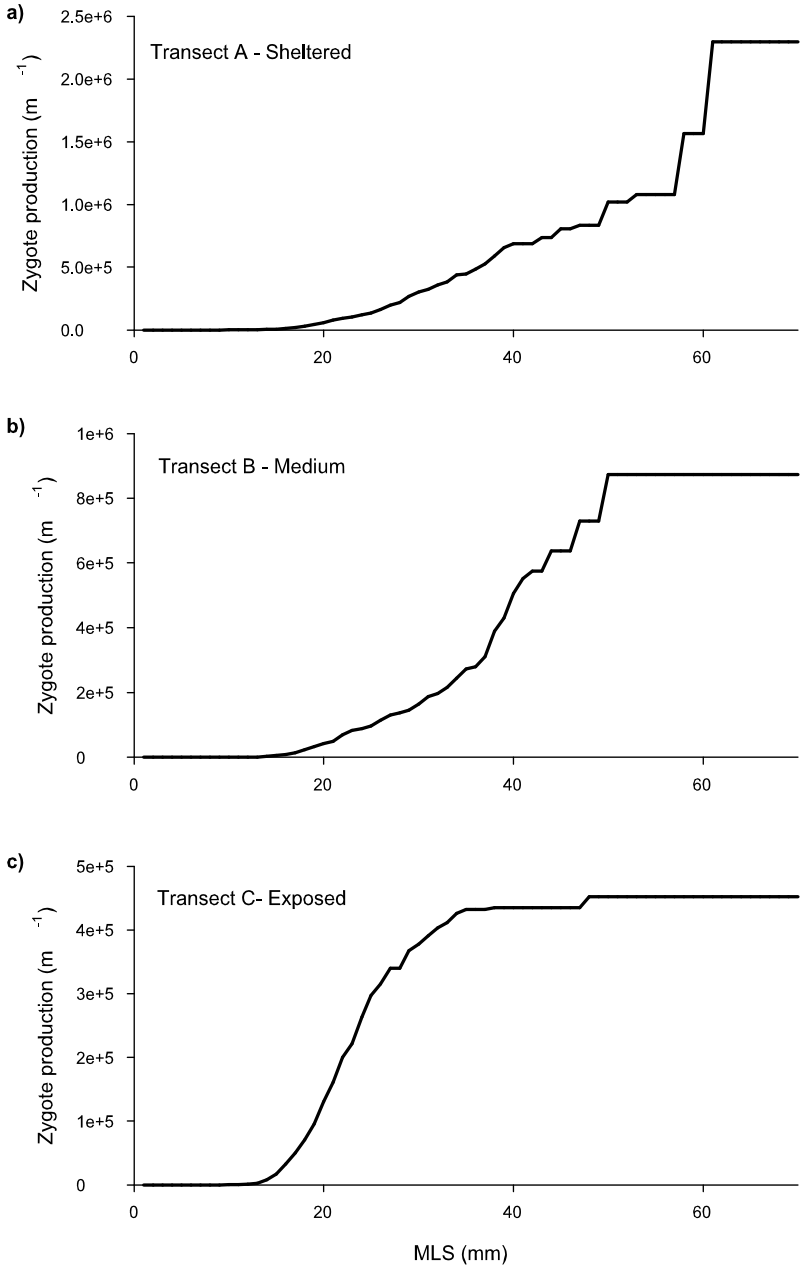

Figure 6.2: Model predictions of zygote production per meter coastline for a) sheltered, b) medium, and c) exposed populations. Note, the y axes are scaled differently.

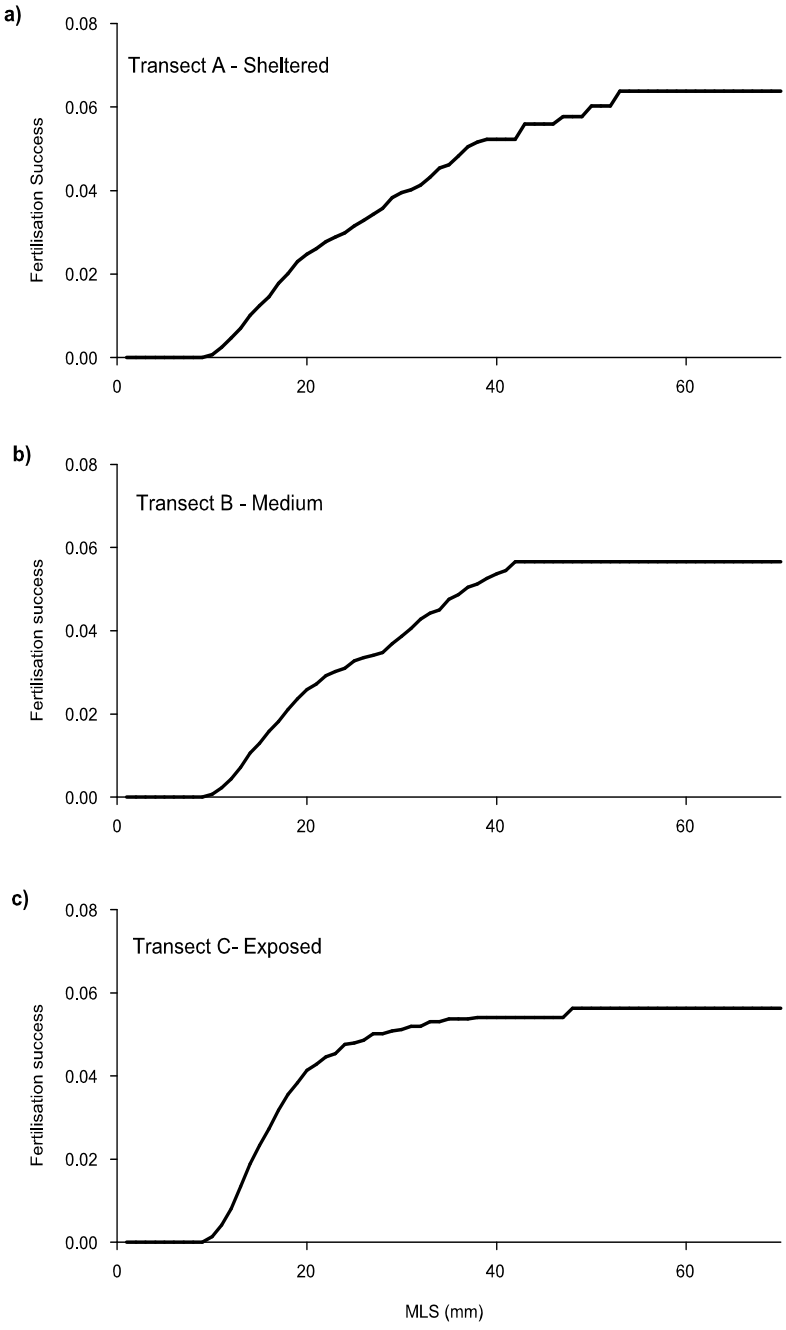

Figure 6.3: Model predictions of the overall proportion of eggs fertilised for a given MLS for a) sheltered, b) medium, and c) exposed populations.

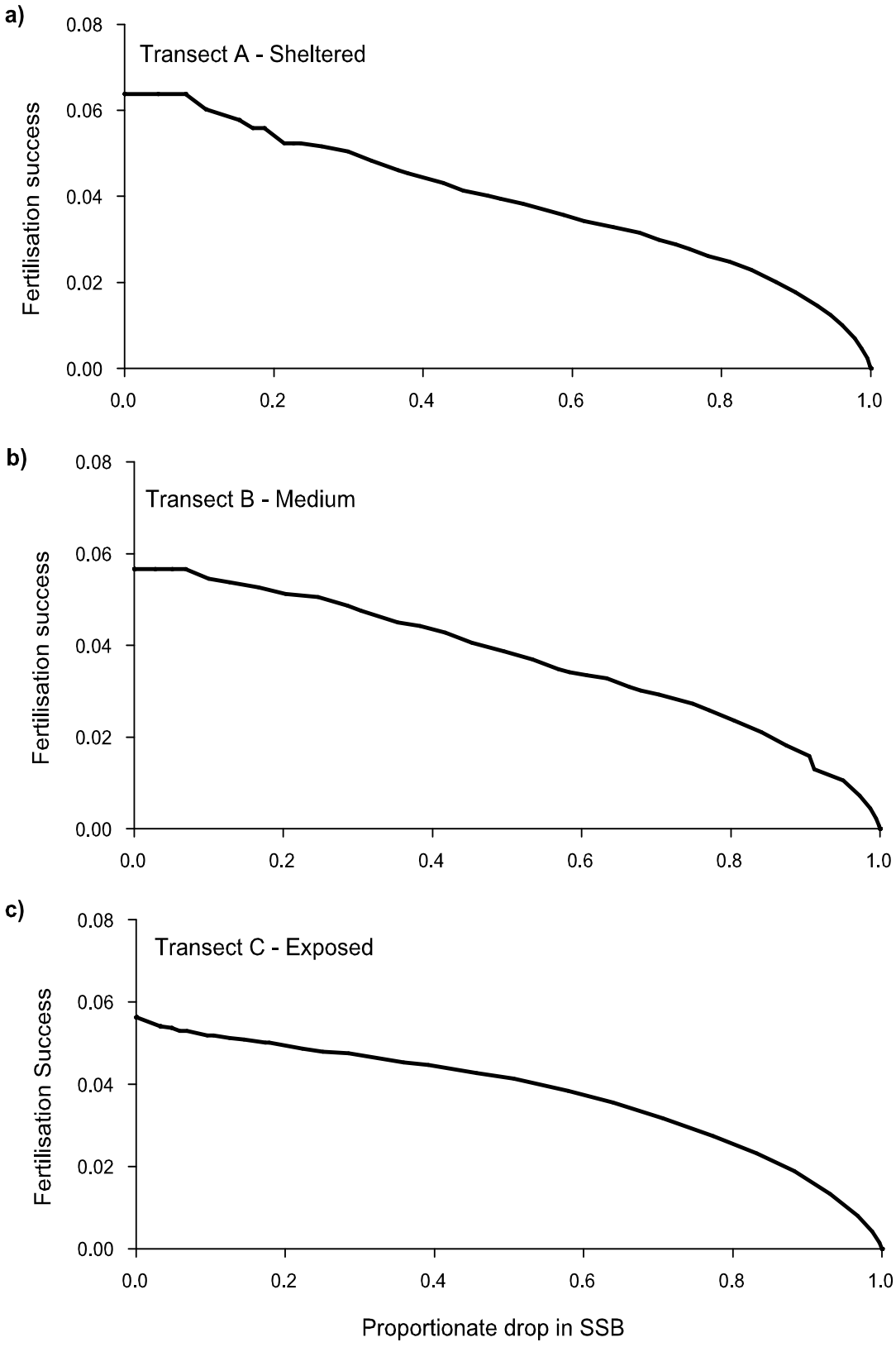

Figure 6.4: Model predictions of the overall proportion of eggs fertilised for a given proportion of the unexploited level of SSB for a) sheltered, b) medium, and c) exposed populations.

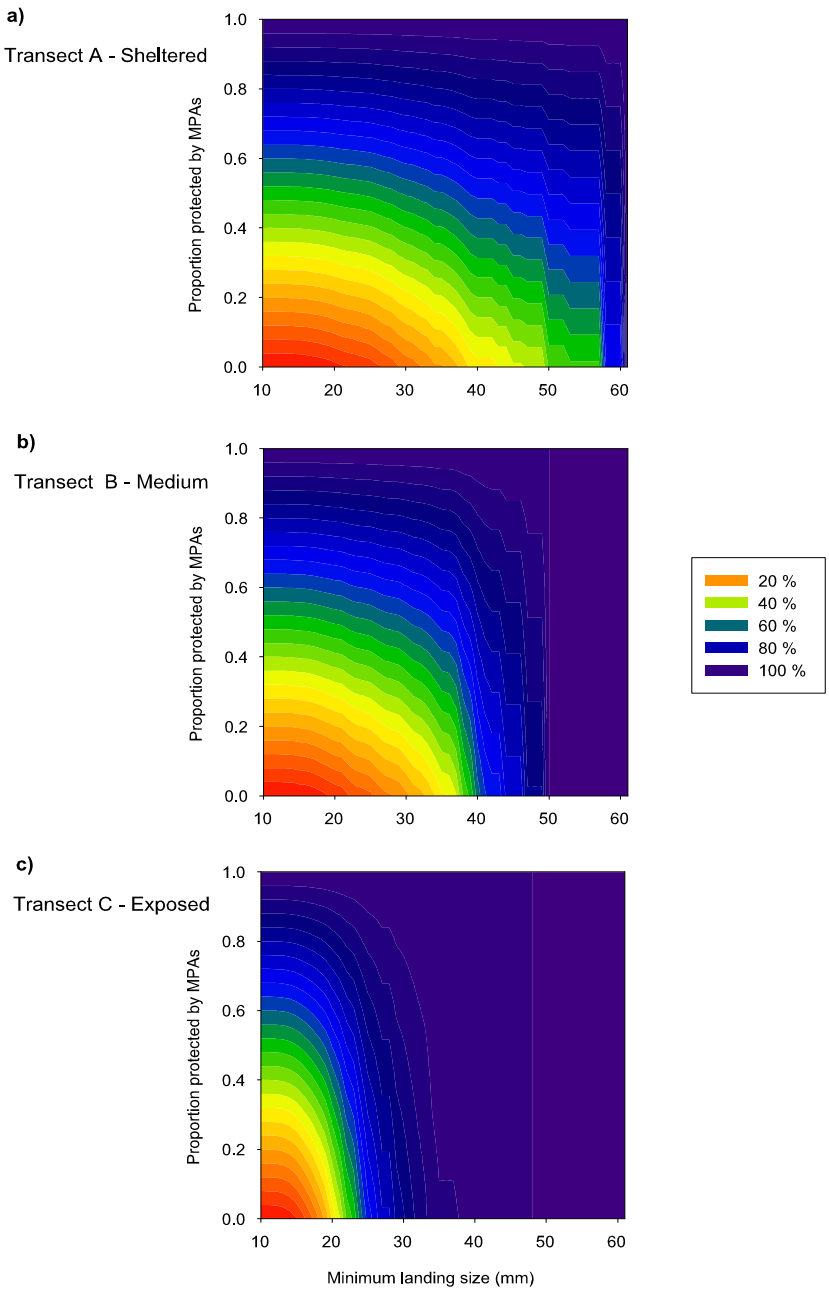

Figure 6.5: Effect of differing management regimes on the proportion of population zygote production conserved for three different limpet populations from across an exposure gradient. For co-management by MPA and MLS, the MLS applies to the section of the population not protected by MPAs.

6.5 Discussion

6.5.1 Is sperm limiting to zygote production?

According to the model predictions sperm is limiting to zygote production under all conditions considered by the model. The maximum fertilisation success achieved under the default model settings is 6.4%. However, as is clear from figure 6.1 the model predictions of fertilisation success are sensitive to the mixed volume of the surf zone and the surf zone half life. The dispersal submodel used relies on simplifying assumptions about surf zone hydrodynamics, whereas empirical studies of surf zone hydrodynamics paint a more complex picture of the likely dispersal of gametes in the surf zone (Denny et al., 1992). It is likely that there are transitory and permanently semi-contained water masses within the surf zone of a rocky shore which can accumulate gametes leading to higher sperm concentrations than achieved in the model for the default physical parameter settings (see section 3.5.2). This would lead to locally elevated levels of fertilisation success which can lead to a significant overall increase in fertilisation success (Denny et al., 1992). However, although the absolute levels of fertilisation success predicted by the model may be inaccurate the model will provide more accurate predictions if the relative change in fertilisation success between populations, and as populations are exploited.

Not only is sperm limiting to zygote production for unexploited populations, the model predicts that sperm becomes increasingly limiting to zygote production as populations are exploited (Figure 6.3). As a population is exploited not only is fertilisation success reduced, but as discussed in chapter 5, population egg production is also reduced. To examine which has a greater effect on zygote production the proportional decrease in egg production and zygote production per MLS are compared in figure 6.6. It is clear from figure 6.6 that the reduction in egg production (M) accounts for most of the decline in zygote production (L) , and on the whole the decline in fertilisation efficiency (N) only accounts for a small fraction of the total decline in zygote production.

To further examine the relative affects of reduced fertilisation success and reduced egg production on the reduction in zygote production, the proportion of the total

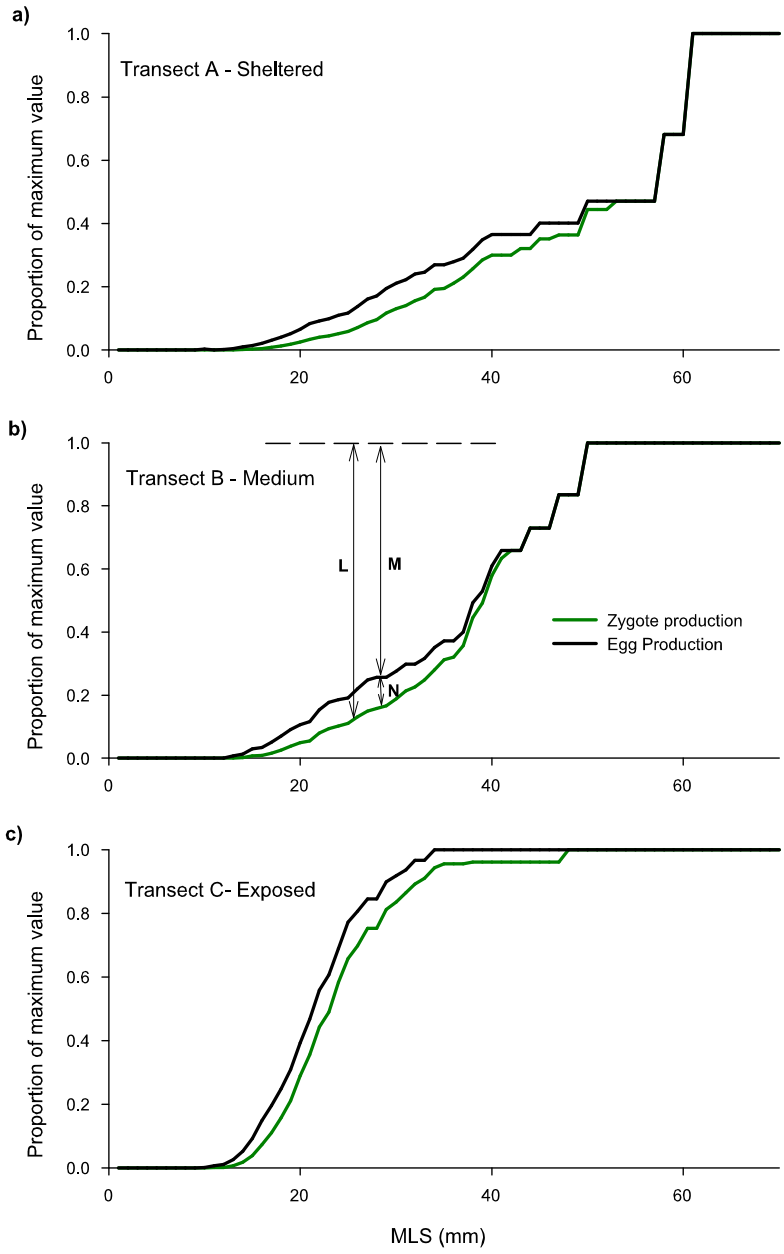

Figure 6.6: The proportional decline in population egg and zygote production per MLS for the for a) sheltered, b) medium, and c) exposed populations. L indicates the full decline in zygote production, M indicates the decline in zygote production due to reduced egg production, and N indicates the decline due to reduced fertilisation success.

decline in zygote production attributable to reduced fertilisation success as SSB is reduced by exploitation was calculated (Figure 6.7). The proportion of the decline in zygote production attributable to the decline in fertilisation success (N) was calculated according to the relationship;

$$N = (L - M)/L \tag{6.4}$$

where L is the proportionate decline in zygote production, and M is the proportionate decline in egg production (Figure 6.6)

For the two most sheltered populations (A and B) reduced fertilisation success never accounted for more than 12% of the total decline in zygote production. For the exposed population(C) reduced fertilisation success accounted for all the reduction in zygote production at low levels of exploitation, due to the presence of large males in the population (Figure 5.4). At higher levels of exploitation, when SSB is reduced to less than half its unexploited value, reduced fertilisation success accounts for less than a fifth of the total decline in population zygote production for the exposed population.

There are two factors underlying this affect. Firstly, due to the protandrous nature of *P. vulgata*, females are concentrated in the larger size classes and males in the smaller size classes. Thus, as a size selective fishery exploits a *P. vulgata* population, females will be selectively removed from the population first, so that population egg production will be more heavily impacted than population sperm production (Figure 5.8). Secondly, over the range of sperm concentrations to which sperm is sensitive, an order of magnitude reduction in sperm concentration only leads to approximately a 50% reduction in fertilisation success (Figure 4.2a.). If it is temporarily assumed that gamete production is linearly related to SSB, then as SSB is reduced by an order of magnitude the fertilisation success will halve, but egg production will be reduced by an order of magnitude. Thus, on a population level, as the population is reduced due to exploitation the affect of egg production on zygote production will be greater than the affect of fertilisation success on zygote production.

This result is inevitable assuming that the sensitivity parameter, S, describing the response of fertilisation success to changing sperm concentration is less than 1, unless the population sex ratio is skewed such that males are removed from the population

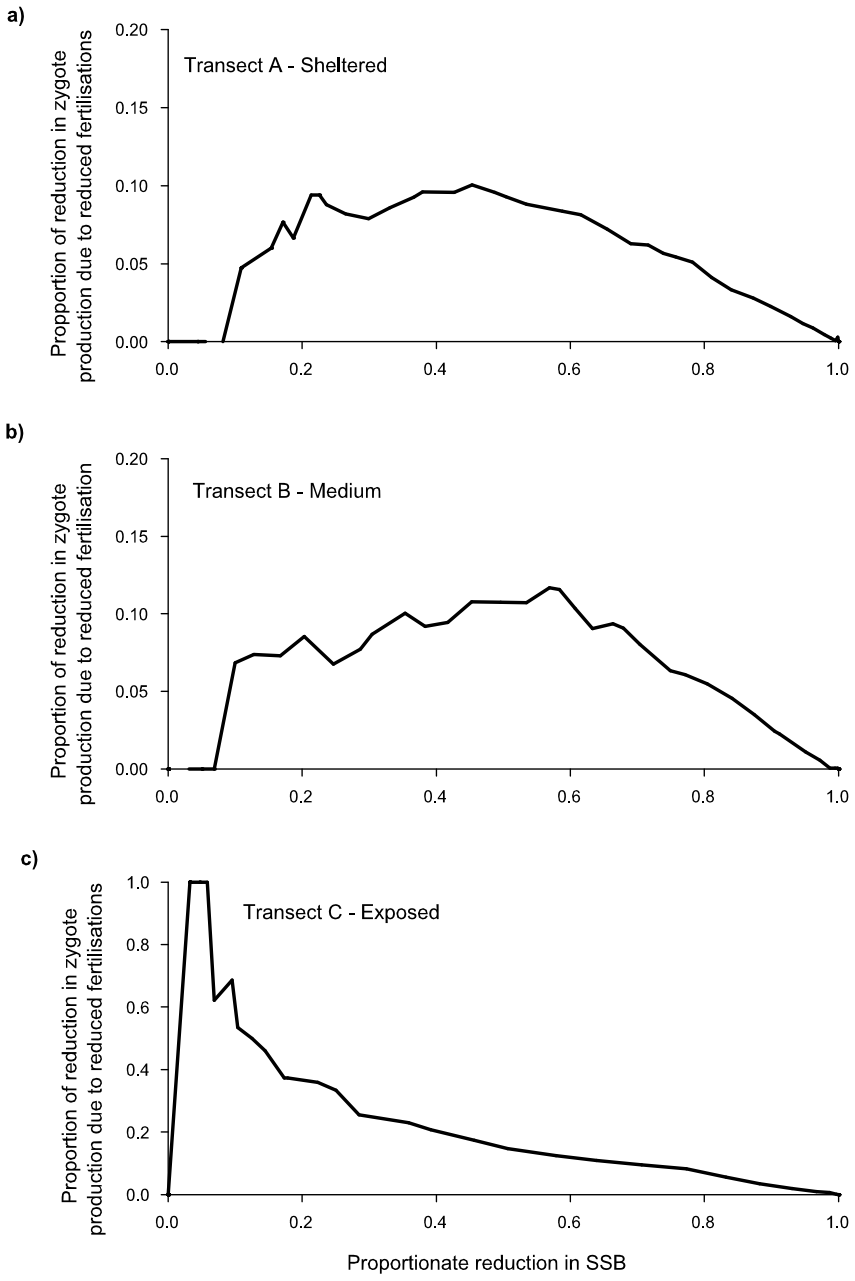

Figure 6.7: The proportion of the reduction in zygote production attributable to reduced fertilisation success as SSB is reduced due to exploitation for the for a) sheltered, b) medium, and c) exposed populations.

much faster than females. In the case of a size selective fishery for protandrous hermaphrodites the situation is reversed and females are removed more rapidly, thus the reduction in population egg production will have a more dominant effect on zygote production than reduced fertilisation success.

Is sperm limitation likely to have significant effects on limpet population dynamics? The main concern in relation to sperm limitation is that it is an example of an Allee effect, leading to increased decline in reproductive output with reduced numbers (Levitan and Petersen, 1995; Levitan and Sewell, 1998; Yund, 2000). Much of the early work that highlighted the potential risk associated with sperm limitation (Denny and Shibata, 1989; Levitan et al., 1991; Pennington, 1985) only considered reduced fertilisation success, and did not consider this effect in relation to the decline in population egg production. However figures 6.8 and 6.7 indicate that reduced egg production is the main cause of reduced reproductive output, even at low population numbers for the situation examined. Previous models have found sperm limitation to be responsible for a larger proportion of the decline in zygote production (Lundquist and Botsford, 2004; Meidel and Scheibling, 2001). That sperm limitation plays a lesser role in limiting population dynamics in this example is partially as a result of protandry in limpets emphasising the affect of decreased population egg production. It is also partially due to differences in the sperm dispersal models used compared to previous models (e.g. Lundquist and Botsford, 2004; Meidel and Scheibling, 2001).

However figure 6.4 illustrates that fertilisation success does indeed decline as SSB declines, indicating that an Allee effect does occur. In this case it is only a weak Allee effect over most of the range of population parameters. It is inevitable though that the reduced fertilisation success will affect the populations at very low levels, and may be responsible for the final total collapse in population reproductive output at very low population numbers and density (Roberts and Hawkins, 1999).

A further concern that has been stated in relation to invertebrate fisheries management is that threshold levels may occur, below which fertilisation success declines rapidly leading to precipitous population collapse (Shepherd and Brown, 1993; Tegner et al., 1996). In agreement with the work of Lundquist and Botsford (2004), this work finds that although reduced fertilisation success does lead to a decline

in zygote production there is no evidence of sharp threshold levels in fertilisation success.

It is hard to directly compare the results of this study with previous models of the effect of exploitation on population zygote production(e.g. Claereboudt, 1999; Lundquist and Botsford, 2004; Meidel and Scheibling, 2001; Quinn et al., 1993) as the different models use different population parameters to measure the effect of exploitation (e.g. population density, population numbers), the effects of simulated exploitation are applied differently and the underlying population models differ. However my study is the first to directly model how population zygote production, population egg production and fertilisation success are related to SSB as simulated fishery pressure is applied. This model indicates that reduced population egg production is the main determinant of reproductive output and that reduced fertilisation success only plays a minor role in modulating this effect with little implication for fisheries managers, although it should be noted that in this example the effect of declining egg production is exacerbated because of the protandrous nature of *P. vulgata*.

6.5.2 Does sperm limitation affect the relationship between zygote production, SSB and fishery yield?

As discussed above, SSB is commonly used in fisheries management on the assumption that SSB is directly related to reproductive output. In chapter 5 the relationship between egg production and SSB was discussed and it was shown that measures of SSB can underestimate declines in reproductive output due to exploitation. To see if sperm limitation leads to further disparity between SSB and reproductive output the relationship between SSB, population egg production and population zygote production were examined.

Under the default parameter settings the model predicts that the maximum fertilisation rate for the unexploited populations are 6.4%, 5.7% and 5.6% for the sheltered, medium and exposed populations respectively. This indicates that measures of egg production will overestimate reproductive output by more than an order of magnitude, similarly attempts to derive an absolute measure of reproductive output on

the basis of SSB would also lead to order of magnitude overestimates of reproductive output. However, SSB is more commonly used as a relative measure of reproductive output, and would thus not be affected by constant systematic error. What is of more interest therefore is how variation in fertilisation success affects the relationship between SSB and reproductive output.

Figure 6.8 shows the proportionate drop in zygote production, egg production and female sex-specific SSB as SSB declines due to simulated fishery pressure. This illustrates that reduced fertilisation success leads to a slightly greater disparity between SSB and reproductive output (v), than that caused by the disparity between SSB and egg production (x). The extent of the further disparity between SSB and reproductive output caused by reduced fertilisation success (w) is the difference between the proportional drop in egg production and the proportional drop in zygote production.

As previously noted, the protandrous nature of *P. vulgata* means that there is a greater disparity between SSB and egg production than would be expected for a gonochronistic species. The effect of protandry can be disregarded by comparing the decline in egg production and zygote production with female sex-specific SSB rather than population SSB. The relationship between egg production, zygote production and female sex-specific SSB varies between populations across an exposure gradient. In the case of the exposed population, for heavy fishery pressure the proportional drop in female SSB corresponds more closely with the drop in zygote production than egg production. This contrasts with the case when population SSB is used, where in all situations, apart for light fishery pressure being applied to the exposed population, SSB provides a worse estimate for zygote production than provided by measures of egg production.

6.5.3 Are SSB and egg production good proxies for population zygote production?

The proportion of the population zygote production that would be conserved if a MLS was set to conserve the arbitrary level of 30% of the unexploited reproductive output (Mace and Sissenwine, 1993; Thompson, 1993) on the basis of SSB, female

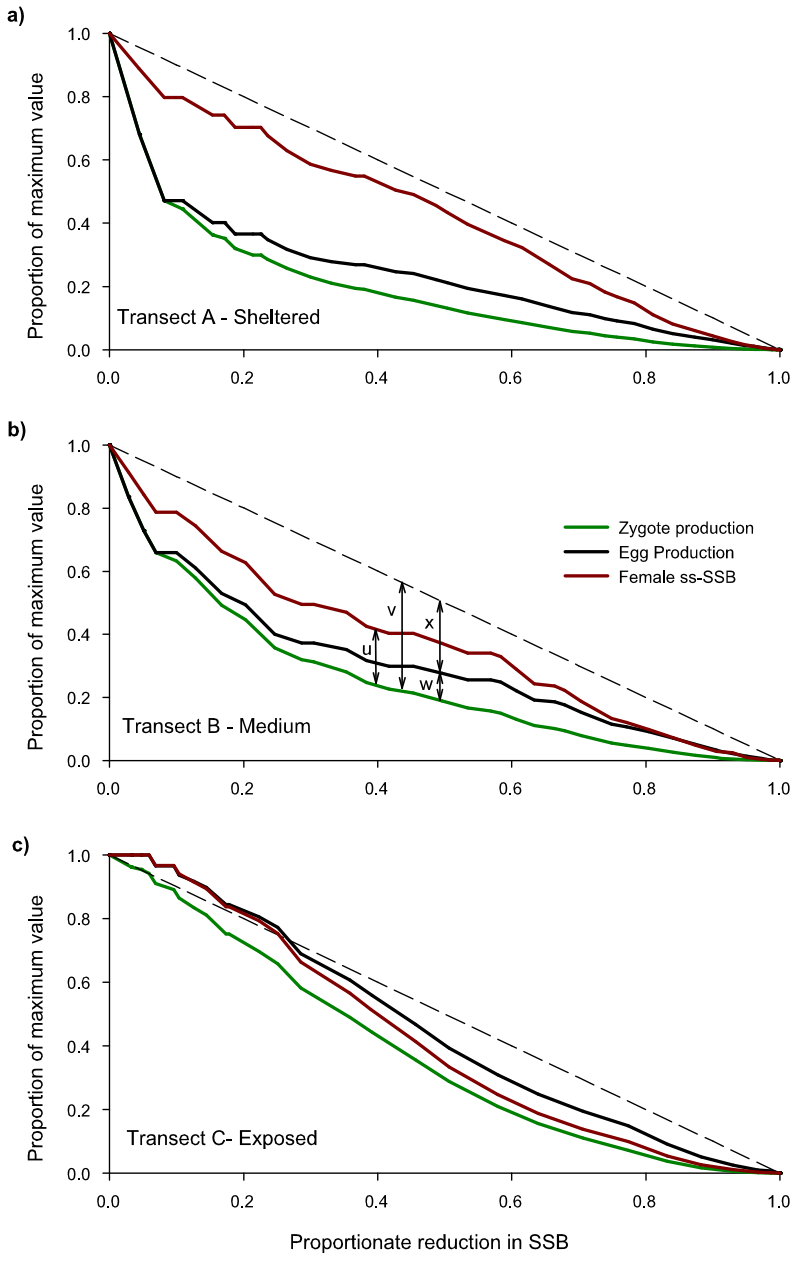

Figure 6.8: The proportionate reduction in female sex-specific SSB, population egg production and population zygote production against reduction in SSB for a) sheltered, b) medium, and c) exposed populations. V indicates the full reduction, W indicates the reduction due to reduced fertilisation success and X the reduction due to reduced population egg production. U indicates the disparity between female SSB and zygote production.

sex-specific SSB, egg production or zygote production is presented in table 6.3. Attempts to conserve 30% of the reproductive output on the basis of SSB would only conserve 6%, 18% and 16% of the zygote production for the exposed, medium and sheltered populations respectively. This demonstrates that attempts to control reproductive output at a given level on the basis of SSB can grossly underestimate the proportion of reproductive output conserved, which may lead to a subsequent failure of management efforts.

If sex-specific female fecundity is used as a proxy for reproductive effort, thereby discounting the affects of protandry, the level of zygote production conserved was still underestimated, although the extent of this underestimate varies greatly between populations. For the sheltered and medium populations there was a shortfall of 21% and 15% respectively in the amount of zygote production protected. Contrastingly in the case of the exposed population the use of female SSB lead to only a 1% shortfall in the level of zygote production protected; thus at this level of fishery pressure sperm limitation has little effect on the decline in reproductive output. The implications for fishery managers is that sperm limitation can be a significant factor in decline of reproductive effort as a result of exploitation, although there may be circumstance where sperm limitation has little effect on the reproductive output of an exploited broadcast spawner.

The effects of protandry and variable fertilisation success on the use of proxies for reproductive effort for limpet, and wider invertebrate fisheries management shall be discussed more fully in chapter 8.

Table 6.3: The different MLS limits that would need to be applied to conserve various proxies for reproductive effort at 30% of their un-exploited level. The actual proportion of zygote production that would be conserved by the MLS limit is in brackets.

	SSB	Female SSB	Egg production	Zygote production
Transect A - Sheltered	25 mm (6%)	27 mm (9%)	38 mm (26%)	43 mm (32%)
Transect B - Medium	23 mm (18%)	27 mm (15%)	31 mm (21%)	35 mm (31%)
Transect C - Exposed	18 mm (16%)	20 mm (29%)	19 mm (21%)	21 mm (36%)

6.5.4 In order to conserve zygote production whilst maximising yield, is the population best managed by MLS or MPAs?

In figure 6.9 the proportionate decline in zygote production averaged across the full exploited range of the species for the spectra of management combinations (Figure 6.5) is overlaid on the plot of the proportion of the yield accessible to the fishery under the same spectra of management options (Figure 5.9 d-f). In figure 6.9 the colour contour plot represents the proportion of the total biomass of the population that is available as yield under the given combination of management conditions. The black contour lines indicate constant levels of larval production, as a percentage of the unexploited level of larval production, that occur for given combinations of management conditions. Thus if it is chosen to manage the population so as to protect a given proportion of the unexploited larval production, the management conditions that will maximise yield can be chosen by following the appropriate contour of larval production to see which management conditions maximise yield.

Figure 6.9 illustrates that when a previously unexploited population is exposed to fishery pressure yield is maximised, whilst protecting a given level of reproductive output, by managing on the basis of MPAs rather than MLS. This affect is more accentuated than when egg production was used as a proxy for reproductive output rather than zygote production (c.f. Figure 5.14). The wider implications of this to fisheries managers, and the wider ecosystem affects of MPAs, will be discussed in chapter 8.

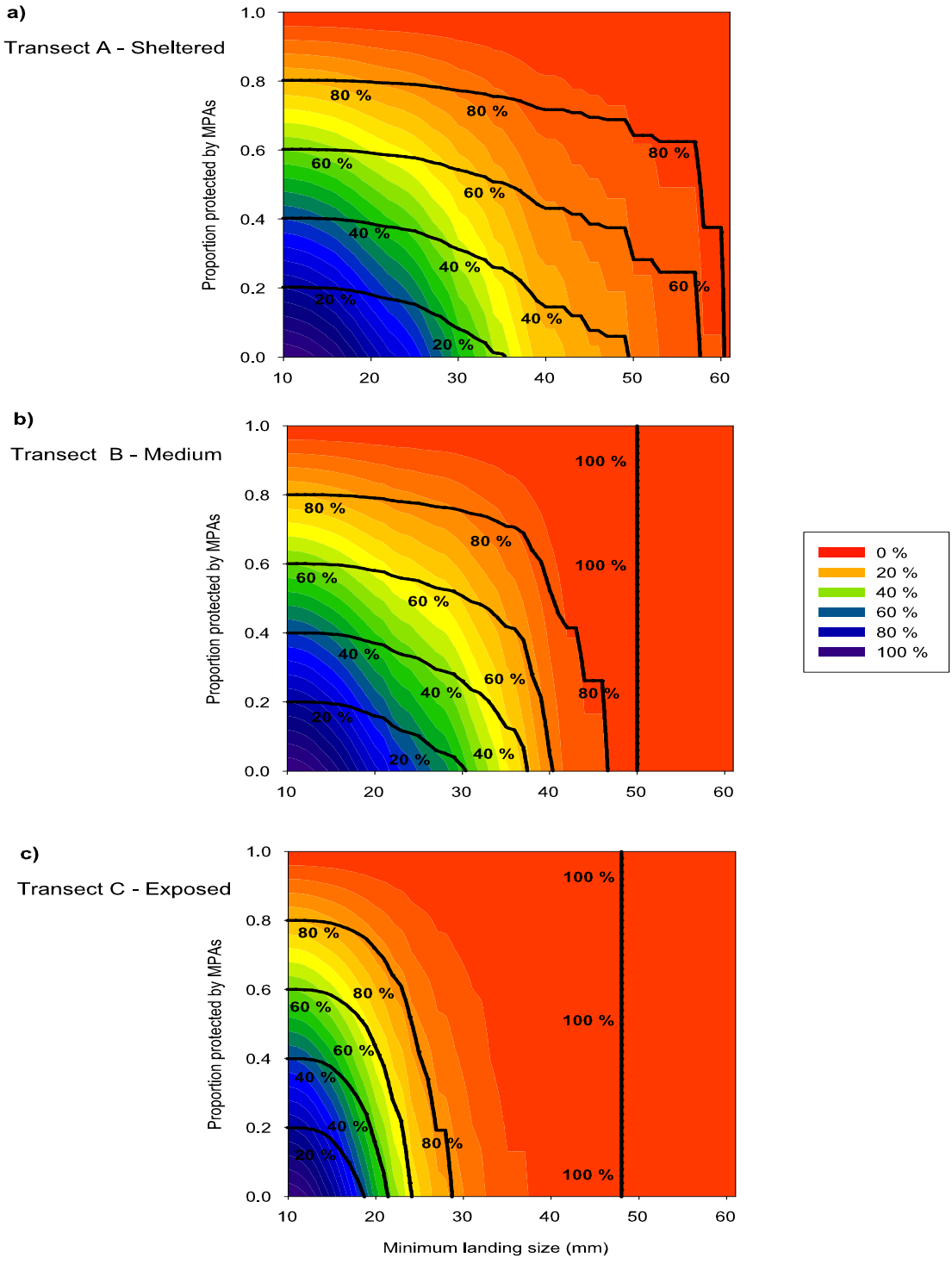

Figure 6.9: The proportion of larval production conserved and yield taken for all combinations of management scenarios. The black contour lines represent the percentage of larval production protected, whilst the colour bar represents the percentage of yield accessible to the fishery under the given management scenario

Chapter 7

Population Genetic Differentiation of *P. candei* in the Azores Archipelago: Management Implications for Stock Identification and Marine Reserve Placement

7.1 Introduction

Attempts to answer the question of how far larvae disperse have been hampered by difficulties in following individual larvae from fertilisation to settlement, and except in exceptional circumstances (e.g. Willis and Oliver, 1990) it is practically impossible. Therefore a variety of genetic methods have been developed to indirectly infer the extent of larval dispersal.

Genetic methods of examining population structure initially generated much excitement as offering a way of turning a light on previously unobservable processes in population dynamics (Palumbi, 1995). However, the initial enthusiasm has been

tempered by the realities of what genetic techniques actually tell us and the limitations to the inferences that can be drawn (Gauldie, 1991; Slatkin, 1987; Waples, 1998). Still, when dealing with larvae which are impractical to tag or directly follow, genetic studies provide one of the only methods to estimate the extent of exchange between populations, and hence the spatial scale of larval dispersal.

Genetic studies of population structure are based on the spatial distribution of allelic or haplotype frequencies, or other marker of genetic mixing, across a species' range (Palumbi, 2003). However patterns of genetic distribution do not reveal how much gene flow is occurring or has occurred (Slatkin, 1987); instead gene flow is inferred by modelling possible scenarios to explain how much larval interchange must have occurred to generate the patterns of genetic differentiation that are observed.

There are two main areas of concern with relation to genetic population studies, firstly that genetic studies are more sensitive to processes that occur over evolutionary timescales than demographic processes of interest to a fisheries manager (Slatkin, 1985); and secondly that the assumptions necessary for many of the models used to explain patterns of genetic diversity are regularly broken with possibly significant implications to the interpretation of the results (Waples, 1998).

It has been noted by several authors that the exchange of only a small proportion of migrants per generation between populations can lead to very similar genetic structure being observed in the different populations (Gauldie, 1991; Maruyama, 1971; Palumbi, 2003; Slatkin, 1985). Therefore seemingly intermixed populations may in fact be demographically separate, with the practical implication that a population identified as a single stock for management purposes may be made up of a number of separate component stocks. This does have the converse implication that when significant genetic structure is found, high confidence can be placed in the interpretation that there is no demographically significant exchange of migrants between populations (Palumbi, 2001).

The F_{ST} value, first proposed by Wright (1943), is commonly used as a measure of population differentiation (Palumbi, 1995). F_{ST} values are standardly calculated on the basis of the departure of homozygous allele frequency from panmictic expectations (Wright, 1943). The Φ_{ST} value is an analogous measure of population differentiation derived from haplotypic data (Weir and Cockerham, 1984) that has

been formally proved to be identical to the F_{ST} statistic (Michalakis and Excoffier, 1996). I will therefore refer to the Φ_{ST} statistic as the F_{ST} statistic. In general F_{ST} values of 0 - 0.05 represent very little genetic differentiation between populations; 0.05 - 0.15 indicates moderate differentiation; 0.15 - 0.25 indicates great differentiation; with over 0.25 indicating very large genetic differentiation between populations Hartl and Clark (1997).

The very low level of genetic separation that would be expected from a spatially structured population with demographically significant larval exchange would probably yield a F_{ST} of $<< 0.01$ (Palumbi, 2003), yet an artifact of calculating F_{ST} values on the basis of a subsample of a population rather than the whole population is expected to generate an error in the F_{ST} value of > 0.01 (Waples, 1998) unless over 100 individuals are sampled per subpopulation. In marine studies samples of over 50 individuals per subpopulation are uncommon, and samples of several hundred individuals per subpopulation would ideally be taken to confidently identify demographically significant population structure. Therefore in this study, where possible, over 100 individuals were sampled per site to try and increase the resolution of estimates of F_{ST}.

Another area of concern for genetic population studies is the assumptions required to interpret genetic results (see Waples, 1998, for review). For any interpretation of patterns of genetic diversity one of the main assumptions is that the genetic structure has achieved equilibrium between genetic drift and migration, following a perturbation to the population it can take 10s to 100s of generation to attain the new equilibrium. F_{st} values are commonly used to make assumptions about the number of migrants per generation on the basis of the simplified formula of Wright (1943);

$$F_{ST} \approx \frac{1}{1 + 4mN_e} \tag{7.1}$$

where N_e is the effective population size and m is the proportion of the population per generation that are migrants. However any F_{st} value can similarly be explained by a scenario where a previously fully mixed population has become split into two separate components. The model calculating how F_{ST} evolves over a number of

generation times (g) for a given N_e can generate any F_{ST} value with an infinite combination of the g and N_e parameters (Waples, 1998). This is merely one example of how breaking the assumptions may lead to incorrect interpretation of the data.

The above problems with genetic approaches to genetic studies do not invalidate their use in population studies. The alternative direct methods of examining larval dispersal are similarly beset with flaws and limitations, and it is only by combining information from a variety of studies that a more accurate picture of larval dispersal will appear.

Direct methods of measuring larval dispersal are based on observations of larval behaviour, planktonic larval duration, plankton sampling, recruitment studies, current data and modelling (Black et al., 1990; Palumbi, 1995). The main problem besetting direct observations of dispersal holds for terrestrial as well as marine studies, and that is the limited temporal and spatial nature of observations (Slatkin, 1985). Dispersal studies rarely last for more than a few generations, if that, and can miss unusual but demographically important dispersal events. Similarly unusual or unexpected dispersal beyond the spatial coverage of the observations will also be missed. Furthermore observations that an organism is capable of a certain range of dispersal does not mean that demographically significant dispersal actually occurs over that distance. Both terrestrial (Ehrlich and Raven, 1969) and marine (Knowlton and Keller, 1986; Slatkin, 1985) studies have found that genetic estimates of gene flow occur over much shorter distances than individuals are capable of moving. This highlights one of the main strengths of genetic studies that they integrate the effects of dispersal over time and space and can provide a synoptic view of population interactions.

7.1.1 Aims and Approach

In this study larval exchange by *P. candei* between and within islands and island groups of the Azores archipelago was studied by examining genetic differentiation between and within populations. Thus I attempted to determine the scale at which populations operate within the archipelago to elucidate the appropriate scale for management of limpet populations within Azores. This should also inform the ap-

propriate size and spacing of any future MPA networks set up in the Azores.

A molecular genetic approach was used to determine the scale of larval dispersal. A fragment of the mitochondrial DNA cytochrome oxidase I gene was chosen as the genetic marker for this study. Mitochondrial genes are considered appropriate for population genetic studies (Avise et al., 1987; Bechenbach et al., 1993; Boore, 1999) and the COI gene in particular has been widely used for population and phylogenetic studies (e.g. Gomez and Carvalho, 2003; Hughes et al., 2004; Marko, 2004). The COI gene contains high levels of genetic variability in molluscs (Hoeh et al., 1997) enabling it to reveal population differentiation over small geographic scales (Bechenbach et al., 1993; Benson et al., 2001), thus it was chosen as a suitable marker for this study.

Genetic variability was assessed by single strand confirmation polymorphism (SSCP) in conjunction with DNA sequencing. SSCP is a simple, inexpensive and sensitive method for detecting differences between DNA sequences (Hayashi, 1999; Sunnucks et al., 2000). Once haplotypes have been identified by SSCP, individual examples of each haplotype can be sequenced to provide information on the exact differences between sequences. Due to its practical advantages the SSCP technique has been widely used in studies of population biology (e.g. Apte and Gardner, 2002; Kyle and Boulding, 2000; Small and Gosling, 2000, 2001).

7.2 Methods

7.2.1 Sample collection

A spatially hierarchical sampling strategy was used to assess genetic differentiation in *P. candei* across the Azores archipelago. The sampling strategy was designed to examine genetic differentiation; i) between island groups, ii) between islands within an island group, iii) between sites within an island, and iv) within sites. Limpets were collected from the intertidal and shallow subtidal during July 2003 from 16 locations across six islands covering each of the island groups (Figure 7.1, and Table 7.1). At each sampling site three separate replicates of 30-50 individuals were collected from non-overlapping consecutive stretches of coastline to examine within site variation.

All samples were stored in molecular grade ethanol prior to DNA extraction. The nomenclature used duringing following discussions is as follows; replicates refer to the individual replicates collected from within a site. A site refers to all the replicates pooled within that site. Islands and island groups refer to the samples from the designated island or island group.

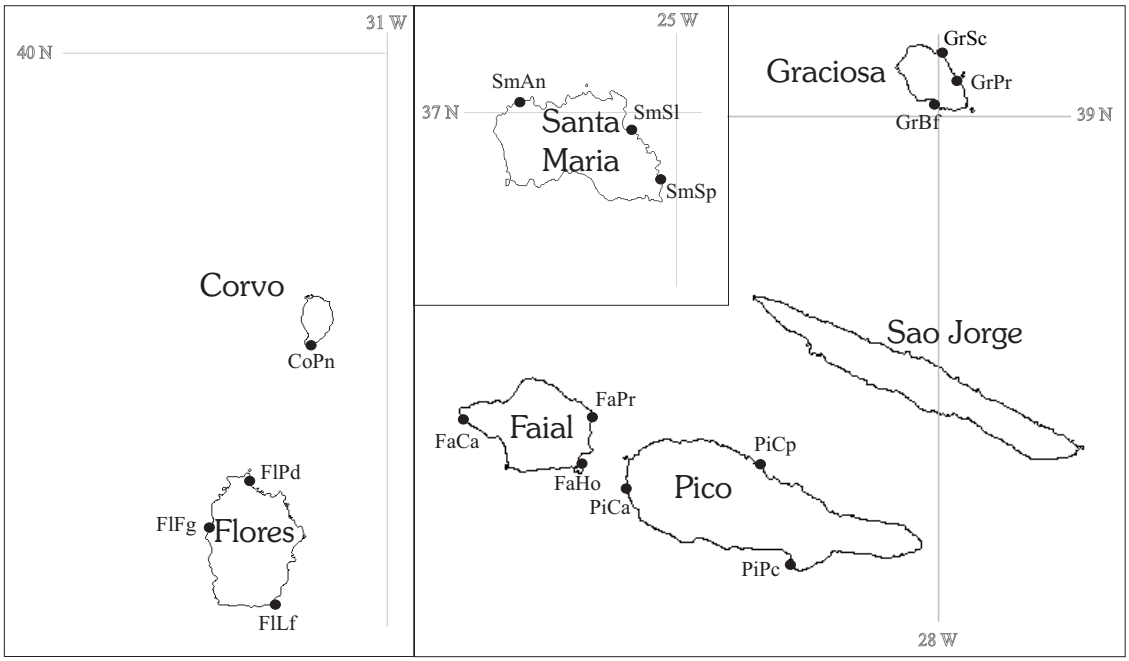

Figure 7.1: Sampling locations of *P. candei* from the Azores archipelago. See Table 7.1 for full location names

7.2.2 Sample analysis

DNA was extracted from a 1 mm^3 section of foot muscle from each individual using a modification of the cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle and Doyle, 1987; Preston, 2003). Following extraction a 226 bp fragment of the mitochondrial COI gene was amplified by PCR using 5'-TTAATTCGAGCCGAA CTTGG-3' and 5'-AGAAACAAGGAAGGGGGAAG-3' as the forward and reverse primers respectively. The haplotype mobility of the amplified gene fragments was analysed by single strand conformational polymorphism (SSCP) analysis (Sunnucks et al., 2000): electrophoresis of denatured PCR product on a polyacrylamide gel. Haplotype frequencies were scored by eye from SSCP banding patterns with reference haplotypes included on each gel. Samples of each haplotype were sequenced

Table 7.1: *P. candei* sampling locations in the Azores with a list of abbreviations used for site names. Latitude and longitude are given as decimal degrees.

Group	Island	Site	Reference	Latitude	Longitude
Occidental	Corvo	Ponta Negra	CoPn	39.668	31.117
	Flores	Ponta Delgada	FlPd	39.518	31.202
		Porto da Faja Grande	FlFg	39.459	31.263
		Lajes das Flores	FlLf	39.375	31.166
Central	Graciosa	Baia da Folga	GrBf	39.018	28.006
		Santa Cruz da Graciosa	GrSc	39.084	27.995
		Praia	GrPr	39.049	27.971
	Faial	Porto da Boca da Riberhina	FaPr	38.591	28.603
		Horta Breakwater	FaHo	38.528	28.624
		Capelinhos Cais	FaCa	38.589	28.830
	Pico	Cais do Pico	PiCp	38.527	28.319
		Ponta da Castelete	PiPc	38.387	28.256
		Calhau	PiCa	38.488	28.545
Oriental	Santa Maria	Sao Lourenco	SmSl	36.987	25.047
		Anjos	SmAn	37.007	25.158
		Senhora dos Prazeres	SmSp	36.944	25.016

to obtain DNA sequence data and to confirm that SSCP banding patterns represented genetic sequence differences. The sequencing was conducted in accordance with manufacturers specifications (ABI Big Dye 3.1) with optimization protocols following Detwiler et al. (2004). Where there was uncertainty in visually ascribing SSCP banding patterns to a haplotype the sample was sequenced to verify the SSCP analysis.

The DNA extraction, amplification and electrophoresis was carried out by myself and co-workers as part of the EUMAR project at the Marine Biological Association laboratories, the DNA sequencing was conducted by M Hall at the Plymouth Marine Laboratory.

7.2.3 Data analysis

Haplotype sequences were edited and aligned using ChromasPro v1.22 sequence editing software and ClustalX sequence alignment software (Thompson et al., 1997).

All primer sites were excluded from the edited sequences before subsequent analysis.

Arlequin ver. 2.000 (Schneider et al., 2000) software for population genetic data analysis was used to calculate:

- proportional haplotype frequency per site
- haplotype diversity (h) per site
- nucleotide diversity (π) per site
- pairwise F_{ST} between all sites, with replicates grouped per site
- pairwise F_{ST} between replicates within each site
- analysis of molecular variance (AMOVA) for the full range of hierarchical clustering; replicates grouped by site, sites grouped by island, and islands grouped by island group
- Tajima's D value (Tajima, 1989b) and probability of significance
- mismatch distributions (Rogers and Harpending, 1992) and the mismatch model parameter τ.

The haplotype diversity, h, is the probability that two randomly chosen individuals from a sample will have different haplotypes. π is the probability that two randomly chosen nucleotides from an individual will be different.

Tajima's D value is a test of neutrality in accordance with the neutral mutation hypothesis (Tajima, 1989b), although it can also indicate that a population has undergone a bottleneck or founder event (Tajima, 1989a). The mismatch distribution is the distribution of pairwise nucleotide differences between all individuals within a breeding population (Rogers and Harpending, 1992), based upon the idea that the average number of pairwise differences will increase over time after initial population expansion. τ is a measure of time since population expansion according to;

$$\tau = 2ut \tag{7.2}$$

where t is the time in years since population expansion, and u is the mutation rate of the sequence according to;

$$u = 2\mu\kappa \tag{7.3}$$

where μ is the mutation rate per nucleotide and κ is the number of nucleotides in the sequence. Mutation rates of between 1.6 and 3.5% per million years have been reported for marine invertebrate COI genes (Lessios et al., 2001, 1999; McCartney et al., 2000).

Haplotype phylogeny was examined by creating a minimum spanning network of the haplotypes present in the samples (Templeton et al., 1992). The minimum spanning network was constructed using TCS 1.18 software (Clement et al., 2000).

The pairwise distances between all sampling locations was calculated to compare genetic distance with physical spatial distance. The distance between sampling locations was calculated using a digital atlas and image analysis software. When tracing the path between sampling locations, bays less than 5km wide were closed off, and the step between islands was measured by following the coastline of the islands round to the point of closest direct separation between the two islands (Figure 7.2).

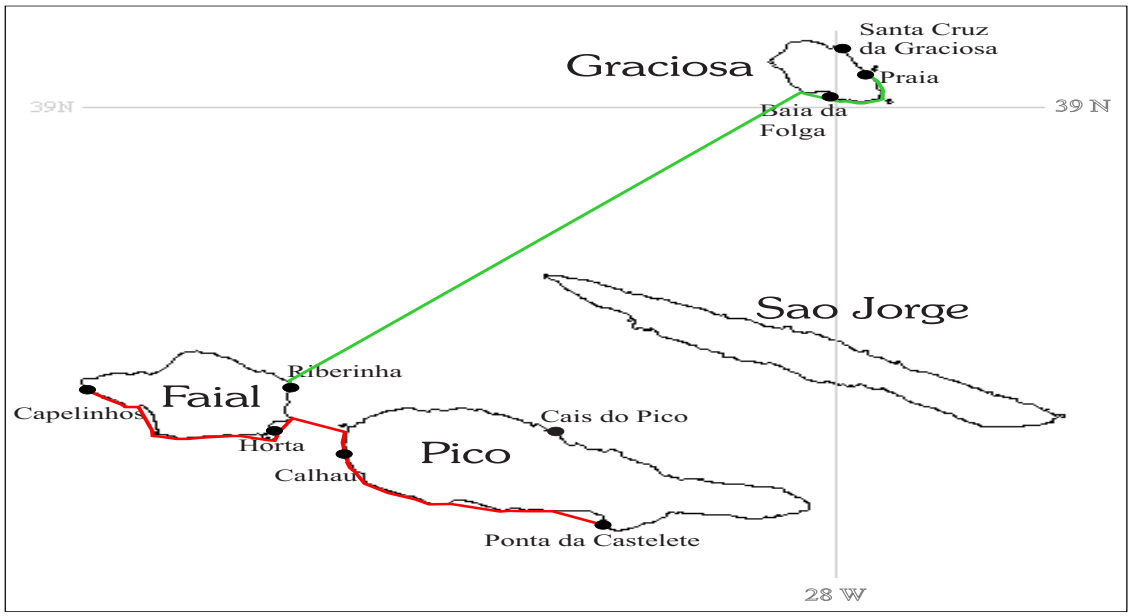

Figure 7.2: Example of pairwise distance measurement, illustrating how distance between islands was measured at point of closest separation.

7.3 Results

7.3.1 Haplotype diversity

1373 individuals were analysed for a 226 bp fragment of the mitochondrial COI gene from 15 locations across 6 Azorean islands, covering the 3 Azorean island groups (Table 6.2). Between 32 and 128 individuals were examined per site (mean = 92), the number of limpets collected from some sites was limited by practical circumstances. Low numbers of limpets at some sites due to recent exploitation also hampered efforts to achieve large, and equally sized samples from all sites.

From the 1373 individuals only 23 different haplotypes were found indicating a low degree of polymorphism. The most common haplotype, PC1, was found in all replicates at all sites. Overall 83% of the individuals were haplotype PC1, ranging between 49% and 94% for individual sites. Two other haplotypes PC4 and PC7 occurred on all of the island groups; they accounted for 7% and 1.3% of the individuals sampled respectively.

Combined, the three haplotypes that occurred across all the island groups, PC1, PC4 and PC7 accounted for 92% of the individuals sampled (Figure 7.3, upper pie charts). Apart from the haplotypes common to all island groups, the western group shared a majority of the remaining haplotypes with the central group (Figure 7.3, lower pie chats). The central group shared haplotypes with both the western and eastern group, although a majority of the haplotypes were unique to the central group. The eastern group shared some haplotypes with the central group, but the majority were unique to the eastern group. The western and eastern groups did not share any haplotypes that were not also present in the central group.

7.3.2 Diversity and population structure

All sites showed low values of haplotype diversity, h, (mean = 0.288) and low levels of nucleotide diversity, π (mean = 0.002). Apart from the common haplotypes (PC1, PC4, PC7) the remaining haplotypes were rare and were unique to individual sites or only occurred at a few sites (Table 6.2 and Figure 7.3). The haplotype frequency-

Table 6.2: Proportional haplotype frequency per a) site, and b) Island.

a)

Island	Site	PC01	PC02	PC03	PC04	PC05	PC06	PC07	PC08	PC10	PC11	PC13	PC14	PC15	PC16	PC17	PC18	PC19	PC20	PC22	PC23	PC24	PC25	PC27	n	h	π
Flores	FlFg	0.789	0.039	0.008	0.031	0.086	0.008	0.039																	128	0.369	0.0025
	FlPd	0.938	0.008		0.008	0.039									0.008										128	0.120	0.0007
	FlLf	0.872			0.098				0.030																133	0.231	0.0011
Corvo	CoPn	0.697		0.013	0.158	0.079		0.053																	76	0.486	0.0026
Graciosa	GrBf	0.625			0.063	0.312																			32	0.524	0.0027
	GrPr	0.867			0.053					0.080															75	0.243	0.0034
Faial	FaPr	0.878		0.026	0.009					0.017	0.017													0.052	115	0.227	0.0031
	FaHo	0.894	0.011		0.075							0.011								0.011					94	0.198	0.0013
	FaCa	0.733			0.158			0.010				0.010				0.040			0.020		0.020	0.010			101	0.044	0.0025
Pico	PiCp	0.772			0.228																				101	0.355	0.0017
	PiPc	0.750			0.094			0.156																	32	0.417	0.0022
	PiCa	0.489										0.067	0.400	0.044											45	0.608	0.0071
Santa Maria	SmSl	0.922			0.052			0.009									0.009						0.009		116	0.148	0.0011
	SmAn	0.884			0.063			0.021								0.021	0.011								95	0.216	0.0011
	SmSp	0.931																0.069							102	0.129	0.0012

b)

Group	Island	PC01	PC02	PC03	PC04	PC05	PC06	PC07	PC08	PC10	PC11	PC13	PC14	PC15	PC16	PC17	PC18	PC19	PC20	PC22	PC23	PC24	PC25	PC27	n	h	π
Western	Flores	0.866	0.015	0.003	0.046	0.041	0.003	0.013	0.010						0.003										389	0.246	0.0015
	Corvo	0.697		0.013	0.158	0.079		0.053																	76	0.486	0.0026
Central	Graciosa	0.794			0.056	0.094				0.056															107	0.357	0.0034
	Faial	0.835	0.003	0.010	0.077			0.003		0.006	0.006	0.006				0.013			0.006	0.003	0.006	0.003		0.019	310	0.296	0.0024
	Pico	0.697			0.146			0.028				0.017	0.101	0.011											178	0.485	0.0040
Eastern	Santa Maria	0.914			0.038			0.010								0.006	0.006	0.022					0.003		313	0.164	0.0012

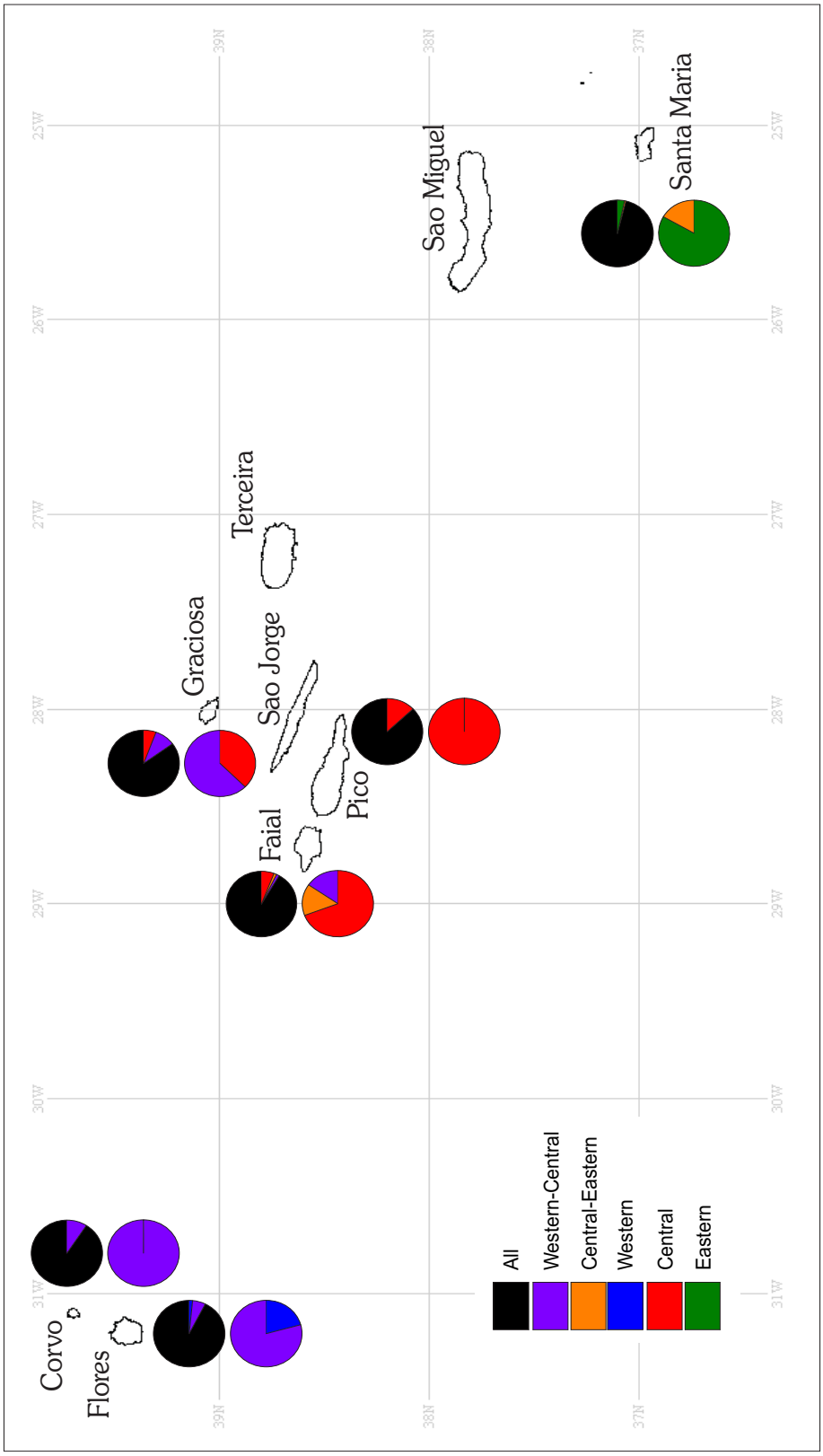

Figure 7.3: Occurrence of haplotypes across the Azores with haplotypes grouped according to the presence on island groups. The upper pie chart for each island shows the overall haplotype frequencies. In the lower pie chart the haplotypes common to all island groups are excluded, and the pie chart shows the relative frequencies of the remaining haplotypes.

frequency plot (Figure 7.4) shows that a majority of haplotypes only occurred in 8 or less individuals from the total sample of 1373 individuals.

The pairwise F_{ST} values (Table 6.3) indicate that there was a high degree of significant population differentiation between islands, and between sites both intra- and inter- island pairwise site comparisons. Pairwise F_{ST} values between replicates within sites (Table 6.4) also indicates that there was significant population differentiation between replicates in 5 of the 12 sites where replicate samples were collected.

The inter-site physical distances (Table 6.5) were plotted against inter-site pairwise F_{ST} values (Figure 7.5) to examine for isolation by distance (Palumbi, 2003). There is a slight trend in reduced F_{ST} value with increasing difference, however the majority of F_{ST} values are between 0 and 0.1 regardless of physical distance separating the sites.

The results of the F_{ST} tests indicate that much of the genetic variation is explainable at small scales (within and between sites) rather than larger scales (between islands and island groups). To further examine the scale at which genetic differentiation occurred hierarchical AMOVA was conducted to see at which scale most of the variation occurred (see Table 6.6). The hierarchical levels of sample clustering were determined *a priori* according to geographical separation. Thus replicates were grouped according to site, sites were grouped according to island, and islands were grouped according to island group. In each case the large majority of the variance was attributable to the smallest spatial scale with less further variance attributable to the larger groupings. In other words some of the variation within the samples can be explained by clustering the samples according to the above defined spatial groupings at all hierarchical levels apart from the inter-island group level.

The haplotype minimum spanning network is predominantly made up of haplotypes with single point mutations, although there are some secondary and tertiary mutations (Figure 7.6). PC1 was considered to be the most likely outgroup root haplotype. The presence of haplotypes on specific islands was overlaid on a stylistic representation of the minimum spanning network (Figure 7.7) to examine if there was any geographic structure to the presence or absence of related haplotypes. As most of the mutations are single step mutations there is evidence of geographic

Table 6.2: Proportional haplotype frequency per a) site, and b) Island.

a)

Island	Site	PC01	PC02	PC03	PC04	PC05	PC06	PC07	PC08	PC10	PC11	PC13	PC14	PC15	PC16	PC17	PC18	PC19	PC20	PC22	PC23	PC24	PC25	PC27	n	h	π
Flores	FlFg	0.789	0.039	0.008	0.031	0.086	0.008	0.039																	128	0.369	0.0025
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	GrPr	0.867			0.053					0.080															75	0.243	0.0034
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	FaHo	0.894	0.011		0.075							0.011								0.011					94	0.198	0.0013
	FaCa	0.733			0.158			0.010				0.010				0.040			0.020		0.020	0.010			101	0.044	0.0025
Pico	PiCp	0.772			0.228																				101	0.355	0.0017
	PiPc	0.750			0.094			0.156																	32	0.417	0.0022
	PiCa	0.489										0.067	0.400	0.044											45	0.608	0.0071
Santa Maria	SmSl	0.922			0.052			0.009									0.009						0.009		116	0.148	0.0011
	SmAn	0.884			0.063			0.021								0.021	0.011								95	0.216	0.0011
	SmSp	0.931																0.069							102	0.129	0.0012

b)

Group	Island	PC01	PC02	PC03	PC04	PC05	PC06	PC07	PC08	PC10	PC11	PC13	PC14	PC15	PC16	PC17	PC18	PC19	PC20	PC22	PC23	PC24	PC25	PC27	n	h	π
Western	Flores	0.866	0.015	0.003	0.046	0.041	0.003	0.013	0.010						0.003										389	0.246	0.0015
	Corvo	0.697		0.013	0.158	0.079		0.053																	76	0.486	0.0026
Central	Graciosa	0.794			0.056	0.094				0.056															107	0.357	0.0034
	Faial	0.835	0.003	0.010	0.077			0.003		0.006	0.006	0.006				0.013			0.006	0.003	0.006	0.003		0.019	310	0.296	0.0024
	Pico	0.697			0.146			0.028				0.017	0.101	0.011											178	0.485	0.0040
Eastern	Santa Maria	0.914			0.038			0.010								0.006	0.006	0.022					0.003		313	0.164	0.0012

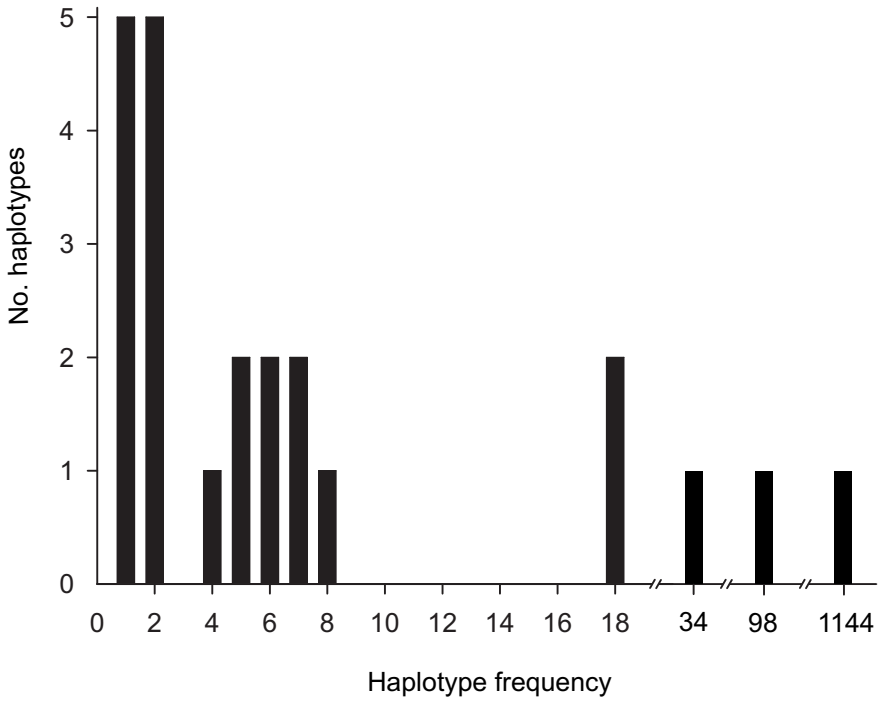


Figure 7.4: Number of haplotypes occurring at a given frequency.

structure in the network. PC23 and its derivatives were confined to the western and central group, and PC14 and PC13 and its derivatives were confined to the central and eastern group. However, PC5, found in the western and central group, has two derivative haplotypes: PC2 which occurred solely in the western group samples and PC19 which solely occurred in the eastern group samples.

The pairwise mismatch distributions of samples per site are plotted in figure 7.8 as bars, the predicted expansion model distribution are overlaid as line plots. Calculated τ and P values are presented along with the values for Tajima's D test and associated P values under the plots. Nearly all the mismatch plots are unimodal with the mode occurring at x = 0. There is no consistent trend as to whether the observed data shows a significant fit with the expansion model. Ten of the sites show a significant fit with the expansion model (the Flores, Faial, Santa Maria, and Pico-Ponta Castelete sites show a significant fit), the other 5 sites do not show a significant fit. The Tajima's D test is a test for neutrality, in the case where the D value is significantly less than zero it indicates that the population has undergone a recent expansion. Five of the population have significantly negative D values (FlFg, FlPd, FaPr, FaHo, SmSl), and all of the other sites apart from 2 (GrBf and PiCp)

Table 6.3: Pairwise F_{ST} values for samples grouped by a) island, and b) site.

a)

	Flores	Corvo	Graciosa	Faial	Pico
Corvo	0.035*				
Graciosa	0.030*	0.029*			
Faial	0.017*	0.019††	0.031*		
Pico	0.072*	0.033*	0.057*	0.032*	
Santa Maria	0.005††	0.053*	0.043*	0.013*	0.071*

b)

	FlFg	FlPd	FlLf	CoPn	GrBf	GrPr	FaPr	FaHo	FaCa	PiCp	PiPc	PiCa	SmSl	SmAn
FlPd	0.021††													
FlLf	0.055*	0.050*												
CoPn	0.027*	0.076*	0.028††											
GrBf	0.053††	0.239*	0.239*	0.090*										
GrPr	0.061*	0.071*	0.057*	0.057*	0.141*									
FaPr	0.040*	0.032*	0.021††	0.035*	0.133*	0.026††								
FaHo	0.032*	0.023*	-0.001	0.023††	0.199*	0.044*	0.009							
FaCa	0.058*	0.077*	0.013††	0.008	0.157*	0.045*	0.025*	0.015						
PiCp	0.108*	0.173*	0.050*	0.020	0.224*	0.091*	0.062*	0.063*	0.009					
PiPc	0.048*	0.133*	0.067*	0.019	0.175*	0.057*	0.038††	0.048††	0.030††	0.084*				
PiCa	0.340*	0.445*	0.418*	0.315*	0.296*	0.281*	0.276*	0.364*	0.325*	0.382*	0.273*			
SmSl	0.041*	0.021*	0.004	0.039*	0.232*	0.049*	0.010	-0.006	0.025††	0.082*	0.060††	0.394*		
SmAn	0.039*	0.027*	0.004	0.033*	0.230*	0.047*	0.015††	-0.005	0.019††	0.079*	0.047††	0.382*	-0.005	
SmSp	0.021††	0.019††	0.067*	0.069*	0.157*	0.069*	0.040*	0.042*	0.080*	0.160*	0.107*	0.383*	0.044*	0.047*

* significantly different to 0.05
†† significantly different to 0.01

Table 6.4: Pairwise F_{ST} values for replicates within sites.

	FlFg1	FlFg2
FlFg2	0.1818††	
FlFg3	0.1364††	0.0506††

	FlPd1	FlPd2
FlPd2	-0.0119	
FlPd3	-0.0188	-0.0168

	FlLf1	FlLf2
FlLf2	0.0769*	
FlLf3	0.0598*	-0.0202

	CoPn2
CoPn3	0.0468

	GrPr1
GrPr2	0.0408

	FaPr1	FaPr2
FaPr2	0.0564*	
FaPr3	0.0440††	-0.0153

	FaHo1	FaHo2
FaHo2	-0.0085	
FaHo3	-0.0052	0.0177

	FaCa1	FaCa2
FaCa2	0.2592††	
FaCa3	0.3417††	0.0148

	PiCp1	PiCp2
PiCp2	0.1725	
PiCp3	0.0046	0.0573

	SmSl1	SmSl2
SmSl2	0.0132	
SmSl3	0.0001	-0.0055

	SmAn1	SmAn2
SmAn2	0.0052	
SmAn3	0.0690	0.0068

	SmSp1	SmSp2
SmSp2	0.1132*	
SmSp3	-0.0144	0.0486

* significantly different to 0.05
†† significantly different to 0.01

Table 6.5: Pairwise distances in Km between sites.

	CoPn	FlPd	FlFg	FlLf	GrBf	GrSc	GrPr	FaPr	FaHo	FaCa	PiCp	PiPc	PiCa	SmSl	SmAn
FlPd	18														
FlFg	27	12													
FlLf	34	21	17												
GrBf	279	287	298	286											
GrSc	279	287	299	286	14										
GrPr	284	292	303	291	10	4									
FaPr	256	270	266	249	71	86	81								
FaHo	256	266	262	245	79	93	89	8							
FaCa	231	242	238	221	97	111	107	26	24						
PiCp	278	295	291	274	73	88	83	32	30	53					
PiPc	298	304	304	287	111	125	121	45	42	66	51				
PiCa	265	278	274	257	81	95	91	15	13	36	27	30			
SmSl	631	621	638	621	353	357	352	379	377	400	342	334	364		
SmAn	618	629	625	608	340	344	339	366	363	387	329	321	351	13	
SmSp	637	648	644	627	359	363	358	385	382	406	348	340	370	6	19

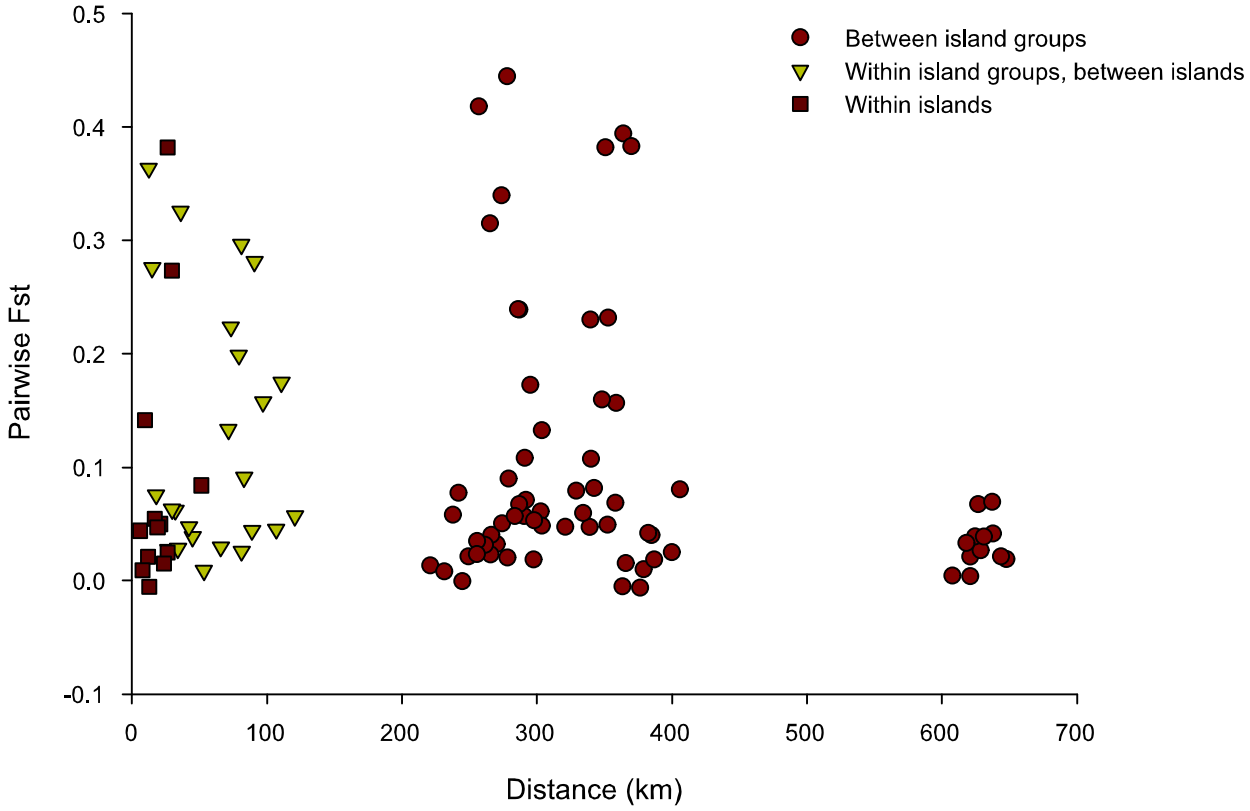


Figure 7.5: Pairwise F_{ST} against pairwise geographic distance between sample locations

have negative D values, although not significantly so.

The data was combined as a single group and re-examined for the mismatch distribution and Tajima's D value (Figure 7.9). As a combined population the samples have a significantly negative Tajima D value indicating that the population was not at equilibrium according to the neutral mutation hypothesis (Tajima, 1989b), and that the population may have undergone a recent population bottleneck (Tajima, 1989a).

The possible age of this bottleneck is calculated using equations 6.2 and 6.3 re-arranged to give:

$$t = \frac{\tau}{4\mu\kappa} \tag{7.4}$$

As noted above μ can vary between 1.6×10^{-8} to 3.5×10^{-8} yr^{-1} and κ is 226. For all the samples combined the Arlequin software estimates τ as 3.00 with estimates to the 0.05 confidence level ranging from 0.471-7.891 (Figure 7.9). τ is an estimate

Table 6.6: Results of hierarchical AMOVA tests.

Level of Grouping	Source of variation	Proportion of variance (%)	F value	P value
Groups	Among groups	-1.8	Fct = -0.018	0.787
	Among islands within groups	4.7	Fsc = 0.046	0.000
	within Islands	97.1	Fst = 0.029	0.000
Islands	Among Islands	-1.3	Fct = -0.013	0.197
	Among sites within islands	11.0	Fsc = 0.109	0.000
	Within Sites	90.3	Fst = 0.097	0.000
Sites	Among Sites	7.6	Fct = 0.076	0.002
	Among replicates within sites	5.8	Fsc = 0.062	0.000
	Within replicates	86.7	Fst = 0.133	0.000

of the mode of the mismatch distribution (Rogers and Harpending, 1992), and it is clearly apparent from a visual examination of the graph that the modal value of the distribution represented by the bars in figure 7.9 is zero. Examination of other Arlequin estimates of τ when the expected τ value is small indicates that the Arlequin package has a tendency to return the answer 3.00 (Figure 7.8, Aboim et al., 2005, J. Preston pers comm.). Therefore the predicted τ value returned by the Arlequin package will be disregarded. Due to the uncertainty in the τ value, a low τ value is assumed, and the final t value should be treated with caution. I shall consider the possible range of results for the time since population expansion for τ values between 0 and 1. If τ is 0, then t=0 and no time has elapsed since the population expansion. The upper limit for the estimate of time since population expansion occurs where where τ= 1 and μ=1.6 $\times$ 10^{-8}. Under these conditions the estimated time since population expansion according to the mismatch distribution hypothesis is 69 000 years (Rogers and Harpending, 1992). Therefore assuming τ lies between 0 and 1, a population expansion is predicted to have occurred sometime in the last 69 000 years.

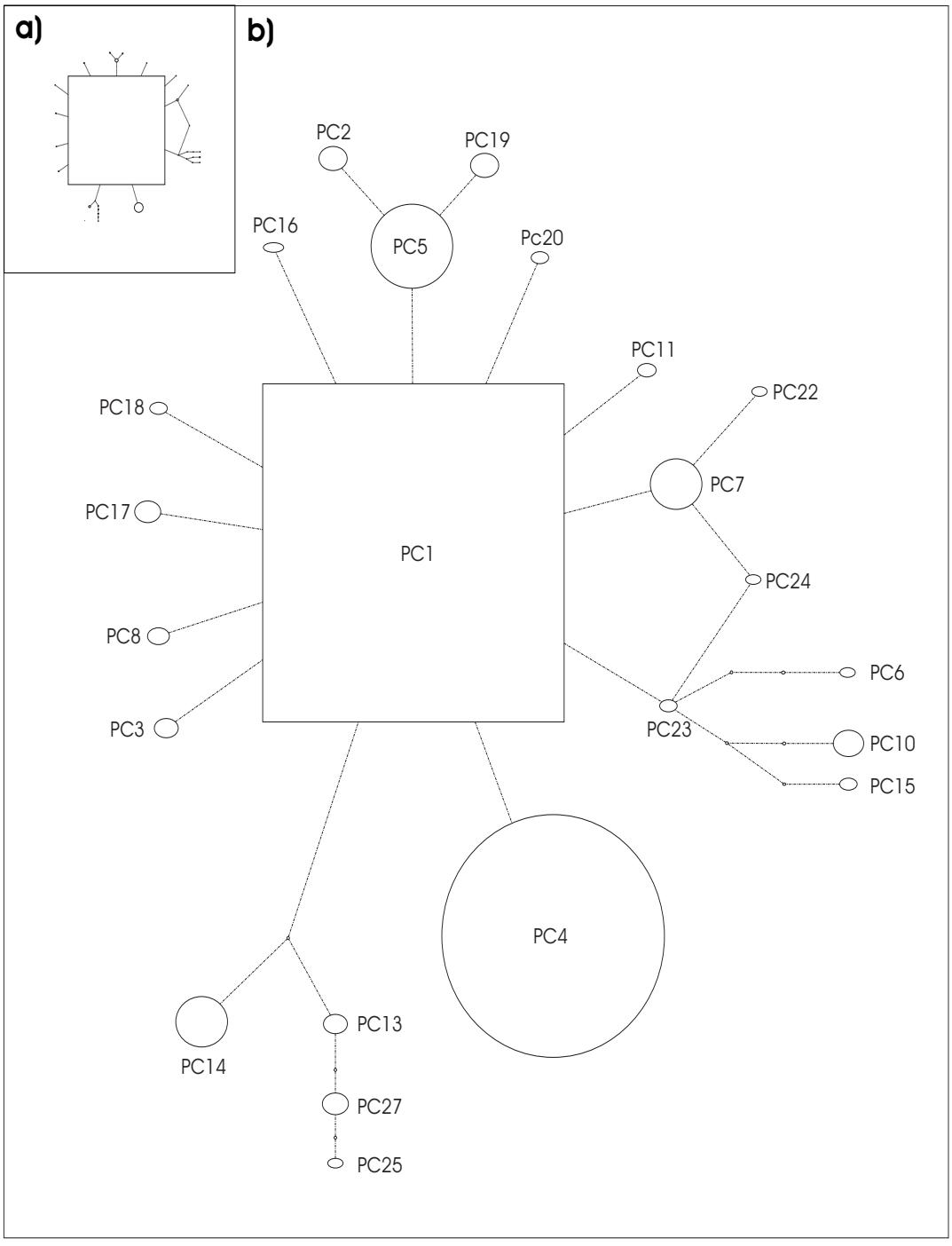


Figure 7.6: Haplotype minimum spanning network for *P. candei* in the Azores. Each step is an equal mutational distance, the unlabelled nodes represent hypothesised missing mutational steps. a) The area of the boxes is proportional to haplotype frequency, b) haplotype PC1 has been reduced in size, all other haplotypes are scaled according to their relative frequency. The square haplotype is considered to be the outgroup.

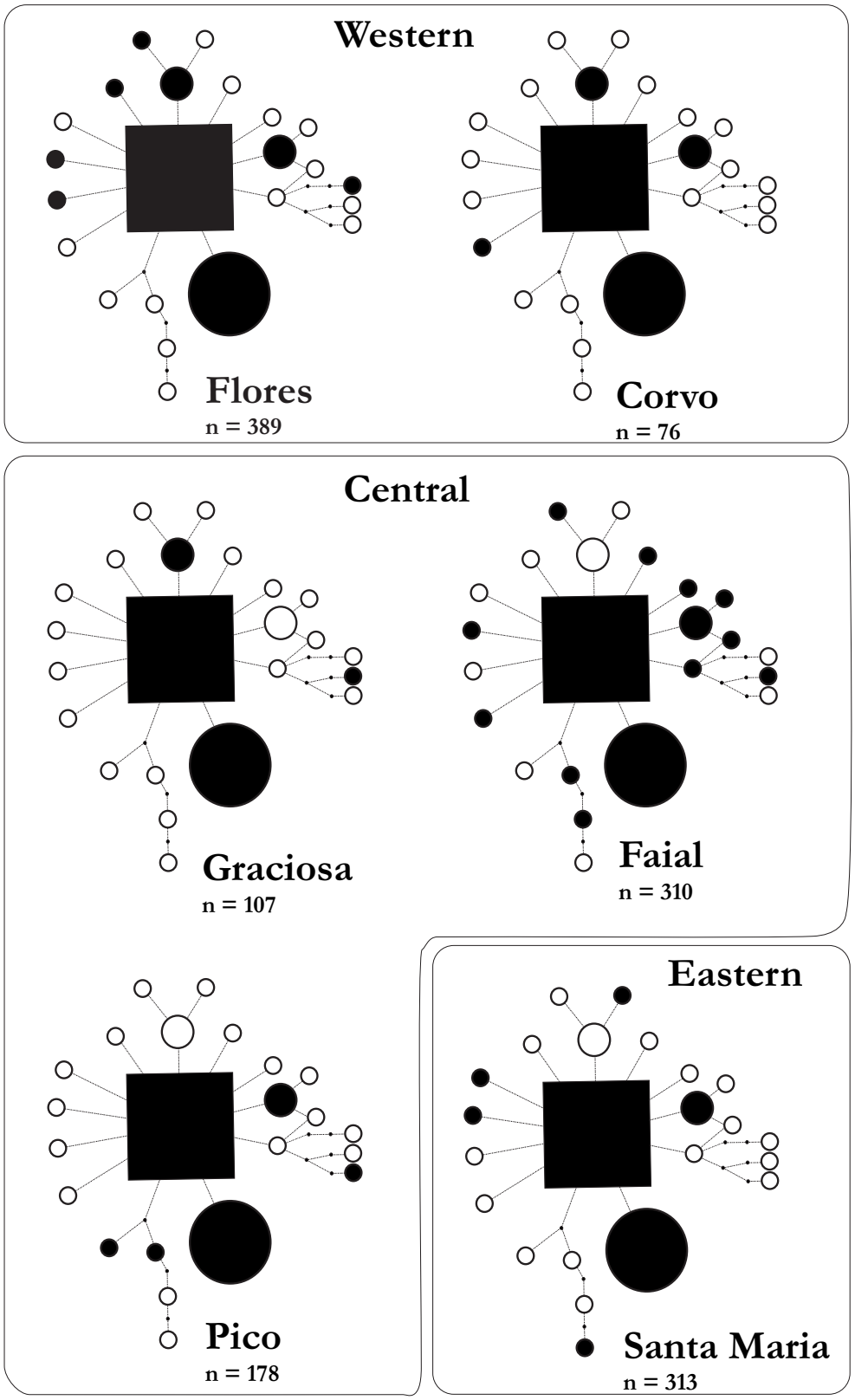
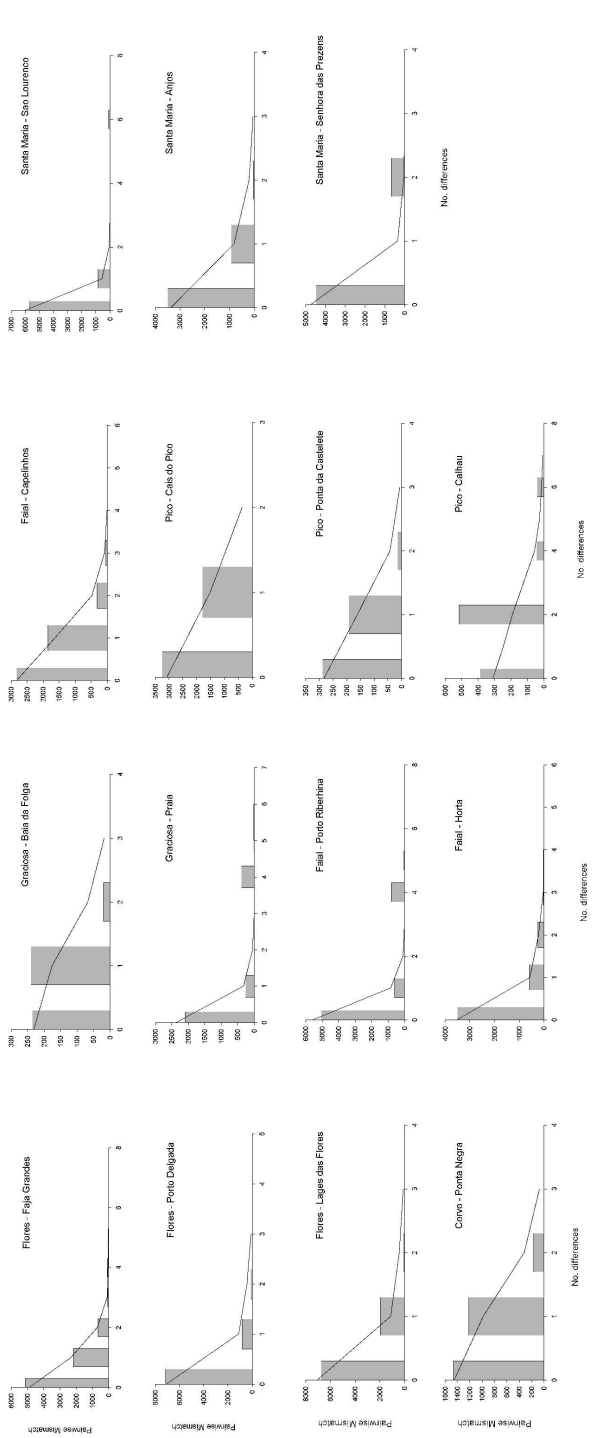


Figure 7.7: The occurrence of haplotypes according to geographic location. The boxes represent the same haplotypes as figure 7.6 although the boxes have been rescaled for clarity. Filled boxes indicate the presence of that haplotype at that location.



	FlFg	FlPd	FlLf	CoPn	GrBf	GrPr	FaPr	FaHo	FaCa	PiCp	PiPc	PiCa	SmSl	SmAn	SmSp
S	9	4	2	4	2	5	10	7	7	1	2	7	8	4	2
τ	0.73	3.00	3.00	0.68	0.74	3.00	3.00	2.99	0.59	0.50	0.56	2.49	3.00	3.00	3.00
P_τ	0.79	0.65	0.69	0.00	0.00	0.04	0.06	0.40	0.10	0.00	0.09	0.01	0.25	0.47	0.06
Tajima D	-1.67	-1.55	-0.52	-0.69	0.27	-0.71	-1.66	-1.91	-1.43	0.96	-0.20	-0.21	-2.00	-1.43	-0.50
P_D	0.035	0.050	0.237	0.261	-0.385	0.256	0.037	0.016	0.068	-0.173	0.440	0.436	0.010	0.070	0.328

Figure 7.8: The plots show the observed pairwise nucleotide mismatch distributions (bars) against the predicted model distributions (line). The table gives the model parameters and values for Tajima's D test. S is the number of haplotypes present at the site, τ is defined in the text, P_τ is the probability that the simulated data provides a better fit than the observed data, P_D is the probability that randomly distributed data would have a lower D value.

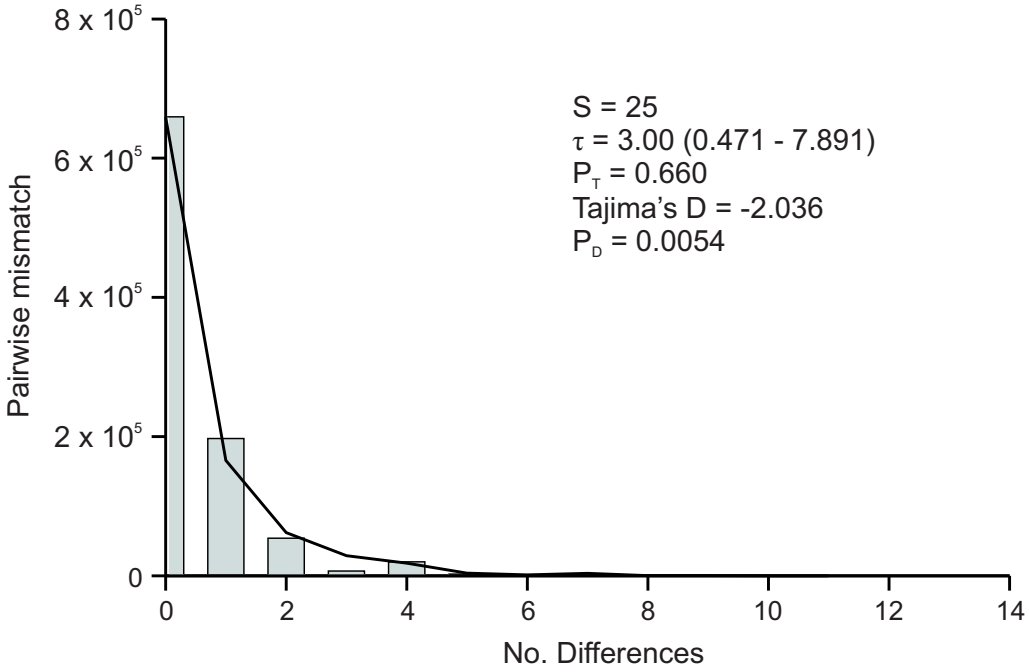


Figure 7.9: Mismatch distribution for all sites combined. The plots show the observed pairwise nucleotide mismatch distributions (bars) against the predicted model distribution (line). The table gives the model parameters and values for Tajima's D test. S is the number of haplotypes present at the site; τ is defined in the text, the values in brackets are the lower and upper limits for 0.01 confidence interval; P_τ is the probability that the simulated data provides a better fit than the observed data; P_D is the probability that randomly distributed data would have a lower D value.

7.4 Discussion

7.4.1 Population structure

An initial visual examination of haplotype diversity and occurrence across the Azores indicates that all the samples are dominated by a few ubiquitous haplotypes (Figure 7.3). This initially suggests that there is either high gene flow between the populations, or that the Azorean *P. candei* population has undergone a recent population bottleneck or founder event and that the ubiquitous haplotypes are the original founding haplotypes (Grant and Bowen, 1998). However, contrastingly, there are many more rare haplotypes that only occur at one or a few sites (Figure 7.4), indicative of a high degree of reproductive isolation between populations. The pairwise F_{ST} values can be used to examine the population structure in more detail.

A traditional analysis of the population structure of *P. candei* across the Azores archipelago on the basis of F_{ST} values for samples grouped by site or island, indicates that there is a high degree of genetic population differentiation (Table 6.3). This population differentiation is not confined to populations on different islands, but is also seen between populations on the same island. This indicates that there is little larval exchange between populations, otherwise the genetic structure would be lost (Palumbi, 1995). Furthermore the trend towards decreasing pairwise F_{ST} value with increasing pairwise geographical distance between populations is suggestive of isolation by distance (Figure 7.5).

The hypothesis of limited larval exchange between local populations is supported by F_{ST} values indicating that some populations show significant population differentiation over short distances, both between (e.g. FaHo-PiCa 13km; FlPd-CoPn 18km), and within islands (e.g. FlPd-FlLf 12km; SmSl-SmSp 6km). This is in agreement with previous studies on population differentiation in shallow water marine invertebrates with pelagic dispersal that have found that significant genetic differentiation can occur between populations only a few kms apart (Brown, 1991; Palumbi, 2003).

In contrast, some pairwise F_{ST} values indicate no significant population differentiation between widely separated populations (e.g. FlFg-SmSp 637km; CoPn-PiPc

298km) suggesting that larval dispersal is highly variable. This is also is agreement with several previous studies of shallow water marine invertebrates that have found highly variability in comparisons of pairwise F_{ST} values and geographic distances (Brown, 1991; Kyle and Boulding, 2000; Reeb and Avise, 1990; Small and Gosling, 2000). This has been attributed to local variations in current patterns or the occurrence of biogeographic boundaries (Brown, 1991; Palumbi, 2003; Reeb and Avise, 1990).

Weber and Hawkins (2002) conducted an allozyme study of *P. candei* across Atlantic archipelagos. In addition to sites outside the Azores, samples were taken from Faial and Santa Maria. The authors report a pairwise F_{ST} between the samples from the two islands of 0.174, compared to a value of 0.013 from this study (Table 6.3). The Weber and Hawkins (2002) F_{ST} value was based on samples of 50 and 47 individuals from Faial and Santa Maria respectively. In my study 310 and 313 individuals were examined from Faial and Santa Maria respectively. The difference between the two F_{ST} values from the two studies could lead to significantly different interpretations of the extent of larval exchange between the populations, the smaller F_{ST} value from my study indicating greater reproductive isolation between the two populations than for Weber and Hawkins (2002).

Notable discrepancies between the results of allozyme and mitochondrial DNA analysis have been reported before for mussels (Karakousis and Skibinski, 1992; Quesada et al., 1995) and oysters (Karl and Avise, 1992; Reeb and Avise, 1990). Differences between allozyme and mitochondrial DNA analyses have been attributed to the insensitivity of allozymes to non-coding third codons (Nei, 1987), higher mutation rates of mitochondrial than nuclear DNA (Brown et al., 1979), differential forcing effects of historical factors on nuclear and mitochondrial DNA (Rigaa et al., 1997), and the difference in effective population size observed with mitochondrial DNA and allozymes coded by nuclear DNA (Apte and Gardner, 2002). The discrepancy between the F_{ST} values found by this and Weber and Hawkins's (2002) study may also be attributable to the larger sample size used in my study, because an increase in sample size decreases the potential error in the F_{ST} value (Waples, 1998). On the basis of these factors the lower F_{ST} value found by this study is considered the more reliable estimate.

The AMOVA analyses conducted at each hierarchical level of geographic grouping indicated that in each case the vast majority of the variance could be accounted for at the smallest spatial scale (Table 6.6). Although in each case a majority of the variance can be explained at the smallest spatial scale, a significant amount of variance can be accounted for by between site variation. The AMOVA tests indicated that grouping the sites according to island, or island group does not account for any of the variance between populations. This suggests two alternative explanations, firstly that genes are shared ubiquitously such that there is no geographic structure to the population and all the small scale genetic differentiation is attributable to sampling noise. The alternative explanation is that the populations are highly isolated and that there is such divergence between geographically close populations that little of the variance can be explained by grouping the samples according to geographic proximity.

The above analysis of the F_{ST} values for samples grouped according to islands and sites supports the second explanation of the AMOVA results, that larval dispersal is so limited that the genetic differentiation across all the samples can be explained due to small scale population differentiation.

The hierarchical nature of the sampling design allows this hypothesis to be more closely examined. A traditional F_{ST} analysis of the samples grouped as replicates within sites allows the genetic diversity over small spatial scales to be examined. The analysis indicates that on the basis of F_{ST} values many of replicates within sites are considered to be separate populations with significant genetic differentiation (Table 6.4). If this is truly the case this means that limpets from immediately adjacent areas should be considered as separate populations, and this conclusion goes strongly against biological understanding of limpet population dynamics. It seems highly unlikely that a broadcast spawning invertebrate with pelagic larvae has significant population differentiation over a few meters.

The unusual and suspect conclusions based on F_{ST} values drawn from the within site pairwise comparison of replicate samples suggests that the application of traditional analysis of F_{ST} values to the *P. candei* populations in the Azores needs to be re-appraised. One of the key assumption for the application of F_{ST} values to understanding population structure is that haplotypes frequencies within the population

have achieved equilibrium between mutation, migration and genetic drift (Waples, 1998), if this assumption is broken then F_{ST} values need to be interpreted with great caution. The Tajima's D value for the population as a whole is significantly negative, indicating that the population is not at equilibrium and has possibly undergone an evolutionary recent population expansion (Tajima, 1989a). Thus the population does not fulfil the assumptions required for a traditional F_{ST} analysis.

What can be concluded about the extent of larval exchange between populations of *P. candei* on the Azores in the face of unreliable F_{ST} values. The population throughout the Azores is dominated by a few ubiquitous haplotypes, but rare or uncommon haplotypes occur at most sites. This pattern of genetic differentiation can be explained by two contrasting interpretations; i) that the Azorean *P. candei* population has undergone a recent founder event and that larval dispersal is widespread. Therefore there are the ubiquitous haplotypes and the rare occasional haplotypes occur widely through the population, but due to limited sample sizes only one or two different rare haplotypes are found at each of one or a few sites; ii) that the Azorean *P. candei* population has undergone a recent founder event and that larval dispersal is limited. Once again the ubiquitous haplotypes are expected, and the occasional haplotypes are local mutations that have only accumulated in small scale local populations.

On the basis of the evidence presented it is not possible to differentiate between these two explanations, and it is therefore hard to draw any conclusions on the distance and extent of larval dispersal. Furthermore due to the unreliability of the F_{ST} values no attempt has been made to evaluate Nm.

As this attempt to determine the extent of larval dispersal and mixing has yielded inconclusive results it is necessary to consider how future attempts may improve on this. Due to the failure of this genetic approach is it should be considered whether it is the best approach. However as discussed above, the practical and logistical difficulties of following larvae from their point of release through to settlement mean that genetic approaches are virtually the only approach available. Therefore there are two alternative ways in which more conclusive results from a genetic assessment of larval dispersal can be achieved. One is to improve the genetic data, and the other is to improve the assessment of the genetic data.

The quality of the genetic data could easily be improved by increasing the number of samples per site and increasing the number of sites sample. Also the samples could be examined for variability over more loci. Additionally a micro-satellite based approach could be used as this offers much greater potential for examining population structure at greater detail (Parker et al., 1998), and drawing stronger conclusions about the inter-relationship between individuals within a populations, and between populations (Groppe et al., 1995; Wilson et al., 1997).

The power of pairwise F_{ST} significance tests can be increased by the use of a sequential Bonferroni test to reduce the likelihood of bias affecting the number of pairwise comparisons deemed significant (Rice, 1989). However as the population breaches the fundamental criteria of neutrality required for the application of F_{ST} tests it is worth considering other approaches to examining genetic data. In addition there are other limitations of the F_{ST} approach to examining population structure on the basis of haplotype frequency distributions, however they will not be further discussed here (see Neigel, 2002; Waples, 1998, for discussions).

Developments of more variable genetic markers, such as micro-satellites, has allowed the development of methods based on individuals rather than population samples (Paetkau et al., 2004). These methods are based on haplotype occurrence likelihood, and aim to identify first generation migrants into a sub-population (Rannala and Hartigan, 1996). A further alternative approach has been developed based on maximum-likelihood and coalescence theory (Nash, 1992). These maximum-likelihood coalescence methods aim to provide better estimates of migrants between sub-populations than F_{ST} values as they integrate over all possible genealogies and migration events rather than relying on simple pairwise comparisons. However even if the whole suite of improvements to the genetic assessment of the extent of larval mixing and dispersal in the Azores was implemented the final result may not be conclusive.

7.4.2 Population history

Both of the possible explanations of the genetic structure of *P. candei* across the Azores rely on the fact that there has been a recent population bottleneck. Does

the data support this conclusion?

There are several aspects of the data that support the hypothesis that the Azorean *P. candei* population has undergone a recent bottleneck. Firstly as noted above Tajima's D value is significantly different to 0 indicating that the population is not at equilibrium (Tajima, 1989b), and as the value is negative this may be due to the population having undergone a recent bottleneck or founder event.

The samples in this study have low values of h and π with a mean and standard deviation of 0.288 $\pm$0.16, and 0.002 $\pm$0.002 respectively. The haplotype minimum spanning network (Figure 7.6) is star shaped with little secondary structure. Both of these are indications that the population has undergone a recent bottleneck or founder event as there has been little time for mutations to build up within the population (Grant and Bowen, 1998).

Analysis of the mismatch distributions also indicates that the population has undergone a recent population expansion (Figures 7.8 and 7.9). As discussed above there is uncertainty regarding the τ value, however a cautious interpretation of the τ value would indicate that population expansion occurred some time within the last 69 000 years.

Does this figure fit in with what is known of the history of the Azorean *P. candei* population? The Azores are oceanic seamounts that occur in oceanic crust. The age of the rocks in the Azores is highly variable; the oldest rocks are 8-10 million years old (Abdel-Monem et al., 1975), and the most recent less than 40 years old. However most of the islands were formed 1-3 million years ago (Azevedo et al., 1991). *Patella* spp. have been reliably reported from the north Atlantic from the Pliocene (2-5 Myr) and limpets have been discovered across Europe from the Cenozoic (2-65 Myr), however they may be lottid limpets (Ridgway et al., 1998). Therefore limpets may have been present on the Azores for 5 million years, which is far longer than the 69 000 years since the bottleneck predicted on the basis of the mismatch distribution.

However it has been noted that the Azores have a lower level of endemism compared to other oceanic islands (Briggs, 1966, 1970) which is attributed to mass extinctions caused by low sea temperatures during the last glacial maximum, and that most

organisms recolonised the islands in the last 17 000 years (Briggs, 1970). This is more consistent with the postdiction that the population expansion of *P. candei* occurred within the last 69 000 years. It should be remembered that the figure of 69 000 years is an upper estimate of the time since population expansion and that the actual time since population expansion may be much less than this. The estimate of time since population expansion based on the genetic data is consistent with the hypothesis that *P. candei* recolonised the Azores after the last glacial maximum during the last 17 000 years. In contrast Weber and Hawkins (2002) calculated that *P. candei* invaded the Azores 2 million years ago on the basis of allozyme data. As discussed above, due to the use of mitochondrial DNA and larger sample sizes the results of this study are considered more robust than the results of Weber and Hawkins (2002).

A second hypothesis for a recent population bottleneck is that it was caused by human fishery exploitation. There has been a long history of Patellid limpet collection by people since the islands were colonised in the 15^{th} Century (Santos et al., 1995). The main commercial limpet fishery is for the larger limpet *P. aspera*, but *P. candei* is also taken, and increasingly so as *P. aspera* stocks decline (Ferraz et al., 2001; Santos et al., 1989). Little is quantitatively known of the present *P. candei* stocks, however *P. candei* stocks have declined as a result of recent human exploitation (R, Ferraz pers comm.). Thus the hypothesis that *P. candei* has undergone a recent bottleneck in the Azores due to human exploitation over the last 30 years is consistent with the genetic data.

As both hypotheses for a recent bottleneck, that it was cause by human exploitation or the last ice age, are consistent with the genetic data it is not possible on the basis of the data available to discriminate between them.

Chapter 8

General Discussion and Conclusions

My thesis has examined aspects of the spawning behaviour, fertilisation kinetics, and population biology of *P. vulgata*. On this basis of this a zygote production model has been developed to examine the relationships between zygote production, egg production, SSB and fishery yield in a protandrous species. In addition the genetic structure of the exploited Azorean *P. candei* population has been examined to try and determine the distance of larval dispersal. The larval production model demonstrated that *P. vulgata* populations are sensitive to sperm limitation, and that population egg and zygote production are decoupled from SSB and yield. No conclusion about the distance of larval dispersal could be drawn from the genetic population study.

Three of the main areas of investigation of this study are discussed below and major conclusions drawn out. These areas are:

- The effect of protandry on the relationship between population egg and zygote production, and SSB and fishery yield.
- The importance, or otherwise, of sperm limitation.
- Management implications of the zygote production model, and the importance of MPAs.

8.1 The Effect of Protandry on the Relationship Between Egg and Zygote Production, and SSB and Yield.

The results of the population fecundity model (chapter 5), and the zygote production model (chapter 6) predict that egg and zygote production do not follow a linear relationship with SSB or yield as a population is exposed to the simulated fishery. For the sheltered population attempts to conserve 30% of the unexploited level of reproductive output by managing on the basis of SSB would only conserve 6% of the population's zygote production. The failure of SSB to provide a proportionate measure of reproductive output is, in this case, due to failure of the assumptions of constant relative fecundity, constant fertilisation success, and a constant sex ratio.

The failure of the above assumptions has the following consequences for the relationship between SSB and zygote production. Firstly, as the sex ratio in the population changes following exploitation, female sex-specific SSB becomes decoupled from SSB. Secondly, due to decreasing relative fecundity with decreasing size, egg production becomes decoupled from sex-specific SSB as the larger individuals are removed from the population. Thirdly, as population sperm production declines fertilisation success declines, and zygote production becomes decoupled from egg production. The relative effect of these different factors on the relationship between SSB and zygote production was examined in figure 6.8 (p.124). This demonstrated that the three different factors had different relative importance on the decoupling of zygote production and SSB depending on the extent of exploitation and wave exposure level of the population.

For the sheltered population, the disparity between sex-specific SSB and E had the greatest effect on the decoupling between SSB and zygote production. This was due to the large proportion of population egg production concentrated in a few large individuals (figure 5.5a, p.85). For the intermediate population the effect of protandry, the decoupling between SSB and sex-specific SSB, was the single greatest factor over virtually the full range of exploitation. Finally, for the exposed popula-

tion reduced fertilisation success was the largest factor at low levels of exploitation, and protandry was the greatest factor at higher levels of exploitation. This is an example of how several processes can simultaneously operate on determining the response of a population to exploitation. But the specific factor that is the main determinant of a population's response at any one time can vary between populations depending on their demographic status, or due to wider factors such as the environment or community assemblage structure. The practical implications of this are that if only the exposed population had been studied, it may have been concluded that female sex-specific SSB provides reasonable measure of zygote production, and that the effect of decoupling between female sex-specific SSB and egg production could be ignored. However the sheltered and intermediate populations show that the decoupling between female sex-specific SSB and egg production can have a very large effect on zygote production. Thus this effect should be accounted for in efforts to assess the reproductive status of the stock. This highlights the caution that must be used when trying to draw out generalised conclusions on demographic processes from limited sample populations.

Despite the variation between populations the effect of protandry leads to a greater disparity between SSB and reproductive output than would be the case for a gonochronist, apart from when the exposed population was lightly exploited.

The decoupling between SSB and reproductive output due to protandry not only means that SSB is a less effective proxy for reproductive output for sequential hermaphrodites than gonochronists, but also that reproductive output of sequential hermaphrodite populations will decline more rapidly as the population is exploited than for gonochronists. As asserted by previous authors (Blaber et al., 1999; Buxton, 1992; Punt et al., 1993), this suggests that sequential hermaphrodites are more susceptible to recruitment overfishing than gonochronists. However this assertion is dependent on the rate at which the size, or age of sex change adjusts to the changing demographic status of the population. Little is known of the factors that regulate the size of sex change in limpets. Wright (1989) found that the size of sex change by the limpet *Lottia gigantea* was mediated by density, although there seemed to be a two year lag between the change in density and the change in size of sex change. A lag of one or several reproductive seasons between the population being exploited, and the

size at sex change responding to the changed demographic status of the population suggests that the reproductive output of the population will be reduced compared to that of a gonochronist. Thus protandrous hermaphrodites are more susceptible to recruitment overfishing than gonochronists. The only case where a protandrous hermaphrodite would be less susceptible is if following exploitation the population sex ratio adjusted to the new optimum ratio before the next reproductively active period.

From this it may be concluded that the management of sequential hermaphrodite stocks must proceed with caution. This is for two main reasons. Firstly, the reproductive output of a sequential hermaphrodite population declines more rapidly than that of a gonochronist as SSB is removed from the population, and secondly SSB provides a less effective proxy of reproductive output for sequential hermaphrodites than gonochronists.

8.2 Does Sperm Limitation Matter?

Sperm limitation is of particular concern to broadcast spawners as it is an example of an Allee effect (Denny and Shibata, 1989; Roberts and Hawkins, 1999). Furthermore, several studies have concluded that sperm limitation can lead to precipitous population collapse for broadcast spawning sedentary and sessile benthic invertebrates (Babcock and Keesing, 1999; Levitan, 1991; Wahle and Peckham, 1999). However few studies have attempted to directly examine the effect of exploitation on sperm limitation and zygote production at the population level (e.g. Claereboudt, 1999). The model developed in this thesis has been used to examine the effect of sperm limitation on population reproductive output by a broadcast spawner when fishery pressure is applied.

The zygote production model (chapter 6) predicts that for the default model parameter settings sperm would be limiting, even in the absence of fishery pressure (table 6.2, p.111). The model also predicted that sperm becomes increasingly limiting as fishery pressure was increased (figure 6.3, p.114). Sensitivity analysis (section 6.3.1) demonstrated that the proportion of eggs fertilised is very sensitive to the physical parameters in the model (figure 6.1, p.109), which are themselves poorly known

(section 3.5.2). Therefore only limited confidence can be placed in the model predictions of the absolute number of eggs fertilised. However, the relative number of eggs fertilised as fishery pressure is increased remains constant, so the predictions of increasing sperm limitation with increasing fishery pressure can be treated as more robust.

That sperm limitation increases as population numbers drop is an example of an Allee effect (Allee, 1931). However, does this sperm limitation lead to rapid declines in population reproductive output? The effect of sperm limitation on the decline in zygote production as fishery pressure is applied was examined in figure 6.8. In figure 6.8 the zygote production line indicates the proportionate decline in zygote production as SSB is removed, and the egg production line indicates the proportionate drop in egg production. The difference between the lines (W) is due to increasing sperm limitation, resulting in zygote production declining faster than egg production. The figure demonstrates that the decline in egg production accounts for the vast majority of decline in zygote production. Furthermore, the theory of the Allee effect (Allee, 1931) predicts that the rate of decline in zygote production increases as population numbers decline. Contrastingly, in this case the rate at which zygote production declines as SSB is removed decreases. This is due to the variable relative fecundity; the decline in egg production for a given decline in SSB decreases as fishery pressure increases, cancelling out the effect of increasing sperm limitation.

In the case of the most exposed population, sperm limitation plays a greater role in the overall decline in zygote production, especially under light exploitation (figure 6.8c, p.124). This is because the exposed population did not have a large proportion of its egg production concentrated into a few large individuals (figure 5.5, p.85) unlike the sheltered and medium populations. However the effect of reduced egg production becomes the main factor controlling zygote production by the exposed population as exploitation increases.

A decline in reproductive output does not necessarily lead to a proportionate decline in recruitment to the adult spawning stock (Beverton and Holt, 1957). The standard stock-recruitment relationships (Beverton and Holt, 1957; Cushing, 1973; Ricker, 1954; Shepherd, 1982) take the general form of a rapid increases in recruitment as reproductive output increases from zero, followed by a levelling off as reproductive

output continues to increase. If the decline in reproductive output due to exploitation occurs over the range of reproductive output at which recruitment is almost saturated then the decline in egg production will have the main effect on zygote production. If, however, the further decline in zygote production due to sperm limitation extends the decline beyond the area of saturation in the S-R curve and into the area of steeper decline, then sperm limitation may have a significant effect on recruitment.

In the case of *P. vulgata* the effect of declining egg production over declining fertilisation success is accentuated due to the effect of protandry. This has the effect of concentrating the egg production in the larger size classes which are exploited first. Thus zygote production may be greatly reduced before the effects of sperm limitation are felt.

The model developed in this thesis demonstrates that zygote production is predominately controlled by population egg production, and that sperm limitation has little further effect on the decline in zygote production. There is no indication that sperm limitation will lead to precipitous population collapse in populations of *P. vulgata*. However fertilisation success does decline as population numbers are reduced, and sperm limitation may be responsible for the final collapse in a marginal population.

8.3 Management Implications of the Zygote Production Model, and the Importance of MPAs.

The model developed in this thesis predicts that in order to protect a given proportion of the unexploited zygote production, a greater proportion of the population is available as yield if management is based on MPAs rather than a MLS (figure 6.9, p.127). As discussed in sections 5.4.3 and 6.5.2 this is due to a combination of, increasing relative fecundity with size, sperm limitation, and concentration of females in the larger size classes.

According to the model, if the population is managed to conserve the arbitrary level of 30% of the unexploited zygote production, for the case of the sheltered

population, 70% of the biomass of the population would be available as yield under a MPA management system, but only 25% would be available as yield under a MLS management system. For the medium and exposed populations the proportion of the biomass available as yield under MPAs and MLS based management systems would be 69% and 43% for the medium population, and 70% and 57% for the exposed population respectively.

Therefore according to the model predictions there is a strong case for proposing MPA based management strategies for sessile and sedentary invertebrates where there is either increasing relative fecundity with increasing size, sperm limitation, or concentration of females in larger size classes. Each of these factors has the effect of making zygote production decline faster than biomass as a sub-population is exploited. According to this model, if zygote production declines faster than biomass, then if a sub-population has been partially exploited it is preferable to fully exploit that sub-population before moving on to exploit a previously unharvested sub-population. However the implications of this model for real world fisheries management must be treated with caution.

One of the main limitations of the model is that it is a static model that only examines what happens when an unexploited population is exposed to a fishery, and only predicts the once off first time yield. The model does not give any indication of the long term yield that may be expected under different proposed management strategies. Furthermore the model takes no account of the loss of future reproductive potential. For example the model predicts that if all the immature limpets were harvested a fishery yield would be generated with no adverse effect on the reproductive status of the population. However a management system based on systematically removing all juveniles from a population would inevitably end in population collapse. The failure of the model to address the loss of future reproductive potential could be rectified by taking a per recruit approach. However, in the case of protandrous species the effect of sex change complicates the application of per recruit methods, so per recruit methods have not been used in this thesis.

Despite its limitations, the main conclusion that can be drawn from the model is that a large proportion of the reproductive output of a population may be concentrated in a small section of the population. By protecting some larger and older individuals

MPAs may open up more of the remaining stock to fishery exploitation than would be the case under MLS based management.

In addition to showing that reproductive output may be concentrated within certain size ranges of the population, the model also indicates that reproductive output may be concentrated within certain geographic areas of the population. The comparison of populations from across a wave exposure gradient indicated that there was a five fold increase in zygote production per unit area between unexploited exposed and sheltered populations (table 6.2, p.111). In terms of egg production, half the biomass of the sheltered population could be removed by the simulated fishery and the sheltered population would still produce more zygotes per unit area than the unexploited exposed population (figure 5.10a, p.91).

Spatial variation in population structure and reproductive output has two main implications for management strategies. Firstly it makes the definition of management reference points harder to define due to the spatial variation in population biology. In the case of a population that shows significant spatial heterogeneity the perceived management reference points may vary depending on the section of the habitat that was examined. It would be possible to determine average reference points to account for the population as a whole, but this would require more detailed knowledge of the population biology of the species in the different habitat types, and the proportional occurrence of the different habitat types.

The second main implication of habitat heterogeneity for management, is that by identifying and protecting areas with high reproductive output it may be possible to increase yield over traditional MLS or effort based management schemes. In the case of egg production, figure 5.14 (p.101) examined three different possible management strategies that could be applied in relation to habitat heterogeneity. The extent to which yield varied between the different management strategies whilst protecting a given level of egg production depended on the level of exploitation. However, for virtually all levels of exploitation the MLS based approach generated the lowest yield, whilst MPA based strategies generated higher yields. For the MPA based strategies, yield was maximised by exploiting the sub-populations with lowest E/SSB ratio first, whilst protecting the sub-populations with the highest E/SSB ratio.

To predict the effectiveness of MPA based strategies it is necessary to know more

about the biology and ecology of the species than just the isolated single species population dynamics as discussed above. For MPAs to be effective for the management of sessile and sedentary species with larval dispersal, there must be significant larval export from the MPAs to seed the surrounding areas (Crowder et al., 2000). Unfortunately the assessment of the genetic structure of the Azorean *P. candei* population (chapter 7) was unable to shed any light on the distance of larval dispersal. However knowledge of the extent of *P. candei* larval dispersal would ideally be required before effective MPAs for the enhancement of the *P. candei* fishery could be proposed.

A further factor that needs to be considered when considering the role of MPAs for limpet management is the role they play in structuring their community (Branch, 1985; Hawkins and Hartnoll, 1983; Jenkins et al., 1999a). Patellid limpet larvae in the Azores settle on lithothamnia encrusting coralline algae (Martins et al., 1987), and grazing by adult limpets keeps the lithothamina clear from being smothered by algal turfs (Morton et al., 1998). Once algal turfs are established they do not relinquish the rock surface unless dislodged by storm activity (Martins et al., 1987; Morton et al., 1998). Therefore as adult limpets are removed, the amount of available settlement habitat may also decline (Martins et al., 1987). The exact relationship between the requirements for settlement habitat, limpet grazing and turf development are not understood so it is not possible to make predictions about additional Allee effects regulating population dynamics of Azorean limpets. Nonetheless this example illustrates the further community interactions that occur and highlights the risk of basing too wider conclusion on a single species analysis of the benefits of MPAs. However, at the same time, this highlights the wider community effects of exploitation, and the use of MPAs would ameliorate the community effects of exploitation on protected areas.

8.4 Limitations and Further Work

There are a number of limitations to the work done in this thesis, and there are opportunities for future work. The main limitation of the combined zygote production model is that it is a static model that only examines the effect of first time

exploitation on a previously unharvested population. A per recruit approach would allow for the long term effect of different levels of harvesting to be examined. For a per recruit approach to be implemented a growth model would need to be developed, and any change in the size at sex change due to exploitation would have to be accounted for. Once these developments had been made a dynamic pool model could be developed that would allow analysis of the effect of exploitation on long term yield, rather than just once off yield, as is the case in this study.

For the population fecundity sub-model, the length fecundity relationship used for all three populations, at different wave exposure, was calculated from a single sample of limpets from a single location. Furthermore, the length fecundity relationships were only based on relatively small sample sizes. The gamete dispersal sub-model is limited by lack of specific information on the synchrony of limpet spawning, the proportion of the population spawning during a single spawning event, and the proportion of gametes released.

This thesis has not examined how size at sex change might alter under exploitation. It would be necessary to determine how size at sex change alters with changing mortality rates and population density to develop a dynamic model of the long term effect of exploitation on reproductive output.

The gamete dispersal sub-model is a very simplified version of the hydrodynamics of the surf zone of a rocky shore in a storm. However, if the assumptions that any longshore losses and additions cancel out, and that the surf zone is fully mixed in the cross-shore direction, are accepted as being reasonable then the model provides a reasonable representation of gamete dispersal, albeit that the physical parameters defining the size and mixing in the surf zone are poorly known.

The model treated the hydrodynamics of the sheltered, medium and exposed shores identically. But the variation in exposure itself is likely to lead to variation in the hydrodynamic characteristics of the different shores. This would effect the relative levels of zygote production between shores at different levels of wave exposure.

The main limitation of the fertilisation sub-model is that the fertilisation experiments were conducted in gently stirred jars that did not imitate natural levels of turbulence. The effect of turbulence on fertilisation rates is uncertain. Turbulence

is predicted to increase the encounter rates of particles (Lewis and Pedley, 2000), however, Mead and Denny (1995) (but see Denny et al., 2002) found that fertilisation rates at high sperm concentrations could be reduced due to high levels of turbulence. Thus further experimental work needs to be conducted to quantify the effect of turbulence to understand fertilisation kinetics under natural hydrodynamic conditions.

The main areas of further work required that have been highlighted by this study are the effect of exploitation on the size at sex change, and surf zone hydrodynamics.

8.5 Concluding Remarks

Protandrous species are likely to be more susceptible to recruitment overfishing than gonochronists. This is because size selective fisheries are likely to reduce the reproductive output of a protandrous population more rapidly than a gonochronistic population. Also standard fisheries metrics of population reproductive output, such as SSB, may provide a less accurate measure of actual reproductive output for protandrous species. This can leave protandrous populations more vulnerable to mismanagement.

Size variable sex ratios, size variable relative fecundity and sperm limitation have the effect that the reproductive output of a population is not spread evenly across a population. Different sections of the population may have different E/SSB ratios. The use of MPA based management strategies taking account of this variation may increase yield, whilst protecting a given level of reproductive output, compared to traditional MLS based strategies. The advantage of MPA based strategies in relation to sessile and sedentary species is that they can be used to protect spawning groups in key habitats, protect high density areas, and protect some larger and older individuals. Furthermore it should be noted that MPAs may have wider benefits in protecting areas from the wider community effects of exploitation, but these factors have not been directly addressed in this study.

Whilst necessarily limited in its scope, the broad conclusions of this study are ex-

pected to be robust, and it is hoped that they may provide useful practical guidance to stock managers, and help identify the most important issues in need of further study.

Bibliography

Abdel-Monem, A., Fernandez, L. A., and Boone, G. M. (1975). K-Ar ages from the eastern Azores group (Santa Maria, S. Miguel and the Formigas islands). *Lithos*, 8:247–254.

Aboim, M. A., Menezes, G. M., Schlitt, T., and Rogers, A. D. (2005). Genetic structure and history of populations of the deep-sea fish *Helicolenus dactylopterus* (Delaroche, 1809) inferred from mtDNA sequence analysis. *Molecular Ecology*, 14:1343–1354.

Allee, W. C. (1931). Animal aggregations. a study in general sociology. *University of Chicago Press, Chicago, Illinois.*

Andre, C. and Lindegarth, M. (1995). Fertilisation efficiency and gamete viability of a sessile, free-spawning bivalve, *Cerastoderma edule*. *Ophelia*, 43:215–227.

Anger, K. and Moreira, G. S. (1988). Morphometric and reproductive traits of tropical caridean shrimps. *Journal of Crustacean Biology*, 18:823–828.

Apte, S. and Gardner, J. P. A. (2002). Population genetic subdivision in the New Zealand greenshell mussel (*Perna canaliculus*) inferred from single-strand conformation polymorphism analysis of mitochondrial DNA. *Molecular Ecology*, 11:1617–1628.

Archambault, P. and Bourget, E. (1999). Influence of shoreline configuration on spatial variation of meroplanktonic larvae, recruitment and diversity of benthic subtidal communities. *Journal of Experimental Marine Biology and Ecology*, 238:161–184.

Archambault, P., McKindsey, C. W., and Bourget, E. (1999). Large-scale shoreline

configuration influences phytoplankton configuration and mussel growth. *Estuarine, Coastal and Shelf Science*, 49:193–208.

Arrontes, J., Arenas, F., Fernandez, C., Rico, J. M., Oliveros, J., Martinez, B., Viejo, R. M., and Alvarez, D. (2004). Effects of grazing by limpets on mid-shore species assemblages in northern Spain. *Marine Ecology Progress Series*, 277:117–133.

Avise, J. C., Arnold, J., and Ball, R. M. (1987). Intraspecific phylogeography: the mitochondrial bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, 18:489–522.

Azevedo, J. M. M., Portugal Ferreira, M. R., and Martins, J. A. (1991). The emergent volcanism of Flores island, Azores (Portugal). *Arquipelago. Life and Marine Sciences.*, 9:37–46.

Babcock, R. C., Bull, G. D., Harrison, P. L., Heyward, A. J., Oliver, J. K., Wallace, C. C., and Willis, B. L. (1986). Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. *Marine Biology*, 90:379–394.

Babcock, R. C. and Keesing, J. (1999). Fertilization biology of the abalone *Haliotis laevigata*: Laboratory and field studies. *Canadian Journal of Fisheries and Aquatic Science*, 56:1668–1678.

Babcock, R. C., Mundy, C. N., and Whitehead, D. (1994). Sperm diffusion models and in situ confirmation of long-distance fertilization in the free-spawning asteroid *Acanthaster planci*. *Biological Bulletin*, 186:17–28.

Bacci, G. (1947). Sex reversal in *Patella coerulea* l. and *Diodora gibberula* (lam). *Nature*, 160:94–95.

Baker, M. C. (2001). Fertilization kinetics in marine invertebrates. *University of Southampton Faculty of Science School of Ocean and Earth Sciences, PhD Thesis, 173pp.*

Baker, M. C. and Tyler, P. A. (2001). Fertilization success in the commercial gastropod *Haliotis tuberculata*. *Marine Ecology Progress Series*, 211:205–213.

Bannerot, S., Fox, Jr, W. W., and Powers, J. E. (1987). Reproductive strategies and the management of snappers and groupers in the gulf of Mexico and Carribean. In

Polovina, J. and Ralston, S., editors, *in: Tropical Snappers and Groupers: Biology and Fisheries Management. Westview Press, Boulder, Colorado.*, pages 561–603.

Barber, B. J., Getchell, R., Shumway, S., and Schick, D. (1988). Reduced fecundity in a deep-water population of the giant scallop *Placopecten magellanicus* in the gulf of Maine, USA. *Marine Ecology Progress Series*, 42:207–212.

Baxter, J. M. (1982). Population dynamics of *Patella vulgata* in Orkney. *Netherlands Journal of Sea Research*, 16:96–104.

Baxter, J. M. (1983). Annual variations in soft-body dry weight, reproductive cycle and sex ratios in populations of *Patella vulgata* at adjacent sites in the Orkney Islands. *Marine Biology*, 76:149–157.

Bayne, B. L. and Worrall, C. M. (1980). Growth and production of mussels *Mytilus edulis* from two populations. *Marine Ecology Progress Series*, 3:317–328.

Beach, D. H., Hanscomb, N. J., and Ormond, R. F. G. (1975). Spawning pheromone in crown of thorns starfish. *Nature*, 254:135–136.

Bechenbach, A. T., Wei, Y., and Liu, H. (1993). Relationships in *Drosphila obscura* species group inferred from mitochondrial cytochrome oxidase II sequences. *Molecular Biology and Evolution*, 10:619–634.

Begg, G. A., Friedland, K. D., and Pearce, J. B. (1999). Stock identification and its role in stock assessment and fisheries management: an overview. *Fisheries Research*, 43:1–8.

Belding, D. L. (1910). A report on the scallop fishery of Massachusetts; including the habits, life history of *Pecten irradians*, its rate of growth and other facts of economic value. *Special Report, Commissioners on Fisheries and Game, Massachusetts, Boston, MA, 150pp.*

Benson, A. J., Marelli, D. C., Frischer, M. E., Danforth, J. M., and Williams, J. D. (2001). Establishment of green mussels, *Perna viridis* (linnaeus 1758), (Mollusca, Mytilidae) on the west coast of Florida. *Journal of Shellfish Research*, 20:21–29.

Berndt, M., Callow, J. A., and Brawley, S. H. (2002). Gamete concentrations and

timing and success of fertilizations in a rocky shore seaweed. *Marine Ecology Progress Series*, 226:273–285.

Beverton, R. J. H. and Holt, S. J. (1957). On the dynamics of exploited fish populations. *Fishery Investigations Series II,Ministry of Agriculture, Fisheries and Food.*, 19, 456pp.

Blaber, S. J. M., Brewer, D. T., Milton, D. A., Sedana Merta, G., Efizon, D., Fry, G., and van der Velde, T. (1999). The life history of the protandrous tropical shad *Tenualosa macrura* (Alosinae: Clupidae): fishery implications. *Estuarine, Coastal and Shelf Science*, 49:689–701.

Black, K. P., Gay, S. L., and Andrews, J. C. (1990). Residence times of neutrally-bouyant matter such as larvae, sewage or nutrients on coral reefs. *Coral Reefs*, 9:105–114.

Boaventura, D., Alexander, M., Della Santina, P., Smith, N. D., Re, P., Cancela da Fonseca, L., and Hawkins, S. J. (2002a). The effects of grazing on the distribution and composition of low shore algal communities on the central coast of Portugal and on the southern coast of Britain. *Journal of Experimental Marine Biology and Ecology*, 267:185–206.

Boaventura, D., Cancela da Fonseca, L., and Hawkins, S. J. (2002b). Analysis of competitive interactions between the limpets *Patella depressa* Pennant and *Patella vulgata* L. on the northern coast of Portugal. *Journal of Experimental Marine Biology and Ecology*, 271:171–188.

Boaventura, D., Da Fonseca, L. C., and Hawkins, S. J. (2003). Size matters: competition within populations of the limpet *Patella depressa*. *Journal of Animal Ecology*, 72:435–446.

Boore, J. L. (1999). Animal mitochondrial genes. *Nucleic Acid Research*, 27:1767–1780.

Bowman, R. S. (1985). The biology of the limpet *Patella vulgata* L. in the British Isles: Spawning time as a factor determining recruitment sucess. *in Moore and Seed (eds) The Ecology of Rocky Coasts, Hodder and Stoughton, London*, pages 178–193.

Bowman, R. S. and Lewis, J. R. (1977). Annual fluctuations in the recruitment of *Patella vulgata* L. *Journal of the Marine Biological Association U.K.*, 57:793–815.

Bowman, R. S. and Lewis, J. R. (1986). Geographical variation in the breeding cycles and recruitment of *patella* spp. *Hydrobiologia*, 142:41–56.

Branch, G. M. (1974). The ecology of *Patella linnaeus* from the Cape Peninsula, South Africa. 2. Reproductive cycles. *Transactions of the Royal Society of South Africa*, 41:111–165.

Branch, G. M. (1975a). Intraspecific competition in *Patella cochlear* Born. *Journal of Animal Ecology*, 44:263–281.

Branch, G. M. (1975b). Notes on the ecoology of *Patella concotor* and *Cellana capensis*, and the effects of human consumption on limpet populations. *Zoologica Africana*, 10:75–85.

Branch, G. M. (1976). Interspecific competition experienced by South African *patella* species. *Journal of Animal Ecology*, 45:507–529.

Branch, G. M. (1981). The biology of limpets: physical factors, energy flow, and ecological interactions. *Oceanography and Marine Biology: An Annual Review.*, 19:235–380.

Branch, G. M. (1985). Limpets: their role in littoral and sublittoral community dynamics. In Moore, P. and Seed, R., editors, *The ecology of rocky coasts.*, pages 97–117. Hodder and Stoughton., London.

Branch, G. M. and Odendaal, F. (2003). The effects of marine protected areas on the population dynamics of a South African limpet, *Cymbula oculus*, relative to the influence of wave action. *Biological Conservation*, 114:255–269.

Briggs, J. C. (1966). Oceanic islands, endemism and marine paleotemperatures. *Systematic Zoology*, 15:153–163.

Briggs, J. C. (1970). A faunal history of the north Atlantic ocean. *Systematic zoology*, 19:19–34.

Brown, L. D. (1991). Genetic variation and population structure in the blacklip

abalone, *Haliotis rubra*. *Australian Journal of Marine and Freshwater Research*, 42:77–90.

Brown, W. M., George, M., and Wilson, A. C. C. (1979). Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 76:1967–1971.

Buxton, C. D. (1992). The application of yield-per-recruit models to two South African sparid reef species, with special considerations to sex change. *Fisheries Research*, 15:1–16.

Caley, M. J., Carr, M. H., Hixon, M. A., Hughes, T. P., Jones, G. P., and Menge, B. A. (1996). Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics*, 27:477–500.

Charnov, E. (1981). Sex reversal in *Pandalus borealis*: effect of a shrimp fishery? *Marine Biology Letters*, 2:53–57.

Charnov, E. L. (1982). The theory of sex allocation. *Princeton University Press, New Jersey. pp. 355.*

Charnov, E. L. and Bull, J. (1977). When is sex environmentally determined? *Nature*, 266:828–830.

Charnov, E. L., Gotshall, D. W., and Robinson, J. G. (1978). Sex ratio: adaptive response to population fluctuations in pandalid shrimp. *Science*, 200:204–206.

Chia, F. S. and Bickell, L. R. (1983). Spermatogenesis and sperm function. *In: Echinodermata. Reproductive Biology of Invertebrates. Vol 2. Adiyodi, K.G. and Adiyodi, R.G. (eds) John Wiley and Sons. New York. p545-620.*

Choat, J. H. (1977). The influence of sessile organisms on the population biology of three species of acmaeid limpets. *Journal of Experimental Marine Biology and Ecology*, 26:1–26.

Choquet, M. (1971). Etude du cycle biologique et de l'inversion du sexe chez *Patella vulgata* L. (mollusque gasteropode prosobranche). *General and Comparative Endocrinology*, 16:59–73.

Claereboudt, M. (1999). Fertilization success in spatially distributed populations of benthic free-spawners: A simulation model. *Ecological Modelling*, 121:221–233.

Claereboudt, M. and Himmelman, J. H. (1996). Recruitment, growth and production of giant scallops (*Placopecten magellanicus*) along an environmental gradient in Baie des Chaleurs, eastern Canada. *Marine Biology*, 124:661–670.

Clavier, J. (1992). Fecundity and optimal sperm density for fertilisation in the ormer *Haliotis tuberculata* L. *In Shepherd, Tegner and Del Proo (eds) Abalone of the World; Biology Fisheries and Culture. Proceedings of the 1st International Symposium on Abalone.*, pages 86–94.

Clement, M., Posada, D., and Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9:1657–1660.

Collin, R., McLellan, M., Gruber, K., and Bailey-Jourdain, C. (2005). Effects of conspecific associations on size at sex change in three species of calyptraeid gastropods. *Marine Ecology Progress Series*, 293:89–97.

Courchamp, F., Clutton-Brock, T., and Grenfell, B. (1999). Inverse density dependence and the Allee effect. *Trends in Ecology and Evolution*, 14:405–410.

Creese, R. G. (1980a). An analysis of distribution and abundance of populations of the high-shore limpet *Notoacmea petterdi* (Tenison-Woods). *Oecologia*, 45:252–260.

Creese, R. G. (1980b). Reproductive cycles and fecundities of four common eastern Australian archaeogastropod limpets (Mollusca: Gastropoda). *Australian Journal of Marine and Freshwater Research*, 31:49–59.

Creese, R. G., Schiel, D. R., and Kingsford, M. J. (1990). Sex change in the giant endemic limpet, *Patella kermadecensis*, from the Kermadec Islands. *Marine Biology*, 104:419–426.

Crisp, D. J. (1958). The spread of *Elminius modestus* Darwin in north-west Europe. *Journal of the Marine Biological Association of the United Kingdom*, 37:483–520.

Crowder, L. B., Lynman, S. J., Figueira, W. F., and Priddy, J. (2000). Source-

sink population dynamics and the problem of siting marine reserves. *Bulletin of Marine Science*, 66:799–820.

Cunliffe, B. and Hawkins, S. J. (1988). The shell midden depositis in Mount Batten Plymouth - a prehistoric Roman port. In Cunliffe, B., editor, *Monograph 26*, pages 35–38. Oxford University Committee for Archaeology.

Cushing, D. H. (1973). The dependence of recruitment on parent stock. *Journal of the Fisheries Research Board of Canada*, 30:1965–1976.

Dalzell, P., Adams, T. J. H., and Polunin, N. V. C. (1996). Coastal fisheries in the Pacific islands. *Oceanography and Marine Biology: An Annual Review.*, 34:395–531.

Denny, M. W. (1988). Biology and mechanics of the wave swept environment. *Princeton University Press, New Jersey. pp. 329.*

Denny, M. W., Dairiki, J., and Distefano, S. (1992). Biological consequences of topography on wave-swept rocky shores: I. Enhancement of fertilisation. *Biological Bulletin*, 183:220–232.

Denny, M. W. and Gaines, S. D. (1990). On the prediction of maximal intertidal wave forces. *Limnology and Oceanography*, 35:1–15.

Denny, M. W., Miller, L. P., Stokes, M. D., Hunt, L. J. H., and Helmuth, B. S. T. (2003). Extreme water velocities: Topographic amplification of wave-induced flow in the surf zone of rocky shores. *Limnology and Oceanography*, 48:1–8.

Denny, M. W., Nelson, E. K., and Mead, K. S. (2002). Revised estimates of the effects of turbulence on fertilisation in the purple sea urchin *Stronglocentrotus purpuratus.*, journal = Biological Bulletin, volume = 203, pages = 275-277,.

Denny, M. W. and Shibata, M. F. (1989). Consequences of surf-zone turbulence for settlement and external fertilization. *American Naturalist*, 134:859–889.

Detwiler, M. M., Hamp, T. J., and Kazim, A. L. (2004). DNA sequencing using the liquid polymer POP-7 on an ABI Prism 3100 Genetic Analyzer. *BioTechniques*, 36:932–933.

Dias, P. C. (1996). Sources and sinks in population biology. *Trends in Ecology and Evolution*, 11:326–330.

Dodd, J. M. (1956). Studies on the biology of limpets. III. Hermaphroditism in the three British species of *patella*. *Journal of the Marine Biological Association U.K.*, 35:327–340.

Doyle, J. J. and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of leaf tissue. *Phytochemistry Bulletin*, 19:11–15.

Dulvy, N. K. and Reynolds, J. D. (2002). Predicting extinction vulnerability in skates. *Conservation Biology*, 16:440–450.

Ehrlich, P. R. and Raven, P. H. (1969). Differentiation of populations. *Science*, 165:1228–1232.

Encena, II, V. C., Capinpin, Jr, E. C., and Bayona, N. C. (1998). Optimal sperm concentration and time for fertilization of the tropical abalone, *Haliotis asinina* Linne 1758. *Aquaculture*, 165:347–352.

FAO (1997). Fisheries statistics: Commodities. *FAO Fisheries Yearbook*, 80:FAO, Rome.

Fasham, M. J. R., Ducklow, H. W., and McKelvie, S. M. (1990). A nitrogen based model of plankton dynamics in the oceanic mixed layer. *Journal of Marine Research*, 48:591–639.

Ferraz, R. R., Menezes, G. M., and Santos, R. S. (2001). Limpet (*Patella* spp.) (Molusca:Gastropoda) exploitation in the Azores, during the period 1993-1998. *Arquipelago. Life and Marine Sciences.*, Supplement 2 (Part B):59–65.

Fletcher, W. J. (1984a). Intraspecific variation in the population dynamics and growth of the limpet, *Cellana tramoserica*. *Oecologia*, 63:110–121.

Fletcher, W. J. (1984b). Variability in the reproductive effort of the limpet, *Cellana tramoserica*. *Oecologia*, 61:259–264.

Frank, K. T. and Leggett, W. C. (1982). Coastal water mass replacement: Its effect on zooplankton dynamics and the predator-prey complex associated with larval

capelin *Mallotus villosus*. *Canadian Journal of Fisheries and Aquatic Science*, 39:991–1003.

Fretter, V. and Graham, A. (1962). *British Prosobranch Molluscs. Their functional anatomy and ecology.* Ray Society, London.

Gaines, S. D. and Lafferty, K. D. (1995). Modelling the dynamics of marine species: The importance of incorporating larval dispersal. *In Ecology of Marine Invertebrate Larvae, Ed. L.R. Mc Edward, CRC Press, Florida.*, pages 389–412.

Galtsoff, P. S. (1938). Physiological reproduction of *Ostrea virginica*. II. Stimulation of spawning in the female oyster. *Biological Bulletin*, 75:286–307.

Galtsoff, P. S. (1940). Physiology of reproduction of *Ostrea virginica*. III. stimulation of spawning in the male oyster. *Biological Bulletin*, 78:117–135.

Garwood, P. R. (1987). The cycle of gonad development in *Patella vulgata* (Mollusca; Gastropoda): The use of oocyte diameter measurements and gravimetric estimates of sexual maturity. *Sarsia*, 72:29–35.

Gauldie, R. W. (1991). Taking stock of genetic concepts in fisheries management. *Canadian Journal of Fisheries and Aquatic Science*, 48:722–731.

Ghiselin, M. T. (1969). The evolution of hermaphroditism among animals. *The Quarterly Review of Biology*, 44:189–208.

Gibbons, E. (1991). Iorras Beag Thair, Port na Feadoige (Dogs Bay) settlement site. *Excavations*, 51:19–20.

Giese, A. C. and Kanatani, H. (1987). Maturation and spawning. *In: Reproduction of Marine Invertebrates Vol IX. Eds Giese, A.C.; Pearse, J.S. and Pearse V.B., Blackwell Scientific Publications, California. 1987. 712pp.*

Glorioso, P. D. and Davies, A. M. (1995). The influence of eddy viscosity formulation, bottom topography, and wind wave effects upon the circulation of a shallow bay. *Journal of Physical Oceanography*, 25:1243–1264.

Gomez, A. and Carvalho, G. R. (2003). Phylogeography and reginal endemism of a passively dispersing zooplankter: mitochondrial DNA variation in rotifer resting egg banks. *Proceedings of the Royal Society of London B*, 267:2189–2197.

Grange, K. R. (1976). Rough water as a spawning stimulus in some trochid and turbinid gastropods. *N.Z. Journal of Marine and Freshwater Research*, 10:203–216.

Grant, W. S. and Bowen, B. W. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, 89:415–426.

Gray, D. R. and Hodgson, A. N. (2003). Growth and reproduction in the high shore South African limpet *Helcion pectunculus* (Mollusca : Patellogastropoda). *African Zoology*, 38:371–386.

Groom, M. J. (1998). Allee effects limit population viability of an annual plant. *American Naturalist*, 151:487–496.

Groppe, K., Sanders, I., Wiemken, A., and Boller, T. (1995). A microsatellite marker for studying the ecology of diverse fungal endophytes (*Epichloe* spp.) in grasses. *Applied and Environmental Microbiology.*, 61:3943–3949.

Gross, F. and Smyth, J. C. (1946). The decline of oyster populations. *Nature*, 157:540–542.

Guerra, M. T. and Gaudencio, M. J. (1986). Aspects of the ecology of *Patella* spp. on the Portuguese Coast., journal = Hydrobiologia, volume = 142, pages = 57-69,.

Gundersen, A. C., Nedreaas, K. H., Kjesbu, O. S., and Albert, O. T. (2000). Fecundity and recruitment variability of northeast Arctic Greenland halibut during 1980-1998, with emphasis on 1996-1998. *Journal of Sea Research*, 44:45–54.

Hall, S. J. (1999). The effects of fishing on marine ecosystems and communities. *Blackwell Science, Oxford.*, page 334pp.

Halpern, B. S. (2003). The impact of marine reserves: do reserves work and does size matter? *Ecological Applications*, 13 Supplement:S117–S137.

Hartl, D. L. and Clark, A. G. (1997). Principles of population genetics. 3rd edition. *Sinauer Associates. Sunderland, MA. 542pp.*

Haven, S. B. (1973). Competition for food between the intertidal gastropods *Acmaea scabra* and *Acmaea digitalis*. *Ecology*, 54:143–151.

Hawkins, J. P. and Roberts, C. M. (2003). Effects of fishing on sex-changing Caribbean parrotfishes. *Biological Conservation*, 115:213–226.

Hawkins, S. J. (1981). The influence of season and barnacles on the algal colonization of *Patella vulgata* exclusion areas. *Journal of the Marine Biological Association of the United Kingdom*, 61:1–15.

Hawkins, S. J., Corte-Real, H. B. S. M., Pannacciulli, F. G., Weber, L. C., and Bishop, J. D. D. (2000). Thoughts on the ecology and evolution of intertidal biota of the Azores and other Atlantic Islands. *Hydrobiologia*, 440:3–17.

Hawkins, S. J. and Hartnoll, R. G. (1982). The influence of barnacle cover on the numbers, growth and behaviour of *Patella vulgata* on a vertical pier. *Journal of the Marine Biological Association of the United Kingdom*, 62:855–867.

Hawkins, S. J. and Hartnoll, R. G. (1983). Grazing of intertidal algae by marine invertebrates. *Oceanography and Marine Biology: An Annual Review.*, 21:195–282.

Hawkins, S. J., Watson, D. C., Hill, A. S., Harding, S. P., Kyriakides, M., Hutchinson, S., and Norton, T. A. (1989). A comparison of feeding mechanisms in microphagous, herbivorous, intertidal, prosobranchs in relation to resource partioning. *Journal of Molluscan Studies*, 55:151–165.

Hayashi, K. (1999). Recent enhancements in SSCP. *Genetic Analysis: Biomolecular Engineering*, 14:193–196.

Hayward, B. W. and Brook, F. J. (1981). Exploitation and redistribution of flax snail (*Placostylus*) by the prehistoric Maori. *New Zealand Journal of Ecology*, 4:33–36.

Heipel, D. A., Bishop, J. D. D., Brand, A. R., and Thorpe, J. P. (1998). Population genetic differentiation of the great scallop *Pecten maximus* in western Britain investigated by randomly amplified ploymorphic DNA. *Marine Ecology Progress Series*, 162:163–171.

Heller, J. (1993). Hermaphroditism in molluscs. *Biological Journal of the Linnean Society*, 48:19–42.

Hesp, S. A., Potter, I. C., and Hall, N. G. (2004). Reproductive biology and protandrous hermaphroditism in *Acanthopagrus latus*. *Environmental Biology of Fishes*, 70:257–272.

Hilborn, R. and Walters, C. J. (1992). Quantitative fisheries stock assesment: Choice, dynamics and uncertainty. *Chapman and Hall, New York*, page 451pp.

Himmelman, J. H. (1975). Phytoplankton as a stimulus for spawning in three marine invertebrates. *Journal of Experimental Marine Biology and Ecology*, 20:199–214.

Hoagland, K. E. (1978). Protandry and the evolution of environmentally-mediated sex change: A study of the Mollusca. *Malacologia*, 17:365–391.

Hobday, A. J., Tegner, M. J., and Haaker, P. L. (2001). Over-exploitation of a broadcast spawning marine invertebrate: Decline of the white abalone. *Reviews in Fish Biology and Fisheries*, 10:493–514.

Hockey, P. A. R. and Branch, G. M. (1994). Conserving marine biodiversity on the African coast: implications of a terrestrial perspective. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 4:345–362.

Hoeh, W. R., Stewart, D. T., Sutherland, B. W., Smith, J. J., and Bush, G. L. (1997). Phylogeny of the genus *Rhagoletis* (Diptera: Tephritidae) inferred from DNA sequences of mitochondrial cytochrome oxidase II. *Molecular Phylogeny and Evolution*, 7:33–43.

Holt, R. D. (1985). Population dynamics in two-patch environments: Some anomalous consequences of an optimal habitat distribution. *Theoretical Population Biology*, 28:181–208.

Hughes, J. M., Baker, A. M., Bartlett, C., Bunn, S. E., Goudkamp, K., and Somerville, J. (2004). Past and present patterns of connectivity among populations of four cryptic species of freshwater mussels *Velesunio* spp. (Hyriidae) in central Australia. *Molecular Ecology*, 13:3197–3212.

Hughes, T. P. (1990). Recruitment limitation, mortality, and population regulation in open systems: A case study. *Ecology*, 71:12–20.

Ihssen, P. E., Booke, H. E., Casselman, J. M., McGlade, J., Payne, N. R., and Utter,

F. M. (1981). Stock identification: materials and methods. *Canadian Journal of Fisheries and Aquatic Science*, 38:1838–1855.

Inman, D. L., Tait, R. J., and Nordstrom, C. E. (1971). Mixing in the surf zone. *Journal of Geophysical Research*, 76:3493–3514.

Itosu, C. and Miki, M. (1983). Field observations of wave-induced circulation in ormer fishing grounds. *Bulletin of the Japanese Society of Scientific Fisheries*, 49:339–346.

Iwasa, Y. (1991). Sex change evolution and cost of reproduction. *Behavioral Ecology*, 2:56–68.

Jackson, J. B. C., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Bourque, B. J., Bradbury, R. H., Cooke, R., Erlandson, J., Estes, J. A., Hughes, T. P., Kidwell, S., Lange, C. B., Lenihan, H. S., Pandolfi, J. M., Peterson, C. H., Steneck, R. S., Tegner, M. J., and Warner, R. R. (2001). Historical overfishing and the recent collapse of coastal ecosystems. *Science*, 293:629–638.

Jara, H. F. and Moreno, C. A. (1984). Herbivory and structure in a midlittoral rocky community: a case in southern Chile. *Ecology*, 65:28–38.

Jenkins, S. R., Coleman, R. A., Della Santina, P., Hawkins, S. J., Burrows, M. T., and Hartnoll, R. G. (2005). Regional scale differences in the determinism of grazing effects in the rocky intertidal. *Marine Ecology Progress Series*, 287:77–86.

Jenkins, S. R. and Hartnoll, R. G. (2001). Food supply, grazing activity and growth rate in the limpet *Patella vulgata* L.: A comparison between exposed and sheltered shores. *Journal of Experimental Marine Biology and Ecology*, 258:123–139.

Jenkins, S. R., Hawkins, S. J., and Norton, T. A. (1999a). Direct and indirect effects of a macroalgal canopy and limpet grazing in structuring a sheltered inter-tidal community. *Marine Ecology Progress Series*, 188:81–92.

Jenkins, S. R., Hawkins, S. J., and Norton, T. A. (1999b). Interaction between a fucoid canopy and limpet grazing in structuring a low shore intertidal community. *Journal of Experimental Marine Biology and Ecology*, 233:41–63.

Jennings, S., Greenstreet, S. P. R., and Reynolds, J. D. (1999). Structural change

in an exploited fish community: a consequence of differential fishing effects on species with contrasting life histories. *Journal of Animal Ecology*, 68:617–627.

Jennings, S. and Kaiser, M. J. (1998). The effects of fishing on marine ecosystems. *Advances in Marine Biology*, 34:201–352.

Karakousis, Y. and Skibinski, D. O. F. (1992). An analysis of allozyme, mitochondrial DNA and morphological variation in mussel (*Mytillus galloprovincialis*) populations from Greece. *Experientia*, 48:878–881.

Karl, S. A. and Avise, J. C. (1992). Balancing selection at allozyme loci in oysters: implications for nuclear RFLPs. *Science*, 256:100–102.

Kautsky, N. (1982). Quantitative studies on gonad cycle, fecundity, reproductive output and recruitment in Baltic *Mytilus edulis* populations. *Marine Biology*, 68:87–98.

Kido, J. S. and Murray, S. N. (2003). Variation in owl limpet *Lottia gigantea* population structures, growth rates, and gonadal production on southern California rocky shores. *Marine Ecology Progress Series*, 257:111–124.

Kiflawi, M., Mazeroll, A. I., and Goulet, D. (1998). Does mass spawning enhance fertilisation in coral reef fish? A case study of the brown surgeonfish. *Marine Ecology Progress Series*, 172:107–114.

Kjesbu, O. S., Witthames, P. R., Solemdal, P., and Greer Walker, M. (1998). Temporal variations in the fecundity of the Arcto-Norwegian cod (*Gadus morhua*) in response to natural changes in food and temperature. *Journal of Sea Research*, 40:303–321.

Knowlton, N. and Keller, B. D. (1986). Larvae which fall far short of their potential: Highly localized recruitment in an alpheid shrimp with extended larval development. *Bulletin of Marine Science*, 39:213–223.

Koljonen, M. L. (2001). Conservation goals and fisheries management units for Atlantic salmon in the Baltic sea area. *Journal of Fish Biology*, 59 Supplement A:269–288.

Komar, P. D. (1998). Beach processes and sedimentation. 2nd edition. *Prentice-Hall Inc. New Jersey. pp.544.*

Kraus, G. H., Tomkiewicz, J., and Koster, F. W. (2002). Egg production of Baltic cod (*Gadus morhua*) in relation to variable sex ratio, maturity, and fecundity. *Canadian Journal of Fisheries and Aquatic Science*, 59:1908–1920.

Kremer, J. N. (1983). Ecological implications of parameter uncertainty in stochastic simulation. *Ecological Modelling*, 18:187–207.

Kutkuhn, J. H. (1981). Stock definition as a necessary basis for cooperative management of Great Lakes fish resources. *Canadian Journal of Fisheries and Aquatic Science*, 38:1476–1478.

Kuwamura, T. and Nakashima, Y. (1998). New aspects of sex change among reef fishes: Recent studies in Japan. *Environmental Biology of Fishes*, 52:125–135.

Kyle, C. J. and Boulding, E. G. (2000). Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. *Marine Biology*, 137:835–845.

Larkin, P. A. (1972). The stock concept and Pacific salmon. In Simon, R. and Larkin, P., editors, *The stock concept in Pacific salmon management.*, pages 34–56. University of British Colombia, Vancover B.C.

Lasiak, T. (1991). The susceptibility and/or resiliance of rocky littoral molluscs to stock depletion by indigenous coastal people of Transkei, Southern Africa. *Biological Conservation*, 56:245–264.

Lessios, H. A., Kessing, B. D., and Pearse, J. S. (2001). Population structure and speciation in tropical seas: global phylogeography of the sea urchin Diadema. *Evoloution*, 55:955–975.

Lessios, H. A., Kessing, B. D., Robertson, D. R., and Paulay, G. (1999). Phylogeography of the pantropical sea urchin Eucidaris in relation to land barriers and ocean currents. *Evoloution*, 53:806–817.

Levitan, D. R. (1991). Influence on body size and population density on fertilisa-

tion sucess and reproductive output in a free-spawning invertebrate. *Biological Bulletin*, 181:261–268.

Levitan, D. R. (1995). The ecology of fertilization in free-spawning invertebrates. *In Ecology of Marine Invertebrate Larvae, Ed. L.R. Mc Edward, CRC Press, Florida.*, pages 123–156.

Levitan, D. R. (1998). Sperm limitation, gamete competition, and sexual selection in external fertilisers. In Birkhead, T. and Moller, A., editors, *Sperm competition and sexual selection.*, page 826pp. Academic Press, San Diego.

Levitan, D. R. and Petersen, C. (1995). Sperm limitation in the sea. *Trends in Ecology and Evolution*, 10:228–231.

Levitan, D. R. and Sewell, M. A. (1998). Fertilization sucess in free-spawning marine invertebrates: a review of the evidence and fisheries implications. *Canadian Specially Publications on Fisheries and Aquatic Science*, 125:159–164.

Levitan, D. R., Sewell, M. A., and Fu-Shiang, C. (1991). Kinetics of fertilization in the sea urchin *Strongylocentrotus franciscanus*: Interaction of gamete dilution, age, and contact time. *Biological Bulletin*, 181:371–378.

Levitan, D. R., Sewell, M. A., and Fu-Shiang, C. (1992). How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. *Ecology*, 73:248–254.

Levitan, D. R. and Young, C. M. (1995). Reproductive success in large populations: Empirical measures and theoretical predictions of fertilisation in the sea biscuit *Clypeaster rosaceus*. *Journal of Experimental Marine Biology and Ecology*, 190:221–241.

Lewis, D. M. and Pedley, T. J. (2000). Planktonic contact rates in homogeneous isotopic turbulence: Theoretical predictions and kinematic simulations. *Journal of Theoretical Biology*, 205:377–4–8.

Lewis, D. M. and Pedley, T. J. (2001). The influence of turbulence on plankton predation strategies. *Journal of Theoretical Biology*, 210:347–365.

Lewis, J. R. (1964). *The ecology of rocky shores.* English Universities Press Ltd., London.

Lewis, J. R. and Bowman, R. S. (1975). Local habitat-induced variations in the population dynamics of *Patella vulgata* L. *Journal of Experimental Marine Biology and Ecology*, 17:165–203.

Lillie, F. R. (1915). Studies of fertilisation. vii. Analysis of variations in the fertilization power of sperm suspensions of *Arbacia*. *Biological Bulletin*, 28:229–251.

Lockwood, D. R., Hastings, A., and Botsford, L. W. (2002). The effect of dispersal patterns on marine reserves: does the tail wag the dog? *Theoretical Population Biology*, 61:297–309.

Longuet-Higgins, M. S. and Stewart, R. W. (1964). Radiation stress in water waves: A physical discussion, with applications. *Deep-Sea Research*, 11:529–562.

Luckhurst, B. E. and Luckhurst, K. (1978). Analysis of the influence of substrate variables on coral reef fish communities. *Marine Biology*, 49:317–323.

Lundquist, C. J. and Botsford, L. W. (2004). Model projections on the implications of the Allee effect in broadcast spawners. *Ecological Applications*, 14:929–941.

MacDonald, B. A. and Thompson, R. J. (1985). Influence of temperature and food avaliability on the ecological energetics of the giant scallop *Placopecten magellanicus*. II. reproductive output and total production. *Marine Ecology Progress Series*, 25:295–303.

Mace, P. M. and Sissenwine, M. P. (1993). How much spawning per recruit is enough? *Canadian Special Publication for Fisheries and Aquatic Sciences*, 120:101–118.

MacLean, J. A. and Evans, D. O. (1981). The stock concept, discreteness of fish stocks, and fisheries management. *Canadian Journal of Fisheries and Aquatic Science*, 38:1889–1898.

Marconato, A. and Shapiro, D. Y. (1996). Sperm allocation, sperm production and fertilisation rates in the bucktooth parrotfish. *Animal Behaviour*, 52:971–980.

Marko, P. B. (2004). 'What's larvae got to do with it?' disparate patterns of

post-glacial population structure in two benthic marine gastropods with identical dispersal potential. *Molecular Ecology*, 13:597–611.

Marshall, C. T., Kjesbu, O. S., Yaragina, N. A., Solemdal, P., and Ulltang, O. (1998). Is spawner biomass a sensitive measure of the reproductive and recruitment potential of northeast Arctic cod? *Canadian Journal of Fisheries and Aquatic Science*, 55:1766–1783.

Marshall, D. J., Semmens, D., and Cook, C. (2004). Consequences of spawning at low tide: Limited gamete dispersal for a rockpool anemone. *Marine Ecology Progress Series*, 266:135–142.

Marshall, D. J., Styan, C. A., and Keough, M. J. (2000). Intraspecific co-variation between egg and body size affects fertilization kinetics of free-spawning marine invertebrates. *Marine Ecology Progress Series*, 195:305–309.

Martins, H. R., Santos, R. S., and Hawkins, S. J. (1987). Exploitation of limpets (*Patella* spp.) in the Azores with a preliminary analysis of the stocks. *ICES Report*, 1987/K:53:1–17.

Maruyama, T. (1971). Speed of gene substitution in a geographically structured population. *American Naturalist*, 105:253–265.

McCartney, M. A., Keller, G., and Lessios, H. A. (2000). Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus *Echinometra*. *Molecular Ecology*, 9:1391–1400.

McCulloch, A. and Shanks, A. L. (2003). Topographically generated fronts, very nearshore oceanography and the distribution and settlement of mussel larvae and barnacle cyprids. *Journal of Plankton Research*, 25:1427–1439.

McEuen, F. S. (1988). Spawning behaviours of northeast Pacific sea cucumbers (Holothuroidea: Echinodermata). *Marine Biology*, 98:565–585.

McLachlan, A. (1980). Exposed sandy beaches as semi-enclosed ecosystems. *Marine Environmental Research*, 4:59–63.

McQuaid, C. D. and Phillips, T. E. (2000). Limited wind-driven dispersal of intertidal mussel larvae: In situ evidence from the plankton and the spread of the

invasive species *Mytilus galloprovincalis* in South Africa. *Marine Ecology Progress Series*, 201:211–220.

McShane, P. E. (1992). Exploitation models and catch statistics of the Victorian fishery for abalone *Haliotis rubra*. *Fishery Bulletin*, 90:139–146.

McShane, P. E. and Naylor, J. R. (1995). Small-scale spatial variation in growth, size at maturity, and yield- and egg-per-recruit relations in the New Zealand abalone *Haliotis iris*. *New Zealand Journal of Marine and Freshwater Research*, 29:603–612.

Mead, K. S. and Denny, M. W. (1995). The effects of hydrodynamic shear stress on fertilization and early development of the purple sea urchin *Stronglyocentus purpuratus*. *Biological Bulletin*, 188:46–56.

Meidel, S. K. and Scheibling, R. E. (1998). The annual reproductive cycle of the green sea urchin, *Stronglyocentrotus droebachiensis*, in differing habitats in Nova Scotia, Canada. *Marine Biology*, 131:461–478.

Meidel, S. K. and Scheibling, R. E. (2001). Variation in egg spawning among subpopulations of sea urchins *Stronglycentrotus droebachiensis*: a theoretical approach. *Marine Ecology Progress Series*, 213:97–110.

Metaxas, A., Scheibling, R. E., and Young, C. M. (2002). Estimating fertilization success in marine benthic invertebrates: a case study with the tropical sea star *Oreaster reticulatus*. *Marine Ecology Progress Series*, 226:87–101.

Michalakis, Y. and Excoffier, L. (1996). A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. *Genetics*, 142:1061–1064.

Milton, D. A., Die, D., Tenakanai, C., and Swales, S. (1998). Selectivity for barramundi (*Late calcarifer*) in the Fly River, Papua New Ginea: Implications for managing gill-net fisheries on protandrous species. *Marine and Freshwater Research*, 49:499–506.

Moore, P. G. and Seed, R., editors (1985). *The ecology of rocky coasts.* Hodder and Stoughton, London.

Morton, B., Britton, J. C., and de Fris Martins, A. M. (1998). *Coastal ecology of the Azores.* Sociedade Afonso Chaves, Associacao de Estudos Acoreanos., Ponta Delgada.

Munday, P. L. (2002). Bi-directional sex change: testing the growth-rate advantage model. *Behavioral Ecology and Sociobiology*, 52:247–254.

Munoz, R. C. and Warner, R. R. (2003). A new version of the size-advantage hypothesis for sex change: Incorporating sperm competition and size-fecundity skew. *American Naturalist*, 161:749–761.

Munoz, R. C. and Warner, R. R. (2004). Testing a new version of the size-advantage hypothesis for sex change: sperm competition and size-skew effects in the bucktooth parrotfish, *Sparisoma radians*, journal = Behavioral Ecology, volume = 15, pages = 129-136,.

Murawski, S. A., Brown, R., Lai, H.-L., Rago, P. J., and Hendrickson, L. (2000). Large-scale closed areas as a fishery-management tool in temperate marine systems: the George Bank experience. *Bulletin of Marine Science*, 66:775–798.

Murawski, S. A., Rago, P. J., and Trippel, E. A. (2001). Impacts of demographic variation in spawning characteristics on reference points for fishery management. *Ices Journal of Marine Science*, 58:1002–1014.

Myers, R. A. (2002). Recruitment: understanding density dependence in fish populations. In Hart, P. and Reynolds, J., editors, *Handbook of fish biology and fisheries. Volume 1: fish biology.*, pages 123–148. Blackwell Publishing.

Myers, R. A. and Barrowman, N. J. (1996). Is fish recruitment related to spawner abundance? *Fishery Bulletin*, 94:707–724.

Naidenko, T. K. (1989). Spawning and development of the abalone *Haliotis ovina* (Linne) in a ship laboratory. *Abalone of the world. Biology, Fisheries and Culture. Supplementary Papers. Proceedings of the 1st International Symposium on abalone. La Paz, Mexico 21-25 th November 1989.*, pages 30–32.

Nakano, T. and Ozawa, T. (2004). Phylogeny and historical biogeography of limpets of the order Patellogastropoda based on mitochondrial DNA sequences. *Journal of Molluscan Studies*, 70:31–41.

Nakaoka, M. (1994). Size-dependent reproductive traits of *Yoldia notabilis* (Bivalvia: Protobranchi). *Marine Ecology Progress Series*, 114:129–137.

Nakashima, Y., Kuwamura, T., and Yogo, Y. (1995). Why be a both way sex changer? *Ethnology*, 101:301–307.

Nash, W. J. (1992). An evaluation of egg-per-recruit analysis as a means of assessing size limits for blacklip abalone (*Haliotis rubra*) in Tasmania. *In Shepherd, Tegner and Del Proo (eds) Abalone of the World; Biology Fisheries and Culture. Proceedings of the 1st International Symposium on Abalone.*, pages 318–338.

Nei, M. (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York, USA.

Neigel, J. E. (2002). Is Fst obsolete? *Conservation Genetics*, 3:167–173.

Orton, J. H. (1919). Sex-phenomena in the common limpet (*Patella vulgata*). *Nature*, 104:373–374.

Orton, J. H. (1928). Observations on *Patella vulgata*. part I. Sex-phenomena, breeding and shell-growth. *Journal of the Marine Biological Association U.K.*, 15:851–862.

Orton, J. H. and Southward, A. J. (1961). Studies on the biology of limpets. iv. The breeding of *Patella depressa* Pennant on the north Cornish coast. *Journal of the Marine Biological Association U.K.*, 41:653–662.

Orton, J. H., Southward, A. J., and Dodd, J. M. (1956). Studies on the biology of limpets. II. The breeding of *Patella vulgata* L. in Britain. *Journal of the Marine Biological Association U.K.*, 35:149–176.

Paetkau, D., Slade, R., Burden, M., and Estoup, A. (2004). Genetic assignment methods for direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology*, 13:55–65.

Palumbi, S. R. (1995). Using genetics as an indirect estimator of larval dispersal. In McEdward, L., editor, *Ecology of marine invertebrate larvae.*, pages 369–387. CRC Press, Florida.

Palumbi, S. R. (2001). The ecology of marine protected areas. In Bertness, M.,

Gaines, S., and Hay, M., editors, *Marine community ecology.*, pages 509–530. Sinauer Associates Inc., Sunderland. M.A.

Palumbi, S. R. (2003). Population genetics, demographic connectivty, and the design of marine reserves. *Ecological Applications*, 13 Supplement:S146–S158.

Parker, P. G., Snow, A. A., Schug, M. D., Booton, G. C., and Fuerst, P. A. (1998). What molecules can tell us about populations: choosing and using a molecular marker. *Ecology*, 79:361–382.

Parry, G. D. (1982). Reprodutive effort in four species of intertidal limpet. *Marine Biology*, 67:267–282.

Pauly, D., Christensen, V., Dalsgaard, J., Froese, R., and Torres, F. (1998). Fishing down marine food webs. *Science*, 279:860–863.

Pawson, M. G. and Jennings, S. (1996). A critique of methods for stock identification in marine capture fisheries. *Fisheries Research*, 25:203–217.

Pennington, J. T. (1985). The ecology of fertilisation of echinoid eggs: The consequences of sperm dilution, adult aggregation, and synchronous spawning. *Biological Bulletin*, 169:417–430.

Petersen, C. H., Summerson, H. C., and Luettich, Jr, R. A. (1996). Response of bay scallops to spawner transplants: A test of recruitment limitation. *Marine Ecology Progress Series*, 132:93–107.

Petersen, C. W. (1991). Variation in fertilisation rate in the tropical reef fish, *Halichoeres bivattatus*: correlates and implications. *Biological Bulletin*, 181:232–237.

Pfister, C. A. and Bradbury, A. (1996). Harvesting red sea urchins: recent effects and future predictions. *Ecological Applications*, 6:298–310.

Polunin, N. V. C. (2002). Marine protected areas, fish and fisheries. In Hart, J. and Reynolds, J. D., editors, *Handbook of fish biology and fisheries. Volume 2: Fisheries*, pages 128–161. Blackwell Publishing.

Pombo, O. A. and Escofet, A. (1996). Effect of exploitation on the limpet *Littia gigantea*: A field study in Baja California (Mexico) and California (U.S.A.). *Pacific Science*, 50:393–403.

Powell, D. K., Tyler, P. A., and Peck, L. S. (2001). Effect of sperm concentration and sperm ageing on fertilisation sucess in the Antarctic soft-shelled clam *Laternula elliptica* and the Antarctic limpet *Nacella concinna*. *Marine Ecology Progress Series*, 215:191–200.

Preston, J. (2003). Allopatric speciation in the littoral gastropod genus *Osilinus philippi*, 1847 (Gastropoda: Trochidae) at the Atlantic/Mediterranean interface. *University of Southampton Faculty of Science School of Ocean and Earth Sciences PhD Thesis, 233pp. and appendices.*

Prince, J. D., Sellers, T. L., Ford, W. B., and Talbot, S. R. (1988). Confirmation of a relationship between the localized abundance of breeding stock and recruitment for *Haliots rubra* Leach (Mollusca: Gastropoda). *Journal of Experimental Marine Biology and Ecology*, 122:91–104.

Pulliam, H. R. (1988). Sources, sinks, and population regulation. *American Naturalist*, 132:652–661.

Punt, A. E., Garratt, P. A., and Govender, A. (1993). On an approach for applying per-recruit methods to a protogynous hermaphrodite, with an illustration for the slinger *Chrysoblephus puniceus* (Pisces: Sparidae). *South African Journal of Marine Science*, 13:109–119.

Quesada, H., Wenne, R., and Skibinski, D. O. F. (1995). Differential introgression of mitochondrial DNA across species boundaries within the marine mussel genus *Mytilus*. *Proceedings of the Royal Society of London B*, 262:51–56.

Quinn, J. F., Wing, S. R., and Botsford, L. W. (1993). Harvest refugia in marine invertebrate fisheries: Models and applications to the red sea urchin, *Strongylocentrous franciscanus*. *American Zoologist*, 33:537–550.

Raitt, D. S. (1933). The fecundity of haddock. *Scientific Investigations*, 1:32–54.

Ramirez Llodra, E. (2002). Fecundity and life-history stratergies in marine invertebrates. *Advances in Marine Biology*, 43:87–170.

Rannala, B. and Hartigan, J. (1996). Estimating gene flow in island populations. *Genetical Research*, 67:147–158.

Reeb, C. A. and Avise, J. C. (1990). A genetic discontinuity in a continually distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. *Genetics*, 124:397–406.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution*, 43:223–225.

Ricker, W. E. (1954). Stock and recruitment. *Journal of the Fisheries Research Board of Canada*, 11:559–623.

Ridgway, S. A., Reid, D. G., Taylor, J. D., Branch, G. M., and Hodgson, A. N. (1998). A cladistic phylogeny of the family Patellidae (Mollusca: Gastropoda). *Philosophical Transactions of the Royal Society of London, Series B*, 353:1645–1671.

Rigaa, A., Cellos, D., and Monnerot, M. (1997). Mitochondrial DNA from the scallop *Pecten maximus*: an unusual polymorphism detected by restriction fragment length polymorphism analysis. *Heredity*, 79:380–387.

Roberts, C. M. (1997). Connectivity and management of Caribbean coral reefs. *Science*, 278:1454–1457.

Roberts, C. M. and Hawkins, J. P. (1999). Extinction risk in the sea. *Trends in Ecology and Evolution*, 14:241–246.

Rodhouse, P. G. (1978). Energy transformation by the oyster *Ostrea edulis* L. in a temperate estuary. *Journal of Experimental Marine Biology and Ecology*, 34:1–22.

Rogers, A. R. and Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic distances. *Molecular Biology and Evolution*, 9:552–569.

Roll, J., Mitchell, R. J., Cabin, R. J., and Marshall, D. L. (1997). Reproductive success increases with local density of conspecifics in a desert mustard (*Lesquerella fendleri*). *Conservation Biology*, 11:738–746.

Rothschild, B. J. and Osborn, T. R. (1988). Small-scale turbulence and plankton contact rates. *Journal of Plankton Research*, 10:465–474.

Rowley, R. J. (1994). Marine reserves in fisheries management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 4:233–254.

Roy, K., Collins, A. G., Becker, B. J., Begovic, E., and Engle, J. M. (2003). Anthropogenic impacts and historical decline in body size of rocky intertidal gastropods in southern California. *Ecology Letters*, 6:205–211.

Russell, E. S. (1909). The growth of the shell of *Patella vulgata*. *Proceedings of the Zoological Society of London*, 79:235–253.

Russell, E. S. (1934). Some theoretical considerations on the "overfishing" problem.

Sala, E., Aburto-Oropeza, O., Paredes, G., Parra, I., Barrera, J. C., and Dayton, P. K. (2002). A general model for designing networks of marine reserves. *Science*, 298:1991–1993.

Santos, R. S., Hawkins, S. J., Monterio, L. R., Alves, M., and Isidro, E. J. (1995). Marine research, resources and conservation in the Azores. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 5:311–354.

Santos, R. S., Martins, H. R., and Hawkins, S. J. (1989). Study report about the state of limpet populations in the Azores and Madeira islands. *DOP Report, Azores Uni*, page 34pp (In portuguese).

Sasaki, R. (1984). Reproduction and larval development of *Septifer virgatus* in Kesennuma Bay Miyagi Prefecture. (in japanese). *Suisan Zoushoku*, 31:214–219.

Sasaki, R. and Shepherd, S. A. (1995). Larval dispersal and recruitment of *Haliotis discus* hannai and *Tegula* spp. on Miyagi coasts, Japan. *Marine and Freshwater Research*, 46:519–529.

Scheltema, R. S. (1986). Long-distance dispersal by planktonic larvae of shoal-water benthic invertebrates among central Pacific islands. *Bulletin of Marine Science*, 39:241–256.

Schmidt, J. (1930). Racial investigations x. the Atlantic cod (*Gadus callaris* L.) and local races of the same. *Comptes-Rendus des Travaux du Laboratoire Carlsberg*, 18:1–71.

Schneider, S., Roessli, D., and Excoffier, L. (2000). Arlequin ver. 2.000: A software for population genetics data analysis. *Genetics and Biometry Laboratory, University of Geneva, Switzland.*

Serrao, E. A., Pearson, G., Kautsky, L., and Brawley, S. H. (1996). Successful external fertilisation in turbulent environments. *Proceedings of the National Academy of Sciences*, 93:5286–5290.

Shaklee, J. B., Beacham, T. D., Seeb, L., and White, B. A. (1999). Managing fisheries using genetic data: case studies from four species of Pacific salmon. *Fisheries Research*, 43:45–78.

Shanks, A. L. (1983). Surface slicks associated with tidally forced internal waves may transport pelagic larvae of benthic invertebrates and fishes shoreward. *Marine Ecology Progress Series*, 13:311–315.

Shanks, A. L. (1998). Apparent oceanographic triggers to the spawning of the limpet *Lottia digitalis* (Rathke). *Journal of Experimental Marine Biology and Ecology*, 222:31–41.

Shanks, A. L. and McCulloch, A. (2003). Topographically generated front, very nearshore oceanography, and the distribution of chlorophyl, detritus, and selected diatom and dinoflagellate taxa. *Marine Biology*, 143:969–980.

Shanks, A. L., McCulloch, A., and Miller, J. (2003). Topographically generated fronts, very nearshore oceanography and the distribution of larval invertebrates and holoplankters. *Journal of Plankton Research*, 25:1251–1277.

Shepherd, J. G. (1982). A versatile new stock-recruitment relationship for fisheries, and the construction of sustainable yield curves. *J Cons int Explor Mer*, 40:67–75.

Shepherd, S. A. and Brown, L. D. (1993). What is an abalone stock: Implications for the role of refugia in conservation. *Canadian Journal of Fisheries and Aquatic Science*, 50:2000–2009.

Shinomiya, A., Yamada, M., and Sunobe, T. (2003). Mating system and protandrous sex change in the lizard flathead, *Inegocia japonica* (Platycephalidae). *Ichthyological Research*, 50:383–386.

Short, A. D. (1985). Rip-current type, spacing and persistence, Narrabeen Beach, Australia. *Marine Geology*, 65:47–71.

Siegel-Causey, D., Lefevre, C., and Savinetsiui, A. B. (1991). Historical diversity of cormorants and shags from Amchitka Island, Alaska. *The Condor*, 93:840–852.

Simpson, A. C. (1951). The fecundity of plaice. *Fisheries Investigations. Series II*, 17:(5).

Slatkin, M. (1985). Gene flow in natural populations. *Annual review of ecology and systematics.*, 16:393–430.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science*, 236:787–792.

Small, M. P. and Gosling, E. M. (2000). Species relationships and population structure of *Littorina saxatalis* Olivi and *L. tenebrosa* Montagu in Ireland using single-strand conformational polymophisms (SSCPs) of cytochtome b fragments. *Molecular Ecology*, 9:39–52.

Small, M. P. and Gosling, E. M. (2001). Population genetics of a snail species complex in the British Isles: *Littorina saxatilis* (Olivi), *L. neglecta* Bean and *L. tenebrosa* (Montagu), using SSCP analysis of Cytochrome-B gene fragments. *Journal of Molluscan Studies*, 67:69–80.

Soong, K., Chang, D., and Chao, S. M. (2005). Presence of spawning-inducing pheromones in two brittle stars (Echinodermata: Ophiuroidea). *Marine Ecology Progress Series*, 292:195–201.

Soong, K. and Chen, J.-L. (1991). Population structure and sex-change in the coral-inhabiting snail *Coralliophila violacea* at Hsiao-Liuchiu, Taiwan. *Marine Biology*, 111:81–86.

Southward, A. J. (1953). The ecology of some rocky shores in the south of the Isle of Man. *Proceedings of the Transactions of the Liverpool Biological Society.*, 59:1–50.

Southward, A. J. and Orton, J. H. (1954). The effects of wave-action on the distribution and numbers of the commoner plants and animals living on the Plymouth breakwater. *Journal of the Marine Biological Association of the United Kingdom*, 33:1–19.

Southward, A. J. and Southward, E. C. (1978). Recolonisation of rocky shores in

Cornwall after use of toxic dispersants to clean up the Torrey Canyon spill. *Journal of the Fisheries Research Board of Canada*, 35:682–706.

Sparck, R. (1927). Studies on the biology of the oyster (*Ostrea edulis*) II-IV. *Report of the Danish Biological Station No.33*, pages pp. 43–65.

Spencer Davis, P. (1969). Effect of environment on metabolic activity and morphology of Mediterranean and British species of *Patella*. *Pubblicazioni della Stazione Marittima di Napoli*, 37:641–656.

Spiegel, M. R. (1961). *Theory and problem of statistics.* Schaum's Outline Series. McGraw-Hill, New York.

Sponaugle, S., Cowen, R. K., Shanks, A. L., Morgan, S. G., Leis, J. M., Pineda, J., Boehlert, G. W., Kingsford, M. J., Lindeman, K. C., Grimes, C., and Munro, J. L. (2002). Predicting self-recruitment in marine populations: Biophysical correlates and mechanisms. *Bulletin of Marine Science*, 70:341–375.

St Mary, C. M. (1994). Sex allocation in a simultaneous hermaphrodite, the blue-banded goby (*Lythrypnus dalli*): the effects of body size and behavioral gender and the consequences for reproduction. *Behavioral Ecology*, 5:304–313.

Starr, M., Himmelman, J. H., and Therriault, J. C. (1990). Direct coupling of marine invertebrate spawning with phytoplankton blooms. *Science*, 247:1071–1074.

Steneck, R. S. and Watling, L. (1982). Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. *Marine Biology*, 68:299–319.

Stephenson, R. L. (1999). Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. *Fisheries Research*, 43:247–249.

Stephenson, T. A. and Stephenson, A. (1972). *Life between the tide marks on rocky shores.* W.H. Freeman and Co., San Francisco, Ca.

Styan, C. A. and Butler, A. J. (2000). Fitting fertilisation kinetics models for free spawning marine invertebrates. *Marine Biology*, 137:943–951.

Sunnucks, P., Wilson, A. C. C., Beheregaray, L. B., Zenger, K., French, J., and Taylor, A. C. (2000). SSCP is not so difficult: the application and utility of

single-strand confirmation polymorphism in evolutionary biology and molecular ecology. *Molecular Ecology*, 9:1699–1710.

Svendsen, I. A. (1992). Surf zone dynamics. *In Breaking Waves. IUTAM Symposium Sydney Australia, 1991. Banner, M.L. and Grimshaw, R.H.J. eds. Springer-Verlag, Berlin Heidelberg, 1992. pp 55-68.*

Symonds, G., Black, K. P., and Young, I. R. (1995). Wave-driven flow over shallow reefs. *Journal of Geophysical Research*, 100:2639–2648.

Tajima, F. (1989a). The effect of change in population size on DNA polymorphism. *Genetics*, 123:597–601.

Tajima, F. (1989b). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123:585–595.

Talbot, M. M. B. and Bate, G. C. (1987a). Distribution patterns of rip frequency and intensity in Algoa Bay, South Africa. *Marine Geology*, 76:319–324.

Talbot, M. M. B. and Bate, G. C. (1987b). Rip current characteristics and their role in the exchange of water and surf diatoms between the surf zone and nearshore. *Estuarine, Coastal and Shelf Science*, 25:707–720.

Tanaka, S., Shepard, M. P., and Bitton, H. T. (1969). Origin of chum salmon (*Oncorrhynchus keta*) in offshore waters of the north Pacific in 1956-1958 as determined from scale studies. *International North Pacific Fisheries Commission Bulletin*, 26:57–155.

Tegner, M. J. (1993). Southern Californian abalones: Can stocks be rebuilt using marine harvest refugia? *Canadian Journal of Fisheries and Aquatic Science*, 50:2010–2018.

Tegner, M. J., Basch, L. V., and Dayton, P. K. (1996). Near extinction of an exploited marine invertebrate. *Trends in Ecology and Evolution*, 11:278–280.

Templeman, W. (1983). Stock discrimination in marine fishes. *NAFO Scientific Council Studies*, 6:57–62.

Templeton, A. R., Crandall, K. A., and Sing, C. F. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease

mapping and DNA sequence data. III. cladogram estimation. *Genetics*, 132:619–633.

Tereshchenko, E. S. (2002). The dynamics of population fecundity in Barents sea capelin. *ICES Journal of Marine Science*, 59:976–982.

Thackeray, J. F. (1988). Molluscan fauna from Klassies River, South Africa. *South African Archaeolical Bulletin.*, 43:27–32.

Thomas, F. I. M. (1994). Transport and mixing of gametes in three free spawning polychaete annelids, *Phragmatopoma californica* (Fewkes), *Sabellaria cementarium* (Moore), and *Schizobranchia insignis* (Bush). *Journal of Experimental Marine Biology and Ecology*, 179:11–27.

Thompson, G. B. (1979). Distribution and population dynamics of the limpet *Patella aspera* (Lamark) in Bantry Bay. *Journal of Experimental Marine Biology and Ecology*, 40:115–135.

Thompson, G. B. (1980). Distribution and abundance of *Patella vulgata* L. in Bantry Bay. *Journal of Experimental Marine Biology and Ecology*, 45:173–217.

Thompson, G. G. (1993). A proposal for a threshold stock size and maximum fishery mortality rate. *Canadian Special Publication for Fisheries and Aquatic Sciences*, 120:303–320.

Thompson, J. D., Gibson, T. J., Plewiak, F., Jeanmougin, F., and Higgins, D. G. (1997). The clustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research*, 24:4876–4882.

Todd, C. D. (1998). Larval supply and recruitment of benthic invertebrates: Do larvae always disperse as much as we believe? *Hydrobiologia*, 375:1–21.

Underwood, A. J., Denley, E. J., and Moran, M. J. (1983). Experimental analyses of the structure and dynamics of mid-shore rocky intertidal communities in New South Wales. *Oecologia*, 56:202–219.

Vogel, H., Czihak, G., Chang, P., and Wolf, W. (1982). Fertilization kinetics of sea urchin eggs. *Mathematical Biosciences*, 58:189–216.

Volman, T. P. (1978). Early archeological evidence for shellfish collecting. *Science*, 201:911–913.

Wahle, R. A. and Peckham, S. H. (1999). Density-related reproductive trade-offs in the green sea urchin, *Stronglyocentrotus droebachiensis*. *Marine Biology*, 134:127–137.

Waples, R. S. (1998). Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, 89:438–450.

Warner, R. R. (1975). The adaptive significance of sequential hermaphroditism in animals. *The American Naturalist*, 109:61–82.

Warner, R. R., Swearer, S. E., and Caselle, J. E. (2000). Larval accumulation and retention: implications for the design of marine reserves and essential fish habitat. *Bulletin of Marine Science*, 66:821–830.

Weber, L. I. and Hawkins, S. J. (2002). Evolution of the limpet *Patella candei* d'Orbigny (Mollosca, Patellidae) in Atlantic archipelagos: human intervention and natural processes. *Biological Journal of the Linnean Society*, 77:341–353.

Weir, B. S. and Cockerham, C. C. (1984). Estimating f-statistics for the analysis of population structure. *Evolution*, 38:323–354.

Williams, M. E., Bentley, M. G., and Hardege, J. D. (1997). Assesment of field fertilization success in the infaunal polychaete *Arenicola marina* (L.). *Invertebrate Reproduction and Development*, 31:189–197.

Willis, B. L. and Oliver, J. K. (1990). Direct tracking of coral larvae: implications for dispersal studies of planktonic larvae in topographically complex environments. *Ophelia*, 32:145–162.

Wilson, G. A., Strobeck, C., Wu, L., and Coffin, J. W. (1997). Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. *Molecular Ecology*, 6:697–699.

Wing, S. R., Gibbs, M. T., and Lamare, M. D. (2003). Reproductive sources and sinks within a sea urchin, *Evechinus chloroticus*, population of a New Zealand fjord. *Marine Ecology Progress Series*, 248:109–123.

Winter, D. F. (1983). A theoretical model of surf zone circulation and diatom growth. *In: Sandy Beaches as Ecosystems (McLachlan, A and Erasmus, T. eds) pp 157-167. Dr W Junk Publishers, The Hague.*

Wolanski, E., Burrage, D., and King, B. (1989). Trapping and dispersion of coral eggs around Bowden Reef, Great Barrier Reef, following mass coral spawning. *Continental Shelf Research*, 9:479–496.

Wolanski, E. and Hamner, W. M. (1988). Topographically controlled fronts in the ocean and their biological influence. *Science*, 241:177–181.

Wolff, M. and Mendo, J. (2000). Management of Peruvian bay scallop (*Argopecten purpuratus*) metapopulation with regard to environmental change. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 10:117–126.

Workman, C. (1983). Comparisons of energy partitioning in contrasting age-structured populations of the limpet *Patella vulgata* L. *Journal of Experimental Marine Biology and Ecology*, 68:81–103.

Wright, J. R. and Hartnoll, R. G. (1981). An energy budget for a population of the limpet *Patella vulgata*. *Journal of the Marine Biological Association U.K.*, 61:627–646.

Wright, S. (1943). Isolation by distance. *Genetics*, 28:114–138.

Wright, W. G. (1989). Intraspecific density mediates sex-change in the territorial patellacean limpet *Lottia gigantea*. *Marine Biology*, 100:353–364.

Wright, W. G. and Lindberg, D. R. (1979). A non-fatal method of sex determination for patellacean gastropods. *Journal of the Marine Biological Association U.K.*, 59:803.

Wright, W. G. and Lindberg, D. R. (1982). Direct observation of sex change in the patellacean limpet *Lottia gigantea*. *Journal of the Marine Biological Association U.K.*, 62:737–738.

Yonge, C. M. (1949). *The sea shore.* The new naturalist: a survey of British natural history. Collins, London.

Yund, P. O. (2000). How severe is sperm limitation in natural populations of marine free-spawners? *Trends in Ecology and Evolution*, 15:10–13.